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THE ANTIOXIDANT CHARACTERISTICS OF THE LIQUID SMOKE OF COCOA SHELL (*THEOBROMA CACAO, L*) IN DIFFERENT WATER CONTENT VARIATIONS

I Ketut Budaraga [*,1], Eva Susanti [1], Asnurita [1], Elliza Nurdin [2], Ramaiyulis [3]

[1]Agricultural Technology Department, Faculty of Agriculture, Ekasakti University, Padang, Indonesia
[2]Animal Production Department, Faculty of Animal Husbandry, Andalas University, Padang, Indonesia
[3]Politeknik Pertanian Negeri Payakumbuh, Payakumbuh, Indonesia

*Corresponding author
Email: budaraga1968@gmail.com

Abstract. *Agricultural and plantation wastes, especially cocoa plants, have not been widely used, although in some conditions they have potential as animal feed ingredients and raw materials for composting. So needs a program potential utilization of waste produced by the plant cocoa is cocoa shell waste such as being liquid smoke. Liquid smoke is a natural food preservative. One of the advantages of liquid smoke is antioxidant compounds. This study aims to know the antioxidant activity of cocoa shell liquid smoke on a variety of different water content. This research is an experimental quantitative descriptive method so that an analysis of the antioxidant activity of liquid smoke from cocoa shell obtained. The results showed that the liquid smoke of cocoa shell at a moisture content of 25%, 20%, 15%, and 10% had strong antioxidant activity because the IC 50 values obtained were below 50 ppm.*
Keywords: *antioxidants, cocoa shell liquid smoke, the water content*

1. Introduction

Cocoa plants (*Theobroma cacao*, L) is one of the plantation commodities whose role is quite important for the national economy, especially the supply of employment, sources of income and foreign exchange of the State (Susanto & Hatta, 1992). In 2016, cocoa production in West Sumatra was 62,623 tons with an area of 152,885 ha, consisting of 60,254 tons of smallholder plantations with an area of 151,123 ha while private plantations 2,369 tons with a total of 1,762 ha. Production and width of cocoa plantations in Padang Pariaman Regency were 9,526 tons with an area of 725 ha with a total of 12,641 farmers (head of family) (Ditjenbun, 2014).

West Sumatra began to proclaim itself as one of the cocoa development areas in Indonesia. In 2014, cocoa plantation area was 129,129 ha, with production reaching 88,967 tons/year (Nasrul, 2015). Cocoa plantations in West Sumatra are mostly managed by the people, with low productivity and quality. Increasing the planting area and increasing cocoa

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production will increase the amount of cocoa pod waste. If the cocoa peel waste is not handled properly it will cause problems for the environment such as odors and others. The utilization of cocoa peel waste is still very limited, the community uses more cocoa shell waste as animal feed and compost fertilizer. Most of the cocoa pod waste produced was only left to rot around the plantation area (Purnamawati & Utami, 2014)

Cocoa plants are used as food, while cocoa pods are just thrown away, whereas cocoa pods can be used as an alternative to animal feed. The availability of cocoa pods in the harvest season is very large and is able to meet the needs of 635,305 livestock units (Puastuti & IWR, 2014).

Cocoa peel is a lignocellulose waste containing the main components in the form of lignin, cellulose, and hemicellulose. The shell of cacao fruit contains cellulose 36, 23 %, hemicellulose 1.14% and lignin 20-27.95% (Purnamawati & Utami, 2014). Cellulose and hemicellulose are polymerized from monosaccharides which can be converted into sugar under certain conditions. Lignin is an aromatic polymer that can be converted into phenolic compounds in the form of liquid smoke (Chen, 2014). Lignin decomposition on cocoa shell can use the pyrolysis method (Mashuni et al., 2017).

Pyrolysis is the heating process of a substance with limited oxygen so that there is a decomposition of heated sample components (Yaman, 2004). The pyrolysis method is carried out by heating at high temperatures and without using organic solvents. In the pyrolysis process heat, energy encourages oxidation so that complex carbon molecules decompose mostly into carbon or charcoal and the rest in the form of liquid smoke (bio-oil) (Hidayat & Qomaruddin, 2015).

Liquid smoke is the result of condensation from a large number of compounds formed due to the pyrolysis of wood constituents such as cellulose, hemicellulose, and lignin. Whereas according to (Darmadji, 2002) research phenol and acid content formed due to the results of pyrolysis of lignin and cellulose. The composition of liquid smoke is very complex and consists of components derived from different groups of chemical compounds, such as Aldehyde, Ketones, Alcohol, Acids, Esters, Furan and Pyran derivatives, Phenolic, Hydrocarbon, and Nitrogen derivatives (Soldera et al., 2008).

The prospect of using this vast liquid smoke has various advantages compared to the use of synthetic chemicals. Liquid smoke is easier to apply because the concentration of

liquid smoke is easily controlled to give the same flavor and color uniformly. Liquid smoke contains various compounds that can be grouped into groups of phenol compounds, acids, and groups of carbonyl compounds. Groups of these compounds act as antimicrobials, antioxidants, flavoring, and coloring. Because liquid smoke can act as an antimicrobial and antioxidant, liquid smoke can be used as a preservative (Yulistiani, 2017).

Base on information about the benefits of liquid smoke brown shell, brown shell that liquid smoke has the potential antioxidant (Lorenzo et al., 2016). Cocoa fruit shell contains compounds of polyphenols and flavonoids. These polyphenols and flavonoids have antioxidant activity. The active compounds extracted from cocoa peel from both ripe and young fruits were determined by antioxidant activity using the DPPH method (Jusmiati et al., 2016). Natural antioxidant compounds in plants are generally in the form of phenolic compounds or polyphenols which can be in the form of flavonoids, cinnamic acid derivatives, coumarins, tocopherols, and polyfunctional organic acids (Ayucitra et al., 2011). Phenolic compounds have diverse biological activities, and many used in enzymatic coupling oxidation reactions as a donor substrate H. Coupling oxidation reactions, besides requiring an oxidizer also requires the presence of a compound that can donate H. Phenolic compounds are ideal examples of compounds that easily donate H. The presence of a hydroxyl group in the phenolic compound group has many benefits. The phenolic hydroxyl group is a good hydrogen (H +) atom donor. As antioxidant hydrogen atoms will react with oxygen (O) reactive or nitrogen (N) reactive species in terminations that break new radical generation cycles (Valentão et al., 2003). The interaction of hydrogen with oxygen-reactive species will inhibit oxidation reactions. The interaction of hydroxyl phenolic groups from the benzene ring provides special properties of molecules, especially the ability to produce free radicals where radicals are stabilized by delocalization (Parra, 2002).

Antioxidants are electron-giving compounds or reductants. This compound has a small molecular weight but is able to inactivate the development of oxidation reactions, by preventing radical formation. Antioxidants are also compounds that can inhibit oxidation reactions, by binding to free radicals and molecules that are very reactive (Winarsih, 2007). One test for determining radical capture antioxidant activity is the DPPH method (1,1-Diphenyl-2- Picrylhydrazyl). DPPH is widely used to measure and compare the antioxidant

activity of phenolic compounds, and evaluate antioxidant activity through DPPH color changes from purple to yellow (Molyneux, 2003).

The best quality of liquid smoke can be found in the cinnamon treatment of raw materials at a temperature of 400 ºC pyrolisis, namely pyrolysis equipment performance 16.29 ml/hour (Budaraga et al., 2016). The quality of liquid smoke is strongly influenced by the quality of raw materials, water content and pyrolysis temperature. So far there has been no study of the characteristics of liquid smoke antioxidants in different water content variations. The purpose of this study is, to find out the antioxidant activity in different water content variations.

2. Materials and Methods

Place of the research

The place of research was carried out at the Agricultural Product Technology Laboratory of the Faculty of Agriculture, Ekasakti University and the Laboratory of Mechanical Engineering Production Process, Faculty of Engineering, Ekasakti University

Material

The materials used in this study were cocoa shell type criollo dried according to treatment obtained from Padang Pariaman Regency and Lubuk Minturun in Padang City, ethanol (pa merc), aquades, DPPH (1,1-diphenyl-2-picrylhydrazyl), cotton, Whatman filter paper, gauze, methylated, aluminum foil and tissue.

Instrument

A set of pyrolysis devices (Budaraga et al., 2016)**,** UV-Vis spectrophotometer (Jasco V-360), laminar air flaw, Erlenmeyer (pyrex), measuring cup (Pyrex), stirring rod, analytical scales (Explorer Ohaus), rubber suction *(filler),* pipette volume, syringes, spray bottle, Bunsen, measure pipette, a volumetric flask *(pyrex),* vortex, pipette, funnel, stative, clamps, *aluminum foil,* scissors, knives, gloves, glass bottles, bottle container as liquid.

Research Procedure

Sample preparation

The raw material in this study is cocoa shell obtained in Padang Pariaman Regency and Lubuk Minturun Kota Padang. The procedure to producing liquid water cacao shell include: Wash the shell cocoa, cocoa shell Enumeration diameter of 5-9 cm 2. Then the cocoa shell is continued by dried under the sun until the water content reaches 25%, 20%, 15%, and 10%.

Pyrolysis

Each cocoa shell sample is weighed as much as 1000 g based on the treatment starting from the water content (25%, 20%, 15%, and 10%) then put into the pyrolysis reactor which is equipped with a series of condensation equipment and cooling condenser (Budaraga et al., 2016). The reactor is equipped with a temperature gauge. Electric heating is in the form of a reactor sheath with a current of 10 amperes. Pyrolysis runs at 400 °C. Pyrolysis was stopped after no liquid smoke dripped into the shelter. The results of pyrolysis in the form of liquid smoke are collected in dark bottles and then left to stand, then filtered using filter paper and activated carbon and gauze to separate tar and liquid smoke (Kadir et al., 2010). After being deposited for 1 (one) week, an analysis of antioxidant activity was carried out.

Determining Antioxidant Activities of the DPPH method (Molyneux, 2003)

The DPPH method is an effective and fast colorimetric method for estimating antiradical/antioxidant activity. This chemical test is widely used in natural product research for antioxidant phytochemical isolation and to test the capacity of extracts and pure compounds to absorb free radicals. DPPH method used to measure a single electron as the hydrogen transfer activity at once to measure the inhibitory activity of free radicals(Pratimasari, 2009).

DPPH (*1,1-diphenyl-2-pikrilhidrazil*) radical is an unstable nitrogen-containing organic compound with strong absorbance at λmax 517 nm and dark purple. after reacting with antioxidant compounds, DPPH will be reduced and the color will change to yellow. These changes can be measured by a spectrophotometer and plotted against concentration. Decreasing color intensity is caused by reduced conjugated double bonds in DDPH. This occured if there is an electron capture by an antioxidant, causing no chance of these electrons to resonate (Pratimasari, 2009). The existence of an antioxidant which can donate electrons to DPPH produces yellow which is a specific characteristic of DPPH radical reactions. Capture of free radicals causes electrons to become pairs which then causes color loss that is proportional to the number of electrons taken (Sunami, 2005). The work procedure measures the antioxidant activity as follows:

- DPPH was weighed as much as 0.0045 and then dissolved with methanol in a 100ml volumetric flask to obtain a DPPH with a concentration of 45 ppm.

- The Sample was weighed as much as 1 gram, diluted in pumpkin 100 for a concentration of 1000 ppm mother liquor.
- The main solution of 1000 ppm is diluted by piping 0.125. 0.5, 1, 1.5, 2. 2.5 ml dissolved in a 10 ml flask with methanol to the boundary mark, a dilution that has been obtained in 1 ml pipette plus 2 ml methanol plus 1 ml DPPH and then in the vortex and incubated for 30 minutes.
- The solution that has been buried is then measured using UV-VIS spectrophotometer.
- The results of the data obtained were analyzed by using Excel 2007 to get a simple genetic line. Formula : APRB = $1 - \frac{\text{Absorbance of sample}}{\text{Absorbance}} \text{x}100\%$

3. Results and Discussion

Antioxidant Activity

After obtaining the optimum wavelength, which is at a wavelength of 515 nm, then the absorbance measurement is done on the tube (blank) containing 1 ml of methanol and 1 mL of DPPH. Then an absorbance meter was measured to measure the absorbance of liquid cocoa peel smoke with DPPH using a UV-Vis spectrophotometer before applying the maximum wavelength of DPPH. The maximum DPPH wavelength used was at a wavelength of maximum wave 515nm. This DPPH wavelength gives the maximum absorbance of the test solution and provides the greatest sensitivity. Furthermore, the amount of the antioxidant activity of cocoa on the shell smoke used was measured at the maximum wavelength.

The comparison used is vitamin C from each representing synthetic antioxidant and natural antioxidants. Vitamin C is used as the comparison because it serves as the secondary as an antioxidant capture free radicals and prevents with a chain. Vitamin C is referred to as a secondary anti-oxidant group capable of counteracting various free radicals that can counteract various extracellular free radicals. because vitamin C has a free hydroxy group that acts as a free radical catcher and if it has a polyhydroxy group it will increase antioxidant activity (Isnindar et al., 2011). The average results of the analysis of the antioxidant activity of cacao shell liquid smoke at different pyrolysis temperatures and water content can be seen in Table 1, 2,3,4 and antioxidant activity of vitamin C in Table 5.

Table 1. The average of the result antioxidant activity at liquid smoke on cocoa shell at 10% moisture content

Concentration	Abs	Aprb

0.25 ppm	0.387	32
0.5 ppm	0.339	42.35
1 ppm	0.285	51.02
1.5 ppm	0.144	75,51
2 pmm	0.079	86.56

Table 2. The average of the result antioxidant activity of liquid cocoa smoke at 15% moisture content

Concentration	Abs	Aprb
0,25 ppm	0,414	21,14
0,5 ppm	0,361	31,32
1 ppm	0,288	45,14
1,5 ppm	0,185	64,76
2 ppm	0,146	72,19

Table 3. The average of the result antioxidant activity of liquid smoke of cocoa peel at 20% moisture content

Concentration	Abs	Aprb
0,125 ppm	0,350	33,33
0,25 ppm	0,273	48,00
0,5 ppm	0,019	64,57
1 ppm	0,111	78,85
1,5 ppm	0,111	92,00

Table 4. The average of the result antioxidant activity in liquid smoke of cocoa peel at 25% moisture content

Concentration	Abs	Aprb
0,25 ppm	0,272	48,19
0,5 ppm	0,247	52,95
1 ppm	0,197	62,47
1,5 ppm	0,036	72,83
2 ppm	0,145	93,14

Table 5. The average of the result antioxidant activity of vitamin C

Concentration	Abs	Aprb
2 ppm	0,350	33,33
4 ppm	0,314	40,19
6 ppm	0,274	47,80
8 ppm	0,242	53,90
10 ppm	0,207	60,57

Furthermore, the results above will calculated by regression equation with the concentration of liquid smoke (ppm) as the abscissa axis (X) and the value of% inhibition (antioxidant) as the ordinate (Y-axis). The $IC_{50\ value}$ from the calculation at% inhibition is 50%. $Y = ax + b$. Based on the analysis above by using simple linear regression to obtain

graphs of linear and linear regression equation between the concentration liquid smoke with an adsorbance such as Figure 1,2,3,4 and antioxidant activity of vitamin C in Figure 1 below.

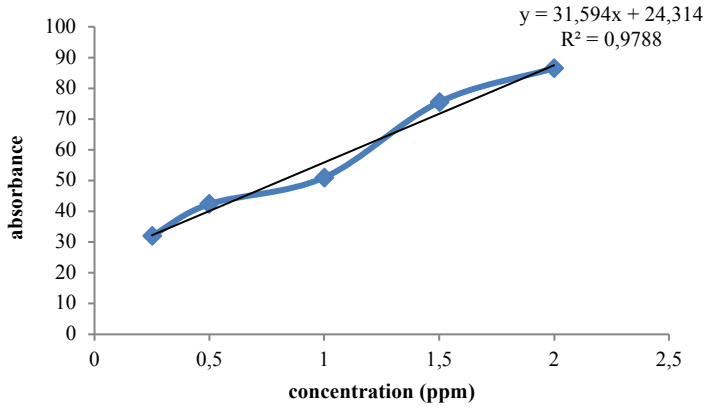

Figure 1. Linear regression graph of antioxidant activity and liquid smoke of cocoa shell at 10% moisture content

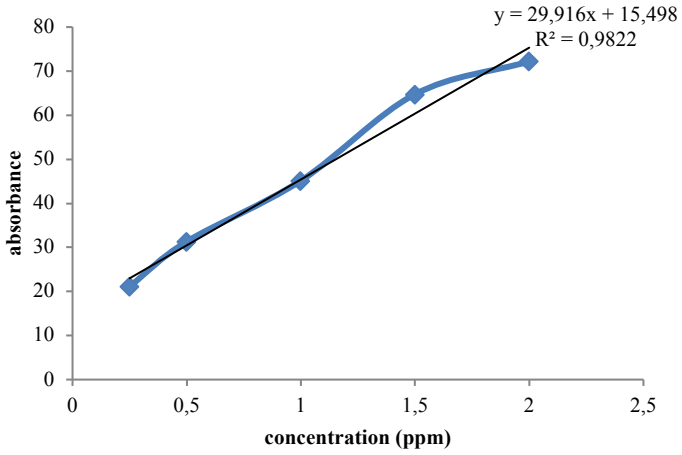

Figure 2. Linear regression graph antioxidant activity of liquid smoke of cocoa shell at 15 % moisture content

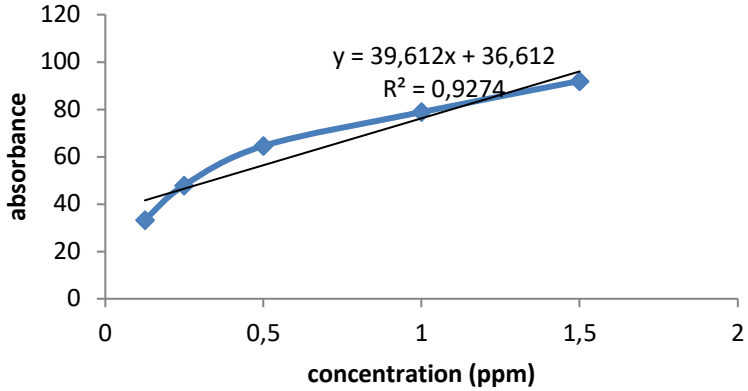

Figure 3. Linear regression graph antioxidant activity of liquid smoke of cocoa shell

at 20% moisture content

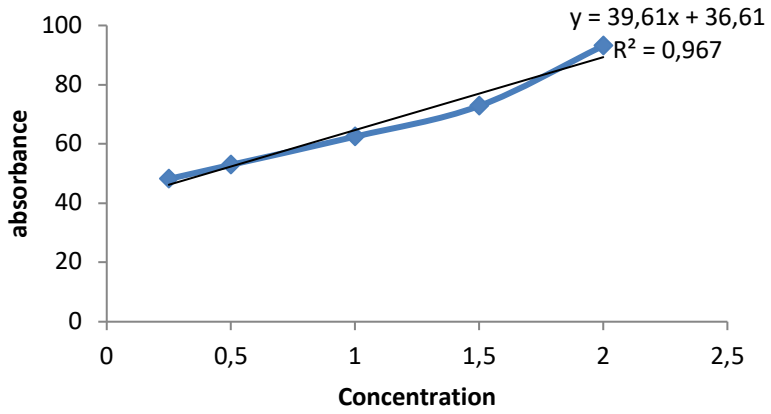

Figure 4. Linear regression graph antioxidant activity of liquid smoke of cocoa shell at 25% moisture content

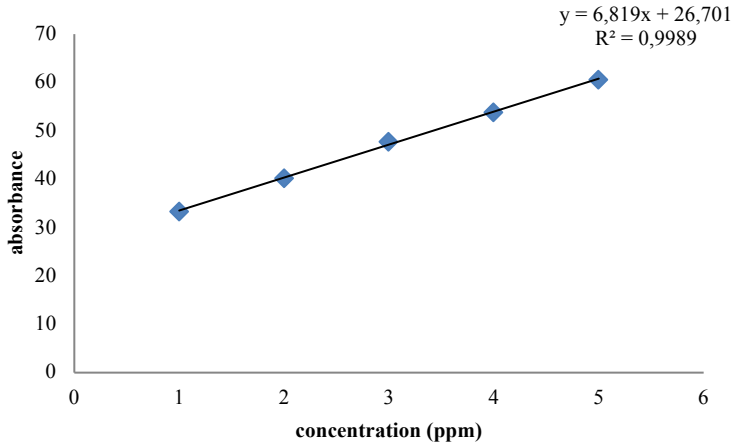

Figure 5. Linear regression graph antioxidant activity of vitamin C

The value of IC $_{50}$ with AAI for the liquid smoke of cocoa peel at different water content and vitamin C variations is presented in Tables 6, 7,8,9 and 10 below.

Table 6. Results of antioxidant activity of cocoa shell liquid smoke moisture content of 10 percent

Concentration (ppm)	Absorbance average (%)	IC $_{50}$ (ppm)	AAI
0,25	32		
0,5	42,35		
1	51,02	0,8132	5,5336
1,5	75,51		
2	86,56		
Form DPPH	0,457		

Table 7. Results of antioxidant activity of liquid cocoa smoke in water content of 15 percent

Concentration (ppm)	Absorban average (%)	IC $_{50}$ (ppm)	AAI
0,25	21,14		
0,5	31,32		
1	45,14	0,0924	4,1193
1,5	64,76		
2	72,19		
Form DPPH	0,482		

Table 8. The results of the antioxidant activity of liquid cocoa smoke in water content of 20 percent

Concentration (ppm)	Absorbance average (%)	IC $_{50}$ (ppm)	AAI
0,125	33,33		
0,25	48		
0,5	64,57	0,4238	0,0010
1	78,85		
1,5	92		
Form DPPH	0,482		

Table 9. Results of antioxidant activity of cacao shell liquid smoke of 25 percent moisture content

Concentration (ppm)	Absorbance average (%)	IC $_{50}$ (ppm)	AAI
0,25	48,19		
0,5	52,95		
1	62,47	0,3137	0,0014
1,5	72,83		
2	93,14		
Form DPPH	0,482		

Table 10. Antioxidant results of vitamin C

Concentration (ppm)	Absorbance average (%)	IC $_{50}$ (ppm)	AAI
0,25	33,33		
0,5	40,19		
1	47,8	0,7375	6,1016
1,5	53,9		
2	60,57		
Form DPPH	0,482		

In Table 6,7,8,9 and 10 showed that the values of IC $_{50}$ of liquid smoke cocoa shell on the graph view IC $_{50}$ values of liquid smoke cacao smaller shell of vitamin C except for liquid smoke water content of 10%. It is because the liquid smoke of cocoa shell is not a pure

compound but still contains other compounds that may have antioxidant activity. IC_{50} value at 10% moisture content greater than the value of the IC_{50} water content 15%, 20% and 25% of small. IC_{50} means that higher antioxidant activity. The compound is said to be an antioxidant very strong if the IC_{50} value is less than 50 ppm, strong for IC is worth 50-100 ppm, medium for 100-150 ppm and weak if IC_{50} a value of more than 150 ppm (Blois, 1958). This means that the antioxidant activity of liquid smoke from each different pyrolysis temperature has a very strong activity because the IC_{50} value obtained from the regression equation is smaller than 50 ppm.

Water content is one of the factors that affect the quality of liquid smoke from the pyrolysis products produced. The moisture content of the raw materials used can affect the result and quality of liquid smoke produced. The water content that is too high will reduce the quality of liquid smoke because it will reduce the level of products produced, such as acid and phenol. The liquid produced will decrease further. The use of high temperatures in making liquid smoke will increase the solubility of phenol. High temperatures can release wall cell phenol compounds or bound phenolic compounds caused by damage to cell elements, causing more phenol compounds found in liquid smoke so that antioxidants will increase. (Luditama, 2006) who obtained liquid smoke phenol levels using old coconut coir materials, which is around 0, 89 % and in coconut shell around 1.40%. The difference in phenol levels produced from this study is caused by the lignin content of the fumigant. Lignin is a wood component which when decomposed will produce phenol compounds. According to (Djatmiko, Ketaren, & Setyakartini, 2009) that lignin content in ripe coconut fruit is around 29.2%, in raw coconut fruit is 20.1%, whereas that coconut shell contains lignin is 33.30%. The difference in the lignin content of the fumigant material affects the phenol content of the liquid smoke produced.

4. Conclusions

Based on the results obtained in the study of cacao liquid smoke at different water levels which were tested for antioxidant activity. IC_{50} value in liquid smoke samples of cocoa peel at 10% moisture content is 0.8132 ppm, at 15% moisture content of 1.0924 ppm, 20% moisture content of 0.4238 ppm, water content of 25% at 0.1411 ppm, and vitamin C of 0.7375. This shows that liquid smoke samples with water content ranging from 10%, 15%,

20%, and 25% have strong antioxidant activity because the IC $_{50}$ values obtained are still below 50 ppm.

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