

ANALYSIS OF THE PHYSICAL PROPERTIES FOR COW NUGGETS MIXED WITH LARD IN ORDER TO IMPROVE HALAL FOOD SAFETY

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ABSTRACT.

The halalness of a food product is very important to be taken into consideration in consuming food products. Processing of beef nuggets must be in accordance with processing standards and not mixed with other compounds (lard). The Lard is often mixed into beef nuggets for reasons of increasing flavor. The n-hexane solvent and maceration time were varied in order to determine the physical properties of the sample. This study aims to determine the effect of n-hexane concentration and maceration time on the analysis of cow nuggets mixed with lard. Complete Randomized Design (CRD) factorial was carried out with 2 (two) replications. Factor 1 was chosen variation of n-hexane concentration (symbol K) consisting of 4 levels, namely K1 = 20%, K2 = 30%, K3 = 40%, K4 = 50%. Factor 2 was chosen maceration time (symbol W) consisting of 4 levels, W1 = 6 hours, W2 = 12 hours W3 = 18 hours, W4 = 24 hours. Parameters observed included Specific Weight, Iodine Numbers, Acid Numbers and Total Microbes. The results showed that the effect of n-hexane concentration had a very significant different effect on the level ($P > 0.01$) of the specific gravity, iodine number, acid number, and total microbes. The maceration time had a very significant effect on the level ($P > 0.01$) of the specific gravity of iodine numbers and total microbes, and the effect was significantly different on the level ($P > 0.05$) of acid numbers. Interaction treatment regarding the effect of n-hexane concentration and maceration time had a very significant different effect on the level ($P > 0.01$) of iodine number, and the effect on the non-significant level ($P > 0.05$) on specific gravity, acid number and total microbes.

Keywords: nugget, cow, lard, concentration, halal food.

A. INTRODUCTION

Meat is a food ingredient that contains lots of nutrients and can be processed into various kinds of dishes [22]. In Indonesia, increased consumption of beef has not been offset by an increase in domestic production, both in quality and quantity. This shows a significant difference between demand and supply. In 2017, beef production in Indonesia reached 354,770 tons, while the estimated demand for beef was 604,968 tons [9]. Beef contains 7.9 g of fat and 73.1 mg of cholesterol in 85.05 g of beef.

The halalness of a food product is very important to be taken into consideration in consuming food products. For the category of processed food, the halalness of a food product is highly dependent on halal and unclean raw materials and additional food. Food security for food production activities to be consumed must be carried out through food sanitation, regulation of food additives, establishment of food packaging standards, provision of food safety and food quality guarantees, as well as guarantees of halal products for those required [8].

Pigs are one of the livestock commodities that have the potential as a mixture of fresh meat. This is because pig has the characteristics of having fast growth, good ration efficiency (75-80%), high percentage of carcass (65-80%) and high number of children per birth (litter size) [26]. The price of pork is cheaper compared to beef. This causes pork

is often used as a mixture in making sausages. Food cases contaminated with unclean additives such as oplosan meatballs are still widely circulating in Indonesia. This, as evidenced by the regulation of the Food and Drug Supervisory Agency (BPOM) of five jerky and shredded meat on the market positive for pork, in early 2009 included beef jerky / savory shredded beef head 250 grams, shredded beef jerky cap Limas 100 gram, abon / beef jerky native cow ACC cap, beef jerky special Beef Jerky Lezaaat brand, special beef jerky No. 1 stamp 999. The recent case of pork mixing is the mixing of beef with pork is rampant reported on mass media both print and electronic. One example in the second news was reported about the mixing of beef with pork in order to gain big profits in Lubuklinggau [29]. In Islam, the halal concept known as food does not contain pigs or their derivatives. The presence of this component of lard causes these foods to be haram for consumption.

Pig fat is obtained from the body parts of pigs that have high concentrated fat tissue. The best quality pork fat is obtained from the parts around the kidneys and inside the waist meat. The best quality pork fat is also obtained from the back, between the muscle and hard fat. The lowest quality pork fat is obtained from the fat found around the digestive organs [18]. The fat in the pig's body can be converted into lard as a food ingredient through two kinds of processes namely wet and dry. The nutritional content of fresh pork is as follows:

70.98% water; 20.79% protein; 0.89% fat; 20.24% ca and 0.21% P (Rompis and Komansilan, 2014). There are some fundamental differences between beef and pork. In plain view there are five aspects that look different between beef and pork, namely:

1. *Color*

Pork has a paler color than beef. The color of pork is close to the color of chicken meat, besides that there are certain parts of pork which are very similar in color to beef so it is very difficult to distinguish them.

2. *Fiber*

In terms of fiber differences are clearly seen between these two meats. In cattle, the meat fibers appear solid and the fiber lines are clearly visible. Whereas in pork, the fibers look faint and very tenuous. This difference becomes clearer when the two meats are stretched together.

3. *Fat appearance*

Pork has a more elastic fat texture while beef fat is more rigid and shaped. In addition fat in pigs is very wet and difficult to remove from meat while meat fat is rather dry and looks fibrous. In certain parts such as the kidneys, the appearance of lard is almost similar to cow fat. Texture Beef has a more rigid and dense texture, compared to soft and stretchable pork. Pork meat is very chewy and easily recycled. While the beef feels solid and hard so it is quite difficult to stretch.

4. *Fragrant*

There is a slight difference between the two. Pork has its own distinctive fragrant, while the fragrant of beef is rancid as we know it. But the ability to differentiate through this fragrant requires repeated practice because the difference is not too significant [20].

Several studies have been conducted to find the right method for detecting the presence of lard in food.

In previous studies lard identification in vegetable oil was carried out with Gas-Liquid Chromatography combined with multivariate analysis. By using GLC, lard of 2% can be detected in vegetable oil. 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 not practical to implement. For this reason, it is necessary to develop a method that is fast and practical for identification of lard.

Another method that can be done 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 cow fat combined with multivariate PLS analysis. Multivariate PLS analysis was used to detect lard in a mixture with goat fat for lard detection in mixtures with cow fat. Lard identification with FTIR is combined with Discriminant Analysis and PLS Analysis (Permana,

2014). The FTIR method has the potential to be used as a fast detection tool for pork fat with consistent results. This is because FTIR can provide fat analysis results from pigs that mix with other fat 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 in accordance with the sample being tested. This becomes very difficult if the food sample to be tested is not clear from its origin, and it is not known exactly what the composition is.

A simple method in testing the physical properties of lard mixed in processed beef will be developed considering this method is very important in checking quickly, accurately and currying on processed beef products mixed with lard. In this study, a maceration method with time variation and n-hexane solvent concentration will be developed. This study aims to determine the effect of n-hexane concentration and maceration time on the analysis of cow nuggets mixed with lard.

B. MATERIALS AND METHOD

The ingredients used in this study were beef sausage and lard. The chemicals used in this study were n-hexane, KOH / NaOH, Na₂SO₄, indicator PP, indicator amylum, diethyl ether, ethanol, aquades, iodine, CHCl₃, saturated solutions of KI, chloroform, and nutrient agar.

The equipment used is erlenmeyer, glass beker, biuret, separating funnel, drop pipette, analytic balance, knife, glove, test tube, clamp, oven, incubator, autoclave, laminar, spreader, flannel, hotplate, stirrer, tube rack, cotton and petridis dishes.

This research method was carried out by factorial completely randomized design method consisting of 2, namely: Factor I: Solvent Concentration consisting of 4 levels, namely: K1 = 20%, K2 = 30%, K3 = 40%, and K4 = 50%. Factor II: Maseration Time which consists of 4 levels, namely: W1 = 6 Hours, W2 = 12 Hours, W3 = 18 Hours, and W4 = 24 Hours

Sample preparation and extraction

The samples to be tested are cow nuggets and lard. Samples were collected at the UMSU Medan Agricultural Products Technology Laboratory. The sample was weighed, mashed, then added n-hexane as a solvent with the appropriate concentration and maceration time, filtered with a flannel cloth. Observation parameters include determination of specific gravity, determination of iodine number, determination of acid numbers and total determination of microbes.

C. RESULTS AND DISCUSSION

Based on the results of the research and statistical tests, it generally shows that the concentration of n-hexane affects the observed

parameters. The average data from the observation of the effect of n-hexane concentration on each

parameter can be seen in **Table 1** below:

Table 1. Effect of N-hexane concentration on the parameters of cow nuggets mixed with pork fat

N-Hexane Concentration (K) (%)	density (g/ml)	Iodin Number (mg/g)	Acid numbers (mg KOH/g)	Number of microbes (CFU/ml)
K ₁ = 20 %	0,949	70,398	1,599	15837.5
K ₂ = 30 %	0,960	71,921	1,655	12737.5
K ₃ = 40 %	0,976	74,966	1,907	11662.5
K ₄ = 50 %	0,994	76,586	2,020	10087.5

Based on **Table 1**. It can be seen that the effect of N-hexane concentration on the weight of species, acid numbers and iodine numbers is increasing, while the number of microbes is

decreasing. After being tested statistically, Maseration Time affects the observed parameters. The average data of observations of maceration time can be seen in **Table 2** below:

Table 2. Effect of Maseration Time on the Parameters of Cow Nugget Mixed with Pig Fat

Maseration time (W) (hour)	density (g/ml)	Iodin Number (mg/g)	Acid numbers (mg KOH/g)	Number of microbes (CFU/ml)
W ₁ = 6	0,943	69,857	1,655	10425.0
W ₂ = 12	0,960	73,254	1,683	11062.5
W ₃ = 18	0,968	74,300	1,823	12650.0
W ₄ = 24	1,009	76,459	2,020	16187.5

Based on **Table 2**, it can be seen that the Effect of Maseration Time on Specific Weights, Acid Numbers, Iodine Numbers, and the number of microbes is increasing. After being tested statistically, the concentration of n-hexane affected

the observed parameters. The average data from the observation of the effect of n-hexane concentration on each parameter can be seen in the following **Table 3**:

Table 3. Effect of N-Hexane Concentration on Parameters of Cow Nuggets without Pork Fat

N-Hexane Concentration (K) (%)	density (g/ml)	Iodin Number (mg/g)	Acid numbers (mg KOH/g)	Number of microbes (CFU/ml)
K ₁ = 20 %	0,949	70,398	1,599	9487,5
K ₂ = 30 %	0,960	71,921	1,655	7762,5
K ₃ = 40 %	0,976	74,966	1,907	5575,0
K ₄ = 50 %	0,994	76,586	2,020	3887,5

Based on **Table 3** above it can be seen that the effect of N-hexane concentration on the weight of species, acid numbers, and iodine numbers is increasing, while the number of microbes

decreases. After being tested statistically, maceration time affects the observed parameters. The average data of observations of maceration time can be seen in **Table 4** below:

Table 4. Effect of Maseration Time on Parameters of Cow Nugget without Pig Fat (P1)

Maseration time (W) (hour)	density (g/ml)	Iodin Number (mg/g)	Acid numbers (mg KOH/g)	Number of microbes (CFU/ml)
W ₁ = 6	0,945	75,000	1,571	5387.5
W ₂ = 12	0,960	77,316	1,627	6200.0
W ₃ = 18	0,968	77,950	1,767	8450.0
W ₄ = 24	1,001	80,263	1,811	9062.5

Based on **Table 4** above, it can be seen that the Effect of Maseration Time on Specific Weights, Acid Numbers, Iodine Numbers, and the number of microbes is increasing.

Specific Weight

Effect of N-Hexane Concentration on Beef Nuggets Mixing Pig Fat

Based on the variance list, it can be seen that the N-Hexane concentration had a significant effect ($P > 0.01$) on the specific gravity. The level of

difference has been tested by the average difference

test can be seen in **Table 5**.

Table 5. Different Test Results on the Average Effect of N-Hexane Concentration on Specific Weights

Distance	LSR		Treatment K	Mean	Notation	
	0,05	0,01			0,05	0,01
-	-	-	20	0.949	cd	CD
2	0.016	0.022	30	0.960	bc	BC
3	0.017	0.023	40	0.976	b	AB
4	0.017	0.024	50	0.994	a	A

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

Based on **Table 5** above, it can be seen that K1 differs not significantly from K2 and differs very significantly from K3 and K4. K2 differs not significantly from K3 and K4. K3 differs not significantly from K4. The highest specific gravity was found in K4 treatment which was 0.994 gr / ml and the lowest was in K1 treatment which was 0.949 gr / ml. The higher the concentration of n-hexane solvent used, the higher specific gravity is 0.994 gr / ml. This is due to the many other

components contained in cow nuggets and mixing with the components contained in lard resulting in increasing specific gravity.

Effect of Maseration Time on Cow Nuggets Mixed with Pig Fat

Based on the variance list, it can be seen that the Maseration Time has a very significant different effect ($P > 0.01$) on the specific gravity. The level of difference has been tested by the average difference test can be seen in **Table 6**.

Table 6. Different Test Results on the Average Effect of Maseration Time on Specific Weights

Distance	LSR		Treatment K	Mean	Notation	
	0,05	0,01			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 indicate the effect significantly different at level ($P < 0.05$) and very different at real level ($P > 0.01$)

Based on **Table 6** above, it can be seen that W1 is not real with W2, and is very real with W3 and W4. W2 differs not significantly from W3 and differs very significantly from W4. W3 differs very significantly from W4. The highest specific gravity was found in W4 treatment, which was 1.009 g / ml and the lowest was in W1 treatment which was 0.943 g / ml.

The longer the extraction time, the greater the specific gravity obtained. Maseration is done by soaking simple powder in the liquid of the dancer. The liquid will penetrate the cell wall and enter the cell cavity containing the active substance, the active substance will dissolve due to differences in concentration between the solution of the active

substance in the cell, the concentrated solution is pushed out. From the graph above, it can be seen that the greater the ratio of maceration time, the resulting yield increases. Armanzah Syarief R et al (2016) stated that the longer maceration time used will increase the amount of dissolved compounds. As a result the extraction rate will increase.

Effect of N-Hexane Concentration on Cow Nuggets without Mixing Pig Fat (P1)

Based on the variance list, it can be seen that the N-Hexane concentration had a significant effect ($P > 0.01$) on the specific gravity. The level of difference has been tested by the average difference test can be seen in **Table 7** below:

Table 7. Different Test Results on the Mean Effect of N-Hexane Concentration on Specific Weights

Distance	LSR		Treatment K	Mean	Notation	
	0,05	0,01			0,05	0,01
-	-	-	20	0.949	abc	ABC
2	0.016	0.023	30	0.960	d	BCD
3	0.017	0.024	40	0.978	ab	AB
4	0.018	0.024	50	0.995	a	A

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

Based on **Table 8**. It can be seen that K1 is not significantly different from K2, K3 and K4. K2 differs not significantly from K3 and K4. K3 differs not significantly from K4. The highest specific gravity was found in K1 treatment which was 0.995 g / ml and the lowest was in K4 treatment which was 0.949 g / ml. The more n-hexane solvent concentration used, the more specific density is 0.995 g / ml. This is most likely related to the non-polar nature of n-hexane solvents. Where according to Wildan Achmat et al (2013), the effect of hexane solvent concentration on the volume of fat

produced is that the heavier the sample used and the higher the concentration of hexane used, the greater the weight of the type of fat produced.

Effect of Maseration Time on Beef Nuggets without Pork Fat (P1)

Based on the variance list, it can be seen that the Maseration Time has a very significant different effect ($P > 0.01$) on the specific gravity. The level of difference has been tested by the average difference test can be seen in **Table 8** below:

Table 8. Different Test Results on the Average Effect of Maseration Time on density

Distance	LSR		Treatment K	Mean	Notation	
	0,05	0,01			0,05	0,01
-	-	-	6	0.945	D	D
2	0.014	0.020	12	0.965	Bc	BC
3	0.015	0.021	18	0.970	B	B
4	0.016	0.021	24	1.004	A	A

Note : Different letters in the notation column indicate the effect significantly different at level ($P < 0.05$) and very significantly different at level ($P > 0.01$)

Based on Table 8 above it can be seen that W1 differs very significantly from W2, W3, and W4. W2 differs not significantly from W3 and differs very significantly from W4. W3 differs very significantly from W4. The highest specific gravity was found in W4 treatment which was 1.004 g / ml and the lowest was in W1 treatment which was 0.945 g / ml. The results of this study indicate that the longer the extraction time, the higher the yield produced. This is consistent with the statement put forward by Irawan (2010), that short extraction times will give low results because not all components are extracted.

Iodin number

Effect of N-Hexane Concentration on Beef Nuggets Mixing Pig Fat

Based on the variance list, it can be seen that the concentration of N-hexane has a significantly different effect ($P > 0.01$) on iodine numbers. The level of difference has been tested with the average difference test can be seen in **Table 9** below:

Table 9. Different Test Results on the Effect of N-Hexane Concentration on Iodine Numbers

Distance	LSR		Treatment K	Mean	Notation	
	0,05	0,01			0,05	0,01
-	-	-	20	70.398	d	D
2	0.589	0.811	30	71.921	c	C
3	0.618	0.852	40	74.966	b	B
4	0.634	0.873	50	76.586	a	A

Note : Different letters in the notation column indicate the effect significantly different at level ($P < 0.05$) and very significantly different at level ($P > 0.01$)

Based on **Table 9** above, it can be seen that K1 differs very significantly from K2, K3, and K4. K2 differs very significantly from K3 and K4. K3 differs very significantly from K4. The highest iodine number is found in K4 treatment which is 76.459 mg / g and the lowest is in K1 treatment which is 69.859 mg / g.

Iodine number is the determination of the level of unsaturation of an oil / fat, therefore the iodine number is the same as the unsaturation of an acid or fat. From the above data in this study produced a high iodine number. Where the more n-hexane

solvent concentration is used, the iodine number increases by 76.459 mg / g. This is because n-hexane solubility or reaction is more perfect.

Effect of Maseration Time on Cow Nuggets Mixed with Pig Fat

Based on the variance list (Appendix 3) it can be seen that the Maseration Time has a very significant different effect ($P > 0.01$) on iodine numbers. The level of difference has been tested by the average difference test can be seen in **Table 10**.

Table 10. Different Test Results on the Average Effect of Maseration Time on Iodin number

Distance	LSR		Treatment K	Mean	Notation	
	0,05	0,01			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

Note : Different letters in the notation column indicate the effect significantly different at level ($P < 0.05$) and very different at real level ($P > 0.01$)

Based on Table 10 above, it can be seen that W1 differs very significantly from W2, W3 and W4. W2 differs very significantly from W3 and W4. W3 differs very significantly from W4. The highest iodine number is found in W4 treatment which is 1.009 mg / g and the lowest is in W1 treatment which is 0.943 mg / g. The longer the maceration process, the higher the number of iodine numbers obtained. A high iodine value indicates that the oil has good quality and low damage. Where according to Margareta et al. (2011) the longer the extraction time, the higher the yield. In this study fat yield with the highest 24-hour extraction time. This indicates that the equilibrium condition has been reached. The equilibrium condition in extraction is a state in which the solute

present in the material cannot dissolve again in the solvent and the concentration of fat in the solution remains the same before decreasing.

The Influence of Iodine Number Interaction Between N-Hexane and Maseration Time Concentration on Processed Products Mixed in Pigs

Based on the variance list, it can be seen that the interaction of the treatments had a very significant effect ($P > 0.01$) on iodine numbers. LSR test results of the interaction effect of iodine number between n-hexane concentration and maceration time on processed products mixed with pork can be seen in **Table 11**.

Table 11. Average Difference Test Results say iodine between n-hexane concentration and maceration time for processed products mixed with pork

Distance	LSR		Treatment K	Mean	Notation	
	0,05	0,01			0,05	0,01
-	-	-	K ₁ W ₁	65.480	P	P
2	1.1775	1.6210	K ₂ W ₁	67.640	O	KLMNO
3	1.2364	1.7035	K ₃ W ₁	72.205	Jk	HIJK
4	1.2678	1.7467	K ₄ W ₁	74.110	fgh	CDEFG
5	1.2953	1.7820	K ₁ W ₂	70.810	N	KLMN
6	1.3110	1.8055	K ₂ W ₂	72.205	Jk	HIJ
7	1.3227	1.8330	K ₃ W ₂	74.110	fgh	CDEF
8	1.3306	1.8526	K ₄ W ₂	75.890	D	C
9	1.3384	1.8683	K ₁ W ₃	71.570	jklm	KL
10	1.3463	1.8801	K ₂ W ₃	72.590	ij	FGHI
11	1.3463	1.8919	K ₃ W ₃	75.630	de	CD
12	1.3502	1.8997	K ₄ W ₃	77.410	bc	ABC
13	1.3502	1.9076	K ₁ W ₄	73.730	ghi	EFGH
14	1.3541	1.9154	K ₂ W ₄	75.250	def	CDE
15	1.3541	1.9233	K ₃ W ₄	77.920	ac	AC
16	1.3581	1.9272	K ₄ W ₄	78.935	a	A

Note : Different letters in the notation column indicate the effect significantly different at level ($P < 0.05$) and very different at real level ($P > 0.01$)

Based on **Table 11**, it can be seen that the highest average value is at a concentration of 50% and an 18-hour maceration time of 77.410 mg / g. The lowest average value is at a concentration of 20% and a maceration time of 6 hours is 65.480 mg

/ g. The relationship between the iodine number between n-hexane concentration and maceration time for processed products mixed with pork can be seen in **Figure 1** below:

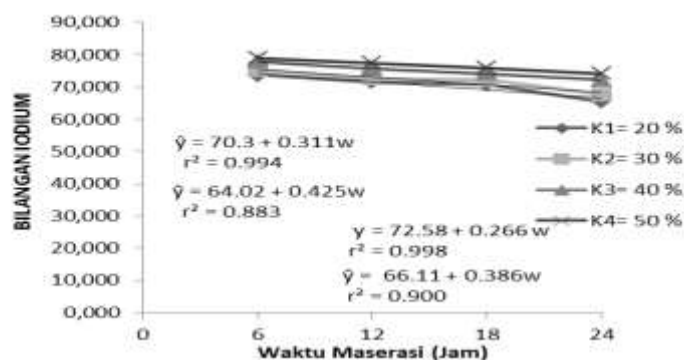


Figure 1. Relationship between the interaction of iodine number between n-hexane concentration and maceration time for processed products mixed with pork

Iodine number is a quality parameter to determine the number of double bonds in the composition of fatty acids in oil / fat. Saturated fatty acids are usually in the form of solids and unsaturated fatty acids in liquid form, hence the higher the iodine number, the less saturated and the softer the fat is. The more measured iodine numbers, the more unsaturated fatty acid content in the oil / fat which indicates that the better the quality of the oil.

Effect of N-Hexane Concentration on Beef Nuggets without Pork Fat (P1)

Based on the variance list, it can be seen that the concentration of N-hexane has a significantly different effect ($P > 0.01$) on iodine numbers. The level of difference has been tested with an average difference test can be seen in **Table 12**

Table 12. Different Test Results for the Mean Effect of N-Hexane Concentration on Iodine Numbers

Distance	LSR		Treatment K	Mean	Notation	
	0,05	0,01			0,05	0,01
-	-	-	20	75,505	d	CD
2	1.393	1.917	30	77,125	bc	BC
3	1.462	2.015	40	78,521	ab	AB
4	1.500	2.066	50	79,379	a	A

Note : Different letters in the notation column indicate the effect significantly different at level ($P < 0.05$) and very significantly different at level ($P > 0.01$)

Based on **Table 12** above, it can be seen that K1 is not significantly different from K2 and differs very significantly from K3 and K4. K2 differs not significantly from K3 and differs very significantly from K4. K3 differs not significantly from K4. The highest iodine number is found in K1 treatment which is 79,379 mg / g and the lowest is in K4 treatment which is 75,505 mg / g.

The higher the n-hexane solvent concentration used, the higher the iodine number is 0.995 mg / g. From the results of the study the obtained iodine numbers are high enough to prove that the fat produced through the maceration

process using hexane is a fat that has an unsaturated bond that is seen from the carbon chain structure has a double bond. The number of different hexans from various samples and affect the balance of iodine numbers.

Effect of Maseration Time on Cow nugget mixed with Pork Fat

Based on the variance list, it can be seen that the Maseration Time has a very significant different effect ($P > 0.01$) on iodine numbers. The level of difference has been tested by the average difference test can be seen in **Table 13**.

Table 13. Different Test Results of the Average Effect of Maseration Time on iodine number

Distance	LSR		Treatment K	Mean	Notation	
	0,05	0,01			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

Note : Different letters in the notation column indicate the effect significantly different at level ($P < 0.05$) and very different at real level ($P > 0.01$)

Based on **Table 13** above, it can be seen that W1 differs very significantly from W2, W3 and W4. W2 differs very significantly from W3 and W4. W3 differs very significantly from W4. The highest iodine number was found in W4 treatment, which was 1.009 gr / ml and the lowest was in W1 treatment, which was 0.943 gr / ml. Margareta et al. (2011) states that the longer the extraction time, the higher the yield. In this study fat yield with the highest 24-hour extraction time. This indicates that the equilibrium condition has been reached. The equilibrium condition in extraction is a state in which the solute present in the material cannot

dissolve again in the solvent and the concentration of fat in the solution remains the same before decreasing.

Acid Numbers

Effect of N-hexane concentration on cow nuggets mixed with pork fat

Based on the variance list, it can be seen that the concentration of N-hexane has a significantly different effect ($P > 0.01$) on iodine numbers. The level of difference has been tested by the average difference test can be seen in **Table 14**.

Table 14. Different Test Results on the Effect of N-Hexane Concentration on Acid Numbers

Distance	LSR		Treatment K	Mean	Notation	
	0,05	0,01			0,05	0,05
-	-	-	20	1.599	cd	CD
2	0.215	0.296	30	1.655	c	BC
3	0.225	0.311	40	1.907	ab	AB
4	0.231	0.318	50	2.020	a	A

Note : Different letters in the notation column indicate the effect significantly different at level ($P < 0.05$) and very significantly different at level ($P > 0.01$)

Based on **Table 14**. It can be seen that K1 is not significantly different from K2 and differs very significantly from K3 and K4. K2 differs not significantly from K3 and differs very significantly from K4. K3 differs not significantly from K4. The highest acid number was found in K4 treatment which was 2.020 g / ml and the lowest was in K1 treatment which was 1.599 gr / ml.

The more n-hexane solvent concentration used, the more acidic number is 0.994 gr / ml. Acid numbers are used to measure the amount of free fatty acids present in fat. Kusnandar (2010) states that the presence of free fatty acids will accelerate the oxidation process, because free fatty acids are

more easily oxidized than the form of the ester. The greater this number means the higher free fatty acid content, while the free fatty acids contained in the sample can come from the hydrolysis process or because of poor processing.

Effect of Maseration Time on Cow Nuggets Mixed with Pig Fat

Based on the variance list, it can be seen that the Maseration Time has a very significant different effect ($P > 0.01$) on iodine numbers. The level of difference has been tested with the average difference test can be seen in **Table 15**

Table 15. Different Test Results on the Average Effect of Maseration Time on Iodine Numbers

Distance	LSR		Treatment K	Mean	Notation	
	0,05	0,01			0,05	0,01
-	-	-	6	1.655	bcd	BCD
2	0.215	0.296	12	1.683	bc	BC
3	0.225	0.311	18	1.823	ab	AB
4	0.231	0.318	24	2.020	a	A

Note : Different letters in the notation column indicate the effect significantly different at level ($P < 0.05$) and very different at real level ($P > 0.01$).

Based on **Table 15**. It can be seen that W1 is not significantly different from W2, W3 and differs significantly from W4. W2 differs not significantly from W3 and differs very significantly from W4. W3 differs not significantly from W4. The highest acid number was found in W4 treatment, which was 2.020 gr / ml and the lowest was in W1 treatment which was 1.655 gr / ml.

The longer the extraction time, the greater the acid number value, this is because the contact time with the solvent is quite long and the storage process is not optimal. The longer the reaction time, the acid number will increase. This is evident from the results of research that showed that the acid number which went through the process of maceration for 24 hours had the highest acid number of 2.020 gr / ml. Lempang Risti Ika (2016), Increasing acid numbers can be caused by incorrect storage due to high humidity conditions or high

temperatures. Because the wrong storage can speed up the hydrolysis process.

Effect of N-Hexane Concentration on Beef Nuggets without Pork Fat (P1)

From the variance list, it can be seen that the N-Hexane concentration has a significantly different effect ($P > 0.05$) on acid numbers. However, further testing is not carried out.

Effect of Maseration Time on Cow Nuggets without Mixing Pig Fat (P1)

From the variance list, it can be seen that the maceration time has a significantly different effect ($P > 0.05$) on acid numbers, so that further testing is not carried out.

Number of microbes
Effect of N-hexane concentration on beef nuggets mixed with pork fat

Based on the variance list, it can be seen that the concentration of N-hexane has a significantly

different effect ($P > 0.01$) on the number of microbes. The level of difference has been tested by the average difference test can be seen in **Table 16**.

Table 16. Different Test Results on the Effect of N-Hexane Concentration on Microbial Amounts.

Distance	LSR		Treatment K	Mean	Notation	
	0,05	0,01			0,05	0,01
-	-	-	20	15837.5	a	A
2	3254.300	4480.086	30	12737.5	b	AB
3	3417.015	4707.887	40	11662.5	bc	ABC
4	3503.796	4827.212	50	10087.5	bd	BCD

Note : Different letters in the notation column indicate the effect significantly different at level ($P < 0.05$) and very significantly different at level ($P > 0.01$)

Based on **Table 16**, it can be seen that K1 is not significantly different from K2, K3 and is significantly different from K4. K2 differs not significantly from K3 and K4. Different K3 is not real with K4. The highest number of microbes was found in K1 treatment which was 1.5×10^4 CFU / ml and the lowest was in K4 treatment which was 1.0×10^4 CFU / ml.

Effect of Maseration Time on Cow Nuggets Mixed with Pig Fat

Based on the variance list, it can be seen that the Maseration Time has a very significant different effect ($P > 0.01$) on the number of microbes. The level of difference has been tested by the average difference test can be seen in **Table 17**

Table 17. Different Test Results on the Average Effect of Maseration Time on Microbial Amounts

Distance	LSR		Treatment K	Mean	Notation	
	0,05	0,01			0,05	0,01
-	-	-	6	10425.0	a	A
2	3254.300	4480.086	12	11062.5	b	B
3	3417.015	4707.887	18	12650.0	bc	BC
4	3503.796	4827.212	24	16187.5	cd	BCD

Note : Different letters in the notation column indicate the effect significantly different at level ($P < 0.05$) and very different at real level ($P > 0.01$)

Based on **Table 17**. It can be seen that W1 differs very significantly from W2, W3 and W4. W2 differs not significantly from W3 and W4. W3 differs not significantly from W4. The highest number of microbes was found in W4 treatment, which was 1.6×10^4 CFU / ml and the lowest was in W1 treatment which was 1.0×10^4 CFU / ml.

Maseration time on the number of microbes in beef sausages mixed with lard. The longer the extraction time, the more microbes that grow in the sample. From the results of the graph research, microbial growth has increased with increasing maceration time. This is possible the longer maceration allows microbes to grow. Microbial growth and enzyme activity require certain water

content. The more water content will enable microbes to grow and enzymes to become more active. Conversely, the less water content of a material will reduce microbial growth and enzyme activity [16].

Effect of N-Hexane Concentration on Cow Nuggets without Mixing Pig Fat (P1)

Based on the variance list, it can be seen that the N-Hexane concentration had a significantly different effect ($P > 0.01$) on the number of microbes. The level of difference has been tested with the average difference test can be seen in **Table 18**.

Table 18. Different Test Results on the Average Effect of N-Hexane Concentration on Microbial Amounts

Distance	LSR		Treatment K	Mean	Notation	
	0,05	0,01			0,05	0,01
-	-	-	20	9487,5	a	A
2	2468.528	3398.341	30	7762,5	ab	AB
3	2591.955	3571.138	40	5575,0	bc	BC
4	2657.782	3661.651	50	3887,5	cd	CD

Note : Different letters in the notation column indicate the effect significantly different at level ($P < 0.05$) and very significantly different at level ($P > 0.01$)

Based on Table 18 above, it can be seen that K1 is not significantly different from K2 and differs very significantly from K3 and K4. K2 differs not significantly from K3 and differs very significantly from K4. K3 differs not significantly from K4. The highest number of microbes found in K1 treatment was 0.94×10^4 CFU / ml and the lowest was in K4 treatment, which was 0.38×10^4 CFU / ml.

Effect of Maseration Time on Cow Nuggets without Mixing Pig Fat (P1)

Based on the variance list, it can be seen that the maceration time has a significantly different effect ($P > 0.01$) on the number of microbes. The level of difference has been tested with the average difference test can be seen in **Table 19.**

Table 19. Different Test Results on the Average Effect of Maseration Time on Microbial Amounts

Distance	LSR		Treatment K	Mean	Notation	
	0,05	0,01			0,05	0,01
-	-	-	6	5387.5	cd	BCD
2	2212.500	3045.875	12	6200.0	bc	ABC
3	2323.125	3200.750	18	8450.0	ab	AB
4	2382.125	3281.875	24	9062.5	a	A

Note : Different letters in the notation column indicate the effect significantly different at level ($P < 0.05$) and very different at real level ($P > 0.01$)

Based on **Table 19** above, it can be seen that W1 is not significantly different from W2, W3, and is significantly different from W4. W2 differs not significantly from W3 and W4. W3 differs not significantly from W4. The highest number of microbes was found in W4 treatment, ie 0.90×10^4 CFU / ml and the lowest was in W1 treatment, which was 0.53×10^4 CFU / ml.

D. CONCLUSION

1. The effect of n-hexane concentration had a very significant different effect on the level ($P > 0.01$) of the specific gravity, iodine number, acid number, and total microbes.
2. The maceration time gives a very real effect on the level ($P > 0.01$) of the specific gravity of iodine and total microbes, and the effect is significantly different on the level ($P > 0.05$) of acid numbers.
3. Interaction of treatment regarding the effect of n-hexane concentration and maceration time had a very significant different effect on the level ($P > 0.01$) of iodine number, and the effect of non-significant levels ($P > 0.05$)

on specific gravity, number acid and total microbes.

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