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Purification and Characterization of Phenolic Antioxidant from Corncob Liquid Smoke

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This study aimed to purify and characterize the antioxidant activity of liquid smoke acquired from corncobs. The smoke of crude liquids was generated through pyrolysis and was purified through fractional and simple distillation. The liquid smoke was analyzed using crude fibres, cellulose, hemicellulose, lignin, antioxidant activity and total phenolic compounds. Gas chromatography-mass spectrometry (GC-MS) was employed to analyze the volatile compounds of liquid smoke. Fractional distillation exhibited the higher carbonyl and phenol content than simple distillation, and the overall acid content of fractional distillation was relatively lower. Moreover, the liquid smoke obtained from fractional distillation exhibited the higher total antioxidant and free radical scavenging activity than simple distillation. The GC-MS results showed different derivatives and phenolic compounds as the most dominant components of the liquid smoke of corncobs. The major antioxidant components of fractional distillation include 2-methoxyphenol, phenol, 2,5-dimethylphenol, 2-methylphenol, 2-methoxy-4-methylphenol, 4-ethyl-2-methoxyphenol and 4-ethylphenol, and those of simple distillation include phenol, 2-methylphenol, 2-methoxy- phenol, 2,6-dimethoxyphenol and 3-ethylphenol.

Keywords: Corn cobs, Liquid smoke, Distillation, GC-MS, Antioxidants.

INTRODUCTION

Corn (*Zea mays* L.) is a food plant type and known for long time and has been cultivated in Indonesia. The utilization of corn kernels as food leads to the production of corncobs. Corncobs, generally discarded as waste or are only used as fuel and animal feed. Corncobs make up the largest part of corn fruits; thus, the large quantities of shelled corn production can generate substantial corncob waste. However, corncobs contain hemicellulose, cellulose and lignin materials and can be converted into liquid smoke through pyrolysis. Corncobs can generate phenols, organic acids and carbonyl compounds used in food preservation and can provide specific colours and flavours [1].

Studies [2-7] have reported that liquid smoke obtained from biomass materials can be utilized for antimicrobial, antioxidant, antidiabetic, lipid peroxidation inhibition and anti-inflammatory activities and for heavy metal reduction. Other studies have found that the crude liquid smoke (CLS) acquired from corncobs comprises phenolic compounds, including phenol, 2,6-dimethoxyphenol, 2-methoxy-4-methylphenol, 4-ethyl-2-methoxyphenol, 2-methoxyphenol and 2-methylphenol that constitutes 30.15% of overall phenols. The dominant phenolic compounds were phenols and 2-methoxyphenols [5]. Additionally, corncobs fractions and extracts contain flavonoids, phenolic compounds, and tannins compounds having a potential as antiphotoxidants, antioxidants and photoprotective [8-10].

Liquid smoke obtained from pyrolysis comprises a group of polycyclic-aromatic hydrocarbons and tar [11]. These toxic compounds are mutagenic, carcinogenic and teratogenic and can react with some food ingredients, including vitamins and amino acids [12]. Distillation is a purification method, and on the basis of their boiling points, crude liquid smoke (CLS) is isolated from tar and toxic components. This study isolated and analyzed phenolic antioxidant compounds from the liquid smoke of corncobs through fractional and simple distillation.

EXPERIMENTAL

The sample used was the corn cobs variety kuning Manado obtained from Tompaso, Minahasa, Indonesia. The chemicals used were hydrochloric acid, acetate buffer (pH 3.5), iron(II) sulfate, iron(III) chloride, trichloroacetic acid, sodium carbonate, Folin-Ciocalteou reagent from Merck (Darmstadt, Germany). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and gallic acid obtained from Sigma-Aldrich Chemical Co. (St. Louis, USA).

Chemical composition analysis of corncob: The chemical composition of corncobs, ash, moisture and crude fibre were analyzed according to the AOAC methods [13]. Cellulose, hemicellulose and lignin contents were estimated through fractionation [14,15].

Pyrolysis of corncob: Liquid smoke was produced through pyrolysis. The pyrolysis furnace equipped with a burn stove of LPG used as a reactor and heater having a diameter and height of 27 and 40 cm, respectively, which can be charged with 4 kg of raw materials. Subsequently, raw materials were cut into uniform and small sizes of 3-5 cm^2. Dry corncobs (1000 g) were place in the pyrolysis reactor attached to two thermometers and a water-cooled condenser. Temperature was determined using the two thermometers placed at the pyrolysis reactor and pipe condenser. Pyrolysis was conducted at a 300-400 ºC for 60 min. Condensation was terminated until no liquid smoke was dripping into the container. Afterwards, the liquid smoke was stored at room temperature for 7 days to separate the supernatant and sediments. By using a filter paper, the supernatant was filtered, and the residue and filtrate were obtained. Before further simple and fractional distillation, the filtrate (CLS) was stored in a bottle at room temperature.

Purificaion of liquid smoke: In a 500 mL round flask having a thermometer and connected to a simple distillation set and cooling condenser, 300 mL of CLS was placed. Furthermore, the flask was heated for 60 min by using an oil heater to 130-150 ºC, and in a 200 mL round-bottom flask, the distillate was collected. Similarly, the CLS was purified through fractional distillation by using a Vigreux column having a 50 cm length. All liquid smoke was stored at room temperature for further analyses.

Determination of total phenolic content: The overall content of phenols of liquid smoke was determined using the modified Folin-Ciocalteu colorimetric method [16]. In a test tube, 0.1 mL of each 1 mg/mL sample solution was added to 0.1 mL of 50% Folin-Ciocalteu reagent, and then for 3 min, the resulting mixture was vortexed. After 3 min intervals, 2 mL of 2% Na_2CO_3 solution was added. After incubation for 30 min at room temperature, the mixture was stored in dark for another 30 min. By using a spectrophotometer, the supernatant was measured at 760 nm. A standard curve was obtained by employing various gallic acid concentrations, and the results were expressed as the equivalents of gallic acid in milligrams per kilogram of liquid smoke.

DPPH free radical scavenger assay: Free radical scavenger activity estimation slightly differed from liquid smoke measured using the method [17]. To 0.5 mL of liquid smoke dissolved in ethanol, 2 mL of 92 μM 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution was added. The colour reduction level of the solution indicated the radical scavenger efficiency. Finally from 5 min of 30 min, the absorbance was determined at 517 nm by using the spectrophotometer. Free radical scavenger activity was calculated as a percentage reduction of DPPH colour using the following equation:

$$\text{Free radical scavenging activity } (\%) = 1\times\left(\frac{\text{Absorbance sample}}{\text{Absorbance control}}\right)\times 100$$

Total antioxidant capacity by FRAP assay: The overall antioxidants were determined through the ferric reducing ability of plasma (FRAP) [18]. Measurements were performed by using 0.1 mL of the liquid smoke dissolved in ethanol and then by mixing it with 3 mL of fresh FRAP reagent. Subsequently, the mixture was shaken using the vortex instrument and then immediately its absorbance was measured at 593 nm. The FRAP reagent was always freshly prepared by mixing 2.5 mL of 10 mM 2,4,6-tripiridil-*s*-triazine (TPTZ) solution dissolved in 40 mM HCl with 2.5 and 20 mL of $FeCl_3{\cdot}6H_2O$ solution and 2.5 mL of 0.3 M acetate buffer at the pH of 3.6. The overall content was expressed in mol/L extract as the equivalent antioxidant Fe^{3+} to Fe^{2+}. Similarly, the standard curve was created using the 100-1000 mol L^{-1} $FeSO_4$ solution.

GC/MS analysis: The obtained yield was measured and the phenolic components were identified through GC-MS. The liquid smoke components were analyzed [19] using GC-MS QP2010S SHIMADZU. The used operational conditions of GC-MS QP2010S were as follows: oven temperature was maintained to 75 ºC for 2 min and then was increased to 130 ºC with an increase rate of 8 ºC/min and maintained at 130 ºC for 3 min. Temperature was further increased to 290 ºC at an increase rate of 10 ºC/min and was maintained at 290 ºC for 3 min. Finally, temperature was increased to 300 ºC and maintained the same for 24 min. Ion source temperature was 200 ºC. Helium with 99.99% purity was used. The gas pressure was set to 75 kPa and the gas flow rate was 0.57 mL/min. Injector temperature was 250 ºC.

Statistic analysis: Statistical data were analyzed with computer software, SPSS version 18 (Illinois, USA) using ANOVA analysis followed by Duncan′s Multiple Range Test to determine the significant differences between the carrying by 5%.

RESULTS AND DISCUSSION

Table-1 presents the results of chemical analysis of corncobs, including ash, moisture, crude fibre, hemicellulose, cellulose, and lignin contents. Corncobs (*Zea may* L. *var*. Manado kuning) exhibit the ash and moisture contents of 0.40% and 8.02%, respectively. The crude fibre content of corncobs was 32.31%. A higher crude fibre content of corncobs can lead to an increase in the cellulose, hemicellulose and lignin contents used as raw materials for carbonyl, phenolic and acid compound production because during thermal degradation, these compounds were commonly obtained in liquid smoke.

The cellulose, hemicellulose and lignin content of corncobs used in this study are 27.43, 27.08 and 28.86%, respectively. Lorenz and Kulp [20] reported that the corncobs comprise high amounts of hemicellulose (36%), cellulose (41%) and lignin (6%) components. This variation resulted from various raw materials and difference in humidity, corncobs and corncob age. The cellulose amount present in raw materials can be used to determine furan, acid and water contents, and the hemicellulose amount affects furfural, furan, acetic acid, and carboxylic acid contents. In addition to hemicellulose and cellulose, the pyrolysis

TABLE-1
CHEMICAL COMPOSITION OF CORNCOB

Components	Content (%)
Moisture	8.02 ± 1.02
Ash	0.40 ± 0.03
Crude fiber	32.31 ± 0,20
Cellulose	42.41 ± 1.05
Hemicellulose	37.89 ± 2.27
Lignin	10.81 ± 1.10

of lignin leads to the production of phenolic compounds, including syringol and guaiacol, which influence the smoke odour and taste [21].

Yield (%) of liquid smoke: Liquid smoke is a result of biomass pyrolysis under atmospheric pressure and at 400 ºC. The liquid smoke of corncobs is produced from corncob smoke condensation through the thermal degradation of hemicellulose, cellulose and lignin [22]. The % yield of CLS acquired from corncob pyrolysis was 54%. Mongan *et al*. [2] found that the liquid smoke yield obtained from corncobs was 31.7%. Another study [21] found that at 400 ºC, corncob pyrolysis leads to 60% CLS. The difference in liquid smoke yields was caused by burning method, corncob type, fuel sources and water content used. Many factors contribute to the maximization of liquid smoke production, such as pyrolysis equipment and the temperature level influences the physical and chemical properties of liquid smoke [23]. Additionally, compared with 425 ºC, the decrease in the temperature of pyrolysis to 300-400 ºC leads to a decrease in polycyclic aromatic hydrocarbon (PAH) levels by up to 10 times [24]. Pyrolysis temperature employed during CLS production was 300-400 ºC.

Purification of liquid smoke: Simple distillation can be employed for the separation and purification liquids having nonvolatile impurities, which differ from the boiling points of the liquids by a minimum of 70-80 ºC. Fractional distillation can be used to separate liquid mixture constituents having a difference in their boiling points of ≥30 ºC [25]. Crude liquid smoke (CLS) can then be purified through fractional and simple distillation. The distillation results indicated that liquid smoke obtained from corncobs was detected only at 100 ºC. The liquid smoke yield obtained from simple and fractional distillation was 50.03 and 49.80%, respectively. The obtained % yield showed that liquid smoke purification achieved using fractional and steam distillation methods provided similar yields to acquire volatile compounds at the boiling point of 100 ºC because at component having boiling points of >100-105 ºC do not contain free water; thus, the evaporation of compounds having high boiling points is difficult. Maga [26] reported that liquid smoke comprises up to 92% water along with the liquid-smoke-dispersed components, including carbonyls, phenols, furans, acids and PAHs, having different boiling points.

Total phenolic contents: Table-2 presents the results of chemical analysis of liquid smoke obtained from fractional and simple distillation. The overall content of the phenol of three liquid smoke types was 0.27-2.01% with the average of 1.04%. The overall phenol content of fractional distillation liquid smoke is higher than that of simple distillation and lower than that of CLS. Fractional distillation increases the compound purity compared with simple distillation. Harwood and Moody [27] reported that a distillation flask passes upwards through a fractionating column, and it condenses column packing and continuously revapourises. Each revapourization of condensates is equivalent to simple distillation, and thus separate distillations result in a condensate, which is successively more abundant in volatile components. Continuous revapourisation and condensation result in vapour; hence, the distillate is collected, which is highly enriched with compounds having lower boiling points. The distillate can even comprise pure compounds.

TABLE-2
PHENOL CONTENT OF CRUDE LIQUID SMOKE, SIMPLE DISTILLATION AND FRACTIONAL DISTILLATION

Sample	Phenolic total content (mg/kg)
Crude liquid smoke (CLS)	207.72 ± 0.02^a
Simple distillation (SD)	26.09 ± 0.97^b
Fractional distillation (FD)	81.19 ± 2.73^c

The phenolic content found in corncob liquid smoke exhibited different components depending on raw materials used and with other biomass. The phenolic content of corncob CLS (2.04%) was compared with coconut shell CLS (4.71%) [28]. This difference was caused by lower lignin contents in corncobs (10.81%) than in coconut shells (36.50%). Pyrolysis temperature and the lignin content influenced the phenol content in liquid smoke [23]. The phenol compounds were formed through the thermal degradation of lignin components. Win an increase in the lignin contents in row materials, the content of phenol in liquid smoke increases. Stolyhwo and Sikorski [24] reported that lignin pyrolysis occurs at 310-500 ºC. If this temperature not reached, lignin degradation does not occur, thereby affecting the phenol content obtained. Many studies [29-31] have reported that the higher lignin content of biomass shows a positive relationship for the overall phenol content. The overall phenol content of CLS, fractional and simple distillation of liquid smoke is eligible for the commercial overall phenol content of 0.2-2.9% [20].

Antioxidant activity: The radicals of DPPH are stable and free, and can dissolve in ethanol or methanol, and exhibit characteristic peaks at a 515-517 nm. In this method, the scavenging abilities of free radicals of liquid smoke can be used to donate electrons for converting free radicals into non-radicals. A decrease in the intensity of the purple colour suggested that the antioxidant capacity for scavenging DPPH free radicals is strong [32]. Fig.1 shows the free radical scavenging capacity of 1 mg/mL crude liquid smoke on 92 µM DPPH in ethanol obtained through simple and fractional distillation.

Crude liquid smoke (CLS) exhibits the highest free radical scavenging capacity compared with fractional and simple distillation ($p < 0.05$). Fractional distillation significantly differed from simple distillation (Fig. 1). Three liquid smokes acquired through different distillation techniques provide various capacities of free radical scavenging. The percentage of free radical scavenging for fractional distillation, simple distillation and CLS are 67.14, 65.66 and 72.22%, respectively. The results indicated that the activity of free radical scavenging the three

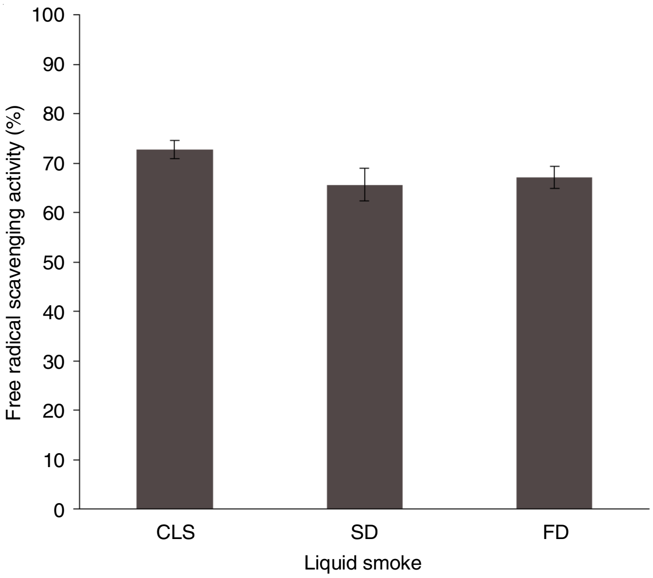

Fig. 1. Free radical scavenging activity of crude liquid smoke (CLS), simple distillation (SD) and fractional distillation (FD)

liquid smokes was in general >50%. This data revealed that in liquid smoke, the overall phenolic content employed during distillation influenced antioxidant compounds. Girard [20] reported that in liquid smoke, phenol compounds are the components functioning as antioxidants. The antioxidant properties of phenols having higher boiling point are better than those having lower boiling points. Other studies [33,34] reported that phenolic antioxidants acquired from the liquid smoke redistillation of coconut shells at 200 ppm exhibited the free radical scavenging activity and reducing power the in a range of 48-55% and 0.12-0.17, respectively. At 200 ppm, the free radical scavenging activity of eucalyptus liquid smoke was 40.2%. The difference in the free radical scavenging activity of DPPH is dependent of raw materials and lignin component amounts present in biomass materials.

The antioxidant activity determined using the DPPH radical technique exhibited a tendency similar to the overall phenolic contents of the three liquid smokes. This difference confirmed that distillation techniques strongly influenced the amounts of phenolic compounds acquired from corncob liquid smoke is. Amic *et al*. [35] reported that phenolic compounds containing hydroxyl groups donate hydrogen atoms to radicals to produce non-radical compounds, and in aromatic rings, the formed phenolic radicals can be stabilized through resonance to make them non-reactive. Thus, phenolic compounds present in liquid smoke can serve as effective antioxidants with a mechanism of free radical scavenging from hydrogen-donating phenolic compounds.

Total antioxidant activity: The FRAP method was used to determine the antioxidant capacity to reduce Fe^{3+} into Fe^{2+}. At low pH and 593 nm, the blue intensity of Fe(III)-TPTZ was changed to Fe(II)-TPTZ [36]. Fig. 2 shows the overall antioxidant capacity of corncob liquid smoke. The overall antioxidant capacity of CLS is higher than that of fractional and simple distillation ($p < 0.05$). The lower activity of antioxidants in fractional and simple distillation is associated with

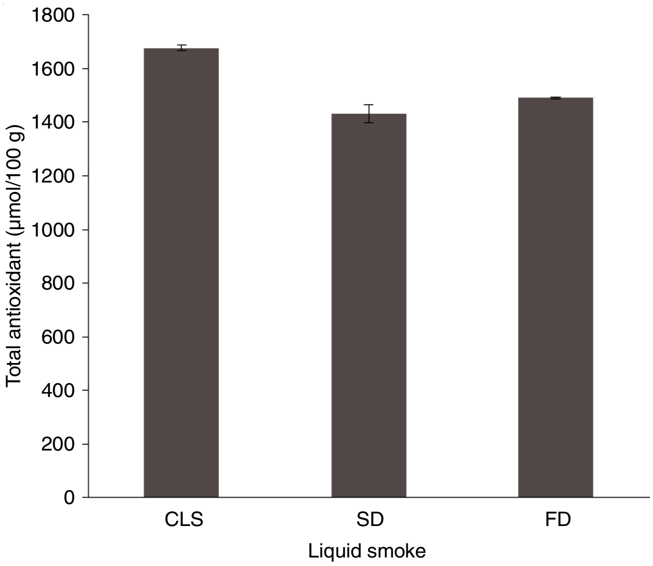

Fig. 2. Total antioxidant of crude liquid smoke (CLS), simple distillation (SD) and fractional distillation (FD)

the lower contribution of phenolic compounds in fractional and simple distillation liquid smoke than in CLS. A higher overall phenolic content indicated high free radical scavenging activity of liquid smoke and reduction capacity.

The high antioxidant content in CLS indicated the presence of more compounds of phenols, which can reduce more Fe^{3+} into Fe^{2+} than fractional and simple distillation. The reductants or reducing compounds of liquid smoke are categorized into phenolic antioxidants. Hence, in liquid smoke, the existence of phenolic compounds can lead to an increase in the antioxidant activity. Phenolic compounds serve as electron donors, which terminate radical chain reactions through the conversion of free radicals into relatively more stable products. According to Shahidi and Nazck [37], phenolic compounds, such as simple flavonoids, phenols and tannins, can be categorized as natural antioxidants. At the same concentration, the overall antioxidant capacity of Fractional distillation was different from that of simple distill-ation ($p < 0.05$). The results of DPPH free radical scavenging and overall antioxidant capacity showed the same tendency for fractional and simple distillation but different tendency for CLS. Several studies [38,39] have reported that the FRAP and DPPH testing methods provide the same results for food plant extracts.

Identification of liquid smoke components: The identification results obtained from GC-MS revealed that the corncob liquid smoke profile containing phenolic compounds after purification through fractional and simple distillation (Table-3). Corncob liquid smoke was acquired through the pyrolysis of hemicellulose, cellulose and lignin and purified using fractional and simple distillation, which indicated that phenol compounds and their derivatives were highly dominant. Girard [20] reported that in liquid smoke, phenol compounds are present due to cellulose and lignin pyrolysis.

The results of CLS analyses were obtained for six phenolic compounds, including phenol, 1,2-benzenediol (pyrocatechol), 2-methoxyphenol (cresol), 1,4-benzenediol (hydroquinone), 2,6-dimethoxyphenol (syringol) and vanillin. The percentage

TABLE-3
PERCENT AREA OF IDENTIFIED PHENOLIC COMPOUNDS IN CRUDE LIQUID SMOKE WITH PYROLYSIS

Retention time (min)	Phenolic compounds	Area (%)
26.208	Phenol	3.13
28.333	2-Methoxy-phenol (guaiacol)	0.93
35.417	1,2-Benzenediol (pyrocatechol)	2.90
37.208	2,6-Dimethoxy-phenol (syringol)	0.27
37.692	1,4-Benzenediol (hydroquinone)	0.47
39.775	Vanillin	0.45

of the overall phenol content was 8.15% (Table-3). Swastawati *et al*. [5] reported that in CLS, phenolic compounds were identified. These compounds included phenol, 2-methoxy-4-methylphenol, 2,6-dimethoxyphenol, 4-ethyl-2-methoxyphenol, 2-methoxyphenol and 2-methylphenol with a total phenolic percentage of 30.15%. The results of CLS analyses conducted through purification by using simple distillation indicated five phenolic compounds, namely 2-methoxy phenol (guaicol), phenol, 3-ethylphenol, 2-methylphenol (cresol) and 2,6-dimethoxyphenol (syringol), with a total phenolic percentage of 21.60% (Table-4).

TABLE-4
PERCENT AREA OF IDENTIFIED PHENOLIC COMPOUNDS IN LIQUID SMOKE FROM SIMPLE DISTILLATION

Retention time (min)	Phenolic compounds	Area (%)
24.739	Phenol	10.59
27.015	2-Methoxy-phenol (guaiacol)	3.01
28.422	2-Methyl-phenol (cresol)	4.65
31.966	3-Ethyl-phenol	1.85
37.809	2,6-Dimethoxyphenol (syringol)	1.50

The CLS analyses conducted through fractional distillation provided seven phenolic compounds, namely 2-methoxyphenol (guaiacol), phenol, 2,5-dimethylphenol, 2-methylphenol (cresol), 4-ethylphenol, 2-methoxy-4-methylphenol and 4-ethyl-2-methoxyphenol, with a total phenol percentage of 21.98% (Table-5). All three identified liquid smokes exhibited similar compounds, such as 2-methoxyphenol (guaiacol) and phenol as well as different components including 2-benzenediol (pyrocatechol), vanillin and 1,4-benzenediol (hydroquinone). By contrast, 2-methoxy-4-methylphenol, 2,5-dimethylphenol, 4-ethyl-2-methoxyphenol and 4-ethylphenol were identified in only the liquid smoke obtained through fractional distillation. 2,6-dimethoxyphenol (syringol) was obtained only in CLS and liquid smoke purified using simple distillation. Siringol was not observed in liquid smoke (fractional distillation) may be because of its high boiling point and dispersed only in simple distillation liquid smoke and CLS. Guaicol and its derivatives are light determinants and provide a moderate characteristic wooden and medicinal aroma. In corncob, liquid smoke giving a strong and pungent smoke odour, acidic components, including acetic acid, were dominant.

TABLE-5
PERCENT AREA OF IDENTIFIED PHENOLIC COMPOUNDS IN LIQUID SMOKE FROM FRACTIONAL DISTILLATION

Retention time (min)	Phenolic compounds	Area (%)
26.863	Phenol	11.37
29.351	2-Methoxy-phenol (guaiacol)	4.87
30.583	2-Methyl-phenol (cresol)	3.02
32.976	2,5-Dimethyl-phenol	0.29
33.673	2-Methoxy-4-methyl-phenol	0.31
34.222	4-Ethyl-phenol	1.80
37.043	4-Ethyl-2-methoxy-phenol	0.32

Among the numerous compounds present in the purified liquid smoke, some compounds were classified into phenolic compounds, which can serve as antioxidants. According to their function in terms of antioxidant activity, flavours, and antimicrobial agent, organic acids and phenolic compounds can serve as biopreservatives, which inhibit lipid peroxidation. Pszezola [12] reported that the liquid smoke components, which serve as antioxidants, include phenol compounds. A positive relationship of the antioxidant activity with amounts of overall phenolic compounds in liquid smoke was observed. All phenolic compounds of corncob liquid smoke can synergistically be used for antioxidant activity, free radical scavenging and lipid peroxidation and have a specific role.

Conclusion

The corncob liquid smoke acquired through fractional distillation exhibited a higher free radical scavenging activity and the most phenolic compounds. The total antioxidants obtained through fractional distillation were compared with those obtained through simple distillation. Crude liquid smoke (CLS) exhibited the highest activity of free radical scavenging. The total antioxidants of CLS were compared with those of fractional and simple distillation. Fractional distillation provided components, including 2-methoxyphenol, phenol, 2-methylphenol, 2-methoxy-4-methylphenol, 2,5-dimethylphenol, 4-ethyl-2-methoxyphenol and 4-ethylphenol. These compounds have a potential for antioxidant activities and as free radical scavengers.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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