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Corn Oil can Lower Levels of Total Cholesterol and MDA (Malondialdehid) in The White Rat (*Rattus norvegicus*)

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Abstract
Repeatedly-heated cooking oil produces trans fatty acids and high free fatty acids that can increase total cholesterol levels. Used cooking oil also contains free radical compounds that can attack the lipid component through the mechanism of cell membrane lipid destruction. Efforts that can be made to minimize the negative impact of using used cooking oil is to replace it with healthy oil. Corn oil is a vegetable oil containing unsaturated fatty acids, high vitamin E, and phytosterols that are very beneficial for body. This study aimed to prove corn oil effect on total cholesterol reduction and malondialdehid levels in wistar rats Rattus norvegicus) treated with used cooking oil. This research was an experimental research with post test control group design. This study used 25 male wistar rats as a sample that divided into 5 groups, namely: (1) negative control group (with standard diet); (2) positive control group (given 0.5 mL used cooking oil); (3) treatment group 1 (given 0.5 mL/day of cooking oil + corn oil 0.321 mL/day); (4) treatment group 2 (given 0 fried oil 0.5 mL/day + corn oil 0.641 mL/day); and (5) treatment group 3 (given 0.5 mL/day of cooking oil + corn oil 0.962 mL/day). The results showed that there was a decrease of mean total cholesterol level in treatment group with significant difference ($p < 0.05$). Mean malondialdehyde levels in the treatment group also decreased with a significant difference ($p < 0.05$). Corn oil treatment could reduce total cholesterol and malondialdehid levels in wistar rats that were given significant used cooking oil.

Keywords*: Corn oil, Total cholesterol, Malondialdehyde*

I. INTRODUCTION

Most Indonesians tend to use cooking oil repeatedly. The use of repeated cooking oil means that cooking oil was heated repeatedly with high temperatures. Cooking oil was usually used as much as 5-7 times. The use of cooking oil would continue to increased at certain times such as Eid, Christmas, and New Year [1].

Heating oil at 170-200°C may lead to oxidation, hydrolysis, and polymerization processes which will result in oil degradation compounds such as ketones, aldehydes, and polymers. This causes the oil to be damaged, such as rancid odor; elevated levels of free fatty acids (FFA) and iodine numbers (IV); raised oil viscosity; formed foam; and turned oil color into brown [2].

The temperature and length of the frying process causes the cooking oil to break down easily and increases trans fatty acids amount. This will increase total cholesterol and affect free fatty acids metabolism that will cause hyperlipidemia, cardiovascular, fatty liver [3], and arterosclerosis [4].

Repeatedly-used cooking oil also has a potential to produce carcinogens that will stick to the next food frying [5]. In addition, heating with high temperatures can oxidize cooking oil and produce free radicals [6]. This free radical will trigger lipid peroxidation to produce malondialdehyde [7]. The increase in malondialdehyde value is one of the signs of oxidative damage by free radicals in cell membranes [8]. Efforts that can be made to minimize the negative impact of using used cooking oil is to replace it with healthy oil.

Corn oil is a very useful oil for health because it contains high unsaturated fatty acids. Corn oil consists of 59% poly-unsaturated fatty acids (PUFA), 24% mono-unsaturated fatty acids (MUFA), and 13% saturated fatty acids (SFA). Corn oil has the highest PUFA level and the main PUFA is linoleic acid C18: 2n-6 with a small amount of linoleic acid C18: 3n-3. High PUFA can lower blood cholesterol levels and high plant sterols in corn oil can reduce cholesterol absorption in the gut [9].

The quality of corn oil is higher because the distribution of fatty acids is balanced, especially oleic and linoleic [10]. Corn oil is effective in lowering blood cholesterol levels because it contains low SFA and high PUFA. The combination of both is more effective in lowering cholesterol than simply reducing the consumption of SFA [11].

Corn oil also contains high amounts of gamma-tocopherol (vitamin E) as a function of stability to rancidity and also as an antioxidant that can counteract free radicals [9].

This study aimed to prove the effect of corn oil on the reduction of total cholesterol and malondialdehid levels in wistar rats treated with used cooking oil.

II. METHODS

This research type was an experimental study using "Random Build Complete Design" with "Post Test Only Control Group" design [12]. The experimental study was conducted at Biochemistry Laboratory of the Faculty of Medicine, University of Airlangga, and started from the maintenance, experimental animals adaptation, animal treatment, and blood sampling of experimental animals in June 2017.

The population in this study were 3-month-old male wistar rats with ± 180 g weight that obtained from the Biochemical Laboratory's animal experimental cage. The research samples were 25 wistar rats which determined by Frederer formula. The sampling technique was done by simple ramdom sampling.

The research material consisted of standard feed and feed treatment. The standard diet was standard formula feed which obtained from the Biochemical Laboratory and given in ad libitum way. Animal feed was using POKPHAND CP 591 brand, with 13.0% water content, 18.0-20.0% protein, 3.0% fat, 6.0% fiber, 7.0% ash, 0.9% calcium, and 0.6% phosphor.

The treatment ingredients were corn oil and used cooking oil. Corn oil dosage was determined based on dosage conversion between organisms [13]. Granting 54 m/day of corn oil in humans had an effect on decreasing cholesterol levels in the blood [14]. 54 g of corn oil dosage for humans was converted for wistar mice in ± 180 g weight and it was 0.8748 g. 0.8748 g of corn oil dosage was converted from gram into volume, so it would be easy to apply in this research. Corn oil unit conversion was used this equation: volume (mL) = mass (g) per density (g/mL). Corn oil density (ρ) was known to be 0.9095 g/mL [15] and corn oil mass (m) was 0.8748 g. Based on the above equation calculation, 0.962 mL corn oil was an optimum dose. The optimum dose was given to the treatment group 3. The dosage for the treatment group 2 was (0.962 mL / 3) x 2 = 0.641 mL. The dose for the treatment group 1 was 0.962 mL / 3 = 0.321 mL. Used cooking oil dose was as much as 0.5 mL.

The 25 wistar rats in 3-months-old were kept for 14 days. Rats were adapted in 5 cages, each in measuring 60 cm x 20 cm x 30 cm and fed a standard diet in ad libitum way. After 7 days adaptation, 25 wistar rats were divided into 5 groups, namely: (1) negative control group (with standard diet); (2) positive control group (given 0.5 mL used cooking oil); (3) treatment group 1 (given 0.5 mL/day of cooking oil + corn oil 0.321 mL/day); (4) treatment group 2 (given 0 fried oil 0.5 mL/day + corn oil 0.641 mL/day); and (5) treatment group 3 (given 0.5 mL/day of cooking oil + corn oil 0.962 mL/day).

After 14 days of treatment, the rats didn't get fed for 12 hours then anesthetized with ether solution. After the rats' eyes began to fade and their body didn't move, the wistar rats' abdomen were incised with a scalpel and was taken ± 3-4 mL of blood from the intracardial (heart). Blood samples were labeled according to the sample sequence in the group. The blood was centrifuged for 15-20 minutes at a rate of 3000 rpm, then the serum was taken.

Total cholesterol levels were determined by the CHOD-PAP (Cholesterol Oxidase-p-aminophenozone) method. The principle of this method was total cholesterol and the ester form is liberated from lipoproteins by detergent. Furthermore, the ester form was hydrolyzed by the enzyme cholesterol esterase. With the help of cholesterol oxidase enzyme, cholesterol would be oxidized to produce hydrogen peroxide. This compound would convert 4-aminoantipirin and phenol (with the aid of catalase peroxidase enzyme) to a colorful qunonamine which its intensity can be measured photometrically.

Measurement of malondialdehid content by colorimetric method was used spectrophotometer at wavelength (λ) 535 nm. Malondialdehyde levels were measured by using a regression line from the standard curve of the malondialdehyde solution [16].

Data from 5 sample groups were processed using SPSS 22.0 software. The data were tested for normality by Kolmogrov-Smirnov test and tested homogenity with Levene test. If there is normal and homogeneous data ($p > \alpha = 0.05$), then the difference test would done by One Way Anova parametric statistic test. If there was a significant difference ($p < \alpha = 0.05$), then could be continue by Tukey statistic test to know where the differences between groups.

III. RESULTS

A. Measurement Results of Trans Fatty Acids, Free Fatty Acids, and Peroxide Numbers of Used Cooking Oil

Table 1. Measurements of Acid Trans Fat Levels, Free Fatty Acids, and Peroxide Numbers

Sample Code	Measurement	Value	Unit
Used cooking oil	*Trans Fatty Acid* (TFA)	1.2	%
	Free Fatty Acid (FFA)	10.52	%
	Peroxide Number	3.75	Miliequivalents/1000 g

Table 1 showed that the test results was 1.2% trans fatty acid; 10.52% free fatty acids; and 3.75 mek/1000 g peroxide number of used cooking oil. The US Dietary Guidelines Advisory Committee (2005) recommended that the consumption of trans fatty acids for each individual was below 1% of total energy [17]. The quality standard of cooking oil in SNI (Indonesian National Standard) 01-3741-2002 requires the maximum free fatty acid content in 0.30%, whereas in SNI-3741-1995 requires peroxide number which is safe to be consumed maximum 2 mek/kg.

B. Results of Total Cholesterol Measurement

Based on the research results, total cholesterol levels of each group had obtained. The descriptive analysis results and total cholesterol levels statistical tests were presented in Table 2.

Table 2. Descriptive Analysis and Anova Statistics Total Cholesterol

Group	n	Mean ± SD (mg/dL)	Min	Max	*p* Value	Interpretation
KN	5	41.00 ± 10.559 mg/dL	30	53	0.001	DifferZent
KP	5	65.40 ± 10.065 mg/dL	57	81		meanings
P1	5	53.40 ± 7.162 mg/dL	45	64		
P2	5	52.60 ± 5.273 mg/dL	44	56		
P3	5	40.00 ± 9.460 mg/dL	31	54		

KN (standard diet), KP (standard diet + used cooking oil 0.5 mL/day), P1 (standard diet + used cooking oil 0.5 mL/day + corn oil 0.321 mL/day), P2 (standard diet + used cooking oil 0.5 mL/day + corn oil 0.641 mL/day), P3 (standard diet + used cooking oil 0.5 mL/day + corn oil 0.962 mL/day)

Table 2 showed that the mean total cholesterol content of the KN group was 41.00 ± 10.559 mg/dL with a minimum value of 30 and a maximum value of 53. The mean total cholesterol content of the KP group after being given 0.5 ml cooking oil for 7 days was increased to 65.40 ± 10.065 mg/dL with a minimum value of 57 and a maximum value of 81. The mean total cholesterol level in the treatment group after being given 0.5 mL cooking oil + corn oil was decreased, where mean total cholesterol level group of P1 was 53.40 ± 7.162 mg/dL with a minimum value of 45 and a maximum value of 64; mean total cholesterol level of group P2 was 52.60 ± 5.273 mg/dL with minimum value 44 and maximum value 56; and mean total cholesterol level of P3 group was 40.00 ± 9.460 mg/dL with minimum value of 31 and maximum value of 56. Normal total cholesterol range was 10-54 mg/dL.

The result of One Way Anova statistic test showed significant difference to total cholesterol level between control group and treatment group with significant value by $p = 0.001$ ($p < \alpha = 0.05$). It had meaning that giving corn oil gave effect to total cholesterol level of wistar rats which given used cooking oil. Anova test result got significant difference ($p < \alpha = 0.05$), so it could continue with Tukey test to know the location of difference between group. Differences between groups were shown in Figure 1.

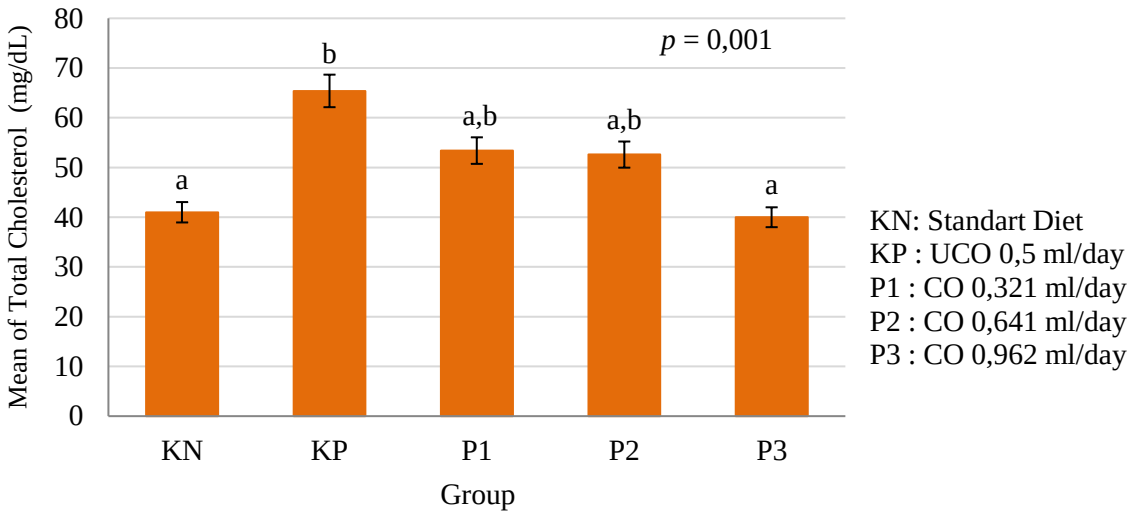

Figure 1. The Difference of Total Cholesterols Levels Between Control and Treatment Group. *UCO = Used-Cooking Oil, CO = Corn Oil. The above letters showed the difference if different letters were based on the Tukey test at α = 0.05. The mean accompanied by the same letters stated no significant difference.

Tukey test result in Figure 1 showed that the mean total cholesterol level of KP group was significantly different with KN group, where the significance value p = 0.002 (p < α = 0.05) and mean total cholesterol level of KP group was significantly different with group P3, in where the significance value p = 0.001 (p < α = 0.05).

C. Measurement Result of Malondialdehid Content

Based on the results of research, malondialdehid levels in each group had been found. The results of descriptive analysis and statistical test of malondialdehyde levels were presented in Table 3.

Table 3. Descriptive Analysis Result and Anova Statistics Test Malondialdehyde Level

Group	n	Mean ± SD (umol/L)	Min	Max	*p* Value	Interpretation
KN	5	31.36 ± 0.888 umol/L	31	33	0.000	Different
KP	5	38.30 ± 1.432 umol/L	37	40		meanings
P1	5	33.39 ± 1.372 umol/L	31	35		
P2	5	29.52 ± 1.034 umol/L	28	31		
P3	5	25.16 ± 0.573 umol/L	24	26		

KN (standard diet), KP (standard diet + used cooking oil 0.5 mL/day), P1 (standard diet + used cooking oil 0.5 mL/day + corn oil 0.321 mL/day), P2 (standard diet + used cooking oil 0.5 mL/day + corn oil 0.641 mL/day), P3 (standard diet + used cooking oil 0.5 mL/day + corn oil 0.962 mL/day)

Table 3 showed the mean rate of malondialdehyde in the KN group was 31.36 ± 0.888 umol/L with a minimum value of 31 and a maximum value of 33. The mean of the grade of malondialdehyde group of KP after being given 0.5 ml of used cooking oil for 7 days was increased to 38.30 ± 1,432 umol/L with a minimum value of 37 and a maximum value of 40. Mean malondialdehyde levels in the treatment group after being given 0.5 ml of used cooking oil + corn oil was decreased, where the mean P1 group malondialdehid content was 33.39 ± 1.372 umol/L with a minimum value of 31 and a maximum value of 35; group P2 mean malondialdehid content was 29.52 ± 1.034 umol/L with a minimum value of 28 and a maximum value of 31; and group P3 mean malondialdehid levels were 25.16 ± 0.573 umol/L with a minimum value of 24 and a maximum value of 26.

The results of One Way Anova statistical test showed that there was significant difference between the malondialdehyde levels between the control groups and the treatment group with significant value, ie p = 0.000 (p <α = 0.05). It had meaning that the provision of corn oil gives effect to the levels of malondialdehid wistar rats which were given used cooking oil. Anova test results obtained a significant difference (p < α = 0.05), so it followed by the Tukey test to determine the location difference between groups. Differences between groups were shown in Figure 2.

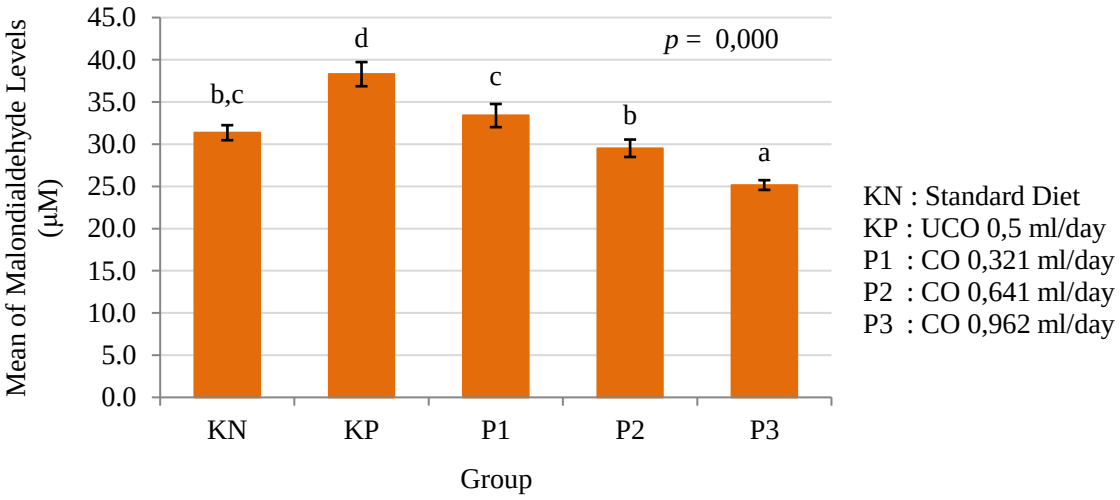

Figure 2. The Difference of Malondialdehyde Levels Between Control and Treatment Group. *UCO = Used Cooking Oil, CO = Corn Oil. The above letters showed the difference if different letters were based on the Tukey test at α = 0.05. The mean accompanied by the same letter stated no significant difference.

The Tukey test results in Figure 2 showed that the kin content mean of the KP groups differed significantly with the KN, P1, P2, and P3 groups, where the significance value p = 0.000 (p < α = 0.05), whereas the P3 group was different meaning with the KN group, where the significance value p = 0.000 (p < α = 0.05).

IV. DISCUSSION

Based on the statistical analysis, the mean total cholesterol and malondiladehid levels in wistar rats in the control group and the treatment group showed that there were significant differences between groups (p <α = 0.05). Mean total cholesterol and malondialdehyde levels in the negative control were significantly different with mean total cholesterol (p = 0,002) and mean malondialdehyde level (p = 0,000) in positive control group. The positive control group showed an increase in mean total and malondialdehyde cholesterol levels. This was because in the positive control group only given used cooking oil without the provision of corn oil.

Used cooking oil that had been tested at Health Laboratory contained 1.2% trans fatty acid of; 10.52% free fatty acid content; and 3.75 meq/1000 g peroxide number which has exceeded normal limits based on SNI. Used cooking oil is known to have been heated many times (more than three times) at high temperatures. Deep frying process will increase free fatty acid content then will form trans fatty acids. New trans fatty acids are formed and their levels will increase with the use of oil [18]; [19]; [20]; [21]; [22]; [4]. As a result, trans fatty acids can increase blood cholesterol levels [23]; [24]. In addition, saturated fatty acids from used cooking oil also result in high blood cholesterol levels [25] as seen in the mean total cholesterol levels of the positive control group, in which the mean total cholesterol level was highest compared to the other groups.

In addition to trans fatty acids and free fatty acids, used cooking oil contains high peroxide numbers. Peroxide number is the most important value in determining the degree of damage to oil. Peroxide numbers are formed due to heating which causes damage to cooking oil due to oxidation and hydrolysis processes. Cooking oil damaging will lead to toxicity in the body and various diseases such as deposition of fat in the blood vessels (atherosclerosis), cancer, and lower fat digestibility [26].

Provision of used cooking oil can lead to increased levels of malondialdehyde. This is because the provision of used cooking oil causes free radicals. Excessive free radicals cause oxidative stress that triggers the peroxidation process of lipids that can be determined by measuring malondialdehyde [27]. Free radicals also have a negative impact on human health, among which is potentially cause cancer [28].

The using of corn oil is very effective in lowering the mean total cholesterol and malondialdehid levels. This was seen in the treatment group, where the group was getting used cooking oil and also corn oil without heating. In most groups, there was a decrease in mean total and malondialdehyde cholesterol levels. Although the mean total cholesterol level between the positive control group and the P1 and P2 groups was statistically significant (p> 0.05), the mean total cholesterol level between the positive control group and P3 group was significantly different (p <0.05). So, the mean total cholesterol level of P3 group resembles the mean of total cholesterol level in negative control group. Nevertheless, it appeared that there were decreased mean total cholesterol levels in all treatment

groups. The mean of malondialdehyde levels between the positive control group and the treatment groups was statistically significant ($p < 0.05$) and showed that the treatment groups had been decreased mean malondialdehyde levels.

Corn oil has the highest PUFA level with C18: 2n-6 linoleic acid as the main PUFA with a small amount of linoleic acid C18: 3n-3 [29]. Polyunsaturated fatty acids, such as linoleic and linolenic acids, also play a role in decreasing cholesterol synthesis. This is due to the synthesis of cholesterol using saturated fatty acid feedstock, while unsaturated fatty acids are not used in cholesterol synthesis [30].

Linoleic acid can reduce the absorption of cholesterol in the digestive system. Reduced cholesterol absorption is done by locking or binding cholesterol molecules and blocking cholesterol molecules from being absorbed by intestinal mucosal cells. Linoleic acid is absorbed by intestinal mucosal cells and will be transported via lipoproteins. Inhibition of cholesterol absorption causes a kilomikron anabolism to be small, causing the entry of cholesterol into the liver also become small. When cholesterol consumption increases, the cholesterol biosynthesis of acetyl CoA in the liver will decrease [31]. The decrease in cholesterol biosynthesis is due to the limitation of HMG CoA reductase (Hydroxy methylglutaril koA reductase) enzyme by cholesterol

V. CONCLUSION

Corn oil treatment affected total cholesterol and malondialdehid levels in wistar rats treated with used cooking oil, whereas in the treatment group given corn oil there was a decrease in total cholesterol and malondialdehyde levels based on the mean values of total cholesterol and malondialdehyde levels. As a suggestion to get the benefits of corn oil, it should be consumed without heating process. It is because the heating process can damage the unsaturated fatty acids in corn oil.

REFERENCES

1. Rifqi, Khaerur, Kadarwati, S., Wahyuni, S. Preparasi, Karakteristik, dan Uji Aktivitas Ni Mo/Zeolit Alam dalam Proses Catalytc Cracking Jelantah Menjadi Biogasoline. Indonesian Journal of Chemical Science No. ISSN No 2252-6951. Kimia FMIPA Universitas Negeri Semarang. 2012
2. Wijana, S., Arif, H. & Nur, H. Teknopangan: Mengolah Minyak Goreng Bekas. Surabaya: Trubus Agrisarana. 2006
3. Suirta, I.W. Minyak Jelantah Berbahaya, Preparasi Biodiesel dari Minyak Jelantah Kelapa Sawit. Jurnal Kimia 3 (1) Januari 2009: 1-6. 2009
4. Sartika, R.A.D. Pengaruh Suhu dan Lama Proses Menggoreng (Deep Frying) Terhadap Pembentukan Asam Lemak Trans. Markara Sains. Vol 13: 23-8. 2009
5. CancerHelps. Bebas Kanker itu Mudah. Jakarta: Fmedia (Imprint AgroMedia Pustaka). 2014
6. Graha, CK.. 100 Questions & Answers: Kolesterol. Jakarta: PT Elex Media Komputindo. 2010
7. Myers, Ronald. K and McGavin, M. Donald. Pathologic Basic of Veterinary Disease 4th Edition. Mosby Elsevier Publishing Company. Illinois. 2007
8. Suryohudoyo, P. Kapita Selekta Ilmu Kedokteran Molekuler. Perpustakaan Nasional RI. Jakarta. Penerbit CV Sagung Seto. hal: 31-47. 2007
9. Dwiputra D., Jagad AN., Wulandari FK., Prakarsa AS., Puspaningrum DA., dan Islamiyah F. Minyak Jagung Alternatif Pengganti Minyak yang Sehat. Jurnal Aplikasi Teknologi Pangan. Januari 2015. Hal.5-6. 2015
10. Rizki, Farah. The Miracle of Vegetables. Jakarta: PT. Agro Media Pustaka. 2013
11. Subroto, M.A. Real Food True Health. Jakarta: Agro Media Pustaka. 2008
12. Kuntoro, H. Dasar Filosofis Metodologi Penelitian, Surabaya: Program Pascasarjana Universitas Airlangga. Pustaka Melati. 2011
13. Laurence, D.R., and Bacharach, A.L. Evaluation of Drug Activities. Academic Press. London. 1964
14. Kevin CM, Andrea LL, Kathleen MK, Valerie NK, Constance JG, dan Mary RD. Corn oil improves the plasma lipoprotein lipid profile compared with extra-virgin olive oil consumption in men and women with elevated cholesterol: Results from a randomized controlled feeding trial, Journal of Clinical Lipidology, Vol. 9, No. 1, p. 49-57. 2015
15. Goering, C.E., A.W. Schwab, M.J. Daugherty, E.H. Pryde, and A.J. Heakin. "Fuel Properties of Eleven Vegetable Oils", Trans. ASAE 25, 1472 – 1477. 1982
16. Pasha, K.V. and B. Sadasivadu. Intracelluler content of thiol compounds, thiobarbituric acid reactive substances and gamma glutamyl transpepidase in rat brain during anoxia. Neurosci. Letter 46: 209-214. 1984

17. Edmond, R.W. Peningkatan Kadar Nitric Oxide Dan Osteokalsin Pada Tikus Sprague Dawley Yang Diberi Asam Lemak Trans Dosis Tinggi. tesis. Fakultas Kedokteran Universitas Diponegoro Semarang. 2014
18. Dhaka, V., Gulia, N., Ahlawat, K.S., Khatkar, B.S. Trans fats—Sources, Health Risks and Alternative Approach - A Review. J Food Sci Technol. Vol 48 : 534–541. 2011
19. Wahab, A.W., Dewang, S., Armynah, B., Ponganan, K. Analisis Spektrum Infra Merah dari Minyak Goreng Kelapa untuk Identifikasi Perubahan Panjang Gelombang Akibat Variasi Temperatur. Makalah Seminar. Ujung Pandang : Universitas Hasanuddin. 2011
20. Fan HY, Sharifudin MS, Hasmadi M, Chew HM. Frying stability of rice bran oil and palm olein. International Food Research Journal 20(1): 403-407. 2013
21. Felix A, Aladedunye, Przybylski R. Degradation and Nutritional Quality Changes of Oil During Frying. J Am Oil Chem Soc 86:149–156. 2009
22. Tsuzuki W, Matsuoka A, Ushida K. Formation of trans fatty acids in edible oils during the frying and heating process. Food Chemistry 123:976–982. 2010
23. Sartika R.A.D. Pengaruh Asam Lemak Jenuh, Tidak Jenuh, dan Asam Lemak Trans terhadap Kesehatan. Jurnal Kesehatan Masyarakat Nasional, Vol. 2, No. 4, Februari 2008. 2008
24. Ilmi I, Khomsan A, Marliyati S. Kualitas minyak goeng dan produk gorengan selama penggorengan di rumah tangga Indonesia. Jurnal Aplikasi Teknologi Pangan Vol 4 (2). 2015
25. Ikrar, T. Nitric Oxide Berperan Dalam Pencegahan dan Penyembuhan Penyakit Jantung dan Pembuluh Darah. http:kabarinews.com/nitric-oxideberperan-dalam-pencegahan-dan-penyembuhan-penyakit-jantung-danpembuluh-darah/49513. 2012
26. Ketaren, S. Pengantar Teknologi Minyak dan Lemak Pangan. Jakarta:Universitas Indonesia Press. 2012
27. Jusup, S.A., dan Raharjo, S.S. Efek Ekstrak Daun Krokot (Portulaca oleracea L.) Sebagai Anti Oksidan Alami Terhadap Kadar Alanin Transaminase (ALT) dan Gambaran Histologi Sel Hepar Rattus norvegicus L. Yang Diberi Minyak Goreng deep frying, tesis, Surakarta. Universitas Sebelas Maret. 2010
28. Mianiko, Adika. Menjaga Kesehatan di Musim Hujan. Yogyakarta: Pustaka Muslim. 2014
29. Almatsier S. Prinsip Dasar Ilmu Gizi. Jakarta: PT Gramedia Pustaka Utama. 2001
30. Montgomery MW, Kersten JR, dan Ghassemi T. Diabetes and hyperglycemia impair activation of mitochondrial K(ATP) channels. Medical. 1: 111-116. 1993
31. Budiyanto, M.A.K. Efek Hipolipidemik dan Hipoglikemik Nata Slgu Pada Tikus Wistar. Disertasi. Program Pascasarjana Universitas Airlangga Surabaya. 1999
32. Linder C.M. Biokimia Nutrisi dan Metabolisme dengan Pemakaian Secara Klinis. Terjemah: Aminuddin Parakkasi. Jakarta: Penerbit Universitas Indonesia (UI-Press). 2006
33. Murray, R. K., Granner, D. K., and Rodwell, V. W. Biokimia Harper. Edisi 29. Jakarta: Buku Kedokteran EGC. 2009
34. Winarsi, H. M. S. Antioksidan Alami dan Radikal Bebas. Cetakan 5. Yogyakarta: Penerbit Kanisius.p.11-37, 49-58, 77-81. 2007
35. Acker S, Koymansm LM, and Bast A. Molecular Pharmacology of Vitamin E : Structural Aspects of Antioxidant Activity. Ncbi.nlm.com . 213-217. 2003