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Chitosan as antimicrobial agent and fatty acid absorber in smoked skipjack tuna processed using coconut shell

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Abstract: Background: Chitosan, a hydrophilic biopolymer industrially obtained by N-deacetylation of chitin, which allow for a wide scope of applications and can be applied as an antimicrobial agent.Theaim of this study was to determine theconcentration of chitosan compound in which it can play an active role in inhibiting the growth of bacteria and also absorb the fatty acid /cholesterol in the smoked fish meat. Method: The research method used in this study wastheRandomized Block Design (RBD) factorial. There were two treatments in this study, namely: Treatment A (fish was dipped in a chitosan solution and then smoked), Treatment B (fish was smoked and then dipped in a 1%, 2% and 3% chitosan solution) and one control, in which the fish was smoked without being dipped in chitosan solution and then stored for five days. Results: The results showed that the chitosan concentration (1%, 2%, 3%) significantly affected the growth of bacteria that occurred on day 3 (p = 0,00) and day 5 (p = 0,000), while day 1 did not differ significantly. There was a difference in the levels of fatty acids between the control and A (p = 0,00) and the control with B (p = 0,000), with the best concentration of chitosan was 3%. Conclusion: Fifteen saturated fatty acids and eleven unsaturated fatty acids were found in fish smoked with coconut shells.

Keywords: Chitosan, Antimicrobial, Smoked Skipjack Tuna

1. Introduction

Chitosan is a polysaccharide obtained from the deacetylation of chitin, which is generally derived from crustacean shell wastes. Chitosan has a relatively more reactive nature compared to chitin and easily manufactured in the form of powder, paste, film and fiber. Chitosan is a bioactive material and its activities can be applied in the field of pharmacy, agriculture and the industry environment. Chitosan as a bioactive material can inhibit the growth of bacteria in dried marinated anchovies. Chitosan compounds can kill bacteria by damaging the cell membrane [1,11]. Chitosan has anti-microbial properties, because its activity can inhibit pathogens and spoilage-causing microorganisms, including fungi, Gram-positive bacteria and Gram-negative bacteria [2]. Chitosan can be used as a coating (film) on a variety of comestibles, the aim is to block oxygen from entering, so it can be used as packaging for various comestibles and can also be consumed directly, because chitosan is not harmful to human health [3,12].

According to the results of a study conducted by Nicholas, the use of chitosan to preserve fishery products using 1% chitosan solution in 1% acetic acid was able to reduce the number of microbes on salmon fillets stored at 4° C for 6 days. Chitosan compound that has the potential as an antimicrobial substance can be added to foodbecause it is harmless to humans [4,13]. This finding was also reinforced by Hardjito, who stated that there has been no negative effects on humans and that human tolerance to chitosan is 1,333 g/kg body weight [5,6]. In humans, chitosan cannot be digested, thus it has no caloric value and directly expelledfrom the body along with feces. Chitosan has metabollic barrier properties of the outer cell membrane [1,14,15]. Chitosan compounds can act as an antioxidant by

forming salts [7]. Chitosan could also mergewithgallic acid to form an antioxidant [8]. Based on the results of this study, it was found that that chitosan is best obtained with the highest degree of deacetylation of 82.98% obtained through thedeacetylation process using 50% NaOH, the mass concentration of chitosan in the volume of fat (g/v) affect the total cholesterol absorption [9].

The results of the coconut shell liquid smoke safety test indicated that the LD50value of coconut shell liquid smoke was greater than5.000mg/kg of mice body weight, thus it is categorized as a non-toxic substance and safe to use for food products [10]. These results are supported by the identification of coconut shell liquid smoke components using GC-MS which showed that there were seven dominant components, namely *2-Methoxyphenol (guaiacol)*; *3,4-Dimethoxyphenol*; *Phenol*; *2-Methoxy-4-methylphenol*; *4-Ethyl- 2-methoxyphenol*; *3-Methylphenol* and *5-Methyl-1,2,3-trimethoxy-benzene*, no PolicyclycAromatic Hydrocarbons (PAHs) were present which are carcinogenic, including benzopyren [10].

Chemical compounds that evaporated are absorbed by fish, especially in the form of vapor. These compounds provide the desired color and taste of the smoked fish. Solid particles are not so important in the process of curing and smoke will preserve food because of the disinfection action of formaldehyde, acetic acid and phenol present in the smoke. The smoke particles play an important role in coloring. Drying has an important function in the preservation of smoked fish, with smoke absorption rate into the flesh of the fish and the drying process itself depend on the amount of smoke that was created, as well as the temperature and water content of the smoked fish. In addition, lignin is broken down into phenol, quinol, guaiacol and pyrogallol derivatives. The percentage of each chemical compound in smoke generated depends on the type of wood used. To obtain a high-quality smoked fish, hardwood species (non-resinous) or coir and coconut shell must be used, because softwood will produce compounds that can cause an odor and other unwanted results.

Food is a basic human need and its fulfillment is a fundamental right of every human being, so that the government is obliged to provide enough, safe, quality, nutritious and varied food every time with prices affordable for the community. For that we need a food safety system that provides protection for the producers (Farmers) and consumers (Society). Food safety is an important requirement for ready for consumption products. Food quality and safety can be produced from the domestic kitchen or food industry. Therefore, the food industry is one of the determining factors of food circulation of certain quality and safety standards set by the government. More than 90% of human disease is associated with food caused by microbiological contamination, which include typhoid, amoebic dysentery, botulism and other bacterial intoxication as well as hepatitis A known as food poisoning. WHO defines it as an infectious disease or toxic caused by agents that enter the body through digested food.

2. Materials and Method

2.1. Total Plate Count (TPC) Method [1]

The colonies that grew in the agar plate culture from the incubator were counted using the Colony Counter and Hand-Counter. For the record, the number of colonies that can be counted range between 30-300 colonies, it is calculated by multiplying the number of colonies counted with its dilution factor.

2.2. Chromatography-Mass Spectrometry (GCMS) Analytical [16]

The method was carried out as follows: smoked skipjack tuna samples that have been dried in an incubator was extracted using petroleum ether to separate the fat component, then the sample was evaporatedby using an evaporator to obtain the fat, then diluted with acetone to be then taken/injected into a set of GCMS apparatus. The result was a peak in the GC spectra and by looking at the retention time the fatty acid type could be determined based on the literature.

2.3. Smoking

Smoking used in this study was hot smoking with temperatures around 70^{o}C - 90°C for 4-5 hours, in the following manner: Samples (A), which were tuna dipped in a 1%, 2%, and 3% solution of chitosan for 5 minutes before the smoking process, samples (B) were tuna dipped in a 1%, 2%, and 3% solution of chitosan for 5 minutes after the smoking process was completed, and control (K) which were smoked tuna that were not dipped in chitosan.

3. Results

3.1. TPC

The results of the total plate count (TPC) of smoked skipjack tuna that were not dipped in a chitosan solution (K) and smoked skipjack tuna that were dipped in a 1%, 2%, and 3%solution of chitosanare grouped into group (A) and (B) which are presented in the following explanation.

3.2. TPCon the First Day

Analysis was conducted on the total plate countfor smoked skipjack tuna not dipped in chitosan (K), skipjack tuna dipped in 1%, 2%, and 3% chitosan solutionbefore being smoked (A) and skipjack tuna dipped in 1%, 2%, and 3% chitosan solutionafter being smoked (B). Based on the analysis results for the total plate count variable (TPC) in the smoked skipjack tuna not dipped in chitosan (K), skipjack tuna dipped in 1%, 2%, and 3%chitosan solution before(A) and after being smoked (B) it was found that there was no difference in the total platecount (TPC) with the value of F = 0,529 and p = 0,778.

3.3. TPC on the Third Day

Analysis was conducted on the total plate countfor

smoked skipjack tuna not dipped in chitosan (K), skipjack tuna dipped in 1%, 2%, and 3% chitosan solution before being smoked (A) and skipjack tuna dipped in 1%, 2%, and 3% chitosan solutionafter being smoked (B). Based on the analysis results for the total plate count variable (TPC) in the smoked skipjack tuna not dipped in chitosan (K), skipjack tuna dipped in 1%, 2%, and 3%chitosan solution before(A) and after being smoked (B) it was found that there was a difference in the total platecount (TPC) with the value of F = 20,78 and p = 0,00.

Further testing of the analysis of variance using the Least Significant Difference (LSD) test, found that there was a significant difference in the total plate count (TPC) between group (A) skipjack tuna dipped in 1%, 2% and 3% chitosan solution before being smoked, (B) skipjack tuna dipped in 1%, 2% and 3% chitosan solution after being smoked and skipjack tuna not dipped in chitosan solution. The test results can be seen in the following table.

3.4. TPC on the Fifth Day

Analysis was conducted on the total plate countfor smoked skipjack tuna not dipped in chitosan (K), skipjack tuna dipped in 1%, 2%, and 3% chitosan solution before being smoked (A) and skipjack tuna dipped in 1%, 2%, and 3% chitosan solutionafter being smoked (B). Based on the analysis results for the total plate count variable (TPC) in the smoked skipjack tuna not dipped in chitosan (K), skipjack tuna dipped in 1%, 2%, and 3%chitosan solution before(A) and after being smoked(B) it was found that there was a difference in the total platecount (TPC) with the value of F = 49,68 and p = 0,000.

Further testing of the analysis of variance using the Least Significant Difference (LSD) test, found that there was a significant difference in the total plate count (TPC) between group (A) skipjack tuna dipped in 1%, 2% and 3% chitosan solution before being smoked, (B) skipjack tuna dipped in 1%, 2% and 3% chitosan solution after being smoked and skipjack tuna not dipped in chitosan solution. The test results can be seen in the following table.

3.5. The Effect of Chitosan Application on Fatty Acid

This analysis aimed to see variations/differences of total protein content in smoked skipjack tuna which wasnot dipped in chitosan (K), skipjack tuna dipped in 1%, 2%, and 3% chitosan solution and then smoked (A), newly smoked skipjack tuna dipped in 1%, 2%, and 3% chitosan solution (B). Based on the analysis results for the fatty acid content variable of the smoked skipjack tuna not dipped in chitosan (K), skipjack tuna dipped in 1%, 2%, and 3%chitosan solution before(A) and after being smoked (B) it was found that there was no significant difference in amino acid content with the value of F = 82,142 and p = 0,000.

Further testing of the analysis of variance using the Least Significant Difference (LSD) test, found that there was a significant difference in the total fat content between group (A) skipjack tuna dipped in 1%, 2% and 3% chitosan solution and (K) skipjack tuna not dipped in chitosan solution. There was also a significant difference in the total fat content between group (B) skipjack tuna dipped in 1%, 2% and 3% chitosan solution and (K) skipjack tuna not dipped in chitosan solution. More details can be found in the following table.

3.6. The Percentage of Fat Absorbed

Analysis of the total fatty acids that were absorbed by chitosan in skipjack tuna dipped in 1%, 2%, and 3%chitosan solutionbefore being smoked (A) and skipjack tuna dipped in 1%, 2%, and 3% chitosan solution after being smoked (B)were compared with the fatty acids contained in the control smoked skipjack tuna. The analysis results for the saturated fatty acid content in skipjack tuna dipped in 1%, 2%, and 3% chitosan solution before (A) and after being smoked (B) compared to the control (K) were as follows: A1 = 16,28%, A2 = 22%, A3 = 37,14%, B1 = 15,14%, B2 = 18,86%, B3 = 44,29%.

4. Discussion

The observation and Anava test results of the first day of the study showed no relationship or growth inhibition of microorganisms on smoked skipjack tuna dipped in 1%, 2%, or 3% chitosan solution, both before (A) and after (B) being smoked compared to the control group. The average microorganism growth rate was 10^1. Microorganism growth on the first day for all treatments were not significantly different may be due to the early stage of microbial growth and that the chitosan compound and the growing microbial colonies were not yet fully in contact.

Meanwhile, the observation and Anava test results of the third day of the study showed a relationship or growth inhibition of microorganisms on smoked skipjack tuna dipped in 1%, 2%, or 3% chitosan solution, both before (A) and after (B) being smoked compared to the control group. The average microorganism growth rate for the control group was 10^4 and for the group with chitosan solution application ranged from 10^2-10^3.

Chitosan can form a film (thin layer) on a variety of food products and functions to obstruct oxygen into the microbial cells, which in turn controls the process of cellular respiration of bacteria to multiply. Chitosan has anti-microbial properties because its activity can inhibit the growth of bacterial pathogens and spoilage microorganisms, including fungi, bacteria, Gram-positive and Gram-negative bacteria [2]. Microbial death also occurs because chitosan compound affects the membrane permeability of Gram-negative and Gram-positive bacterias so that their metabolic activity is disrupted, with the highest activeness in Gram-negative bacterias.This is caused by the properties of chitosan that hasan NH_3^+ group (Chung, 2004). Research conducted by Hui Liu stated that, damage to the walls of *Escherichia coli* and *Staphylococcus aureus*were caused by the interaction of NH_3^+ groups in chitosan compound with phospholipid compound in the cell membrane [1]. Graph 3

above shows that the bacteria in the control group grew well because nothing impeded the metabolic process. While bacterial growth in treatment group A and B was relatively low compared to the control group. This shows that, the growth of bacteria in the control group was influenced by the chitosan compound of various concentrations applied to it.

The quality of chitosan as a preservative is determined by the high degree of deacetylation. The higher the degree of deacetylation, the bigger the number of amino groups(-NH2) on the side chain of chitosan molecules, thus the chitosan becomes more reactive. The activeness of chitosan as a preservative is because of the amino groups.

This polysaccharide coating is a good barrier, because this type of coating can form a strong and compact matrix that serves as a shield. Chitosan is soluble in organic acids and has a strong positive charge that can bind the negative charge of other compounds, including those contained in the bacterial membrane. In the smoking process, not all bacteria present in smoked fish died. Bacteria that died during the application of chitosan solution was also caused by the relatively low water activity as a result of the smoking process. One use of chitosan in the processing of smoked tuna is as an anti-microbial substance. In general terms, anti-microbial substance is defined as a substance that interfere with the growth and metabolism of microbes.

The results of further testingof the analysis of variance usingLeast Significant Difference (LSD) test showed that, treatment K (smoking without chitosan application) was significantly different from treatment A (Dipped in 1%, 2%, and 3%of chitosan solutionbefore being smoked), and treatment B (Dipped in 1%, 2%, and 3%ofchitosan solutionafter being smoked). This study also found 26 types of weak acids (including cholesterol) which consists of 15 saturated fatty acids and 11 unsaturated fatty acids. Of the 15 types of saturated fatty acids, 5 fatty acids; Isopropyl myristic acid, Lauric acid, Tridecanoic acid, Oleic acid and Behenic acid were not found in treatment A and B (treatment with chitosan application).Meanwhile, of the 11 types of unsaturated fatty acids only one type of unsaturated fatty acid was found in treatment A and B (treatment with chitosan application). In addition, fatty acids that were present in all treatments were also found (Control, treatment A and treatment B) namely: Heptadecanoic acid, Nonadecanoic acid (unsaturated fatty acids) and 9-Nonadecanoic acid (saturated fatty acids).

5. Conclusion

It was concludedthat chitosan can inhibit the growth of bacteria in smoked skipjack tuna at a concentration of 1%, 2%, and 3%, where there was a difference in bacterial growth between control and treatment (p = 0,01) during the storage process and the best chitosan concentration was 3%. Chitosan can also serve to absorb fatty acids, in which there were differences in the levels of fatty acids between the control (K) and treatment A2 (p = 0,000), A3 (p = 0,000), B2 and B3 (p = 0.000) with the best concentration was 3%. In addition, 15 saturated fatty acids and 11 unsaturated fatty acids were found.

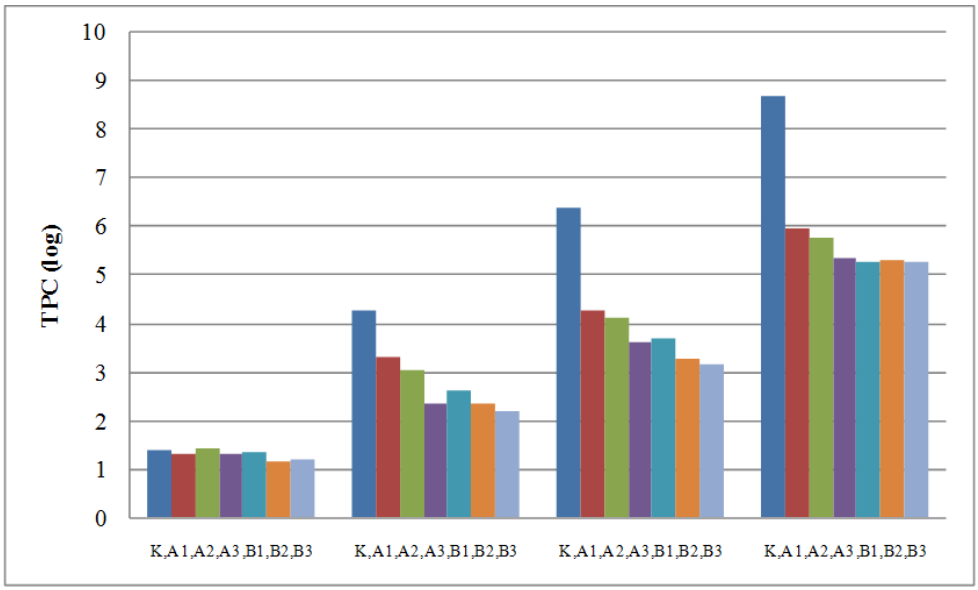

Figure 1. *Graph of Microbial Growth, TPC (Log) Day 1, 3, 5, 7.*

K= without chitosan
A1= Chitosanconcentration of1%, beforeSmoked, B1= Chitosanconcentration of1%, after Smoked
A2= Chitosanconcentration of2%, beforeSmoked, B2 = Chitosanconcentration of2%, after Smoked
A3= Chitosanconcentration of3%, beforeSmoked, B3 = Chitosanconcentration of3%, after Smoked

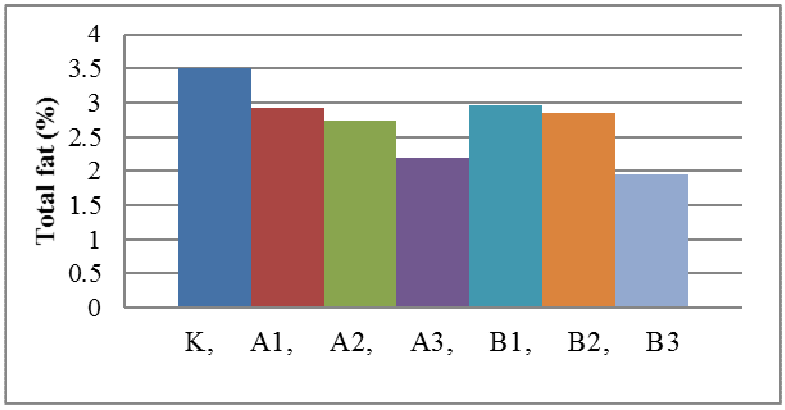

Figure 2. *Graph of Total Fat Percentage (%).*

K= without chitosan
A1= Chitosanconcentration of1%, beforeSmoked, B1= Chitosanconcentration of1%, after Smoked
A2= Chitosanconcentration of2%, beforeSmoked, B2 = Chitosanconcentration of2%, after Smoked
A3= Chitosanconcentration of3%, beforeSmoked, B3 = Chitosanconcentration of3%, after Smoked

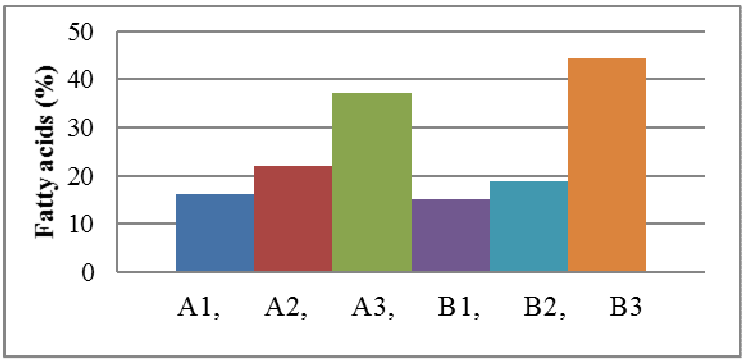

Figure 3. *Graph of the Percentage offatty acids Absorbed (%).*

A1= Chitosanconcentration of1%, beforeSmoked, B1= Chitosanconcentration of1%, after Smoked
A2= Chitosanconcentration of2%, beforeSmoked, B2 = Chitosanconcentration of2%, after Smoked
A3= Chitosanconcentration of3%, beforeSmoked, B3 = Chitosanconcentration of3%, after Smoked

Table 1. *Anava of the First Day on Skipjack Tuna TPC.*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.163	6	.027	.529	.778
Within Groups	.719	14	.051		
Total	.882	20			

Table 2. *Anava of the Third Day on Skipjack Tuna TPC.*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.934	6	1.656	20.782	.000
Within Groups	1.115	14	.080		
Total	11.049	20			

Table 3. *Further Testing Using LSD of Total Plate Count (TPC), Third Day.*

N= 21	K	A1	A2	A3	B1	B2	B3
K		0,001	0,000	0,000	0,000	0,000	0,000
A1	0,001		0,290	0,001	0,011	0,001	0,000
A2	0,000	0,290		0,008	0,88	0,009	0,002
A3	0,000	0,001	0,008		0,229	0,966	0,526
B1	0,000	0,011	0,88	0,229		0,244	0,077
B2	0,000	0,001	0,009	0,966	0,244		0,499
B3	0,000	0,000	0,002	0,526	0,077	0,499	

Table 4. *Anava of the Fifth Day on Skipjack Tuna TPC.*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	21.832	6	3.639	49.683	.000
Within Groups	1.025	14	.073		
Total	22.857	20			

Table 5. *Further Testing Using LSD of Total Plate Count (TPC), Fifth Day.*

N= 21	K	A1	A2	A3	B1	B2	B3
K		0,000*	0,000*	0,000*	0,000*	0,000*	0,000*
A1		0,000*	0,429	0,008*	0,021*	0,000*	0,000*
A2		0,000*	0,429	0,040*	0,097	0,002*	0,001*
A3		0,000*	0,008*	0,040*	0,637	0,161	0,700
B1		0,000*	0,021*	0,970	0,637	0,070	0,028*
B2		0,000*	0,000*	0,002*	0,161	0,070	0,637
B3		0,000*	0,000*	0,001*	0,070	0,028*	0,637

Tabel 6. *LSD Test of the Effect of Chitosan Application on Fatty Acid.*

N= 21	K	A1	A2	A3	B1	B2	B3
K		0,000*	0,000*	0,000*	0,000*	0,000*	0,000*
A1		0,000*	0,046*	0,000*	0,602	0,262	0,000*
A2		0,000*	0,046*	0,000*	0,015*	0,345	0,000*
A3		0,000*	0,000*	0,000*	0,000*	0,000*	0,006*
B1		0,000*	0,620	0,015*	0,000*	0,107	0,000*
B2		0,000*	0,262	0,345	0,000*	0,107	0,000*
B3		0,000*	0,000*	0,000*	0,006*	0,000*	* 0,000*

Table 7. *Composition of Fatty Acids Found in Smoked Skipjack Tuna.*

No.	Type of Fatty Acids	K	A1	A2	A3	B1	B2	B3
1.	Isopropyl myristic acid	+	-	-	-	-	-	-
2.	Heptadecanoic acid	+	+	+	+	+	+	+
3.	Methyl hexadecanoate	+	-	+	+	+	+	-
4.	Ethyl pentadecanoate	+	-	+	+	-	-	+
5.	Pentyldecanoate	+	-	- +	-	-	-	-
6.	Heptyl decanoate	+	-	-	+	-	-	
7.	Lauric acid	+	-	-	-	-	-	-
8.	Nonadecanoic acid	+	+	+	+	+	+	+
9.	Tridecanoic acid	+	-	-	-	-	-	-
10.	Tetradecanoic acid	+	+	+	+	+	-	-
11.	Palmitate acid	+	+	-	-	+	-	+
12.	Oleic acid	+	-	-	-	-	-	-
13.	Stearic acid	+	-	+	-	+	-	-
14.	Ricinoleic acid	+	-	-	-	-	+	-
15.	Behenic acid	+	-	-	-	-	-	-
16.	6,9-Heptadecenoic acid	+	-	-	-	+	+	-
17.	9, 11-Heptadecenoic acid	+	+	-	+	-	-	+
18.	9,12-Heptadecenoic acid	+	+	-	-	-	-	-
19.	9- Nonadecanoic acid	+	+	+	+	+	+	+
20.	6, 11, 13, 15-Eicosanoic acid	+	-	-	-	-	-	-
21.	9,11, 13, 15-Eicosanoic acid	+	+	-	-	+	-	-
22.	6-Heptadecenoic acid	+	+	+	-	+	-	-
23.	11-Nonadecanoic acid	+	+	+	+	+	-	+
24.	11-Eicosanoic acid	+	-	+	-	-	-	-
25.	9, 12-Nonadecanoic acid	+	-	+	-	-	-	-
26.	Cholesterol	+	+	+	-	+	+	-

Note: (+) = Fatty acids found, (-) = No fatty acids found

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