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Research article

Aroma-active compounds of *Melaleuca cajuputi* essential oil, a potent flavor on *Cajuputs Candy*

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Abstract: *Melaleuca cajuputi* essential oil (MCEO) from Pulau Buru (PBR), Indonesia, has been used as a functional flavor on *Cajuputs Candy* for years. Our recent study has explored thirteen other alternative MCEO sources to be developed as food flavor. However, not all of the MCEO had similarities to PBR MCEO both in their sensory and volatile profiles. This study aimed to identify the aroma-active compounds which would affect the overall aroma perception of the most- and the least-liked MCEO as a flavor ingredient based on the nasal impact frequency (NIF) method. Initial screening was performed to evaluate the overall liking of thirteen MCEO on *Cajuputs Candy* through a hedonic test, conducted by seventy-eight untrained panelists. The sample obtained from Mojokerto (MOJ) was the most-liked alternatives MCEO in a similar degree of liking to the currently used PBR, while Belu (BEL) was the least-liked. Further analysis using Gas chromatography–Mass spectrometry/Olfactometry (GC-MS/O) was carried out on the MCEO from PBR, MOJ, as well as BEL. The response of nine panelists showed that the overall aroma perception of PBR was contributed by 1,8 cineole (eucalyptus-like, mint, fresh), α-pinene (pine, green, fresh), and ylangene (spicy, fresh, woody) as the main aroma-active compounds. In addition to α-pinene and 1,8 cineole, the unique aroma of MOJ was dominantly contributed by caryophyllene, possessing a woody, sweet, and spicy aroma. The strong floral odor of linalool and nerolidol, the aroma-active compounds of BEL, generated distinct sensory characteristics in comparison to the reference, PBR.

Keywords: *Melaleuca cajuputi*; essential oil; natural flavor; aroma-active compound; nasal impact frequency; GC-MS/O

Abbreviations: *MCEO:* Melaleuca cajuputi *essential oil*; *PBR: Pulau Buru*; *MOJ: Mojokerto*; *BEL: Belu*; *GC-O: Gas chromatography-Olfactometry*; *GC-MS: Gas chromatography-mass spectrometry*; *NIF: Nasal impact frequency*

1. Introduction

Melaleuca cajuputi subsp. *cajuputi* is one of the broad-leaved paperbark subspecies that belongs to the Myrtaceae family and is endemic to Pulau Buru, Indonesia. This plant has been used for a wide range of purposes, in particular, its foliage to produce the essential oil as the main commercial product [1]. Furthermore, Indonesian people commonly use *Melaleuca cajuputi* essential oil (MCEO) for personal care as a liniment to reduce colds and to relieve the effect of insect bites. In addition to being widely reported to have therapeutic activities [2–4], this essential oil has also been permitted as one of the natural flavoring agents added to food by the U.S. Food and Drug Administration (FDA, 2018). It was even declared as *Generally Recognized as Safe (GRAS)* by the expert panel of the Flavor and Extract Manufacturers Association (FEMA GRAS, 1965). Hence, Indonesian MCEO is potential to be developed as a promising native flavor ingredient.

MCEO from Pulau Buru (PBR) was the first utilized MCEO as the main flavor in *Cajuputs Candy* production, which was known as Indonesian lozenges. This product is an emerging functional food to maintain oral cavity health due to its antimicrobial capacity against oral pathogenic microbes [5]. Various candy variants, such as hard candy, non-sucrose hard candy, and soft candy, have been developed for years by primarily utilizing PBR MCEO. Unfortunately, the natural forest in Pulau Buru (Maluku) was slowly ruined as a result of illegal gold mining, which caused a limitation in PBR MCEO availability [6]. On the other hand, Indonesia also had several other MCEO sources, which were still limitedly developed as pharmaceutical ingredients. Therefore, we tried to explore various Indonesian MCEO from different origins that could replace PBR as the flavoring ingredient in *Cajuputs Candy*. Based on our preliminary study, not all the alternative MCEOs had similar sensory characteristics from that obtained from PBR, which could be related to the variety of volatiles compositions in MCEOs [7].

The volatile constituents of MCEO have been widely studied, Fall [8] reported the presence of forty-three volatile compounds in MCEO which were classified in monoterpenes, sesquiterpenes, and sesquiterpenols. 1.8-cineole, α-terpineol, caryophyllene were identified as the main compounds in MCEO [9,10]. Despite the characterization of volatile composition in MCEO was highly important, not all of the volatiles could give a specific impact on the overall aroma perception (aroma-active compounds) [11]. In flavor analysis, Gas Chromatography-Olfactometry (GC-O) is the most common method for the characterization of the aroma-active compounds [12,13]. Nasal impact frequency (NIF) has been widely used to identify various aroma-active compounds by using GC-O analysis with good repeatability as reported in *asam sunti* and several other essential oils obtained from bitter orange, sweet orange, and *Chrysanthemum* [13–16]. The main advantage of this method is its simplicity since highly trained panelists are not required. Untrained panelists could be recruited without further training sessions, therefore it could reduce the required time for the analysis [17–19].

This study aimed to characterize the aroma-active compounds responsible for the unique flavor of the most- and least-liked MCEO based on the NIF method. This information is expected to be useful in the selection of alternative MCEO and its further development as a flavoring ingredient.

2. Materials and methods

2.1. MCEO samples

MCEOs were obtained from different manufacturers, including PT. Bukit Asam (Tanjung Enim), Perhutani (Indramayu, Ponorogo, Mojokerto, Gundih, Kuningan, and Pasuruan), Department of Forestry in the Province of Yogyakarta (Gelaran and Sendangmole), and several home-scale distilleries (Pulau Buru, Namlea, Bupolo, and Belu). The details of the sample origins presented in Table 1. These MCEOs were then kept in dark bottles at 4 ℃ prior to the analysis.

2.2. Sensory analysis

Non-sucrose Cajuputs Candy was used as the carrier to evaluate the flavor characteristics of each MCEO to replace MCEO from Pulau Buru (PBR). The candy was prepared based on a previous patent [20] with modification using thirteen kinds of MCEO. Isomalt (Beneo-Palatinit GmbH, Mannheim, Germany), acesulfame K (Anhui Jinhe Industries, China), and water were mixed and heated to 150 ℃ while then stirred continuously. A flavor mixture of MCEO and peppermint oil (PPO) (Brataco Chemika, Jakarta, Indonesia) was added as temperature decreased to 135 ℃. PPO was the secondary flavor which was added in fewer amounts than MCEO. The dough was then molded accordingly.

Table 1. *Melaleuca cajuputi* essential oil origins and manufacturing.

MCEO Sample	Code	Origin	Cultivation	Production
Pulau Buru	PBR	Maluku	Natural forest	Home scale
Bupolo	BUP	Maluku	Natural forest	Home scale
Namlea	NAM	Maluku	Natural forest	Home scale
Belu	BEL	East Nusa Tenggara	Natural forest	Home scale
Pasuruan	PAS	East Java	Silviculture	Large scale factory
Ponorogo	PON	East Java	Silviculture	Large scale factory
Mojokerto	MOJ	East Java	Silviculture	Large scale factory
Gundih	GUN	Central Java	Silviculture	Large scale factory
Indramayu	IND	West Java	Silviculture	Large scale factory
Kuningan	KUN	West Java	Silviculture	Large scale factory
Sendang mole	SEN	Yogyakarta	Silviculture	Large scale factory
Gelaran	GEL	Yogyakarta	Silviculture	Large scale factory
TanjungEnim	TAN	South Sumatera	Silviculture	Large scale factory

The acceptability of numerous MCEO obtained from different origins was evaluated by performing an affective rating test on thirteen kinds of *Cajuputs Candy* using *Balanced In-completed Block Design* [21]. The test block was provided for 0.81% data strength, t = 13, k = 4, r = 4, b = 13, and λ = 1. A group of seventy-eight untrained panelists was recruited for this test. The panelists were then asked to provide a preference response on the aroma and taste attributes based on a 7-point hedonic scale, ranging from dislike extremely to like extremely [22]. Furthermore, a set of plain crackers and lukewarm water were used as palate cleansers.

2.3. GC-O analysis

MCEO obtained from Pulau Buru (PBR) as well as MOJ and BEL, which were respectively defined as the most-liked and the least-liked MCEO, were subsequently subjected to GC-O analysis on Agilent Technologies 7890A Gas Chromatography coupled with an Olfactory Detection Port (ODP2 GERSTEL GmbH & Co. KG, Germany). Each sample was injected into a DB-5 capillary column (30 m × 250 µm × 0.25 µm, Agilent Technology, USA) in split mode (1:175). Hydrogen was used as carrier gas at a flow rate of 2.3 mL/min. The injection temperature was 250 ℃. The initial column temperature was set at 40 ℃ for 2 min followed by a constant temperature rate (3 ℃/min) to achieve 220 ℃ final temperature. The sniffing ran for 35 minutes for each person.

The aroma-active compounds in each MCEO were selected using the nasal impact frequency (NIF) method [11]. Nine untrained panelists (6 females and 3 males, aged between 22–35 years old) were recruited for this test. An initial aroma familiarization was performed before this analysis. Panelists recorded the perceived aroma by pressing the olfactory pad button and described the aroma description simultaneously. The odorants which were detected in the same retention time with a similar odor description by at least 60% of total panelists (six out of nine) were then identified as the aroma active compounds [14].

2.4. GC-MS analysis

Volatiles identification was performed through GC-MS analysis on an Agilent Technologies 7890A Gas Chromatography coupled with 5975C Mass Spectrometer. The condition of the GC-MS was similar to those described in previous GC-O analysis. The MS system was used in Electron Ionization (EI) mode with 70 eV ionization energy voltage. The GC-MS peaks were tentatively identified by comparing the mass spectra to the NIST 14 library. Further confirmation was performed by calculating the linear retention index (LRI) of each volatile compound compare with the literature [8,23–25]. The LRI was calculated using an alkane series (C_6–C_{17}) as the external standard. The aroma-active compounds on MCEO were characterized by comparing the aroma peaks on GC-O with those peaks which appeared in GC-MS in similar retention time.

2.5. Statistical analysis

All of the hedonic data were presented as the means of panelists' liking responses. The Statistically significant variation on each attribute was evaluated by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test at $p < 0.05$ to determine the effect of MCEO sources. Data analysis was performed using SPSS 22 Statistics software (IBM Corp., New York, USA).

3. Results and discussion

3.1. Sensory preferences of MCEO as the main flavor on Cajuputs Candy

MCEO has been reported to possess biological activities, including antimicrobial and antioxidant capacity [3,26]. However, the characteristics of MCEO as a food flavoring is little known, although it has been recognized as a permitted flavor ingredient (FDA, 2018). Specifically, MCEO from Pulau Buru, Indonesia has been utilized as the main flavor ingredient in *Cajuputs Candy*. Although the use of PBR MCEO as an emerging functional flavor is greatly promising, the availability of this MCEO is becoming limited due to the land conversion in Pulau Buru [6]. Hence, our recent study explores other MCEO from different origins as possible alternatives to PBR [7].

The hedonic results showed significantly different levels of liking in terms of aroma and taste attributes amongst the *Cajuputs Candy* obtained from various MCEO ($p < 0.05$). *Cajuputs Candy* made from PBR MCEO had the highest score compared to others (Table 2). This result confirmed the role of PBR as the reference MCEO. In addition to BUP and NAM obtained from the same origins with the reference, MOJ, PON, PAS, and KUN had a similar degree of liking to PBR in the aroma attribute (Table 2). This was of interest since these MCEOs were produced in Java. In terms of taste, it was established that all samples tend to provide various liking responses. This might be related to the mastication process in the oral cavity allowed the flavor release from the candy, which was then perceived by the panelist. However, MOJ and PON had the highest levels of liking in both attributes which were similar to BUP and PBR, obtained from Maluku.

The response of seventy-eight panelists revealed that *Melaleuca cajuputi* cultivated in Mojokerto and Ponorogo were able to produce sensory characteristics that are similar to the original plants in Pulau Buru, despite they had different cultivation and production methods (Table 1). Furthermore, the high score of MOJ confirmed our preliminary study in which revealed this MCEO had the most similar sensory characteristic with PBR regarding the sweet taste and the cooling aftertaste [7]. The principal component analysis further described its classification to the reference group, due to the composition of ylangene, α-gurjunene, and β-longipinene, in addition to the dominant volatiles, such as 1,8-cineole, α-terpineol, caryophyllene, and humulene [7]. Therefore, MOJ was then selected over PON as the most liked alternatives MCEO for further assessment. Although BUP obtained from Maluku also had a high liking score, it was discredited as a result of its imminent limitation in availability due to obtained from the same origins with the reference.

On the contrary, BEL and IND were observed to be the least-liked MCEO with the lowest hedonic score in terms of aroma and taste attributes. Based on GC-MS data in our previous report, BEL was found as the most distinct MCEO due to the high content of linalool and nerolidol, which generated distinct sensory characteristics among others. This sample was strongly characterized by floral, metallic, soapy, and iodophor-like attributes [7]. The hedonic test also confirmed the tendency for these unfavorable attributes to significantly impair the liking levels. Even though IND also showed a similarly low degree of liking, our previous data revealed the most distinct volatiles composition features, as well as the sensory attributes, were shown in BEL [7]. Therefore, BEL was selected to represent the most significantly different MCEO compared to PBR in the aroma-active compound analysis.

Table 2. The hedonic test of *Cajuputs Candy* made by MCEO from different origins.

MCEO Source	Code	Hedonic score	
		Aroma	Taste
Pulau Buru*	PBR	5.04[a]	5,38[a]
Bupolo	BUP	5.04[a]	5,08[ab]
Namlea	NAM	4.63[ab]	4,88[abc]
Belu	BEL	4.00[b]	3,67[de]
Pasuruan	PAS	4.50[ab]	4,79[abc]
Ponorogo	PON	4.54[ab]	5,25[ab]
Mojokerto	MOJ	4.79[ab]	5,13[ab]
Gundih	GUN	4.08[b]	4,42[bcd]
Indramayu	IND	4.13[b]	3,42[e]
Kuningan	KUN	5.13[a]	4,75[abc]
Sendang Mole	SEN	4.58[ab]	4,33[bcd]
Gelaran	GEL	4.58[ab]	4,13[cde]
Tanjung Enim	TAN	4.67[ab]	4,79[abc]

Note: *: The existing MCEO (PBR) was performed as the reference. Different superscript letters represented significantly different values among the groups ($p < 0.05$).

3.2. Aroma-active compounds in MCEO

A comprehensive volatile analysis has been conducted in our previous study [7]. There were sixty putatively identified compounds on thirteen MCEOs consisted of the oxygenated monoterpenes group (48–55%) followed by sesquiterpenes (7–18%), and hydrocarbon monoterpenes (2–18%). 1,8-cineole (40.07–54.09%), followed by α-terpineol (3.70–6.99%), caryophyllene (1.47–6.26%), α-pinene (0.81–11.98%), and γ-terpinene (0.80–3.73%) were identified as the dominant volatiles [7]. These terpene compounds in MCEO had previously been cited in several studies [8,10,26]. Even though a large number of studies have reported the volatile profiles of MCEO, there is limited information related to the aroma-active compounds in Indonesian MCEO, which is responsible for the overall flavor characteristics. Therefore, we used a combination of GC-O and GC-MS to characterize the aroma-active compounds on PBR as the target reference, as well as MOJ and BEL, as the most-liked and least-liked MCEO respectively.

Among the sixty compounds, only thirty-three which exhibited aroma activity on the GC-O (Table 3), of which twenty-three aroma-compound peaks were detected in PBR and twenty-six in MOJ, while, BEL was only characterized by twenty aroma peaks (Figures 1–3). A total of twelve aroma-compounds were detected in all three MCEOs, including α-pinene (pine, green, fresh), 1,8-cineole (eucalyptus-like, mint, fresh), γ-terpinene (fresh, herbal, woody), linalool (floral, sweet, woody, fruity), δ-terpineol (metallic, woody, mint), α-terpineol (floral, fruity, citrus, woody), ylangene (spicy, fresh, woody), caryophyllene (woody, sweet, spicy), humulene (woody, acid, burnt), α-muurolene (woody, floral, sweet), germacrene B (woody, earthy, fresh), β-eudesmol (woody, burnt). Furthermore, four peaks were detected in both PBR and MOJ, and three aroma-compound peaks that were only detected in the BEL sample.

Table 3. Aroma compounds in *Melaleuca cajuputi* essential oil by GC-MS/O.

No	LRI Exp.	LRI Lit.	Compound	NIF* PBR	MOJ	BEL	Odor Description	Identification**
1	930	927	a-Thujene	3	3	0	woody	MS, LRI, O
2	939	936	α-Pinene	6	7	3	pine, green, fresh	MS, LRI, O
3	971	971	β-Pinene	0	3	4	sweet, fruity, pine	MS, LRI, O
4	992	990	β-Myrcene	5	2	0	woody, spicy	MS, LRI, O
5	1012	1013	α-Phellandrene	0	3	3	burnt, woody	MS, LRI, O
6	1042	1039	1,8-Cineole	8	7	9	eucalyptus-like, mint, fresh	MS, LRI, O
7	1063	1060	γ-Terpinene	4	4	0	fresh, herbal, woody	MS, LRI, O
8	1106	1101	Linalool	4	3	9	floral, sweet, woody, fruity	MS, LRI, O
9	1122	-	Unknown	0	4	5	floral, fruity	-
10	1170	1166	δ-Terpineol	2	4	4	woody, mint, metallic	MS, LRI
11	1190	1177	Terpinen-4-ol	2	4	0	sweet, fruity, herbal	MS, LRI, O
12	1205	1195	α-Terpineol	5	4	3	woody, floral, fruity	MS, LRI, O
13	1237	-	Unknown	0	0	4	floral	-
14	1267	-	Unknown	0	4	0	woody, mint, fruity	-
15	1276	-	Unknown	4	0	0	mint, woody, floral	-
16	1292	-	Unknown	0	4	0	acid, mint, fresh	-
17	1329	-	Unknown	3	4	0	woody, herbal	-
18	1357	1354	α-Terpinyl acetate	4	3	0	woody, burnt, herbal	MS, LRI, O
19	1383	1373	Ylangene	6	3	4	spicy, fresh, woody	MS, LRI, O
20	1393	1391	α-Copaene	2	0	0	woody	MS, LRI, O
21	1402	1390	β-Elemene	0	3	0	fresh, woody	MS, LRI, O
22	1421	1407	α-Gurjunene	0	0	3	woody	MS, LRI, O
23	1434	1424	Caryophyllene	3	6	2	woody, sweet, spicy	MS, LRI, O
24	1468	1454	Humulene	4	4	4	woody, acid, burnt	MS, LRI, O
25	1501	1498	α-Muurolene	4	4	4	woody, floral, sweet	MS, LRI, O
26	1518	1521	β-Cadinene	4	4	0	woody	MS, LRI, O
27	1527	1523	δ-Cadinene	0	4	3	woody, sweet, fruity	MS, LRI, O
28	1549	1544	α-Calacorene	0	3	0	floral, fresh, woody	MS, LRI, O
29	1557	1557	Germacrene B	2	3	4	woody, earthy, fresh	MS, LRI, O
30	1572	1564	Nerolidol	4	0	6	floral, sweet, fruity, woody	MS, LRI, O
31	1602	1592	Viridiflorol	0	4	0	green, sweet	MS, LRI, O
32	1628	1630	γ-Eudesmol	3	0	0	woody	MS, LRI, O
33	1649	1649	β-Eudesmol	3	3	3	woody, burnt	MS, LRI, O

Note: LRI exp: calculated linear retention index (LRI) by a relative comparison of the compound and *n*-alkane series retention time on DB-5 capillary column, LRI lit: LRI value from the literature [8,23–25]. *Frequency of detection by panelists (n/9). **identification method was performed by comparing the mass spectrum (MS), linear retention index on DB-5 with the literature (LRI), and odor description in literature data (O).

In this study, the aroma-active compounds were identified by the NIF method which recognized an aroma active compound if it was detected by at least six out of nine panelists [14]. There were six aroma-active compounds detected in all of the samples (Figure 4). 1,8-cineole (NIF 8/9), α-pinene (NIF 6/9), and ylangene (NIF 6/9) were found as the aroma-active compounds on PBR (Figure 1, Table 3). 1,8-

cineole which had over 51.50% abundance on PBR [7], has also been widely reported in the literature as the main volatile component in MCEOs [8–10]. Therefore, it was not surprising that this volatile served as the major aroma-active compound. Moreover, the strong eucalyptus-like, mint, and fresh characters of 1,8-cineole dominantly contributed to the overall character of MCEOs. This odor description was also reported by several studies [27,28].

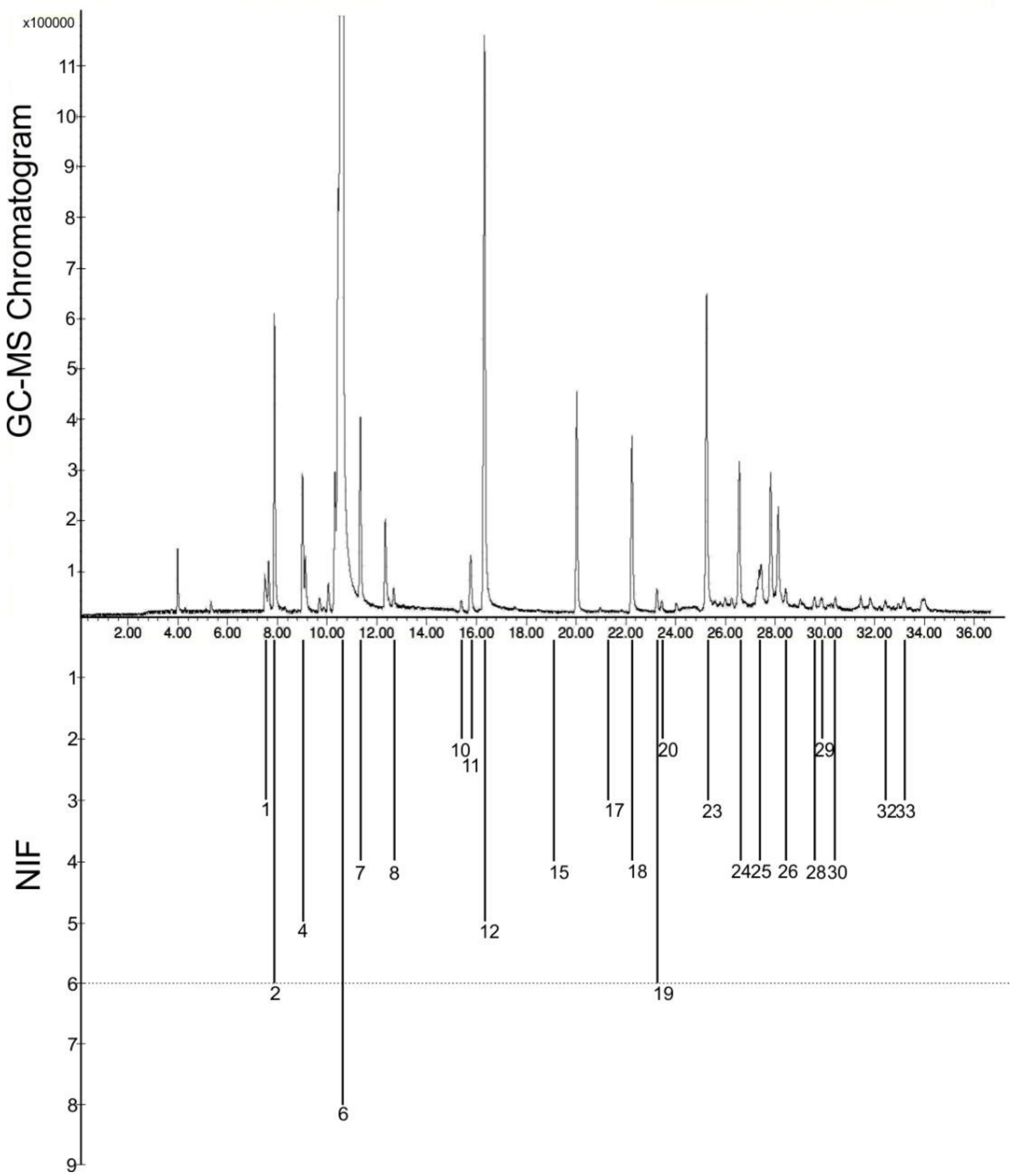

Figure 1. GC-MS chromatogram (upper figure) and nasal impact frequency (NIF) profile of corresponding aroma-compounds (lower figure) in *Melaleuca cajuputi* essential oil from Pulau Buru (PBR). The numbers on peaks were related to the compound numbers presented in Table 3.

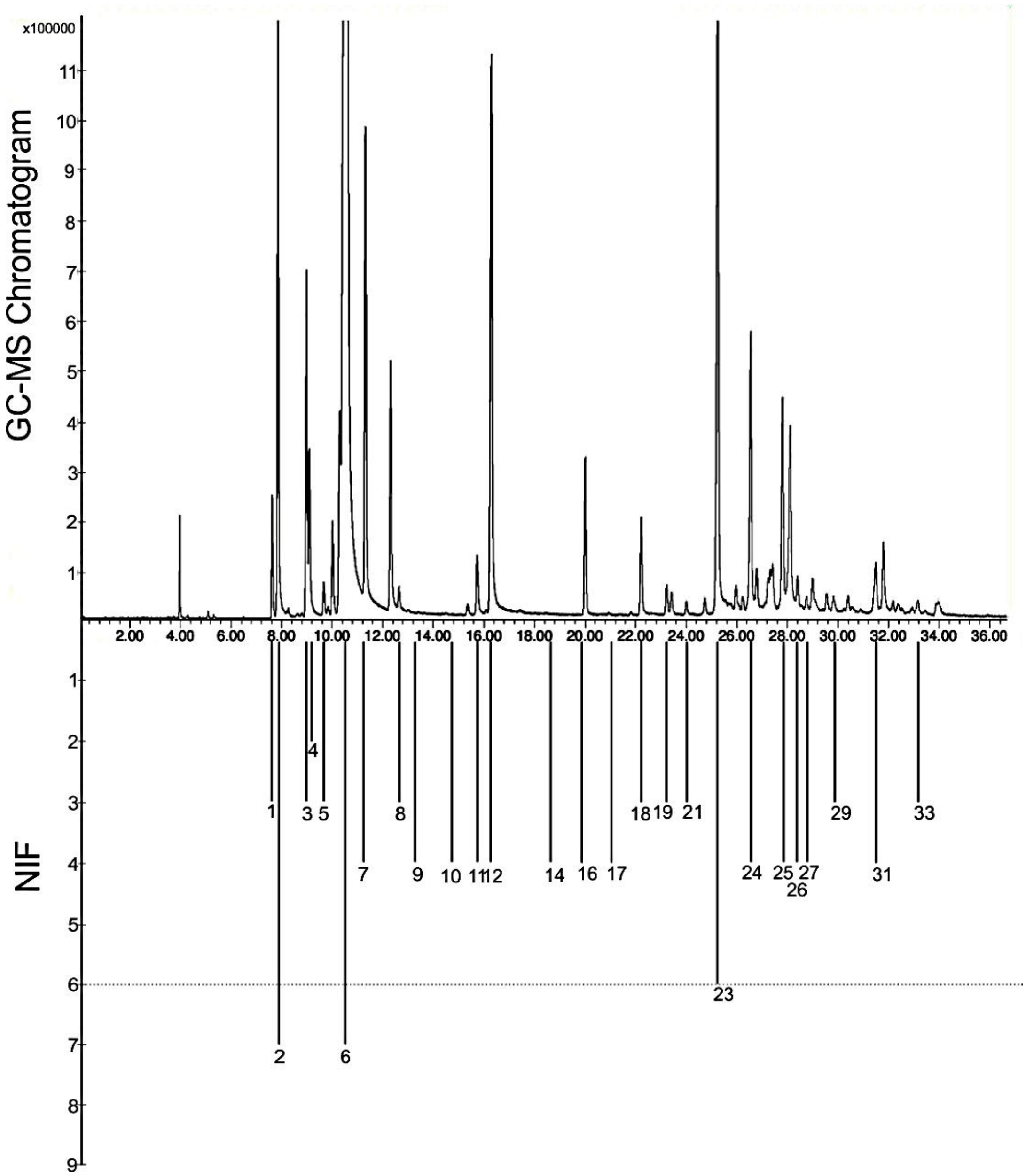

Figure 2. GC-MS chromatogram (upper figure) and nasal impact frequency (NIF) profile of corresponding aroma-compounds (lower figure) in *Melaleuca cajuputi* essential oil from Mojokerto (MOJ). The numbers on peaks were related to the compound numbers presented in Table 3.

The fourth dominant volatile on PBR, α-pinene, also showed a strong aroma impact with the odor of pine, green, woody, and fresh, which was in agreement with the previous report [29]. The woody and green character of α-pinene was also found in dill herbs [30]. This major monoterpene in pine pollen essential oil was also identified as the aroma-active compound in bitter orange essential oils. It was detected by all of the panelists as floral, pine, and green [14,31].

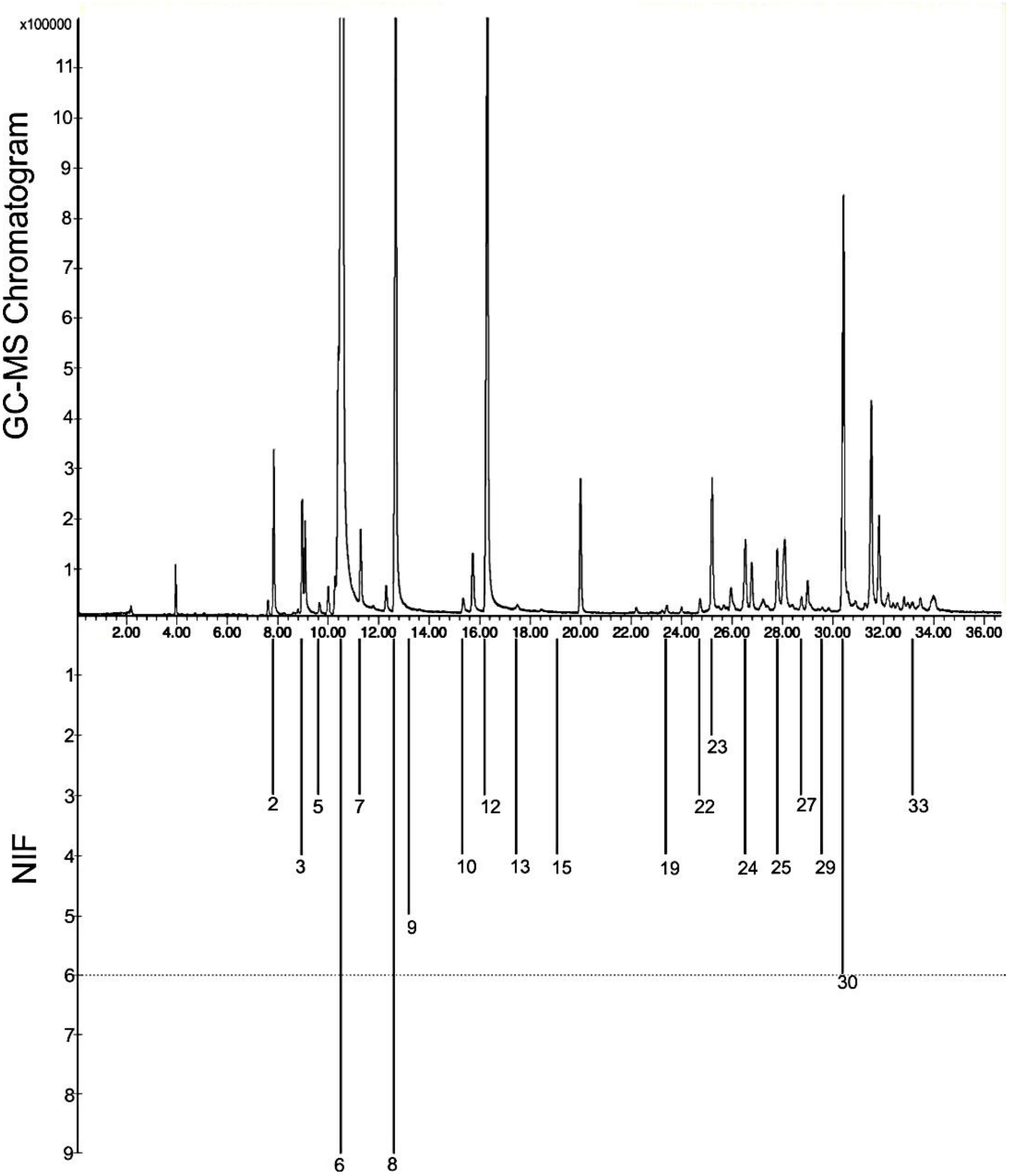

Figure 3.GC-MS chromatogram (upper figure) and nasal impact frequency (NIF) profile of corresponding aroma-compounds (lower figure) in *Melaleuca cajuputi* essential oil from Belu (BEL). The numbers on peaks were related to the compound numbers presented in Table 3.

Ylangene with the spicy, fresh, and woody odor was identified as the third aroma-active compound on PBR, demonstrating a high aroma impact, despite it had a low abundance in PBR (0.26%). It was defined as the discriminant volatile of the reference group amongst the others MCEO based on our previous study [7]. This sesquiterpene was also found on dried Omija fruits and Shiraz grape, however, it was not declared as the aroma-active compound [32,33]. Interestingly, ylangene was reported to have a strong correlation with the pepper aroma on the Shiraz grape (correlation coefficient > 0.98). Although it did not have a strong peppery or spicy aroma, this compound might have performed as a pepper flavor precursor [33]. To the best of our knowledge, this is the first study reporting ylangene as the aroma-active compound on MCEO. This result revealed that the unique aroma of PBR was derived from the combination of 1,8-cineole, α-pinene, and ylangene, rather than a single aroma-active compound.

In addition to 1,8-cineole and α-pinene, MOJ as the most-liked MCEO had caryophyllene as the aroma-active compound (Figure 2, Table 3). Based on the volatile profiles in our preliminary study [7], this sesquiterpene was the third major compound amongst the thirteen MCEO samples and its highest amount was found in MOJ (6.00%). Therefore, it was reasonable that caryophyllene was found as one of the main aroma-active compounds in MOJ, described as having a strong sweet odor, followed by woody and spicy, as reported in mint [30]. This strong sweet odor might correlate with our metabolomics study which reported caryophyllene was the key aroma compound of sweet taste [7], while the spicy character of this sesquiterpene was similar with the reports in black pepper and *Evolvulus alsinoides* essential oils [34,35].

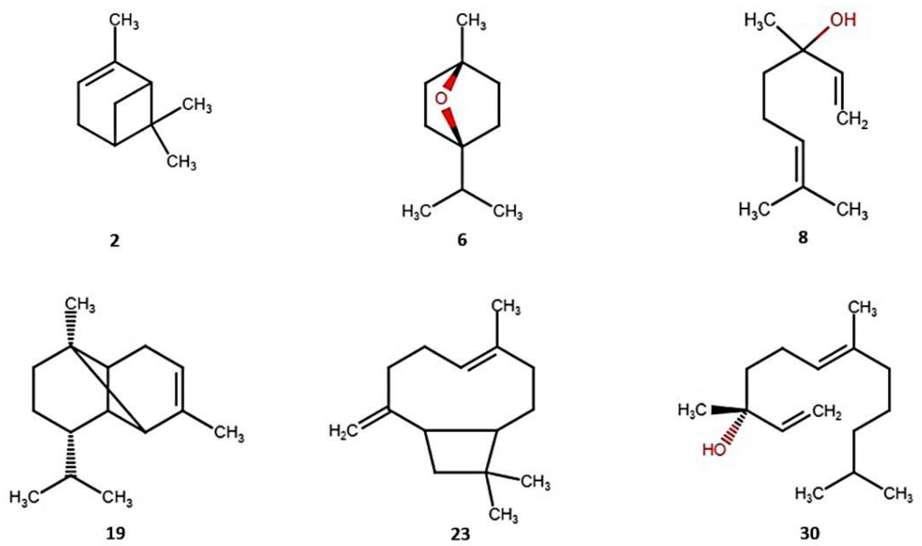

Figure 4. The aroma-active compound identified in *Melaleuca cajuputi* essential oil. The numbers below the chemical structures correspond to the compound numbers presented in Table 3.

Regarding the odorants in BEL, twenty aroma compounds were detected by at least three panelists. Among these odorants, 1,8-cineole, linalool, and nerolidol had the highest detection frequency by nine panelists and were then declared as the aroma-active compounds in BEL (Figure 3, Table 3). 1,8-cineole was specifically recognized by all panelists as a result of the strong eucalyptus-like, mint, and fresh odor. In general, the whole eucalyptus-like aroma perception of MCEO was determined by this oxygenated monoterpene. Interestingly, there were additional trace volatiles compounds that demonstrated a high detection frequency.

Linalool and nerolidol which were found as the minor compounds on other MCEO had the highest amount on BEL sample (5.65% and 4.94% for linalool and nerolidol, respectively) [7]. In this current study, linalool was detected by all of the panelists with a strong floral sweet odor, as well as the woody and fruity notes. This description was confirmed by several studies regarding the odor description of linalool in coriander, mint, wines, and also lychee which tended to possess floral characteristics [28,30,36]. Linalool was also identified as the most aroma-active terpene in bitter orange (essential oils and heat cut) and *Chrysanthemum* essential oils with a strong floral note [15,37]. The high detection frequency of linalool was related to its low threshold, 6 mg/L [38,39].

Nerolidol also contributed to the generation of sweet floral character on BEL, in addition to the woody and fruity odor. Despite known as a minor compound in common MCEO, BEL had the highest content of this compound [7]. Therefore, it was reasonable that nerolidol could give an impactful character on BEL, which was different from common MCEO. Similar to linalool, nerolidol was also frequently detected by the strong floral note in parsley, red wine, and floral-based products [30,40,41]. Nerolidol was also identified as the strong aroma active compound in Longjing tea which was responsible for the rose odor with a low odor threshold (15 µg/g) [42]. A similar result was reported in sun-dried Pu-erh tea leaves, in which linalool had a floral, woody, and fruity odor note, while nerolidol described by rose-like and sweet notes [43]. In general, the combination of linalool and nerolidol described the intense floral character on BEL.

Despite linalool and nerolidol have a pleasant aroma as a single compound, their combination with other volatile compounds on MCEO could decrease the liking response given by the panelists. The aroma-active analysis was in agreement with our recent study, which predicted the most correlated compound with the sensory attributes using the orthogonal partial least square (OPLS). It was found that linalool and nerolidol were identified as the key compounds of floral, which confer the unfavorable attributes on MCEO [7]. Therefore, it was reasonable that the higher content of these aroma-active compounds reduced the liking score when applied on *Cajuputs Candy*, although the strong eucalyptus-like character from 1,8-cineole was still also detected.

This GC-MS/O result was confirmed our previous metabolomic study, in which 1,8-cineole and caryophyllene served as the key metabolites of the favorable attributes, due to the strong collective correlation with the cooling and sweet taste in MCEO [7]. γ-terpinene was also predicted as the key metabolites for the cooling aftertaste in our metabolomics study. Although it was not identified as the aroma-active compound in this study, four out of nine panelists detected the fresh character of γ-terpinene, which possibly contribute to the generation of the overall cooling aftertaste. The floral flavor, the unfavorable attributes in MCEO, was contributed by the presence of linalool and nerolidol, subsequently confirming our prediction.

4. Conclusion

This study provides a novel aroma-active compounds characterization on MCEO as a flavoring ingredient on *Cajuputs Candy.* 1,8-cineole (eucalyptus-like, mint, and fresh), α-pinene (pine, green, and fresh), and ylangene (spicy, fresh, and woody) were identified as prominent aroma-active compounds in PBR. MOJ, the most-liked alternative MCEO on *Cajuputs Candy*, had caryophyllene (woody, sweet, spicy) as its aroma-active compound in addition to 1,8-cineole, α-pinene. The aroma-active compounds on BEL, the least-liked MCEO, were characterized by linalool and nerolidol (floral, sweet, woody, and fruity) as well as 1,8-cineole. These results were in accordance with our previous prediction by a metabolomics approach.

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Conflict of interest

All authors declare no conflict of interest in this paper.

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