Analysis of Lard Adulterated in Beef Sausages: Influence of n-hexane Concentration and Maceration Time

Desi Ardilla[1*], Muhammad Taufik[2], Dafni Mawar Tarigan[1], Muhammad Thamrin[1], Mariany Razali[3], Revi Trisna Siregar[1], Irfan Kurniawan[1], Nur Waridah Angriani Nasution[1], Rika Astuti Pulungan[1] and Sri Dewi Sihotang[4]

[1]*Agricultural Technology Department, Universitas Muhammadiyah Sumatera Utara, Medan, Indonesia*
[2]*Chemistry Department, Universitas Sumatera Utara, Medan, Indonesia*
[3]*Pharmacy Department, Universitas Tjut Nyak Dhien, Medan, Indonesia*
[4]*Pharmacy Department, Universitas Muslim Nusantara Al Washliyah, Medan, Indonesia*

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Abstract: Prime food products must pay attention to product halal factors that have an impact on consumers. some products that are circulating in the general public are often adapted with other ingredients. Beef sausages is a processed product that is often adulterated with lard. This work aims to study the comparison of solven concentration (n-hexane) and maceration time on the analysis of beef sausage hat were added with lard. The Factorial Completely Randomized Design (CRD) was developed used two (2) replications. Factor I was the Solvent Concentration (C) which consists of four levels : C_1 = 20%, C_2 = 30%, C_3 = 40%, C_4 = 50% and Factor II was the maceration time (W) consisting of four levels : W_1 = 6 Hours, W_2 = 12 Hours, W_3 = 18 Hours, W_4 = 24 Hours. The results showed that the concentration of n-hexane in beef sausages adulterated with lard gave a significant difference (P> 0.01) on specific gravity, a significantly different effect (P> 0.01) on iodine numbers. Real and tangible realization (P <0.05) to iodine numbers. The maceration gives a significant difference (P> 0.01) to the specific gravity, the difference is significantly different (P> 0.01) with respect to iodine number. The Concentration of lard at C_1W_1, C_2W_2, C_3W_3 and C_4W_4 were analyzed using UV spectroscopy respectively 23, 35, 41, and 54%.

1 INTRODUCTION

At the present time it often happens important food ingredients in the form of processed or raw from other countries to Indonesia without going through in-depth testing. The rate of increase in population followed by improvement in living standards and changes in consumer tastes have changed consumption patterns that lead to animal protein (Fadzlillah et al., 2011). Meat is a food that is widely used as an ingredient for food diversification, because meat contains many nutrients and can be processed into a variety of dishes (Liu et al., 2018). The increase in beef consumption has not been offset by an increase in domestic production, both in quality and quantity, resulting in an increasingly large gap between demand and supply. Based on the prognosis of beef production in the country in 2017 amounted to 354.770 tons, while the estimated domestic demand for beef in 2017 amounted to 604.968 tons (Doosti & Dehkordi, 2014).

The halalness of a food product is very important to be considered in consuming food products (Sukmawati, 2018). For the category of processed food, the halal status of a food product is highly dependent on halal and the prohibition of raw materials and additives about food (Hilda & Si, 2014). Explanation of the Food Law states that the implementation of food safety for activities or processes of food production for consumption must be done through food sanitation, regulation of food additives, setting food packaging standards, providing food safety guarantees and food quality, and guaranteeing halal products for the required (Burlian, 2013).

One of the halal concepts in Islam is that food does not contain "lard" or food fat derived from pigs.

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The presence of this component of lard, as low as any of its ingredients in food, will bring the food to be forbidden for consumption (Mayasari, 2019). Several studies have been conducted to find the right method to detect the presence of lard in food. In previous studies identification of lard in vegetable oils was carried out with Gas-Liquid Chromatography combined with multivariate analysis. Using GLC, lard of 2% can be detected in vegetable oils (Naquiah et al., 2017). In addition to GLC, HPLC has also been used to identify lard by 5% in meat products. However, most of these methods require a lot of time and are impractical to apply. This requires the development of a fast and practical method for identification of lard (Lopes et al., 2017).

Another method that can be used to identify the presence of lard in beef is the Fourier Transform Infra Red (FTIR) method to analyze the presence of lard in goat and beef fat combined with multivariate PLS analysis. Multivariate PLS analysis was used to detect lard in the mixture with goat fat for detection of lard in the mixture with beef fat. Lard identification with FTIR combined with Discriminant Analysis and PLS Analysis (Rohman & Fadzillah, 2018). The FTIR method has the potential to be used as a tool to detect lard quickly with consistent results. This is because FTIR can provide analysis results of fat from pigs that are mixed with other fats consistently, even with a low content . The weakness of the FTIR method is only able to detect contamination if the prediction model is made according to the sample being tested. This becomes very difficult if the food sample to be tested is unclear, and its exact composition is not known (Fadzlillah et al., 2011). This work aims to study the comparison of hexane concentration and time to the analysis of beef sausage which has been adulterated with lard

2 METHOD

Materials

The ingredients used in this research are processed sausage and lard products. The chemicals used in this study were n-hexane, PP indicator, KOH / NaOH, amylum indicator, Diethyl Ether, Na_2SO_4, Ethanol, Aquades, Iodine, KI, $CHCl_3$, Saturated Solution, , Nutriet Agar, and Chloroform.

Equipment

In this work, we used erlenmeyer, biuret, glass beaker drop pipette separating funnel, analytical balance, gloves, test tube, knife, clamp, oven, laminar, incubator, autoclave, spreader, flannel, tube racks ice cream cup, hotplate, stirrer, cotton, plastic wrap and petridish.

Research method

This research method was described a factorial completely randomized design (CRD) method consisting of 2: Factor I: Solvent Concentration consisting of 4 levels, They are : C_1 = 20%, C_2 = 30%, C_3 = 40%, C_4 = 50% . Factor II: Maceration Time consisting of 4 levels, namely: W_1 = 6 Hours, W_2 = 12 Hours, W_3 = 18 Hours, W_4 = 24 Hours. The number of treatment combinations (Tc) is 4 x 4 = 16, so the number of replications (n) wasv as follows:

$$Tc\ (n-1) \geq 15$$
$$16\ (n-1) \geq 15$$
$$16\ n-16 \geq 15$$
$$16\ n \geq 31$$
$$n \geq 1{,}937 \text{.....rounded to } n = 2$$

then for the accuracy of the study, repeated 2 (two) times.

Preparation and maceration

Samples to be tested are processed products of pork sausage and lard. Both samples were mixed. Sample Extraction Preparation, including weighing the sample (pork sausage and lard), smoothed material (cut into cubes), put into a container, then add the solvent according to the concentration and maceration according to the specified factors.

Observation parameters

Observation parameters are based on analysis which includes: specific gravity and iodine numbers.

Spectrofotometry UV analysis

UV spectroscopy Beckman DU640 UV / Vis was used analyzing the lard contained in beef corneds. In this work, the wavelangth was used in the range 200 - 400 nm. The standard of lard was determined at 5, 10, 15, 20, 25%.

3 RESULT

3.1 Statistical Test Measurement

Base of the statistical tests, in general it shows that the concentration of n-hexane affects the observed parameters. Data on the average observations of the effect of n-hexane concentration on each parameter can be seen in Table 1.

Table 1: Effect of N-Hexane concentration.

n-hexanne Concentration (C) (%)	Specific gravity (g/ml)	Iodine number (mg/g)
K_1 = 20 %	0.950	70.397
K_2 = 30 %	0.959	71.920
K_3 = 40 %	0.975	74.965
K_4 = 50 %	0.993	76.584

Maceration time after being tested statistically ifluences the observed parameters. Data on the average observation results of maceration time can be seen in Table 2.

Table 2: Effect of maceration time.

n-hexanne Concentration (C) (%)	Specific gravity (g/ml)	Iodine number (mg/g)
W_1 = 6	0.942	69.850
W_2 = 12	0.958	73.251
W_3 = 18	0.972	74.314
W_4 = 24	1.002	76.452

Table 2 shown the effect of maceration time on specific gravity and iodine number acid number, iodine number, and the number of microbes was increasing.

3.2 Specific Gravity

Effect of N-Hexane Concentration

Based on the list of variance it can be seen that the concentration of N-Hexane has a significantly different effect (P> 0.01) on specific gravity. The level of difference has been tested with the average difference test can be seen in Table 3.

Table 3: Average difference test results on the effect of maceration time on specific gravity.

Distance	LSR		Treatment	Average	Notation	
	0,05	0,01	C		0,05	0,01
-	-	-	20	0.950	cd	CD
2	0.017	0.021	30	0.962	bc	BC
3	0.018	0.022	40	0.975	b	AB
4	0.018	0.023	50	0.993	a	A

Table 3 shown that C_1 is not significantly different from C_2 and very significantly different from C_3, and C_4. C_2 is not significantly different from C_3 and C_4. C_3 is not significantly different from C_4. The highest type of weight is in the K4 treatment that is 0.994 gr / ml and the lowest is in the K1 treatment that is equal to 0.945 gr / ml. For more details can be seen in Figure 1.

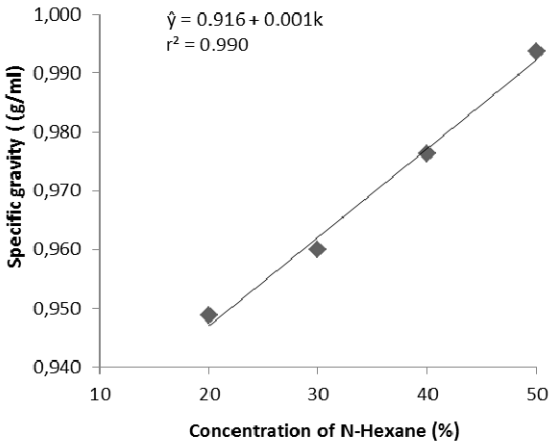

Figure 1: Relationship of N-Hexane solvent concentration to weight.

Figure 1 shown the concentration of n-Hexane to specific gravity. The more concentration of n-hexane solvent used, the specific gravity is also increasing, namely 0.994 g / ml. This is because the number of other components contained in beef sausage and mixing with the components contained in lard causes the specific gravity increases. The substance has a large mass then the probability of its specific gravity also becomes greater. The high specific gravity produced from the sample is caused by other compositions contained in lard.

Effect of maceration time

Based on the list of variance, it can be seen that the maceration time has a very significant effect (P> 0.01) on specific gravity.

The level of difference has been tested with the average difference test can be seen in Table 4.

Table 4: Average difference test results on the effect of maceration time on specific gravity.

Distance	LSR		Treatment	Average	Notation	
	0,05	0,01	W		0,05	0,01
-	-	-	6	0.943	d	CD
2	0.016	0.022	12	0.960	bc	BC
3	0.017	0.023	16	0.968	b	B
4	0.017	0.024	24	1.009	a	A

Note: Different letters in the notation column show significantly different effects at the level (P <0.05) and very significantly different at the level (P> 0.01).

Table 4 shown that W_1 is not significantly different from W_2, and very significantly different from W_3 and W_4. W_2 is not significantly different from W_3 and very significantly different from W_4. W_3 is very different from W_4. The highest type of weight was found in the W_4 treatment that was 1,009 gr / ml and the lowest was in the W_1 treatment which was 0,943 g / ml. For more details can be seen in Figure 2.

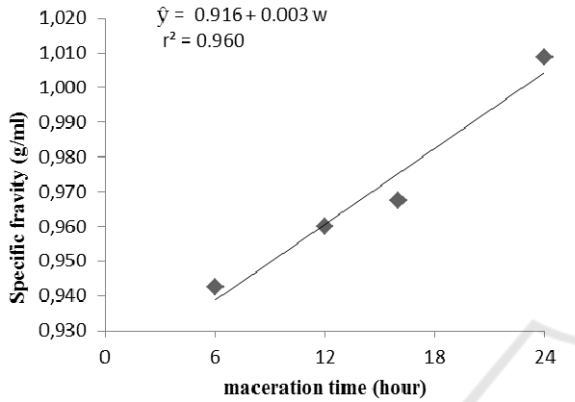

Figure 2: Relationship of macerated time to weight of types of processed products of beef sausage adulterated with lard.

Figure 2 shown the maceration time of specific gravity in beef sausages adulterated. The longer of the extraction time, the greater the specific gravity value obtained. Maceration is done by immersing simplex powder in a liquid solution. The search liquid will penetrate the cell wall and enter the cell cavity which contains the active substance, the active substance will be dissolving at the difference concentration. The active substance solution was pushed out. Figure 2. shown the greater the comparison of maceration time, the resulting yield increases. The longer the maceration time used would also increase the amount of dissolved compounds (Mas et al., 2019). As a result, the extraction rate will increase.

3.3 Iodin Number

The concentration N-hexane effect

Based on the list of variance, it can be seen that the concentration of N-Hexane (solvent) has a significantly different effect (P> 0.01) on the iodine numbers. The level of difference has been tested with the average difference test can be seen in Table 5.

Tabel 5: Average difference test results effect of the concentration of N-Hexane on iodine numbers.

Distance	LSR		Treatment	Average	Notation	
	0,05	0,01	C		0,05	0,01
-	-	-	6	69.859	d	D
2	0.589	0.811	12	73.254	c	C
3	0.618	0.852	18	74.300	b	B
4	0.634	0.873	24	76.459	a	A

Table 5 shown that C_1 (Concentration 1) differs very significantly from C_2 (Concentration 2), C_3 (Concentration 3) and C_4 (Concentration 4). C_2 (Concentration 2) was very different than C_3 (Concentration 3) and C_4(Concentration 4). The C_3 was very different than C4 (Concentration 4). The highest iodine number in C_4 treatment is 76.459 mg /g and the lowest in C_1 treatment is 69.859 mg /g. This data was showed at Figure 3.

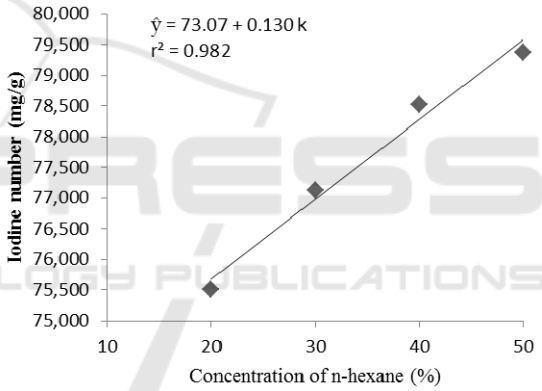

Figure 4: Relationship of N-Hexane solvent concentration to iodine numbers.

Figure 4 shown the concentration of n-hexane with iodine number. Iodine number gave a determination of the level of unsaturation of a fat or oil, therefore iodine number is equal to the unsaturation of an acid or fat. From the above data in this study produced a fairly high of iodine number. Where the more the concentration of n-hexane solvent is used, the iodine number was increasing at 76.459 mg / g. This is due to the fact that n-hexane has better solubility or reaction (Nees et al., 2017). If iodine number is getting higher, then the double bond contained in lard will also be more. The more of double bonds in the melting point of lard will be lower and cause lard to be more easily oxidized so that the number of peroxides is higher (Liu et al., 2018).

Effect of maceration time

Based on the list of variance it can be seen that the maceration time has a very significant effect (P> 0.01) on iodine numbers.

3.4 Analysis UV Spectroscopy Data

UV spectroscopy using DU640 UV / Vis type using a wavelength of 200-400 nm. The standard lard solution is made in concentrations of 5, 10, 15, 20, 25%. This method measured relative light energy if the energy obtained from the sample is transmitted, reflected or emitted as a function of the wavelength. In this work, UV-Vis (Ultra Violet-Visible) spectrophotometer was chosen from many instruments commonly used in analyzing lard in animals. Spectrophotometers are commonly used because of their ability to analyze so many chemical compounds and their practicality in terms of sample preparation when compared with several methods of analysis. The concentration of the solution analyzed will be proportional to the amount of light absorbed by the substance contained in the solution. The optimum wavelength is obtained at 270 nm with the absorbance value obtained in Figure 5.

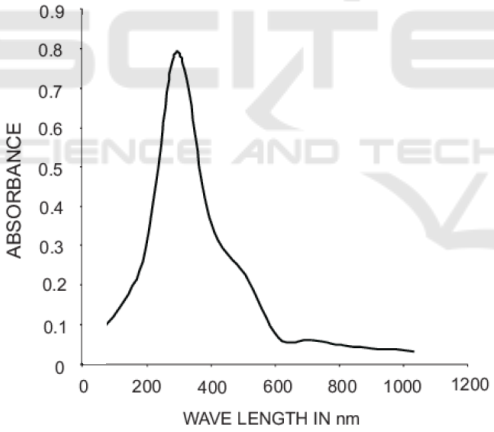

Figure 5: UV spectrum at 270 nm.

Figure 5 shown that the UV spectral at 270 nm of the optimum wavelength. The Concentration of lard at W1K1, W2K2, W3K3 and W4K4 were analyzed using UV spectroscopy respectively 23, 35, 41, and 54%.

4 CONCLUSIONS

Beef sausages was adulterated with lard have spesification of physical properties. This product that is often adulterated with lard. The concentration of n-hexane in beef sausages adulterated with lard was a significant difference (P> 0.01) on specific gravity, gave a significantly different effect (P> 0.01) on iodine numbers, and not significant (P <0.05) of acid numbers. Real and tangible realization (P <0.05) to iodine numbers. The maceration gives a significant difference (P> 0.01) to the specific gravity, the difference is significantly different (P> 0.01) with respect to iodine, the difference was not significant (P <0.05) with respect to the acid number. The Concentration of lard at W_1K_1, W_2K_2, W_3K_3 and W_4K_4 were analyzed using UV spectroscopy respectively 23, 35, 41, and 54%.

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