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Antioxidant Properties of Liquid Smoke Production Variation of Pyrolysis Temperature Raw and Different Concentration

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Abstract : This study aims to determine the antioxidant properties of liquid smoke which is obtained from pyrolysis of various raw materials with temperature levels and different concentrations of liquid smoke. This research is conducted experimentally using a completely randomized factorial design 3 x 4 x 6 with three replications to obtain 216 units trial. A factor is the type of raw material (coconut fiber, coconut shell and cinnamon), B factor is the temperature of pyrolysis (temperature of 100±10°C; 200±10°C; 300±10°C; and 400±10°C) and C factor concentration of liquid smoke (1 ppm , 10 ppm, 100 ppm, 500 ppm, 1000 ppm and 1500 ppm). The observed parameters measured is % inhibition (inhibition) and IC_{50} (inhibitor concentration) of liquid smoke is the third combination of treatment results. The result of research show a significant interaction (P <0.05) in the treatment combination of differences in raw material liquid smoke with a temperature pyrolysis against the percentage of inhibition (inhibition) of liquid smoke as well as in combination treatment for different concentrations with the difference of pyrolysis temperature, while for the combination treatment of liquid smoke raw materials with different concentrations show no significant difference (P> 0.05) as well as the interaction of a combination of three (3) treatment of the raw material, pyrolysis temperature and concentration show no significant difference (P> 0.05). Based on the result of the research, a conclusion can be drawn. The percentage of the largest inhibition is got in the combination of cinnamon raw materials treatment on pyrolysis temperature of 400 ± 10 ° C of 23.865% with IC_{50} value of 35.52 ppm. b. The percentage of the largest inhibition values is got in the combination of cinnamon raw materials at 1500 ppm concentration of liquid smoke of 27.173% is not significantly different from other treatments with IC50 value of 6.08 ppm. c. the percentage of the largest inhibition value is got in the combination of the pyrolysis temperature treatment of 400 ± 10 ° C in liquid smoke concentration of 1500 ppm of 30.559% with IC_{50} value of 4.96 ppm. d.the percentage of the largest inhibition is got in the combination of cinnamon raw materials treatment on pyrolysis temperature of 400±10°C in liquid smoke concentration 1500 ppm of 35.091% is not significantly different from other treatments with IC_{50} value of 8.19 ppm.

Key words: antioxidants, raw materials, temperature, liquid smoke, concentration.

1. Introduction

Advancement of Science later find out that many factors that cause premature old process that is partly due to genetic factors, lifestyle, environment, gene mutations, immune system damage and free radicals. From all the causes the free radical theory is a most often expressed theory[1]. Free radicals can come from pollution, dust and continuously produced as a consequence of normal metabolism[2]. Therefore our body needs a body of important substances and antioxidants that can help to protect the body from free radical attack to reduce the negative impact this compounded. Antioxidants can have some functions to overcome or neutralize free radicals that are expected by giving these antioxidants so old process is inhibited or at least not "accelerated" and can prevent damage of the body from the onset of degenerative diseases[1].

The sources of antioxidants can be either synthetic or natural antioxidant. But now the use of synthetic antioxidants begins to be restricted because in fact from the results of research which has been done that synthetic antioxidants such as BHT (*ButylatedHydroxy Toluene*), in fact it can poison animal testing and carcinogenic. Therefore, the food industry and medicine change to develop natural antioxidants and find sources of new natural antioxidants[3]. There are many foods that can be a source of natural antioxidants, such as spices, tea, chocolate, foliage, Cereal grains, vegetables, enzymes and proteins. Most sources of natural antioxidants are plants and generally a phenolic compound that is scattered in all parts of the plant either in wood, seeds, leaves, fruits, roots, flowers and pollen[4]. Phenolic compounded or polyphenolic among others can be a flavonoid. The ability of flavonoids as antioxidants have been widely observed during recent years, which flavonoids have the ability to change or reduce radical free and also as anti-free radical[5].

Liquid smoke is a liquid of wood smoke disperse in water, which is made by condensing the smoke of the incomplete combustion of wood. Liquid smoke contains many compounds that can be grouped into phenol, acids and carbonyl. That compounds are able to act as an antimicrobial, antioxidant, giving flavor and color formers[6,7]. Antioxidative components of the smoke are phenolic compounds that act as hydrogen donors and are usually effective in very small amounts to inhibit the oxidation reaction[8]. Liquid smoke can act as an antioxidant through preventing the oxidation of fat by stabilizing free radicals and effectively inhibit the formation of off flavors oxidative[7].

According[7], phenol is a major antioxidant in liquid smoke. Antioxidative role of liquid smoke is showed by high-boiling phenolic compounds, especially 2,6-dimetoksifenol; 2,6 dimethoxy-4-metilfenol and 2.6-dimethoxy-4-etilfenol, which acts as a hydrogen donor to free radicals and inhibit the chain reaction [10,8,7]. The use of liquid smoke to the food product has several advantages than traditional fogging, including: saving required cost for timber and equipment manufacturing of smoke, can set the flavor of products as desired, can reduce component berhahaya (compound benzo (a) pyrene is carcinogenic) , can be used exclusively on the food which can not be solved by traditional methods, can be applied to the general public, reducing air pollution and the composition of the liquid smoke is more consistent to use repetitive[11,12,7]. The use of liquid smoke to the food products have been widely applied. Its application is done by adding in products such as sausages, immersion for pork products as well as sausage, penyuntiknn (injection) as in pork products and atomization for pork products and sausages[8].

All kinds of wood distillate contain compounds that can be extracted as a phenol derivative that can act as antioxidants. Liquid smoke of wood is used as a preservative because of the similarity of chemical components of wood distillation is got in certain kinds of preservatives, where that act as preservatives is phenol and its derivatives. Effort to give plus value from waste crop plantations that are still yet to get optimal treatment such as coconut husk, coconut shell and cinnamon in West Sumatra is necessary to do a research about the antioxidant properties. Information about antioxidant properties of various types of materials and raw materials in the pyrolysis liquid as well as smoke concentration has not been much different. Thus this study aims to know about the antioxidant properties of liquid smoke which is obtained from pyrolysis of various raw materials with temperature levels and different concentrations of liquid smoke.

2. Materials and Methodology

2.1. Sample

Tools are used such as tools of laboratory glassware, test tube rack, stir bar, dark bottles, micro pipette, oven. analytical balance, label paper, rulers, pencils, aluminum foil, plastic, filter paper, cotton, Erlenmeyer flask, glass becker, measuring glass, funnels, test tubes, shaker waterbath, kuvets, UV-VIS T-70 spektrofometer Hitachi Solution 2.2 and 1 set tool of maker laboratory-scale liquid smoke[13] as the figure 1.

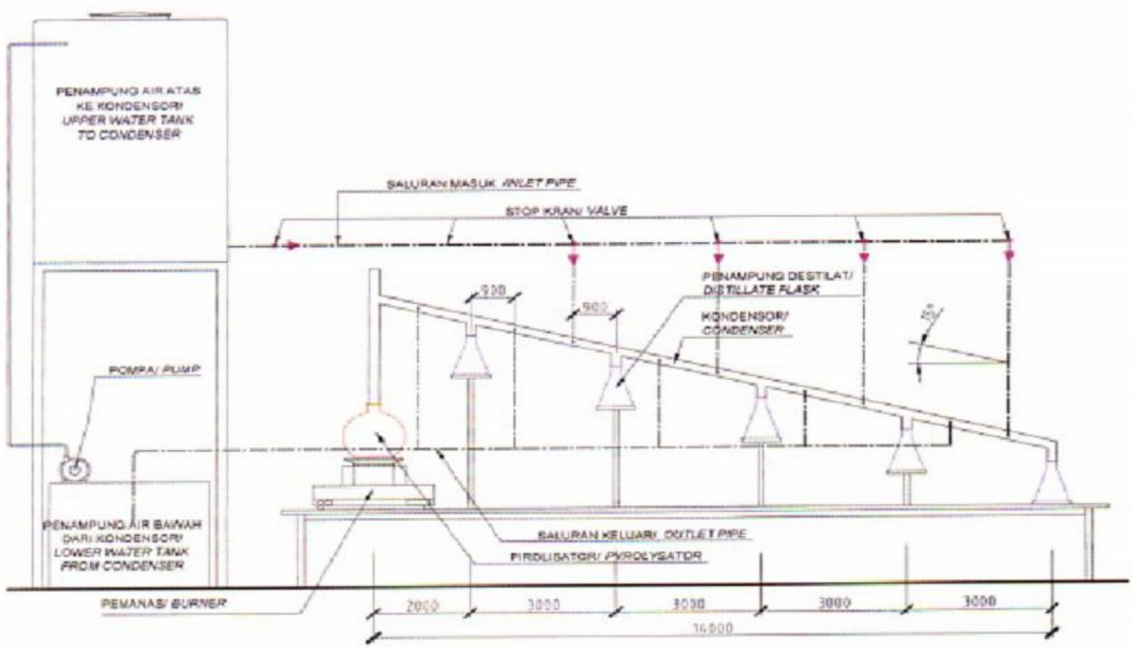
Materials and chemical reagents used in this research are a waste of coconut husks, coconut shells which are obtained from Raya market in Padang and cinnamon desert highway that is already taken superficially derived from cinnamon farmers in Tanah Datar, methanol, DPPH, distilled water.

2.2.Implementationof Research

The stages of this research implementation consist of three phases:

2.2.1.Arrangcment means of pyrolysis liquid smoke

Circuit extraction tool of liquid smoke made at laboratory scale refers to the results of research and the characteristics of liquid smoke[13]. In this research, it uses the tool place maker of liquid smoke which consist of one unit of condenser equipment complete with water in the form of a drum with capacity of 100 liters equipped with a water pump to help circulate cooling water along the 14 meter equipped with a hose for water circulation, a container place of liquid smoke in the form of a tube Erlenmeyer with capacity of 500 ml amounted to 5 pieces, stainless steel kiln with capacity of 3 kg and a burner stove LPG fueled at the end of the pipe pyrolysis include a vacuum pump to draw the smoke of burning in order to be obtain a liquid smoke as shown in Figure 1 below.



Gambar 1. Alat penghasil asap cair skala laboratorium.

Figure 1. Liquid smoke device of laboratory scale

2.2.2. The process of pyrolysis (manufacture) of liquid smoke

Research about the manufacture of liquid smoke of pyrolysis refers to research activities to provide input to redesign the tool of liquid smoke laboratory scale. After the tool maker of liquid smoke strung well then continued with producing liquid smoke. This process is started from raw material preparation providing coconut fiber, coconut shell and dry cinnamon dry with each weighing about 40 kg with a moisture content ranging from 4-10%, cleanedfrom dirt. Raw materials cut in small size with size ± 4- 9 cm2. Next activities put the raw materials into pyrolysis reactor during the five (5) hours with each weighing 3 kg sample at a temperature of 100 ±10°C; 200± 10°C; 300±10°C; 400±10°C. using fuel burner LPG stove. The water pump is used to drain water from the water source to the condenser. Burner and water pump switched on simultaneously. distillate container (liquid smoke) accommodated using glass bottles. After 5 hours, will be obtained three fractions, they are solid fraction like arangand light fraction like smoke and gas methan. Next light fraction is passed to pipe condensation in order to be obtained a liquid smoke while methane remained the gas and condensed. Liquid smoke areallowed to stand for one week, a new analysis.The purpose of the deposition during 1 (one) week to precipitate impurities that exist in liquid smoke. After 1 (one) week liquid smoke is allowed to stand next to analysis of antioxidants in the form% inhibition and IC_{50}.

2.2.3.Test Antioxidant activity, DPPH method[14]modified

2.2.3.1. The maker of solution

Prepare the DPPH 634 ml. The trick is DPPH weigh as much as 0.0014 gram dissolved in 14 ml of methanol, the solution is shaken so homogeneous and then put into a dark bottle. The absorbance is measured by using UV-Vis spectrophotometer T-70 to obtain the maximum wavelength.

2.2.3.2. Make of control solution.

The trick is in 1500 µL of methanol was added to 500 mL of DPPH solution, the solution was shaken until homogeneous.

2.2.3.3. Make of test solution.

Main liquid (10.000 ppm); the trick is take 100 ml of liquid smoke which the result of a combined treatment of raw materials with different temperature pyrolysis dissolved into 10 ml of methanol = 100 ml / 10 ml = 10 000 mL / ml = 10.000 ppm

Series liquid

1. 1 ppm; the trick is take 20 mL of methanol main liquid added to 1500 mL volume then added 500 mL solution of DPPH
2. 10 ppm, the trick is take 200 mL of methanolmain liquid added to 1500 mL volume then added 500 mL solution of DPPH
3. 100 ppm, the trick is take 200 mL of methanol main liquid added to 1500 mL volume then added 500 mL solution of DPPH
4. 500 ppm, the trick is take 1000 mL of methanol main liquid added to 1500 mL volume then added 500 mL solution of DPPH
5. 1000 ppm, the trick is take 2000 mL of methanol main liquid added to 1500 mL volume then added 500 mL solution of DPPH
6. 1500 ppm, the trick is take 2500 mL of methanol main liquid added to 1500 mL volume then added 500 mL solution of DPPH

2.2.3.4. Absorbance measurement

All of the control solution, test solutions is shaken using a water bath shaker and incubated at 37 ° C for 30 minutes in the dark (closed aluminum foil). This is done because DPPH radical easily degraded by light. Then the absorbance is measured using a UV-Vis spectrophotometer at a wavelength of 515.8 nm. After an absorbance values obtained is calculated barrier (%) of each solution by using the formula[15,16,17]:

$$\%\ \text{Barrier} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100\%$$

Information :

Control Abs = absorbance of the sample does not contain
Sample abs = absorbance of the sample

Having obtained the percentage of barner activity then search IC 50 values through the linear regression equation y = a + bx

2.2.3.5. Data analysis of antioxidants

Antioxidants data on DPPH radicals (% inhibition/inhibition) of liquid smoke from a combination of raw materials to the pyrolysis temperature and different concentrations are analyzed and calculated the value of the IC 50. The smaller the IC 50 value means stronger antioxidant activity. In this research is IC50 values are calculated using a linear regression equation[15,17,18].

Percentage data of obstacles, type of raw material, pyrolysis temperature and concentration of liquid smoke is used to find the value of IC 50 with the linear regression equation y = a + bx, where y is the% inhibition of 50 ($ 50) and x is the value of IC 50 [19,20].Here is a table regarding the classification of the antioxidant activity according to[21]:

Table 1. Classification of antioxidant activity

No.	IC 50	value of Antioxidants
1.	<50 ppm	Very strong
2.	50-100 ppm	Strong
3.	100 -150 ppm	Medium
4.	150 -200 ppm	Weak

3. Resullts and Discussion

3.1. Effect of combination treatment of raw material differences with pyrolysis temperature %inhibition of liquid smoke.

Results of varience analysis shows that the combination treatment of differences in raw material liquid smoke with different a pyrolysis temperature of significant effect on the value of antioxidants (percentage inhibition) of liquid smoke (P <0.05) as well as in combination treatment of the difference concentration to the different temperature pyrolysis, whereas for the combination treatment raw material liquid smoke with different concentrations showed no significant difference (P>0.05) as well as the interaction of a combination of three (3) treatment of the raw material, pyrolysis temperature and concentration showed no significant difference (P>0.05).

The measurement of extract absorbance using a UV-Vis spectrophotometer previously conducted to determine the maximum wavelength DPPH. DPPH maximum wavelength used was at a wavelength of 515.5 nm. This gives the maximum wavelength of maximum absorbance of the test solution and provides the greatest sensitivity. The result of average of the type of raw material treatment and different pyrolysis temperature which are obtained can be seen in the following table 2.

Table 2. Summary (%) Inhibition (antioxidant) liquid smoke for the combination treatment of raw materials with different pyrolysis temperature.

KodeSampel	(%) Inhibisi	IC50 (ppm)
SKT1 (coconut fiber, temperature 100 ±10°C)	14.227±7,38 c	2,97
SKT2 (coconut fiber, temperature 200 ±10°C)	14.322±7,36 c	
SKT3 (coconut fiber, temperature 300 ±10°C)	15.044±8,72 bc	
SKT4 (coconut fiber, temperature 400 ±10°C)	16.797±10,35 b	

TKT1 (coconut shell, temperature 100 ±10°C)	14.039±7,36 c	9,52
TKT2 (coconut shell, temperature 200 ±10°C)	14.507±7,98 bc	
TKT3 (coconut shell, temperature 300 ±10°C)	14.644±8,53 bc	
TKT4 (coconut shell, temperature 400 ±10°C)	14.827±8,68 bc	
KMT1(cinnamon, temperature 100 ±10°C)	15.98±6,88 bc	35.52
KMT2 (cinnamon, temperature 200 ±10°C)	16.05±7,74 bc	
KMT3 (cinnamon, temperature 300 ±10°C)	16.259±7,38 bc	
KMT4 (cinnamon l, temperature 400 ±10°C)	23.865±10,07 a	

Information : * different superscript alphabet in average column shows real different (P<0,05)

Based on Table 2 shows the% inhibition of the largest found in the combination treatment of raw materials cinnamon on pyrolysis temperature of 400 ± 10 ° C of 23.865% is significantly different from other treatments with IC50 value of 35.52 ppm. The smallest percentage of inhibition value is got in the combination treatment of the coconut shell raw material on pyrolysis temperature of 100±10°C of 14.039% with IC50 of 9.52 ppm. The value of the percentage inhibition of liquid smoke cinnamon with pyrolysis temperature of 400±10°C is suspected because there are many chemical components that can be outlined at the time of pyrolysis so that the percentage of inhibition becomes great. By the standards of[21] states that the antioxidant activity of the three raw materials with four different pyrolysis temperature as grouped very powerful is with IC_{50}<50 ppm.

To view the strength of the relationship of raw material with the pyrolysis temperature to the percentage of inhibition (antioxidant) next is made the regression equation. Figure relationship of Average% inhibition (antioxidant) liquid smoke for treatment combination of raw materials with pyrolysis temperature Figure 2 below, such as.

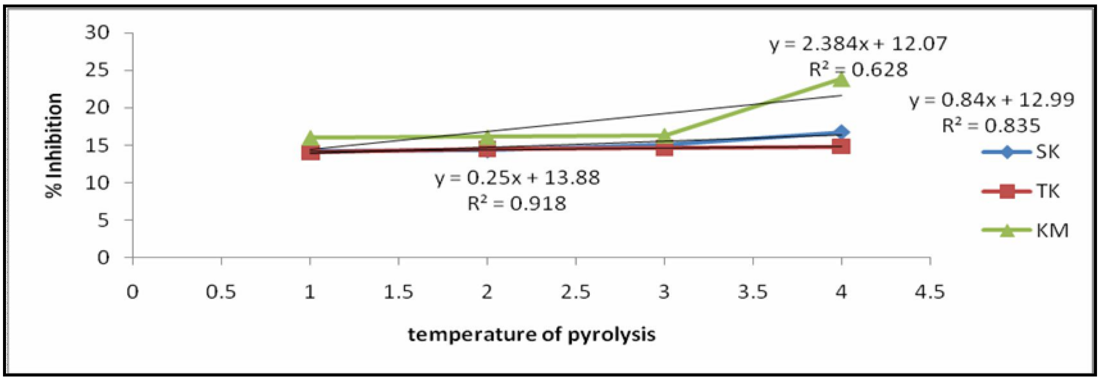


Figure2. Average (%) for Inhibition (antioxidant) from some types of raw material with different pyrolysis temperature

Based on Figure 2 above that the raw at material cinnamon different pyrolysis temperatures show a strong correlation to the percentage of inhibition (antioxidant) with R2 of 0.9188 means that 91.88% inhibition percentage are affected by the combination treatment Kabu material cinnamon on pyrolysis temperature of 400±10°C. For the combination treatment of the raw material coconut shells and coconut fiber at different temperatures show the value of R2 respectively 0.835 and 0.6285. The high raw cinnamon material relationship at pyrolysis temperature 400°C means cinnamon liquid smoke has the ability in dampen the free radicals in the form of DPPH. Antioxidant activity test quantitatively done by using DPPH (2,2-diphenyl-1-picrylhydrazyl). DPPH method is chosen because that method is simple, easy, quick and sensitive and method only few sample to evaluate of the antioxidant activity from method of natural materials[15].

The measurement principle of the antioxidant activity quantitatively using this DPPH method is the change in the intensity of the purple color DPPH proportional with the concentration of the DPPH solution. DPPH free radicals have unpaired electrons will give the purple color. The color will change to yellow when the electron pairs. The change of the purple color intensity happens because there is the reduction of free radicals generated by DPPH molecules reacting with hydrogen atoms released by the sample compounds molecule

Diphenylpikril hydrazine to from compounds and cause decay of DPPH color from purple to yellow. This color change will provide a change of absorbance at a wavelength of maximum DPPH using UV-Vis spectrophotometry so they will know the value of the activity of free radicals reduction expressed with IC50 value (inhibitory concentration)[15].

3.2. The combination effect of the raw materials with different liquid smoke concentration against% inhibition (inhibition) of liquid smoke.

The Activity is measured by calculating the amount of the reduction of the intensity of the purple color DPPH which is proportional to the reduction of DPPH solution concentration. The damping is produced by reacting *PikrilHidrazilDiphenyl* molecules with hydrogen atoms that are released one sample component molecules to form DiphenylPikril Hydrazine compounds and cause decay DPPH color from purple to yellow. Average test results of antioxidant activity for the treatment of raw materials with different concentrations of liquid smoke which is obtained can be seen in the following table 3.

Table 3. Activity (%) Inhibition (antioxidant) liquid smoke for the combined treatment of raw materials with different concentrations of liquid smoke.

Kodesampel	(%) Inhibition	IC 50 (ppm)
SKK1 (coconut fiber,consentrationof liquid smoke 1 ppm)	2.275±1,60 a	2,46
SKK2 (coconut fiber, consentrationof liquid smoke10 ppm)	7.937±0,61 a	
SKK3 (coconut fiber, consentrationof liquid smoke 100 ppm)	14.918±1,15 a	
SKK4(coconut fiber, consentrationof liquid smoke 500 ppm)	18.47±0,68 a	
SKK5 (coconut fiber, consentrationof liquid smoke 1000 ppm)	21.459±4,00 a	
SKK6 (coconut fiber, consentrationof liquid smoke 1500 ppm)	25.524±5,18 a	
TKK1(coconut shell, consentrationof liquid smoke 1 ppm)	1.965±1,60 a	7,77
TKK2 (coconut shell, consentrationof liquid smoke 10 ppm)	7.735±0,81 a	
TKK3(coconut shell, consentrationof liquid smoke 100 ppm)	14.126±0,66 a	
TKK4 (coconut shell, consentrationof liquid smoke 500 ppm)	18.351±1,20 a	
TKK5 (coconut shell,consentrationof liquid smoke 1000 ppm)	20.712±1,62 a	
TKK6 (coconut shell,consentrationof liquid smoke 1500 ppm)	24.137±4,76 a	
KMK1 (cinnamon,consentrationof liquid smoke 1 ppm)	5.557±2,20 a	6,08
KMK2 (cinnamon,consentrationof liquid smoke 10 ppm)	11.038±2,47 a	
KMK3 (cinnamon, consentration of liquid smoke 100 ppm)	17.411±2,97 a	
KMK4 (cinnamon, consentration of liquid smoke 500 ppm)	22.298±5,11 a	
KMK5 (cinnamon, consentration of liquid smoke 1000 ppm)	24.756±4,75 a	
KMK6 (cinnamon, consentration of liquid smoke 1500 ppm)	27.173±5,34 a	

Information : * different superscript alphabet in average column shows real different (P<0,05)

According to the table 3 indicates the largest % inhibition (antioxidant), which is found in the combination treatment of cinnamon raw materials at 1500 ppm concentration of liquid smoke of 27.173% is not significantly different from other treatments with IC50 value of 6.08 ppm. The smallest percentage inhibition value is got in the combination treatment of the coconut shell raw material at a concentration of liquid smoke 1 ppm of 1.965% with IC50 of 7.77 ppm. The value of the percentage inhibition of cinnamon liquid smoke with the concentration of liquid smoke1500 ppm allegedly because at high concentrations there will be a lot of chemical components that can be outlined at the time of pyrolysis so that the percentage of inhibition become great. Based on standards of[21] states that the antioxidant activity of the three raw materials by four different pyrolysis temperature are classified as very powerful like with IC50 <50 ppm.

To view the strength of the raw material relationship with the pyrolysis temperature to the percentage inhibition (antioxidant) made the regression equation. The image of % inhibition (antioxidant) liquid smoke average for treatment of a combination of types of raw materials with different concentrations of liquid smoke as figure 3 below.

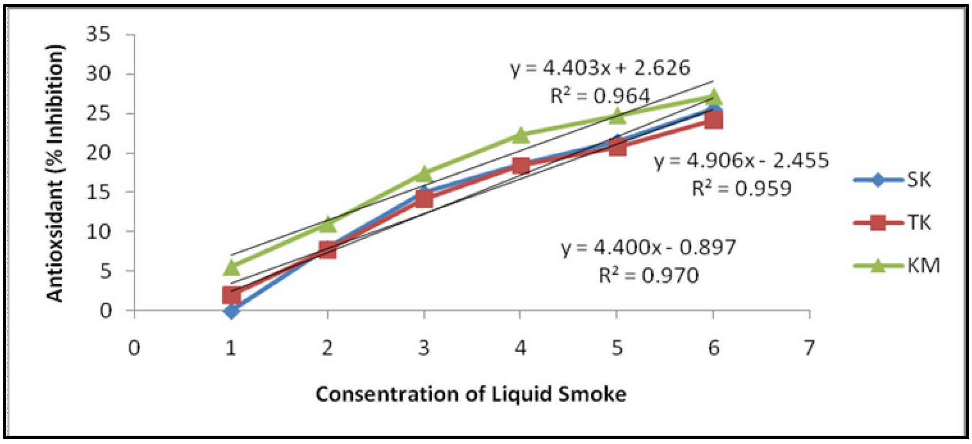


Figure 3. Average (%) for Inhibition (antioxidant) from some types of raw material with different pyrolysis temperature

Based on Figure 3 above that cinnamon at different concentrations show a strong correlation to the percentage inhibition (antioxidant) with R^2 of 0.9701 means that 97.01% the percentage of inhibition is affected by the combination treatment of cinnamon raw material at different concentrations. For the combination treatment of the coconut shells and coconut fiber raw material at different temperatures show R^2 value respectively 0.9599 and 0.9643. The high raw material cinnamon relationship in different concentrations of liquid smoke to the antioxidant (% inhibition) means cinnamon liquid smoke has a greater quality in reducing free radicals in the form of DPPH compare with coconut husk and coconut shell. [24-30]Antioxidants is substances in lower levels are able to inhibit the oxidation rate of molecular targets, thus it is often called as magic compound because it can counteract premature aging and various diseases that accompany it. Compounds that dwells in fruits, vegetables, fish, spices, and grains can stop the chain reaction of free radical formation in the body believed as the mastermind of premature aging[22].

3.3.Effect for combination treatment of liquid smoke concentration with different pyrolysis temperature to % inhibition (inhibition) of liquid smoke

Average test results of antioxidant activity for the treatment of liquid smoke concentration with different pyrolysis temperatures which are obtained can be seen in the following table 4.

Table 4. Activity (%) Inhibition (antioxidant) for the combination treatment of liquid smoke concentration and temperature pyrolysis different.

Kodesampel	(%) Inhibition	IC_{50} (ppm)
K1T1 (concentration of liquid smoke 1 ppm, temperature 100 ±10°C)	3.028±1,91 ij	4,95
K1T2 (concentration of liquid smoke 1 ppm, temperature 200 ±10°C)	2.6784±1,66 gh	5,62
K1T3 (concentration of liquid smoke 1 ppm, temperature 300 ±10°C)	3.1863±2,37 ef	4,08
K1T4 (concentration of liquid smoke 1 ppm, temperature 400 ±10°C)	4.170±3,49 cde	4,96
K2T1 (concentration of liquid smoke 10 ppm, temperature 100 ±10°C)	8.5778±1,32 cd	4,95
K2T2 (concentration of liquid smoke 10 ppm, temperature 200 ±10°C)	8.5241±1,37 bcd	5,62
K2T3 (concentration of liquid smoke 10 ppm, temperature 300 ±10°C)	8.1290±1,20 ij	4,08
K2T4(concentration of liquid smoke 10 ppm, temperature 400 ±10°C)	10.381±3,41 gh	4,96
K3T1(concentration of liquid smoke 100 ppm, temperature 100 ±10°C)	15.117±1,19 ef	4,95
K3T2(concentration of liquid smoke 100 ppm, temperature 200 ±10°C)	14.678±1,29 cde	5,62
K3T3(concentration of liquid smoke 100 ppm, temperature 300 ±10°C)	14.737±0,97 bcd	4,08
K3T4(concentration of liquid smoke 100 ppm, temperature 400 ±10°C)	17.409±3,70 bc	4,96

K4T1(concentration of liquid smoke 500 ppm, temperature 100 ±10°C)	19.072±1,59 ef	4,95
K4T2(concentration of liquid smoke 500 ppm, temperature 200 ±10°C)	18.997±1,14 cde	5,62
K4T3(concentration of liquid smoke 500 ppm, temperature 300 ±10°C)	18.246±1,09 de	4,08
K4T4(concentration of liquid smoke 500 ppm, temperature 400 ±10°C)	22.510±6,04 bcd	4,96
K5T1(concentration of liquid smoke 1000 ppm, temperature 100 ±10°C)	20.642±1,25 cd	4,95
K5T2 (concentration of liquid smoke 1000 ppm, temperature 200 ±10°C)	21.376±1,88 bcd	5,62
K5T3(concentration of liquid smoke 1000 ppm, temperature 300 ±10°C)	21.268±1,43 bcd	4,08
K5T4(concentration of liquid smoke 1000 ppm, temperature 400 ±10°C)	25.949±6,57 ab	4,96
K6T1(concentration of liquid smoke 1500 ppm, temperature 100 ±10°C)	22.054±2,82 bcd	4,95
K6T2(concentration of liquid smoke 1500 ppm, temperature 200 ±10°C)	23.505±2,74 bc	5,62
K6T3(concentration of liquid smoke 1500 ppm, temperature 300 ±10°C)	26.328±3,76 ab	4,08
K6T4(concentration of liquid smoke 1500 ppm, temperature 400 ±10°C)	30.559±6,11 a	4,96

Information : * different superscript alphabet in average column shows ral different (P<0,05)

Based on Table 4 shows the largest % inhibition (antioxidant), and is found in the combination treatment of the pyrolysis temperature of 400±10°C in liquid smoke concentration of 1500 ppm of 30.559% is not significantly different from other treatments with IC_{50} value of 4.96 ppm. The value of smallest percentage inhibition value is got in the combination treatment of the pyrolysis temperature of 200±10°C in liquid smoke 1 ppm concentration of 2.6784% with IC_{50} of 5.62 ppm. The value of the percentage inhibition of pyrolysis temperature of 400±10 C with concentration of 1500 ppm suspected with high temperature pyrolysis followed by a high concentration of liquid smoke will be more widely available in chemical components that can be outlined at the time of pyrolysis so that the percentage of inhibition become great. By the standards of[21] states that the antioxidant activity of the three raw materials by four different pyrolysis temperature are classified as very powerful like with IC_{50}<50 ppm.

To see the strength of the relationship of raw material with the pyrolysis temperature of the percentage inhibition (antioxidant) made the regression equation. The image of % inhibition (antioxidant) average for liquid smoke because pyrolysis temperature treatment combination of pyrolysis temperature with different concentrations of liquid smoke as figure 4 below.

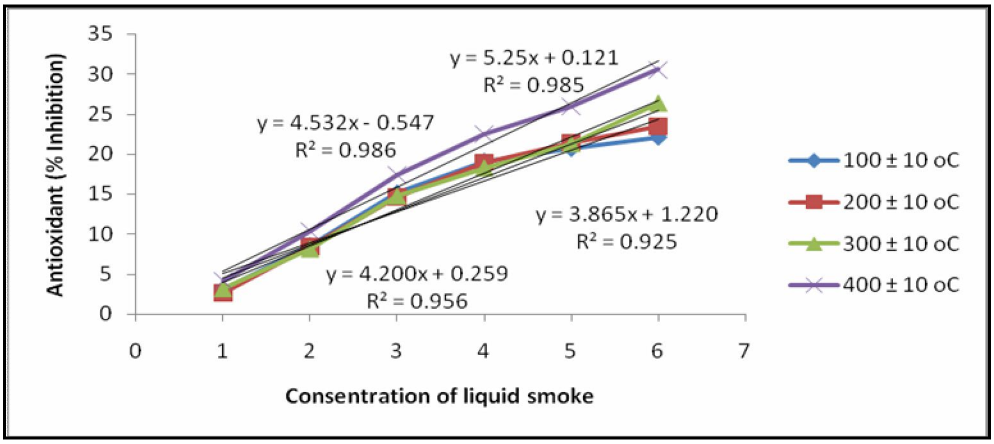


Figure 4. Average (%) for Inhibition (antioxidant) from treatment combination of pyrolysis temperature level and different concentration of liquid smoke

Based on the figure 4 above that the pyrolysis temperature of 200±10°C at different concentrations show a very strong correlation to the percentage of inhibition (antioxidant) with R^2 of 0.9866 means that 98.66% percentage of inhibition is affected by a combination treatment of the pyrolysis temperature of 200±10°C at different concentrations , For the combination treatment of the pyrolysis temperature of 100 ±10°C, 10 ° C ± 300 and 400 ± 10 ° C in different concentrations of liquid smoke indicates the value of R^2 each 0.955, 0.9258 and 0.9564. The high correlation of pyrolysis temperature of 200 ± 10°C in different concentration of liquid smoke differently to the antioxidants (% inhibition) means liquid smoke temperature pyrolysis of 200±10°C has a strong ability in reducing free radicals in the form of DPPH compared pyrolysis temperature of 100 ± 10°C, 300 ±10°C and 400±10°C, but based on the correlation value is obtained that four pyrolysis temperature at different concentrations of liquid smoke relationships equally strong against antioxidants (% inhibition).

3.4. Effect of combination of raw materials, the pyrolysis temperature with different liquid smoke concentration against% inhibition (inhibition) of liquid smoke

The average of % inhibition (antioxidant) for liquid smoke which is given treatment combination of raw material, pyrolysis temperature and concentration of different liquid smoke as well as IC_{50} value in Table 5 as follows:

Table 5. Results of the % inhibition average (antioxidant) which is given different treatment of raw materials, pyrolysis temperature and different concentrations.

Raw material	Temperature	Concentration (ppm)	Rerata % Inhibition	IC_{50} (ppm)
Coconut Fiber	100 + 10 °C	1	2.3133±1,87 a	92,737
		10	7.8821±0,72 a	43,80
		100	14.5235±0,75 a	38,61
		500	18.2089±0,51 a	43,95
		1000	20.2585±1,03 a	2,65
		1500	22.174±3,27 a	8,19
	200 + 10 °C	1	2.4429±1,87 a	92,73
		10	7.997±0,71 a	43,80
		100	14.620±0,75 a	38,61
		500	20.335±0,51 a	43,95
		1000	20.335±1,03 a	2,65
		1500	22.246±3,26 a	8,19
	300 + 10 °C	1	2.1741±1,87 a	92,737
		10	7.7348±0,72 a	43,80
		100	14.368±0,76 a	38,61
		500	18.056±0,51 a	43,95
		1000	20.913±2,18 a	2,65
		1500	27.017±5,27 a	8,19
	400 + 10 °C	1	2.1712±1,87 a	92,737
		10	8.1324±0,59 a	43,80
		100	16.161±1,57 a	38,61
		500	19.324±0,52 a	43,95
		1000	24.330±8,02 a	2,65
		1500	30.662±4,76 a	8,19
Cocunut shell	100 + 10 °C	1	2.1682±1,87 a	92,737
		10	7.714±0,72 a	43,80
		100	14.329±0,75 a	38,61
		500	18.010±0,51 a	43,95
		1000	20.053±1,03 a	2,65
		1500	21.959±3,26 a	8,19
	200 + 10 °C	1	1.8994±1,87 a	92,737
		10	7.4515±0,72 a	43,80
		100	14.078±0,76 a	38,61
		500	19.913±1,48 a	43,95
		1000	21.098±2,94 a	2,65
		1500	22.602±2,19 a	8,19
	300 + 10 °C	1	1,8969±1,87 a	92,737
		10	7.4413±0,71 a	43,80
		100	14.059±0,76 a	38,61

		500	17.752±0,51 a	43,95
		1000	20.652±0,83 a	2,65
		1500	26.063±5,20 a	8,19
	400 + 10 °C	1	1.8943±1,87 a	92,737
		10	8.3328±1,14 a	43,80
		100	14.040±0,76 a	38,61
		500	17.729±0,51 a	43,95
		1000	21.044±1,73 a	2,65
		1500	25.925±7,83a	8,19
Cinnamon	100 + 10 °C	1	4.6024±1,42 a	92,737
		10	10.138±0,70 a	43,80
		100	16.497±0,49 a	38,61
		500	20.996±1,09 a	43,95
		1000	21.616±1,39 a	2,65
		1500	22.031±3,23 a	8,19
	200 + 10 °C	1	3.683±1,15 a	92,737
		10	10.124±0,70 a	43,80
		100	15.336±2,08 a	38,61
		500	18.787±0,81 a	43,95
		1000	22.694±0,31 a	2,65
		1500	25.667±2,02 a	8,19
	300 + 10 °C	1	5.488±1,87 a	92,737
		10	9.211±1,42 a	43,80
		100	15.785±0,24 a	38,61
		500	18.930±1,75 a	43,95
		1000	22.238±0,76 a	2,65
		1500	25.905±0,81 a	8,19
	400 + 10 °C	1	8.445±0,77 a	92,737
		10	14.678±1,78 a	43,80
		100	22.027±0,63 a	38,61
		500	30.478±0,64 a	43,95
		1000	32.474±1,12 a	2,65
		1500	35.091±1,51 a	8,19

Information : * different superscript alphabet in average column shows real different ($P<0,05$)

In Table 5 shows the greatest % inhibition which is got the combination treatment of cinnamon raw material at pyrolysis temperature of 400 ± 10°C in liquid smoke concentration of 1500 ppm of 35.091% is not significantly different from other treatments with IC_{50} value of 8.19 ppm. The smallest percentage inhibition value is found in the combination treatment of the coconut shell raw material on the pyrolysis temperature of 200 ±10°C in liquid smoke 1 ppm concentration of 1.8943% with IC_{50} of 92.737 ppm. The value of the percentage inhibition in cinnamon raw materials at a temperature pyrolysis of 400 ± 10°C in a concentration of liquid smoke 1 500 ppm alleged cinnamon raw material with high pyrolysis temperature high followed by concentration of high liquid smoke that will be more available in chemical components that can be outlined during pyrolysis so that the percentage of inhibition becomes great. Based on standard of[21] states that the antioxidant activity of the three raw materials to four pyrolysis temperature and six different concentrations of different liquid smoke is classified as very strong with $IC_{50}<50$ ppm.

To view the strength of raw material relationship with the temperature pyrolysis and different concentrations of liquid smoke to the percentage of inhibition (antioxidant) made the regression equation. The following image for the average of antioxidant relationship (%) inhibition in combination treatment of raw material with pyrolysis temperature level and concentration of different liquid smoke found in Figure 5 below.

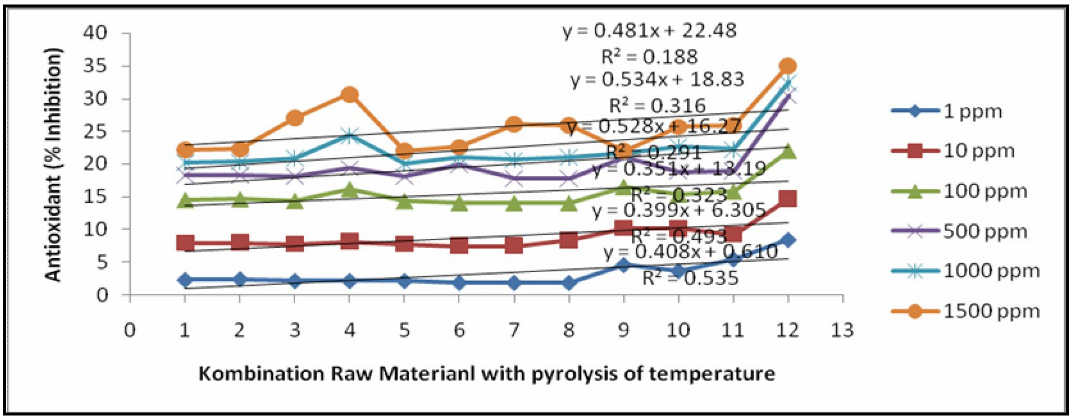


Figure.5. Average (%) Inhibition (antioxidant) from combination treatment for some types of raw material with pyrolysis temperature level as well as different concentration of liquid smoke

Based on the figure 5 above that all relationships combination treatment of raw materials and the pyrolysis temperature with liquid smoke concentration of 1 ppm, 10 ppm, 100 ppm, 500 ppm, 1000 ppm and 1500 ppm show a weak correlation to the percentage of inhibition (antioxidant) with R^2 of 0.1885 , 0.3189, 0.2919, 0.3231, 0.4935, 0.535. Weak tie of raw materials at a pyrolysis temperature at different concentrations of liquid smoke to the antioxidant (% inhibition) suspected that liquid smoke with raw material and pyrolysis temperature and the concentration of different liquid smoke haven't had a strong ability in reducing free radicals in the form of DPPH. Antioxidant effects are mainly cause because there are phenolic compounds such as flavonoids, phenolic acids. Usually, compounds which have antioxidant activity is a phenolic compound that a hydroxy group substituted in the ortho position and the -OH group and -or. After doing examination of the chemical content, liquid smoke contains phenolics. Testing of antioxidant activity is done by using DPPH free radical reduction method[23,24] that bases its principles on the sample (containing compounds are antioxidants) that can reduce free radicals (DPPH).

4. Conclusion

1. The largest percentage of inhibition is found in the combination treatment of cinnamon on pyrolysis temperature of 400 ± 10 ° C of 23.865% with IC_{50} value of 35.52 ppm.
2. The largest percentage of inhibition is found in the combination treatment of cinnamon raw materials at 1500 ppm concentration of liquid smoke of 27.173% is not significantly different from other treatments with IC_{50} value of 6.08 ppm.
3. The largest percentage of inhibition is found in the combination treatment of cinnamon on pyrolysis temperature of 400 ± 10 ° C in liquid smoke concentration of 1500 ppm of 30.559% with IC_{50} value of 4.96 ppm.
4. The largest percentage of inhibition is found in the combination treatment of cinnamon on pyrolysis temperature of 400 ± 10 ° C in liquid smoke concentration of 1500 ppm of 35.091% is not significantly different from other treatments with IC_{50} value of 8.19 ppm.

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