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Project title Electrolysis of agarwood oil and analysis of the chemical profiles with TLC and GC-MS

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Program Bachelor of Science in Applied Chemistry

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by

Miss Punyanuch Jenthamnukul

In Partial Fulfillment for the Degree of Bachelor of Science

Program in Applied Chemistry (International Program)

Department of Chemistry, Faculty of Science

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Abstract

This study develops electrolysis approach to treat agarwood oil. The oil sample was added with water enhancing conductivity of the sample and enabling electrolysis. The samples before and after the electrolysis were initially analyzed using thin layer chromatography (TLC). The TLC method applied the mobile phase system of DCM and Hexane with 7:3 v/v ratio. This method was used to investigate the sample undergoing electrolysis under different conditions. The electrolysis time of 1 h, electrolysis voltage of 9 V and addition of 1 %v/v of water were selected as the suitable conditions resulting in significant change of the TLC result after the electrolysis. The samples were also analyzed with solid phase micro extraction (SPME) and gas chromatography-mass spectrometry (GC-MS) in order to identify the volatile compounds according to comparison with MS and retention index database of NIST17. The analysis showed the decreasing amount of γ-Eudesmol, Toluene, 1-Pentanol, 4-methyl, cis-Thujopsene with the increasing amounts of 2-Butanone, 4-phenyl, Khusimone, Propanoic acid, 2-methyl, 1-Hexanol, Benzaldehyde, Cyclohexane, 1-methylene-4-(1-methylethenyl), Cadrene after the electrolysis. The developed approach is expectedly applicable for either adjusted quality of essential oil or study of agarwood stability in the future.

Keywords: Agarwood oil, Agarwood oil treatment, TLC, Electrolysis, GC-MS

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List of Abbreviations

Abbreviation	Definition
TLC	Thin Layer Chromatography
SPME	Solid Phase Micro Extraction
HS-SPME	Headspace Solid Phase Micro Extraction
GC-MS	Gas Chromatography Mass Spectrometry
EC	Electrocoagulation
DC	direct current
MB	mobile phase
CAR	Carboxane
PDMS	Polydimethylsiloxane
DVB	Divinyl benzene
DCM	Dichloromethane

Chapter 1
Introduction

1.1 Introduction to the research problem and significance

Nowadays, our world is facing the problem of deforestation with the subsequent problem of the increasing price of wood products worldwide as well as other related issues such as the greenhouse effect and global warming. To this end, the old-age trees have been cut down [1], especially that can be refined to make essential oils. However, only the inner layer of the agarwood is commonly used and has valuable to merchants with the expense of cutting down the whole tree. In general, 10 trees are cut down in order to find an agarwood tree. So, the unsustainable harvesting of the agarwood in natural forests has resulted in almost extinction of trees in many areas of South East Asia. Agarwood is one of the resinous woods belonging to the Aquilaria species and has a high demand from its high valued and wide usages such as in medicine, incense, and perfume. Agarwood has been widely applied in many countries not just in Europe, India or Japan, but also in Thailand. There were 4 species of Aquilaria in Thailand which are Aquilaria Malaccensis (Mai Hom), Aquilaria Crassna (Krissana), Aquilaria Subintegra (Gaharu), Aquilaria Hirta (Janae). The oils obtained from agarwood are among the most expensive ones. This essential oil from agarwood which has the scientific name, Aquilaria Crassna or known in Krissana as a local name was used in this research. In addition, the prices of Krissana oils depend on their ages where the older age leads to the higher price. Instead of cutting down the old-age agarwood trees, a great challenge is to develop alternative approach to improve quality and price of agarwood oil obtained from younger-age agarwood trees (>15 years) in order to reduce the number of deforested old-age agarwood trees.

In this study, electrolysis approach with two stainless spring electrodes was developed and applied to adjust chemical profiles including odor active compounds of agarwood oil. Two-phase system of water and the oil was applied. The electrolysis system was applied to treat agarwood oil. The samples before and after treatment were analyzed by thin layer chromatography (TLC) to monitor the volatile and nonvolatile chemical profile change [2]. Headspace solid phase micro extraction (HS-SPME) and gas chromatography hyphenated with mass spectrometry (GC-MS) were further applied to investigate the volatile components including

the odor active compounds [3]. The results were discussed, and possible odor active compound changes were proposed.

1.2 Research objectives

To find the changes of the essential oil composition after electrolysis treatment.

To analyze the odor of agarwood oil.

1.3 Literature search

Agarwood oil is widely used in aromatherapy, medicinal applications, religious ceremonies (especially in Southeast Asia and the Middle East) and as perfume. The oil can be produced by either infecting Aquilaria tree with various fungi or natural wounding of wood followed by the oil extraction, *e.g.* by means of hydrodistillation [4]. The genus Aquilaria has >15 species which belong to the Thymelaeaceae family being indigenous to Southeast Asian countries such as Indonesia, Malaysia, Myanmar, Philippines, Vietnam and Thailand [5]. Agarwood contains a wide range of volatile compounds with the major class of terpenes mostly with the woody based smells.

Analysis of volatile compounds in agarwood can be performed using GC-MS. GC is a high performance technique for separation of mixtures based on different boiling points of volatile components and interactions between the components and stationary phase. After separation, MS provides highly sensitive and accurate compound identification based on different characteristic *m/z* for quantitative and qualitative analysis. The use of GC also allows compound confirmation based on comparison between the experimental retention indices with that from library [6]. Among different approach, solid phase microextraction (SPME) can be applied as a simple technique requiring a small amount of sample allowing effective removal of solvent. Volatile compounds are adsorbed onto a SPME fiber, concentrating the compounds prior to the desorption into the GC instrument [7]. SPME GC-MS has been widely applied for analysis and database construction for a broad range of essential oils.

Electrochemical synthesis is widely recognized as effective technique with the capabilities to be recycled and monitored online [8]. This technique was reported for synthesis of a wide

range of organic compounds such as epoxidation, halogenation, hydrodimerization and ozonolysis [9]. Since usable electrochemical system requires conductive media throughout an electrical circuit, direct of electrolysis of essential oil is not likely to occur. Addition of conductive phase (such as salt in aqueous solution) resulting in a two-phase system of aqueous and oil can be applied where electrical current is maintained by the aqueous phase and the reactants were continuously supplied from the oil phase. Similar 2-phase systems were applied to perform a range of organic reactions such as oxidations, epoxidations, ozonolysis, halogenation processes, reductions or hydrodimerizations [10]. A challenge is to apply this 2-phase technique to improve quality of agarwood oils.

An electrolysis system contains two metal electrodes linked with a power supply and an electrolyte. When sufficiently high voltage is applied, electrolysis occurs. The system allows electrochemical conversion of chemicals in sample. If corrosion of the applied electrodes occurs, metal ions and the solid metal complexes (sludge) can also be formed [11]. Electrolysis approach (specifically called as 2-phase electrocoagulation) has been applied for adjustment of odor active compounds in the perfume extract sample leading to the softer, sweeter and fresher smells of the electrochemically treated sample [12]. The approach has been successfully applied for treatment of many standard solutions, which can be straightforward for the application in this thesis. Also, the study reported the major products at the anode of the electrolysis system as methyl benzoate, 2-phenylethanol and benzyl acetate; whilst, phenylmethanol was mainly produced at the cathode. Application of electrolysis for adjusted quality of agarwood has not been reported.

Chapter 2

Experiment

2.1 List of instruments

Digital Control DC Power Supply, maximum voltage and current 30V 5A (Korad, Korea), Fluorescence Analysis Cabinet, Model CM-10A (Spectroline, United States), TLC Silica gel 60 F (Merck, Germany), Vortex-Genie 2 (Scientific industries, United States)

2.2 List of materials

Acetone, Dichloromethane, Ethanol, and H_2SO_4 were purchased from Merck KGaA, Germany. Aquilaria crassna oil (Almas Oudh Al, Thailand), Hexane (RCI Labscan, Thailand), Toluene (J.T.Baker, United States), Vanillin (Fisher scientific, United States)

2.3 Experimental procedure

2.3.1 Preparation of vanillin solution

Vanillin (5 g) was dissolved in ethanol (500 mL). H_2SO_4 was then added into the solution (dropwise) into the mixture until reaching the total volume of 5 mL. Finally, the mixture was kept in fridge before spraying onto a TLC plate for band detection with naked eyes.

For electroanalysis reaction, the power supply was used in this experiment as direct current (DC).

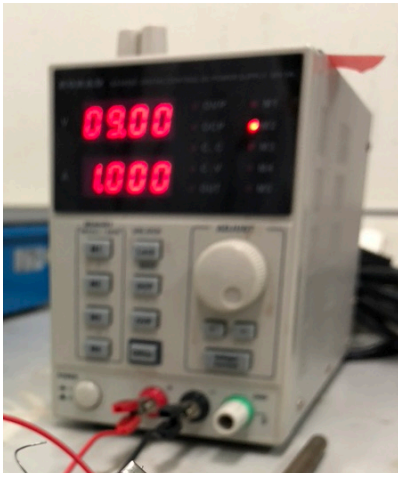

Figure 1 Digital Control DC Power Supply

2.3.2 Electrolysis and Electrocoagulation (EC) using Spring Electrodes

Diluted agarwood in hexane in 1:10 v:v ratio, stirred for 10 s and left the solution for 10 min for precipitation. Prepared the mobile phase DCM:Hexane (7:3 v:v) for the TLC separation.

Figure 2 Electrolysis on Coil Spring Electrodes

Transferred control agarwood oil before EC without diluting (500 µL) into three centrifuge tubes. Added the solvent into a 1 mL beaker, followed by two aluminium springs with different sizes (the bigger is anode, and the smaller is cathode). Applied the voltage of 9 V with the power supply. Performed the treatment for 1, 3, 5, 30, and 60 min, and collect the samples into centrifuge tubes. Put filter paper into a MB beaker, and left for 10 min. After spotting solvents (agarwood oil after EC) on TLC plates, put them into the MB beaker, and run the separation for about 3 min. Then, transferred the TLC plates out, dry for 5 min, and dip TLC plates into the vanillin solution. Dried the TLC plates for 5 min. Used the Stirring Hot Plate to heat TLC plates at 60°C until colors of the bands appeared.

2.3.3 Gas chromatography-Mass spectrometry (HS-SPME/GC-MS)

2.3.3.1 Sample preparation

The crassna samples derived from before and after 2-phase (hexane and water phases) electrocoagulation for 1 h were collected and left until the hexane phase (from dilution) totally evaporated in order to make higher concentration of the crassna in the hexane phase.

2.3.3.2 HS-SPME

Each derivatized crassna (before and after electrocoagulation reaction) were transferred with 15 microliters into 20mL headspace vial and capped with vial cap. The samples were equilibrated for 10 min in the vials. SPME with a CAR/PDMS/DVB fiber was used to extract volatile compounds from the samples for 45 min of extraction time at room temperature before further analysis by GC-MS.

Figure 3 20mL headspace vial

2.3.3.3 Gas chromatography-Mass spectrometry (GC-MS)

The GC-MS technique was used in term of analysis and identify compounds from the samples with separation of the compounds would accordingly to the GC-MS condition. The extracted samples attached onto the SPME fiber were desorbed into the GC-MS injection port and the results were shown as chromatograms.

Figure 4 Gas chromatography-Mass spectrometry (GC-MS)

Chapter 3

Result and discussion

3.1 TLC analysis of Crassna oil without dilution

Figure 5 showed example TLC results of agarwood oils without dilution.

Figure 5 Crassna oil without dilution

There were no changes between before and after applying the electrical current to the crassna oil no matter how much time has been used. This is because the oil was too viscous and concentrated, with the crassna oil sample showing the long bands on the TLC plate.

3.2 Dilution effect

Hexane can be used to dilute the crassna oil in order to clearly observe different bands of the samples on the TLC plate. However, only hexane cannot be used as the mobile phase due being nonpolar. DCM, which has higher polarity, was added into the mobile phase with the suitable DCM:hexane v/v ratios of 7:3. The TLC results for the samples with 1:5 dilutions before and after the electrolysis for 30 and 60 min are shown in **Figure 6**. The results after 60 min

electrolysis showed the faded colors of bands at the top, with the shorter distance of the bands below for the electrolyzed samples. However, the separation of the bands with different colors has not been clearly observed.

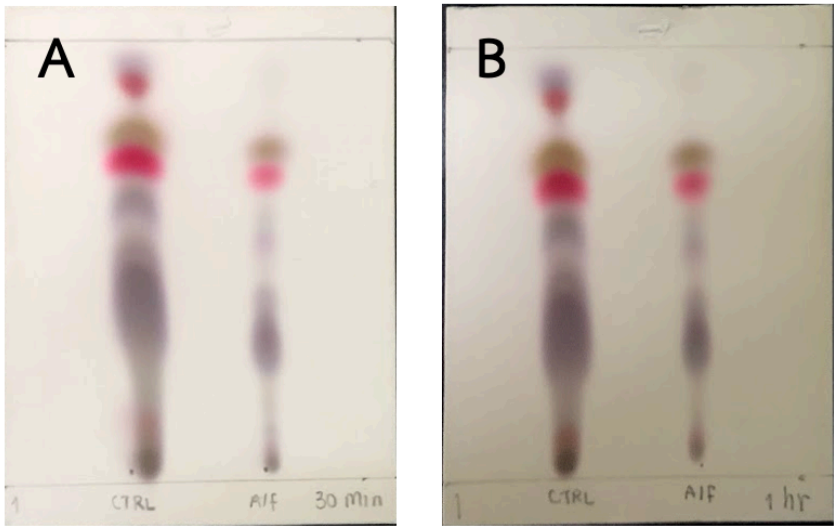

Figure 6 Crassna oil and hexane in 1:5 ratio after electrified current for (A) 30 min (B) 60 min

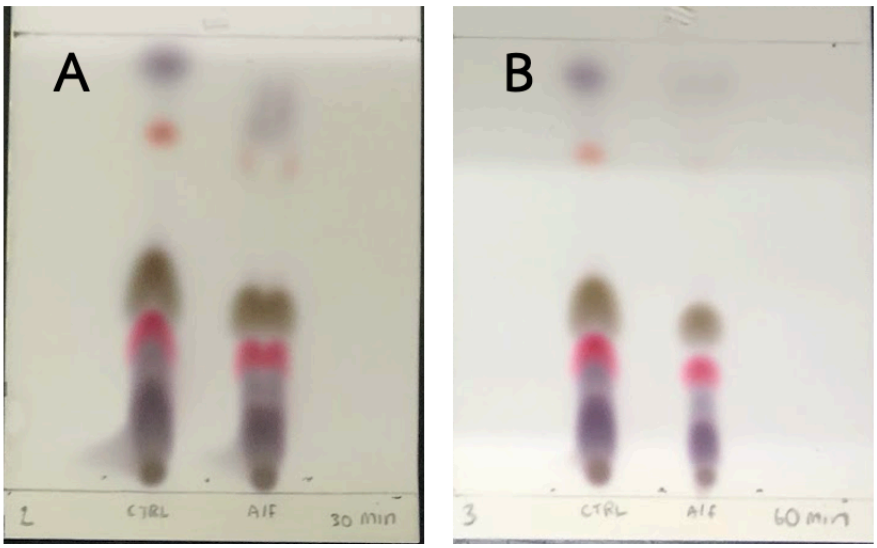

Figure 7 Crassna oil and hexane in 1:10 ratio after electrified current for (A) 30 min, (B) 60 min

1:10 dilution was then performed showing more clearly separated bands on the TLC plates. From these results, 1:10 ratio was selected for further analysis below.

3.3 TLC analysis of water and oil phase

The crassna in water and hexane phases (**Figure 8**) were analyzed with TLC before and after electrolysis in order to investigate the compound loss into the water phase after the electrolysis.

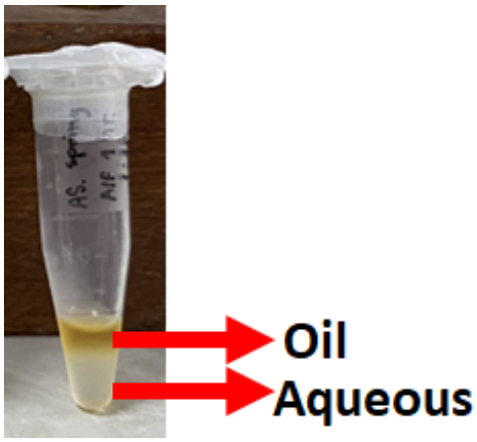

Figure 8 Water and oil phase

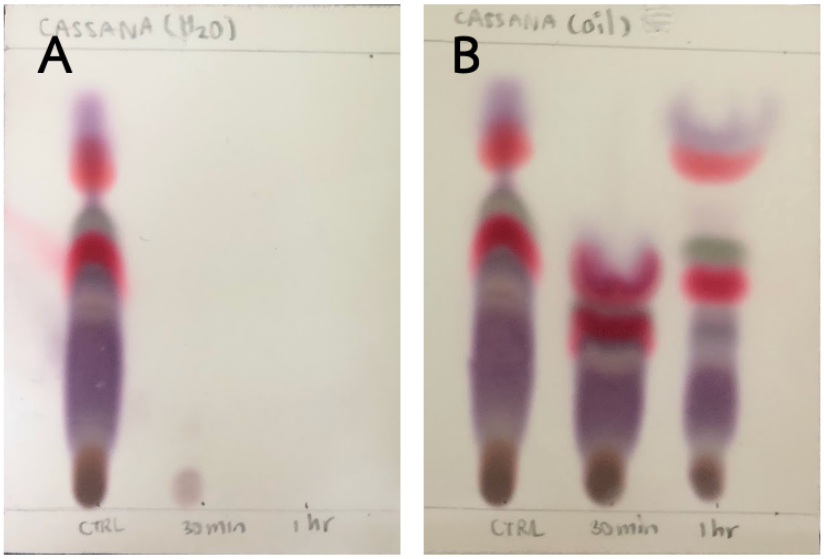

Figure 9 Crassna solvent of (A) water and (B) oil phase

Figure 9 A and **B** showed the TLC results. In each figure, the control (before the electrolysis) and the samples after 30 and 60 min electrolysis were shown from left to right, respectively.

There were only a single spot observed with the 30 min electrolysis and no peaks on the TLC plate of the sample in water phase after the 60 min electrolysis. This indicates that there was insignificant loss of the detectable compounds into the water phase after the electrolysis.

On the other hand, the samples in the hexane phase showed most of the bands of the samples with the difference between the control and the electrolyzed sample. The result showed that there was the compound profile change after applying the electrical current for 30 min and 60 min, which can be seen as the faded spots at the top after of the 30 min electrolysis.

3.4 GC-MS results

For the selected condition of 60 min electrolysis, the compound profiles of the sample before and after the treatment were obtained with GC-MS with the result shown in **Table 1** and **2**, respectively.

Table1: Volatile compound profile in the control of agarwood sample (2-phase) without the electrolysis.

Compound	Time	RI_{Lit}^{*}	Area	Match	R Match
n-Hexane	2.32	600	335910	847	868
Toluene	3.97	763	34846	600	659
1-Pentanol, 4-methyl	6.01	846	76961	831	851
Benzaldehyde	8.67	962	12644	999	999
1-Hexanol, 4-methyl	8.96	953	26205	685	840
Cyclohexane, 1-methylene-4-(1-methylethenyl)	10.85	1004	64479	702	711
6β-Hydroxy-oral turinabol	11.19	3142	2943	622	624
Decane, 3-methyl-	11.88	1071	51405	754	826
1-Octyl trifluoroacetate	12.34	1059	65281	720	771
1-Undecene, 4-methyl	13.45	1085	30772	641	798
Acetic acid, trifluoro-, nonyl ester	14.64	1157	19345	634	689
Octane, 3-ethyl-2,7-dimethyl	17.01	1180	11959	706	816
Undecane, 2,6-dimethyl	17.32	1210	8884	609	684
Undecane, 2,7-dimethyl	17.60	1216	8006	618	812
2-Butanone, 4-phenyl	18.28	1232	271750	858	864
1-Dodecanol, 3,7,11-trimethyl	19.57	1275	59220	650	747
1-Chloroeicosane	19.86	1571	8509	631	752
Retinoic acid, methyl ester	20.30	2264	9394	612	657
Triacetin	21.10	1300	7991	645	798
Retinal	21.94	1344	18250	680	846

β-Longipinene	23.23	2396	11585	648	658
Caryophyllene	23.95	1415	18697	621	672
β-Longipinene	24.08	1419	30211	620	686
2-(3-Pyridyl)piperidine, N-acetyl	25.14	1454	27545	682	746
γ-HIMACHALENE	25.81	1471	12691928	704	704
Cyclohexanamine, N-(phenylmethylene)	26.02	1477	98006	604	634
Alachlor	29.18	1712	46270	618	681
Cubenol	30.06	1631	125669	687	777
Zizanal	30.51	1649	48602	622	735
(+)-Cyperotundone	30.75	1662	44028	619	712

Table2: Volatile compound profile in the agarwood sample obtained after the 2-phase electrolysis treatment for 60 min.

Compound	Time	RI_{Lit}^{*}	Area	Match	R Match
n-Hexane	2.31	600	1844582	893	910
Propanoic acid, 2-methyl	3.97	774	173030	628	799
Butanoic acid	4.67	805	188127	855	868
Butanoic acid, 4-chloro	5.65	1063	28460	552	688
1-Hexanol	6.04	868	112960	832	850
Pentanoic acid	6.73	904	33563	694	893
Benzaldehyde	8.62	967	121910	763	841
1-Hexanol, 4-methyl	8.93	953	49712	723	846
Cyclopentanone, oxime	9.26	972	8699	455	579
1-Aminoanthraquinone, N-trimethylsilyl	9.97	2452	565837	605	609
p-Cymene	10.69	1025	101242	679	799
Cyclohexene, 1-methyl-4-(1-methylethenyl)	10.84	1031	160433	740	795
Nonane, 4,5-dimethyl	11.88	1046	32966	679	788
Acetophenone	12.11	1065	25842	759	847
1-Octyl trifluoroacetate	12.34	1059	98907	697	757
Linalool	13.33	1099	19259	622	676
Hydroxylamine, O-decyl	13.45	1100	30485	666	829
Anthranilic acid, 2TMS derivative	14.07	1612	5644	654	693
Anthranilic acid, 2TMS derivative	14.28	1612	19476	641	668
Acetic acid, trifluoro-, nonyl ester	14.64	1157	23738	655	721

Anthranilic acid, 2TMS derivative	15.44	1612	1898321	711	846
1-Nonanol	15.85	1173	39029	610	815
cis-5,8,11,14,17-Eicosapentaenoic acid	16.30	2334	3452	602	651
Undecane, 2,4-dimethyl	16.84	1208	18435	692	831
Octane, 3-ethyl-2,7-dimethyl	17.02	1180	14407	658	804
Undecane, 2,6-dimethyl	17.32	1210	15161	625	885
1-Chloroeicosane	17.59	2263	11328	620	644
Benzene, (1,3-dimethylbutyl)	18.16	1220	71785	601	680
2-Butanone, 4-phenyl	18.29	1232	785288	826	863
1-Dodecanol, 3,7,11-trimethyl	19.50	1754	28362	646	674
2(3H)-Furanone, dihydro-5-tetradecyl	20.05	2264	8509	627	666
Megastigma-4,6(E),8(E)-triene	20.23	1275	12180	847	876
Betulin	23.23	1391	23146	641	678
3Z)-4-(2,7,7-Trimethylbicyclo[3.2.0]hept-2-en-1-yl)-3-buten-2-one	23.96	1415	31730	611	654
Dihydro-β-agarofuran	25.14	1454	36027	620	676
α-Bulnesene	25.82	1466	18351112	735	735
β-Vetivenene	26.56	1493	299083	636	725
Zizanal	30.05	1605	124848	636	660
Debrisoquine	30.51	1649	43091	629	789
(11Z,14Z,17Z)-Methyl icosa-11,14,17-trienoate	31.51	1540	56337	624	674

According to the chromatograms shown in **Figure 10**, the relative contents of 2-Butanone, 4-phenyl, Khusimone, Propanoic acid, 2-methyl, 1-Hexanol, Benzaldehyde, Cyclohexane, 1-methylene-4-(1-methylethenyl), Cadrene significantly increased after the electrolysis treatment (see also the data in **Table 3**). They contribute to the odors of floral balsamic sweet herbal fruity, vetiver woody, Acidic sour cheesy dairy buttery rancid, pungent ethereal fusel oily fruity alcoholic sweet green, strong sharp sweet bitter almond cherry, fresh fruity citrus green nutmeg spicy, Cedarwood woody, respectively, of the agarwood oil. On the other hand, the contents of γ-Eudesmol, Toluene, 1-Pentanol, 4-methyl, cis-Thujopsene decreased after the electrolysis corresponding to the smells of waxy sweet, sweet pungent benzene-like odor, nutty, woody, respectively.

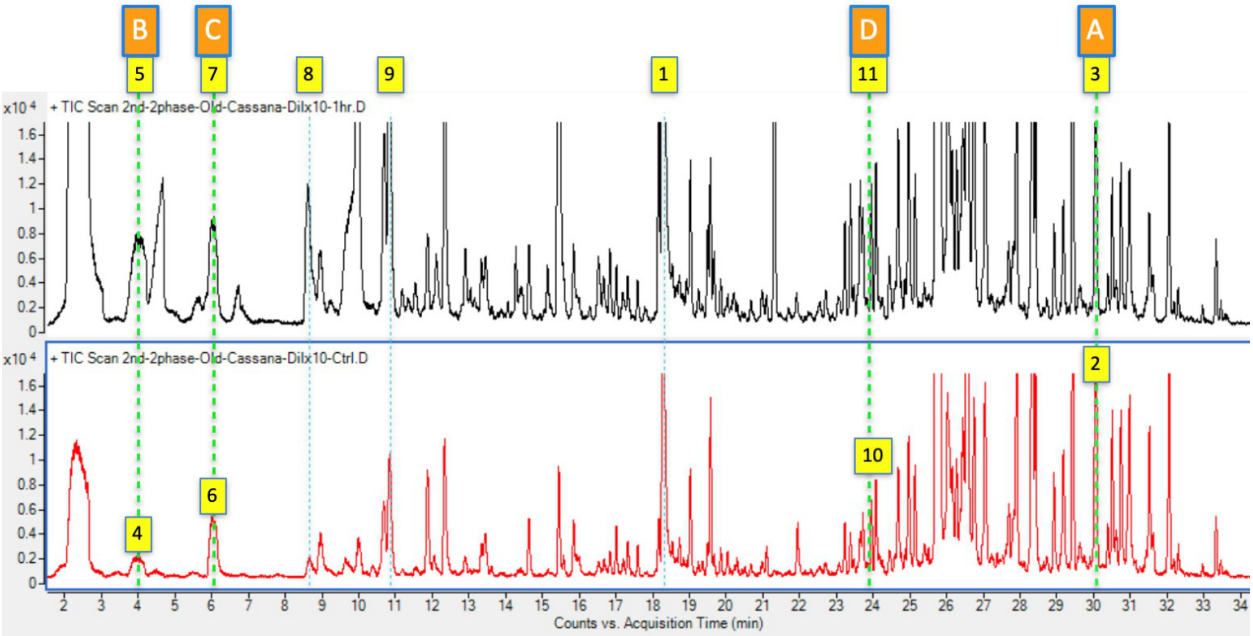

Figure 10 SPME-GC-MS results of (above) agarwood oil in hexane before (below) and after (above) the electrolysis. The related volatile compound profiles were given in **Table 3**.

Table 3: Comparison of volatile compounds area percentages in the agarwood sample before and after the 2-phase electrolysis treatment for 60 min.

No.	Compound	RI_{Lit}^{*}	Odor description	Area percentage	
				Control	60 min electrolysis
1	2-Butanone, 4-phenyl	1232	Floral balsamic, sweet, herbal, fruity	1.805	2.904
2	γ-Eudesmol	1631	Waxy sweet	0.835	0
3	Khusimone **(A)**	1605	Vetiver woody	0	0.462
4	Toluene	763	Sweet, pungent, benzene-like odor	0.231	0
5	Propanoic acid, 2-methyl **(B)**	774	Acidic sour cheesy dairy buttery rancid	0	0.711
6	1-Pentanol, 4-methyl	846	Nutty	0.511	0
7	1-Hexanol **(C)**	868	Pungent ethereal fusel oily fruity alcoholic sweet green	0	0.464

8	Benzaldehyde	962	Strong sharp sweet bitter almond cherry	0.084	0.501
9	Cyclohexane, 1-methylene-4-(1-methylethenyl)	1004	Fresh fruity citrus green nutmeg spicy	0.428	0.659
10	cis-Thujopsene	1419	Woody	0.297	0
11	Cadrene **(D)**	1429	Cedarwood woody	0	0.284

Table 3 Significant compounds area percentages before and after 60 min of EC reaction from **Figure 10** consisting of (A) Khusimone, (B) Propanoic acid, 2-methyl, (C) 1-Hexanol, (D) Cadrene.

The compounds without the highlight in **Table 3** represented the undesirable smells of the crassna oil that can be diminished after the electrolysis. Thus, it could be concluded that the electrolysis approach could improve crassna oil odorant properties.

Chapter 4
Conclusion

Electrolysis approach was developed with the potential application for adjustment of the overall odor of the agarwood oil. The smell was sweeter, softer and milder after electrolysis. This corresponds to the decrease/increase in the amounts of the odor active compounds after the electrolysis. Since small amount of water was added into the electrolysis system, electrical current was maintained by the aqueous phase; whilst, the reactants were continuously supplied from the organic to the aqueous phase undergoing electrolysis. The potential contaminants from the electrode corrosion (e.g. the metal complexes and sludge) were clearly separated into the aqueous phase after the electrolysis. They were easily removed from the system after the electrolysis. TLC approach was successfully applied for selection of electrolysis condition where the selected condition resulted in decrease in the amounts of the low polar components of the agarwood oils. SPME GC—MS further revealed the volatile compound profile confirming the overall smell change after the electrolysis. Since the developed system employed two stainless steel spring electrodes, this is simple, cost effective and applicable for improvement towards the industrial scale for electrochemical adjustment of essential oil compositions in the future.

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Biography

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