Analysis Antioxidant IC50 Liquid Smoke of Cocoa Skin with Several Purification Method

by Rera Aga Salihat

Submission date: 30-Jun-2022 06:40PM (UTC-0500)
Submission ID: 1865198802
File name: Budaraga_2021_IOP_Conf._Ser.__Earth_Environ._Sci._757_012053.pdf (450.19K)
Word count: 3939
Character count: 20835

PAPER · OPEN ACCESS

Analysis Antioxidant IC50 Liquid Smoke of Cocoa Skin with Several Purification Methods

To cite this article: I K Budaraga and D P Putra 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **757** 012053

Analysis Antioxidant IC_{50} Liquid Smoke of Cocoa Skin with Several Purification Methods

I K Budaraga[1], D P Putra[1]

[1] Department of Agricultural Product Technology, Faculty of Agriculture, Universitas Ekasakti,Veteran Dalam Street No. 26 B Padang, Indonesia

Corresponding author's email address: budaraga1968@gmail.com

Abstract. Cocoa skin waste can be used as the raw material for making liquid smoke by pyrolysis. Liquid smoke has a function as the preservative in food. The use of liquid smoke as the preservative must go through several purification processes. The purpose of this study was to determine the antioxidant content of cocoa skin liquid smoke in several purification methods. The purification process uses several methods in purifying liquid smoke, such as distillation, purification with zeolite, purification by active charcoal and the mixture of zeolite and active charcoal. The results of IC50 antioxidant activity of cocoa skin liquid smoke by distillation, purification with zeolite, purification with active charcoal and combined purification between active charcoal and zeolite obtained IC50 values of 217.55 ppm for the distillation method, 164.15 ppm for purification with active charcoal and zeolite, 143.43 ppm for purification with zeolite and 44.05 ppm for purification with active charcoal. Purification with active charcoal is the best treatment and has a very strong category of Antioxidants compared to the other three treatments.
Keywords: IC50 Antioxidant, Cocoa Skin, Liquid Smoke, Purification

1. Introduction

Cocoa skin is one of the agricultural wastes that its utilization is still very limited. Cocoa farmers usually only discard and let the skin of the cocoa skin rot and dry around the cocoa plantations. Cocoa skin has many benefits if further processed. Some reports mention the content of cocoa skins have active compounds that are beneficial to humans. Cocoa skins contain phenolic compounds and flavonoids [1]. The polyphenol content includes cinnamic acid, tannin, pyrogallol, quercetin, resorcinol, and epicatechin-3-galat [2]. Previous studies have reported that cocoa skins have antibacterial compounds to inhibit bacterial growth, including research by [3] that extracts bioactive components from cocoa skin waste and their effects on antioxidant and antimicrobial activities, [4] conducted antibacterial activity tests of cocoa skin extracts (Theobroma cocoa L.) against Escherichia coli, Bacillus subtilis, and Staphylococcus aureus, [5] analyzing the chemical substance of cocoa skin liquid smoke with GC-MS method. Seeing the potential of the cocoa skin, this study utilizes the cocoa skin to produce liquid smoke.

Liquid smoke is a result of pyrolysis or condensation of vapor from indirect or direct combustion of materials that contain lots of carbon and other compounds. Raw materials that are widely used are wood,

palm oil humps, pulp produced by wood, and others. According to [6], liquid smoke is compounds that evaporate simultaneously from heat reactors through pyrolysis techniques and condensed in the cooling system.

Liquid smoke contains various compounds that can be grouped into groups of phenol compounds, acids, and carbonyl compound groups. Groups of these compounds play the role of the antimicrobial, antioxidant, flavoring, and coloring. Because liquid smoke can act as an antimicrobial and antioxidant, liquid smoke can be used as a preservative [7]. Antioxidants are compounds that can inhibit oxidation reactions, by binding to free radicals and highly reactive molecules. As a result, cell damage can be inhibited [8]. Based on the source, antioxidants can be classified into 2 types, namely natural antioxidants and antioxidant synthesis. However, there are concerns about the side effects of synthetic antioxidants making natural antioxidants the chosen alternative [9].

The antioxidant activity of liquid smoke from rubber fruit shells can be determined from the inhibitor concentration value of 50% (IC50). IC50 is a value that indicates the concentration of the sample that can inhibit free radical activity by 50%. Each of these values and the percent inhibition of known concentration plotted on the graph so obtained linear equation $y = mx + c$ to change the value of y with 50 of linear line equation, then it will get the value of x that becomes the value of IC50. IC50 values obtained from the straight-line equation of 101.27 ppm. The smaller IC50 value, the stronger the antioxidant activity of the compound. A compound is said to be a very strong antioxidant if IC50 value is less than 50 ppm, active if it is worth 50-100 ppm, while if it is 101-250 ppm and weak if it is worth 250-500 ppm. The obtained IC50 values can indicate that the liquid smoke of rubber fruit shells has moderate antioxidant activity [10].

Research on the antioxidant effect of liquid smoke is very important considering that liquid smoke is currently being used commercially by the food industry [11]. Purification of liquid smoke has been carried out by [12] using active charcoal to purify the liquid smoke of durian skin. Furthermore [13] purifies liquid smoke from a coconut shell using a filtration method with active charcoal and zeolite and distillation. Research by [14], which purifies liquid smoke with different types of active charcoal. Research by [15], purifies liquid smoke by the distillation method.

Based on the description above, this research was conducted the purification of smoke liquid by several methods and analyze the value of antioxidants IC50 liquid smoke cocoa skin with some methods of purification. The purpose of this method is to determine the results of the antioxidant activity of IC50 liquid smoke of cocoa skin by distillation, purification with zeolite, purification with activated charcoal and a combination of zeolite and activated charcoal, so that it can be used as a natural preservative in food.

2. Materials and Methods

This research was conducted at the Agricultural Product Technology Laboratory of Ekasakti University in July - September 2019. This study uses an exploratory design by looking at the IC50 content antioxidant activity of liquid smoke with several purification methods. Liquid smoke can be obtained by the pyrolysis method.

The material used in this study was cocoa skin obtained from Lubuk Minturun Village, Padang City, and pyrolysis was carried out until liquid smoke of cocoa skins was obtained. Aquades, active charcoal, zeolite, methanol (p.a), diphenyl pycril hidrazil (DPPH) (Sigma Aldrich).

The equipment used is a series of pyrolysis tools, distillation equipment series, 250 ml filter, test tubes, gels cups, glass funnels, filter paper, micropipettes, vortex, plastic wrap, aluminum foil and photometer Spectro.

2.1 Purification of liquid smoke

2.1.1. Distillation [13]

This process is carried out to separate components from a mixture on the basis that some components can evaporate faster than other components. The resulting steam contains more volatile components, so the process of separating the components from the mixture can occur. Distillation smoke liquid can be carried out at a temperature of 100oC to 150oC. The liquid smoke distillation process can also eliminate unwanted compounds, namely tar compounds and aromatic polycyclic hydrocarbons.

2.1.2. Active charcoal [16]

The liquid smoke filtrate from active zeolite filtration is refiltrated using active charcoal derived from activated cocoa shell charcoal. The liquid smoke filtrate is put into a column containing activated carbon then the filtrate obtained is collected in a container for testing.

2.1.3. Zeolites [17]

Liquid smoke resulting from filtering is then filtered using active zeolite by flowing distillate liquid smoke into the active zeolite column to obtain the purified liquid smoke filtrate using zeolite. The filtrate is stored in a container for testing.

2.1.4. Mix of active charcoal and modified zeolite [17]

Liquid smoke produced from the pyrolysis process is added to active charcoal and zeolite, then allowed to stand for overnight. After 24 hours the liquid smoke is filtered using filter paper, then the value of its antioxidant activity is observed.

2.2. Testing of IC_{50} Antioxidant Activity [18]

2.2.1. Sample preparation

Liquid smoke samples used for the antioxidant activity test were taken from the filtering results with several purification methods, namely with active charcoal, purification with zeolite, purification by distillation and purification with a mixture of active charcoal and zeolite. Weigh the sample up to 0.1 gr plus 10 ml of methanol (pa) as mother liquor. Samples from the mother liquor are weighed and made in various concentrations, namely: 15, 30, 45, 60, and 75 ppm into 15 test tubes. Pipette 1 ml solution of each concentration plus 2 ml of methanol and 1 ml dpph then let stand for 30 minutes, then read using a UV-VIS spectrophotometer at a wavelength of 517 nm. As a blank, 3 ml of methanol was used and added with 1 ml dpph.

2.2.2. Testing IC50 antioxidant activity of liquid smoke

The method used in determining the antioxidant activity of liquid smoke of cocoa skin is using the DPPH method (2,2-diphenyl-1-pikrihidrazil). DPPH method used DPPH (2,2-diphenyl-1-pikrihidrazil) as free radicals. This method was chosen because the method is simple, fast, and easy for screening the radical capture activity of several compounds, besides this method has proven to be accurate and practical [19] The measured absorbency of DPPH is the absorbance of DPPH that remains after being reacted with the test solution. From the residual DPPH absorbance value, it can be seen the antioxidant activity of each test solution in inhibiting DPPH free radicals. From the absorbance value obtained then calculated percent inhibition (damping) against DPPH free radicals, namely the amount of activity of antioxidant compounds that can capture DPPH free radicals. Calculation of percent DPPH antioxidant activity used the following formula:

$$\text{Antioxidant activity (\%)} = \frac{(\text{A blank} - \text{A sample})}{\text{A blank}} \frac{(\text{A blank} - \text{A sample})}{\text{A blank}} \text{ x } 100\%$$

Information:

A blank = radical DPPH absorption of 0.4 mM

A sample = radical DPPH 0.4 mM uptake after sample treatment.

The amount of antioxidant activity from cocoa skin smoke liquid can be determined from the inhibitor concentration values of 50% (IC50). IC50 is the value that indicates the concentration of the sample that can inhibit radical activity by 50%. Each of the inhibition % value and known concentration plotted on the graph so obtained linear equation y = mx + c to change the value of y with 50 of linear line equation, it will get the value of x as the value of IC50.

3. Results and Discussion

Liquid smoke used in this study is liquid smoke from the skin of cocoa fruit taken from the pyrolysis without purification (grade 3). In this study, it carryout the purification of cocoa skin smoke liquid by using several methods of purification, such as purification with active charcoal, with zeolite, with active charcoal + zeolite and distillation. In testing the antioxidant activity, it employs DPPH radical compounds as indicators to see the percentage of free radical inhibition. This method is commonly used because it has simple advantages and the work is carried out very simply. Measurement of the antioxidant activity of the sample is done by looking at the wavelength path which is the maximum wave DPPH.

The presence of antioxidant activity from the sample results in a change in color in the DPPH solution in methanol which was originally dark purple to brownish-yellow. This is a change in color or what is called reacting. The results of the antioxidant activity test at all concentrations obtained must be measured using a UV-VIS spectrophotometer. Antioxidants activity is indicated by the value IC50 (Inhibitory Concentration 50%). The IC50 value is the value of the concentration of antioxidants to reduce 50% of free radical activity. The test results a percentage inhibition of antioxidant using several methods of purification of liquid smoke in Table 1 and the linear equation antioxidant activity or inhibition of free radicals using the DPPH method, these can be seen in Figures 1, 2, 3 and Figure 4.

Tabel 1. Percentage data on antioxidant inhibition of cocoa skin liquid smoke after purification.

Treatment	Solution concentration (ppm)	Abs	Blank	%inhibition
Distillation	15	0,358	0,526	17,8
	30	0,328	0,526	20,8
	45	0,306	0,526	23,0
	60	0,282	0,526	25,4
	75	0,263	0,526	27,3
Mix	15	0,356	0,526	18,0
	30	0,334	0,526	20,2
	45	0,301	0,526	23,5
	60	0,266	0,526	27,0
	75	0,226	0,526	31,0
Zeolites	15	0,159	0,526	37,7
	30	0,146	0,526	39,0
	45	0,133	0,526	40,3
	60	0,117	0,526	41,9
	75	0,101	0,526	43,5
Active Charcoal	15	0,062	0,526	47,4
	30	0,046	0,526	49,0

	45	0,035	0,526	50,1
	60	0,022	0,526	51,4
	75	0,011	0,526	52,5

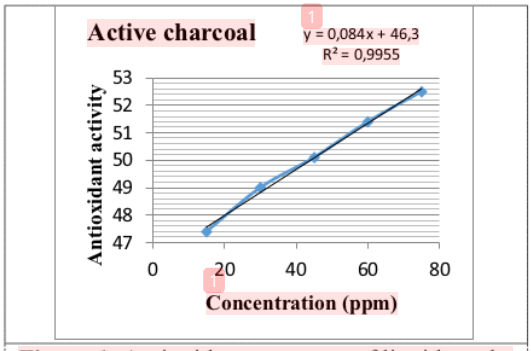

Figure 1. Antioxidant test curve of liquid smoke from active charcoal filtering

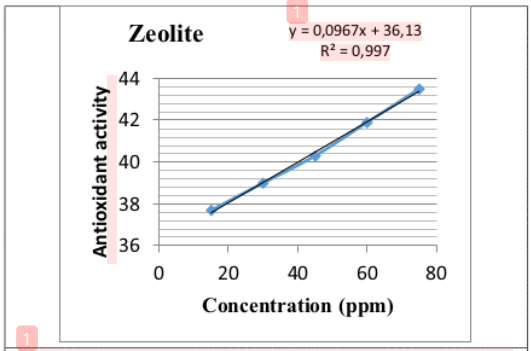

Figure 2. Antioxidant test curve of liquid smoke from zeolite filtering

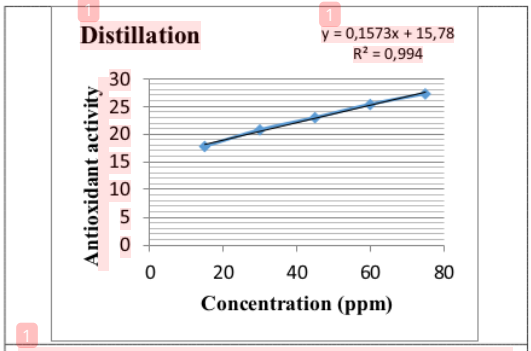

Figure 3. Antioxidant test curve of liquid smoke from Distillation

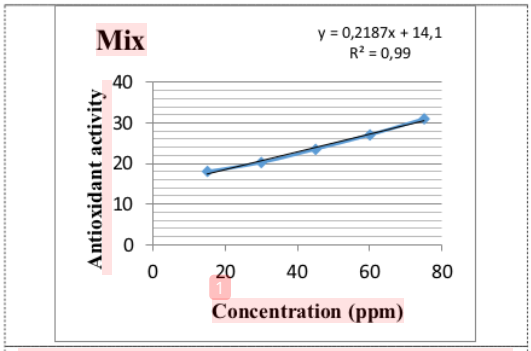

Figure 4. Antioxidant test curve of liquid smoke from active charcoal and zeolite filtering

From the curves above, then it can be calculated the value of IC50 with each inhibition percent value and known concentration is plotted on the graph so obtained linear equation y = mx + c to change the value of y with 50 of linear line equation, it will get the value of x as the value of IC50. Furthermore, the results of probit analysis are compared with the level of antioxidant power to classify into the strength category in counteracting free radicals. IC50 value calculation data is contained in Table 2.

Tabel 2. IC_{50} values of cocoa skin liquid smoke with different purification methods

Treatment	Linear equation	a	b	y	x (ppm)	Information
Distillation	y = 0,1573x + 15,78	0,1573	15,78	50	217,55	intermediate
Mix	y = 0,2187x + 14,1	0,2187	14,1	50	164,15	intermediate
Zeolites	y = 0,0967x + 36,13	0,0967	36,13	50	143,43	intermediate
Active charcoal	y = 0,084x + 46,3	0,084	46,3	50	44,05	very strong

Based on the data in the table above, IC50 values are obtained from each purification method. Value of antioxidant activity IC50 ranging between 44.05 ppm - 217.55 ppm. The highest value is in the purification treatment using active charcoal with IC50 antioxidant activity value of 44.05 ppm and classified into a very strong category, while the lowest value is in the purification treatment using distillation with an antioxidant activity value of 215.55 ppm and classified into the medium category. The antioxidant activity test by the DPPH method was stated with IC50 (Inhibition Concentration), which is the concentration of the extract or sample that inhibited DPPH activity by 50%. The smaller IC50 value means a higher antioxidant activity. IC50 values <50-100 indicate highly active antioxidants, IC50 values <101-250 indicate moderate antioxidant activity, and IC50 values <250-500 indicate weak antioxidants, and IC50 values <500 less active as antioxidants [20].

The antioxidant activity value of IC50 liquid smoke of cocoa skin with purification using activated charcoal has the highest IC50 value due to the absorption of activated charcoal. Absorption is determined by the surface area of the particles and this ability can be higher if the charcoal is activated by active factor chemicals or by heating at high temperatures. Thus, the charcoal will experience changes in physical and chemical properties. Such charcoal is referred to as active charcoal. The absorption of active charcoal is very large, which is 25-100 % of active charcoal weight [14]. According to [12], there is a tendency to decrease the pH value of liquid smoke from pyrolysis with the purification results with active charcoal. This is due to the content of acetic acid and phenol compounds which continue to increase during the purification process. The higher the total phenol content in liquid smoke, the lower the pH value or acidic. This is directly proportional to the increase in antioxidant activity of cocoa skin liquid smoke.

Purification by using active zeolites can separate compounds that are not part of the antioxidant group such as benzopyrene so that purified liquid smoke has higher antioxidant content. This is consistent with the results of research [13], the use of active zeolite aims to obtain liquid smoke which is completely free of harmful substances such as benzopyrene. The use of zeolites is very effective in absorbing the content of benzo(a)pyrene in liquid smoke. Zeolites function as filters because they have smaller pore holes than tar and benzo (a) pyrene compounds.

Whereas the distillation process only separates compounds based on their volatile properties. It is consistent with the statement of [21], this process is done to separate the components of a mixture by using the basis that some of the components may evaporate faster than the other components. The resulting steam contains more volatile components, so the process of separating the components from the mixture can occur. Besides that, the temperature used in the distillation process is also high so that the components of antioxidant compounds will be damaged during the distillation process. According to [16], the temperature of distillation used in the purification of liquid smoke that is 100ºC – 150ºC.

4. Conclusions

Based on the results of the research, cocoa skin liquid smoke that is purified using active charcoal has the value of antioxidant activity IC50 by 44.05 ppm compared with the three other purifications. This value entered into antioxidant activity is very strong because if the IC50 value is less or equal to 50 ppm then it is classified as having very strong antioxidant activity, while the other three purification methods are classified as having moderate antioxidant activity.

Acknowledgements

Thank you to the Chancellor of Ekasakti University, Chair of the LPPM of Ekasakti University, Dean of the Faculty of Agriculture, Ekasakti University, Head of the Laboratory Chemistry Agriculture Fakulty of the Padang and team.

References

[1] A. Jusmiati. R. Rusli. and L. Rijai. (2016). "Aktivitas antioksidan kulit buah kakao masak dan kulit buah kako muda." *J. Sains dan Kesehatan* vol. 1. no. 2. pp. 34–39.

[2] W. A. Asis. (2018). "Penentuan Kandungan Fenolik Total Asap Cair Limbah Kulit Kakao Hasil Pirolisis dan Uji Aktivitasnya Dalam Menghambat Pertumbuhan Jamur Fusarium oxysporum."

[3] Sartini. N. M. Djide. and G. Alam. (2007). "Ekstraksi komponen bioaktif dari limbah kulit buah kakao dan pengaruhnya terhadap aktivitas antioksidan dan antimikroba."

[4] A. S. Mulyatni. A. Budiani. and D. Taniwiryono. (2012). "Aktivitas antibakteri ekstrak kulit buah kakao (Theobroma cacao L .) terhadap Escherichia coli . Bacillus subtilis . dan Staphylococcus aureus." *Menara Perkeb*. vol. 80. no. 2. pp. 77–84.

[5] M. M. Wijaya. M. Wiharto. and M. Anwar. (2017). "Kandungan kimia asap cair kulit kakao dengan metode GC-MS." *J. Kim. dan Pendidik. Kim.* vol. 2. no. 3. pp. 191–197.

[6] I. K. Budaraga and D. P. Putra. (2019). "Liquid Smoke Antimicrobial Test of Cocoa Fruit Peel Against Eschericia Coli and Staphylococcus Aureus Bacteria." *Conf. Ser. Earth Environ. Sci.*. vol. 365. p. 12049.

[7] Muratore, G., Mazzaglia, A., Lanza, C.M., Licciardello, F. 2007. Process Variables on the Quality of Swordfish Fillets Flavored with Smoke Condensate. J of Food Processing and Preservation 31: 167–177.

[8] H. Winarsih. (2007). Antioksidan Alami dan Radikal Bebas Potensi dan Aplikasi dalam Kesehatan.

[9] W. Trilaksani. (2003). "Antioksidan: jenis. sumber. mekanisme kerja dan peran terhadap kesehatan." *Term Pap. Introd. Sci. phylosophy (PPS702). IPB. Bogor*

[10] Sumpono. H. D. Putri. and L. R. Sari. (2017). "Uji Aktivitas Antibakterial dan Antioksidan Asap cair Cangkang Buah Karet (Hevea brassiliensis) serta Implementasinya Sebagai Pengawet dan Penghambat Ketengikan Daging." *Pros. Semin. Nas. Kim. UNY*. pp. 215–228.

[11] S. Soldera. N. Sebastianutto. and R. Bortolomeazzi. (2008). "Composition of Phenolic Compounds and Antioxidant Activity of Commercial Aqueous Smoke Flavorings." *J. Agric. Food Chem.*. vol. 56. no. 8. pp. 2727–2734.

[12] A. Rinaldi. A. Alimuddin. and A. S. Panggabean. (2015). "Pemurnian Asap Cair Dari Kulit Durian Dengan Menggunakan Arang Aktif." *Molekul*. vol. 10. no. 2. p. 112.

[13] Fauzan and M. Ikhwanus. (2017). "Pemurnian asap cair tempurung kelapa melalui distilasi dan filtrasi menggunakan zeolit dan arang aktif." *J. Muhammadiyah Jakarta*. pp. 1–5.

[14] S. Jamilatun and S. Slamah. (2015). "Peningkatan kualitas asap cair dengan menggunakan arang aktif SNTT FGDT 2015." *Simp. Nas. Teknol. Terap.*. vol. 3. pp. 23–28.

[15] E. Erawati. T. W. Kirana. E. Budiyati. W. B. Sediawan. and P. Mulyono. (2015). "Distilasi asap cair hasil pirolisis limbah serbuk gergaji kayu glugu." *Simp. Nas. RAPI*. vol. 16. pp. 213–219.

[16] P. Darmadji. (2002). "Optimasi Pemurnian Asap Cair Dengan Metoda Redistilasi." *J. Teknol. dan Ind. Pangan*. vol. XIII. no. 3. pp. 267–271.

[17] M. Lisa. P. L. Hariani. and M. Faizal. (2015). "Uji persamaan Langmuir dan Freunlich pada penyerapan Mn (II) oleh komposit Fe3O4- zeolit." *J. Tek. Lingkung. UNAND*. vol. 12. no. 2. pp. 114–119.

[18] M. Jun. H. Y. Fu. J. Hong. X. Wan. C. S. Yang. and C. T. Ho. (2003). "Comparison of antioxidant activities of isoflavones from kudzu root (Pueraria lobata Ohwi)." *J. Food Sci*.

[19] A. Prakash. F. Rigelhof. and E. Miller. (2001). "Antioxidant Activity." Medallion Lab. Anal. Prog..vol. 19. no. 2. pp. 46–55.

[20] H. Faustino. N. Gil. C. Baptista. and A. P. Duarte. (2010). "Antioxidant activity of lignin phenolic compounds extracted from kraft and sulphite black liquors." *Molecules*. vol. 15. pp. 9308–9322.

[21] R. L. Earle. (2009). "Unit Operations in Food Processing Water."

Analysis Antioxidant IC50 Liquid Smoke of Cocoa Skin with Several Purification Method

ORIGINALITY REPORT

95%	36%	94%	19%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	I K Budaraga, D P Putra. "Analysis Antioxidant IC50 Liquid Smoke of Cocoa Skin with Several Purification Methods", IOP Conference Series: Earth and Environmental Science, 2021 Publication	89%
2	Submitted to Universitas Andalas Student Paper	6%

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	Off		