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Original research article

Chemical Composition and Physicochemical Properties of Beeswaxes of *Apis mellifera* and *Apis cerana*

Aman Dekebo[1,3] and Chuleui Jung[2,3,*]

[1]Department of Applied Chemistry, Adama Science and Technology University, P.O.Box 1888, Adama, Ethiopia
[2]Agricultural Science and Technology Research Institute, Andong National University, Andong GB 36729, Republic of Korea
[3]Department of Plant Medicals, Andong National University, Andong GB 36729, Republic of Korea

Abstract Beeswax, an important structural material of honeybees is utilized for the formation of comb, and is becoming important biomaterial important in medicine, cosmetics, food, engineering and industry. This study analyze chemical composition and physicochemical properties of beeswax of *Apis mellifera* and *A. cerana*. Hydrocarbons fraction of beeswax was extracted using the SPE technique and analyzed by GC-MS. The hydrocarbon fraction constitutes 88.6% hydrocarbon compounds. Moreover, the beeswax samples were esterified using the BF_3 solution in methanol and analyzed by GC-MS. The physicochemical characteristics evaluated included melting point, acid value, saponification value, ester value, and the acid-to-ester ratio. Melting points was not significantly different among beeswaxes of the bees species ranging from 62–64°C. The acid value and iodine absorption values were higher for *A. mellifera* beeswax samples than *A. cerana*. The saponification and ester values were higher for *A. cerana* than *A. mellifera* beeswax samples. From the chemical compositional analysis, hydrocarbons (48%) and monoesters (21.5%) were found as the major classes of compounds after derivatization of beeswax solution. Methyl palmitate was abundant in the derivatized fraction of beeswax samples, ranging from 8.46% to 28.66%. The other principal compound identified after derivatization of the beeswax was heptacosane (10.21–17.35%). The physicochemical properties and composition were more influenced by the species than the floral resources, environment or location of honeybee colonies.

Keywords Heptacosane, Methyl palmitate, SPE, Floral resources, Forest

INTRODUCTION

Beeswax is a naturally occurring wax produced by honeybees in wax glands located under their abdomen (Jiménez *et al*., 2004). Unless the bee source is specified, the term 'beeswax' generally refers to the wax produced by *Apis mellifera*. However, it's worth noting that *A. dorsata*, *A. florea*, and *A. cerana* are also sometimes used as commercial sources of wax (Tulloch, 1970).

In the earliest time it was exploited for various purposes such as preservation of mummies, wax tablets, bending agent, and for sealing and waterproofing (Warth, 1956; Crane, 1983; Heron *et al*., 1994; Luo *et al*., 2012). Although beeswax is now partly replaced by synthetic or fossil products, it played an important role in a number of fields such as polymer technology, symbolic and artistic fields, preparation of cosmetics or medicinal commodities, food, pharmaceutical, and pesticides (Bonet Rosado and Mata Parreño, 1997; Bernal *et al*., 2005; Chao *et al*., 2010; Kheradmandnia *et al*., 2010; Soazo *et al*., 2011; Nguyen *et al*., 2012; Pérez Gallardo *et al*., 2012).

Beeswax generally consists of a complex mixture of aliphatic hydrocarbons, mono-, di- and poly esters,

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*Corresponding author. E-mail: cjung@andong.ac.kr

hydroxy esters, free fatty acids, free fatty alcohols and minor other compounds (Jiménez *et al*., 2004, 2009; Hepburn *et al*., 2009; Bonvehi and Bermejo, 2012). Based on literature reports the chemical composition of beeswax varies depending on geographic location of production, species of honey bees, age of the wax, climatic conditions and the time of its production (Nong *et al*., 2023).

There were several reports on chemical composition of *A. mellifera*. However, there are relatively limited information on chemical composition analysis of *A. cerana* and even comparison of beeswax samples from the same locality. Therefore, this study present the results on chemical composition of *A. mellifera* and *A. cerana* beeswax after SPE extraction and derivatization techniques. Additionally, we evaluated physicochemical properties of these beeswax samples, and discussed the possible sources of variations for the chemical compositions, if the floral resource or environmental conditions would be influential.

MATERIALS AND METHODS

1. Bee wax samples

Bee wax samples were collected from local *Apis mellifera* apiaries in Daegu (Am1), and Andong National University bee lab (Am2), and *A. cerana* apiaries in Cheonan (Ac1) and Yean, Andong (Ac21, Ac22, Ac23) in 2021 (Fig. 1).

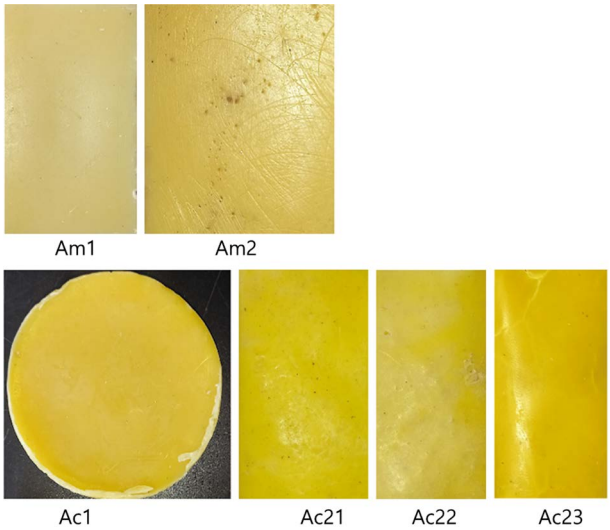

Fig. 1. Beeswax samples (Photo take by Aman Dekebo on September 21, 2021) of *Apis mellifera*; Am1 from Daegu, Am2 from ANU bee lab, and *Apis cerana*; Ac1 from Cheonan, Ac21–23 from yean, Andong, Korea in 2021.

2. Beeswax sample preparation

Extraction, fractionation and derivatization of beeswax were done based on the method reported by Svečnjak *et al*. (2019) with some modification.

3. TLC and GC-MS analysis

Isolation and analysis of different beeswax samples is a time and resources consuming process. Tulloch (1980) reported a quicker method which could be used to compare large numbers of beeswax samples by thin-layer chromatography (TLC) and gas liquid chromatography (GLC). The advantage of TLC is that it is the equipment is simple and inexpensive, and that all classes of components can be screened however its disadvantage is that the composition is analyzed only qualitatively. Additionally, TLC has the advantage in that a small amount of sample (less than 100 μg) is applied on a TLC plate, and solvent (chloroform or benzene) is used as a developing solvent to separate the components and 50% sulfuric acid is used as spraying reagent.

GC-MS analysis was done by using a GC (7890B, Agilent Technologies, USA) coupled with an MS (5977A Network, Agilent Technologies). The GC had an HP 5MS column (non-polar column, Agilent Technologies), 30 m × 250 μm internal diameter (i.d.) and 0.25 μm film thickness. The injection volume was 1 μL. The carrier gas was helium flowing at a rate of 1 mL/min. The injector temperature was 280°C and the injection mode was split mode with split ratio 20 : 1. The initial oven temperature was 120°C and it was raised to 320°C at 10°C/min held at this temperature for 5 min. Mass spectra were recorded in EI mode at 70 eV, scanning the 50–550 m/z range. The identification of compounds was performed by comparing the mass spectra of the compounds with those in the database of NIST11 (National Institute of Standards and Technology, Gaithersburg, USA). Relative amounts of detected compounds were calculated based on the peak areas of the total ion chromatograms (TIC).

4. Physicochemical properties

Acid value, ester value, melting point and saponifica-

tion values were done according to procedure reported by Svečnjak *et al*. (2019) and Iodine number was performed based on thee method of Bernal *et al*. (2005). The acid value determination is one of the most effective and simple methods for detecting beeswax adulteration especially with paraffin and stearic acid. Acid value decreases with the addition of paraffin (Bernal *et al*., 2005; Maia and Nunes, 2013; Svečnjak *et al*., 2015), and increases with the addition of stearic acid (Bernal *et al*., 2005).

RESULTS AND DISCUSSION

1. Physicochemical properties

Physicochemical properties of beeswax samples of two *A. mellifera* and four *A. cerana* were shown in Table 1. Melting point of the beeswax analyzed were comparable to each other. The acid value was higher for *A. mellifera* beeswax samples (18.1±0.23–19.5±0.43). The saponification and ester values were higher for *A. cerana* than *A. mellifera* beeswax samples. Iodine absorption values of *A. mellifera* is relatively higher than those of *A. cerana*. Our physicochemical parameters analysis results for all samples are comparable with those of international standard and the samples do not have adulterants. As the beeswax is the honeybee product from wax gland of worker bees, beeswax profiles would be more influenced by the honeybee species than floral, environmental or surrounding vegetation structures which are their foraging resources. Physicochemical properties were shown more influential between species. Biochemical or functional properties could be differed among honeybee species and relative to the local conditions such as floral composition and season.

2. TLC analysis of Beeswax

Fig. 2 shows TLC profile of beeswax samples from *A. mellifera* and *A. cerana*. Our TLC analysis profile shows presence of typical spots of beeswax components comparable with those of Tulloch (1980).

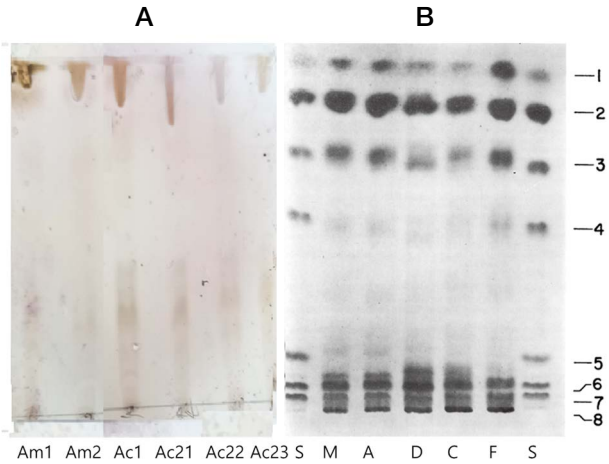

Fig. 2. Thin layer chromatography (TLC) profiles of Beeswaxes of *Apis mellifera*; Am1 from Daegu, Am2 from ANU bee lab, and *Apis cerana*; Ac1 from Cheonan, Ac21–23 from yean, Andong, Korea in 2021 (A), compared with Tulloch (1980, B), where M; European *A. mellifera*; A; African *A. m. adansonii*; D, C, F; Asiatic (*A. dorsata*, *A. cerana*, *A. florea*, respectively). S is a mixture of 1, C_{30} hydrocarbon (12 mg); 2, C_{46} monoester (14 mg); 3, C_{58} diester (5 mg); 4, C_{76} triester (3 mg); 5, C_{26} alcohol (3 mg); 6, C_{40} hydroxy monoester (2 mg); 7, C_{58} hydroxy polyester (2 mg); 8, C_{24} acid (6 mg). Adsorbent is silica gel diluted twice with benzene; components were detected by spraying with 50% sulphuric acid.

Table 1. Physicochemical properties of beeswax samples of *A. mellifera* and *A. cerana* collected from different locations

Species	Sample code	Parameter					
		Melting point (°C)	Acid value (mg KOH/g sample)	Saponification value (mg KOH/g sample)	Ester value	Ester/Acid ratio	Iodine absorption value (g/100 g sample)
A. mellifera	Am1	62.84±0.41	18.1±0.23	89.5±6.52	71.4±1.30	3.94±0.10	5.49±0.56
	Am2	63.54±0.43	19.5±0.43	90.4±8.63	70.9±0.60	3.63±0.16	5.32±0.33
A. cerana	Ac1	64.05±0.41	7.2±0.43	102.4±7.23	95.2±1.24	13.22±1.35	4.71±0.25
	Ac21	63.9±0.32	8.3±0.12	103.5±8.32	95.2±2.33	11.46±1.41	4.60± 0.55
	Ac22	64.4±0.28	6.9±0.61	103.6±9.58	96.7±0.70	14.01±1.30	4.56± 0.44
	Ac23	64.6±0.51	6.3±0.44	103.7±7.61	97.4±1.33	15.46±1.10	4.59± 0.26

*Beeswax samples of *Apis mellifera*; Am1 from Daegu, Am2 from ANU bee lab, and *Apis cerana*; Ac1 from Cheonan, Ac21–23 from yean, Andong, Korea in 2021

3. Chemical composition of beeswax

GC-MS analysis of the hydrocarbon fraction showed the alkane C27 (heptacosane) was the most abundant and common (14.9–27.2%). The highest composition of the compound was recorded in Am1 and the lowest in Ac21. Another predominant compound in Am1 were nonacosane (C29), 1-docosene and octadecane (Table 2, Fig. 3). Similar trend of the percentage composition of the hydrocarbons were observed for those samples of ACs, but the detailed composition was differed even among those from the same apiary but different hives (Fig. 4).

A. cerana beeswax constitute 88.6% hydrocarbon and the remaining 11.4% include nitrogen and silicone derivatized hydrocarbons. Hydrocarbon compounds such as heptadecane (C17), tetracosane (C24), pentacosane (C25), eicosane (C20), nonadecane (C19), heptacosane (C27), octadecane, 2-methyl (C19), heneicosane (C21), hexadecane (C16), 2-methyloctacosane (C29), octadecane (C18), docosane, 11-butyl-(C26), 6,6-diethylhoctadecane (C22), tetratriacontane, 17-hexadecyl-(C50), Anthracene and 9-ethyl-9,10-dihydro-9,10-dimethyl-(C18) were identified in the beeswax (Table 2). The principal compounds in the hydrocarbon fraction were heptacosane (15.7%) and 2-methyloctacosane (9.0%) (Table 2 and Fig. 4). The previous report indicated heptacosane (C27) is a wasp queen pheromone that decreased ovary activation in queenless workers (Oi, 2022). Since the structural basis of the cell consists only of wax, the wax must have suitable physical properties. Presumably some degree of plasticity and knead ability are also desirable. The unsaturated hydrocarbons of beeswax may act as plasticizers (Tulloch, 1970). Live bees are warm and produce a wide range of hydrocarbons. Dead bees cool rapidly and some of the volatile hydrocarbon levels decrease in the immediate vicinity of the corpse. The undertaker bees specifically monitor the levels of (at least) heptacosane and nonacosane as a means of discriminating between live and dead bees. Within 30 min of death local heptacosane and nonacosane levels have dropped below a level associated with life and the undertaker bee removes the corpse. This study was conducted on *Apis cerana*. *A. mellifera*, may use the same necrophoresis signals (The Apiarist, https://theapiarist.org/the-scent-of-death/).

Additionally, we detected presence of some Si-derivatized compounds as minor constituents of the beeswax of *A. cerana* sample. Zimnicka and Hacura (2006) analyzed chemical composition of beeswax of *Apis mellifera* using vibrational spectroscopy (FT-IR and Raman) and reported presence of esters and hydrocarbons as principal compounds together with alcohol and amides, inorganic compounds of Si, P, and S. The source of inorganic compounds of Si, P, and S were detected from samples collected from industrial area which was attributed to pollution of bees' environment (Zimnicka and Hacura, 2006). Therefore, the presence of Si in the beeswax of *A. cerana* might be attributed by some environmental contaminants.

GC of the derivatized fraction of beeswax samples solution using the BF_3 solution in methanol enable identification of several class of compounds with methyl palmitate was the most abundant and common com-

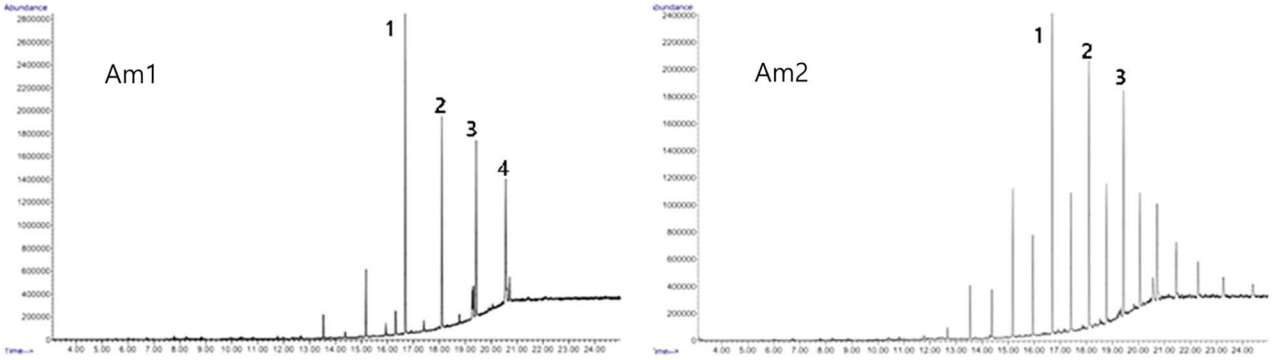

Fig. 3. GC of the hydrocarbon fraction of *Apis mellifera* beeswax sample, Am1 collected from Daegu, South Korea; 1: Heptacosane (27.18%); 2: Nonacosane (18.35%); 3: Octadecane (15.92%); 4: 1-Docosene (16.11%); Am2 from Andong National University bee lab); 1: Heptacosane (15.41%); 2: Heneicosane (13.90%); 3: Octadecane (11.93%).

Table 2. Percentage composition of hydrocarbon fraction of beeswax samples of two species of honey bees

RT (min)	Compound name	Am1	Am2	Ac1	Ac21	Ac22	Ac23
12.671	Docosane		0.59				
13.535	Heptadecane	2.04	2.56	1.22	0.89	0.94	0.91
14.376	Tetracosane		2.41	1.27	0.88	1.04	0.62
15.172	Pentacosane	5.79	7.13	4.01	3.00	3.71	5.67
15.944	Eicosane	1.38	5.07	2.54	2.34	3.08	1.86
16.322	Pentadecane, 2,6,10,14-tetramethyl-	2.92		12.39			
16.494	Z-8-Pentadecen-1-ol acetate						0.84
16.551	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-						1.00
16.694	Heptacosane	27.18	15.41	15.68	14.87	15.18	23.07
17.409	6,6-Diethylhoctadecane	1.33		4.16	4.66	5.66	2.84
17.415	Octacosane		7.18				
17.941	Cyclopentane, 1,1′-[3-(2-cyclopentylethyl)-1,5-pentanediyl]bis-				1.11	1.04	1.92
17.993	11,14,17-Eicosatrienoic acid, methyl ester						1.63
18.102	Nonacosane	18.35		9.54	9.13	9.48	6.19
18.107	Heneicosane		13.90				
18.777	Hexadecane	0.95	7.06	5.69	6.05	7.28	4.20
19.275	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	1.92		0.47			
19.315	Cyclotrisiloxane, hexamethyl-	3.08					
19.418	2-methyloctacosane			9.00	8.04	8.77	5.37
19.423	Octadecane	15.92	11.93				
19.738	Benzo[h]quinoline, 2,4-dimethyl-					1.78	
19.818	1,1,1,3,5,5,5-Heptamethyltrisiloxane				1.00	1.22	
20.047	Heptadecane, 9-octyl-		6.58	6.45	8.33	7.98	5.16
20.39	3-Phenyl-2H-chromene						
20.453	1,4-Bis(trimethylsilyl)benzene					1.28	
20.562	1-Docosene	16.11	2.66	3.95	2.26	2.50	
20.573	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-						2.54
20.705	Nonadecane						5.64
20.711	Docosane, 11-butyl-	3.04	6.71	6.53	8.80	7.52	
21.443	6,6-Diethylhoctadecane			4.13	7.06	5.15	4.63
21.455	Octadecane, 3-ethyl-5-(2-ethylbutyl)-		3.74				
22.055	Benzo[h]quinoline, 2,4-dimethyl-						
22.124	17-Pentatriacontene			4.35	6.22	5.48	13.99
22.279	Tetratriacontane, 17-hexadecyl-			3.89			4.41
22.284	2-methyloctacosane				6.28	4.74	
22.296	Eicosane, 9-octyl-		3.08				
23.251	Octadecane, 3-ethyl-5-(2-ethylbutyl)-			2.63	5.16	3.47	
23.246	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-						2.95
23.263	Cyclotrisiloxane, hexamethyl-		2.06				
24.178	2-Ethylacridine						1.97
24.379	6,6-Diethylhoctadecane			2.11	3.94	2.72	2.59
24.396	Anthracene, 9-ethyl-9,10-dihydro-9,10-dimethyl-		1.95				
	Total						

*Beeswax samples of *Apis mellifera*; Am1 from Daegu, Am2 from ANU bee lab, and *Apis cerana*; Ac1 from Cheonan, Ac21−23 from yean, Andong, Korea in 2021

pound (8.46−28.66%). The percentage of this compound was higher for *Apis mellifera* (Am1). Heptacosane was also common and among major compounds in all beeswax analyzed.

The *n*-hexane fraction of sample of beeswax obtained from a local market in Sudan was reported to have heptacosane (37%) (Ebrahim, 2015) as a major compound along with other constituents even though its honey bee

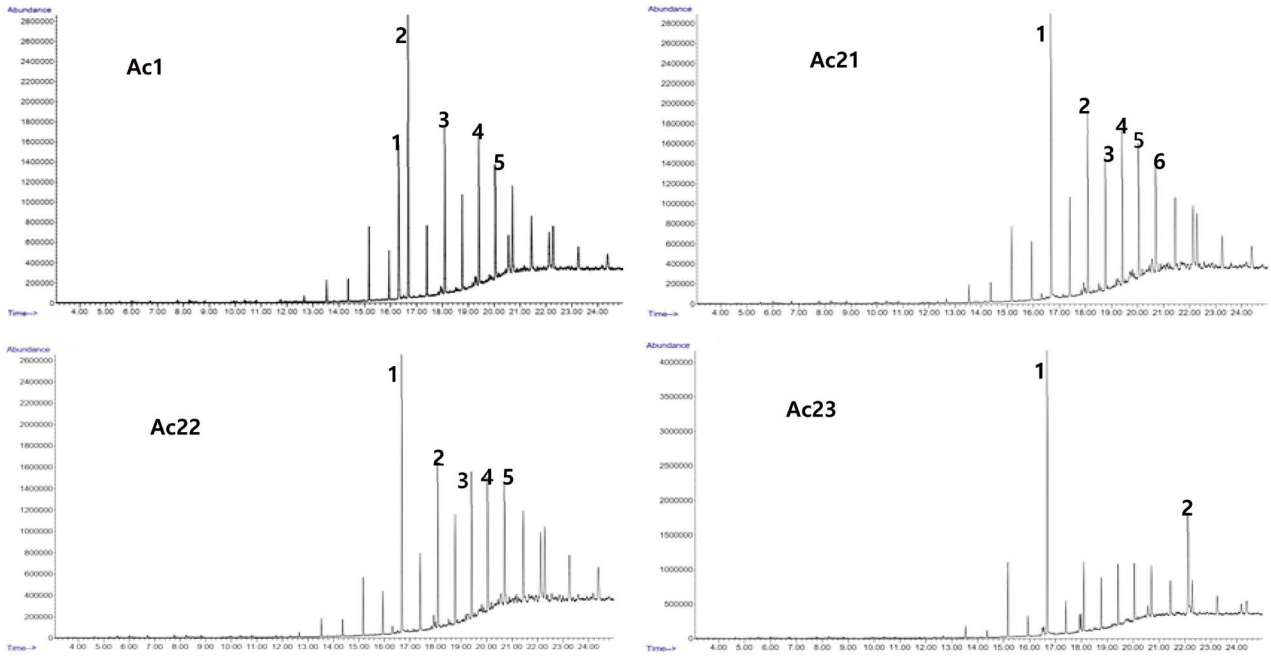

Fig. 4. GC of the hydrocarbon fraction of *Apis cerana* beeswax samples Ac1 from Cheonan, Korea; 1: Pentadecane, 2,6,10,14-tetramethyl- (12.39%); 2: Heptacosane (15.68%); 3: Nonacosane (9.54%); 4: Octadecane (9.00%) 5: Heptadecane, 9-octyl (6.45%). Ac21 collected from hive 1, Yean, Andong, South Korea; 1: Heptacosane (14.87%); 2: Nonacosane (9.13%); 3: Octadecane (8.04%); 4: Heptadecane, 9-octyl (8.33%); 5: Docosane, 11-butyl- (8.80%); 6: Docosane (6.4%). Ac22 collected from hive 2, Yean, Andong, South Korea; 1: Heptacosane (15.18%); 2: Nonacosane (9.48%); 3: Hexadecane (7.28%); 4: 2-methyloctacosane (8.77%); 5: Heptadecane, 9-octyl (7.98%). Ac23 collected from hive 3, Yean, Andong, South Korea; 1: Heptacosane (23. 07%); 2: Nonacosane (6.19%).

source was unknown. Aichholz and Lorbeer (1999) investigated the chemical composition of beeswax honeybee species *Apis mellifera*, *Apis cerana*, *Apis dorsata*, *Apis laboriosa*, *Apis florea* and *Apis andreniformis* using high-temperature GC/MS and reported the alkanes C_{27} greater than 5% in beeswaxes of *A. mellifera*, *A. cerana* and *A. florea* (Aichholz and Lorbeer, 1999). Heptatriacontene (C_{37}) (5.4%) was reported as a major compound in *A. cerana*. However, this compound was absent in the beeswax sample we investigated. Previous reports showed that variation in chemical composition of beeswax depends on type the genetics of bees, the age of the wax and the climatic circumstances of its product (Negri *et al*., 2000; Maia and Nunes, 2013) which might be the reason for the absence of heptatriacontane in the *A. cerana* beeswax we analyzed.

Zhang *et al*. (2019) reported the number of hydrocarbons extracted from larvae and larval food at different larval instars of worker larvae of *Apis mellifera*. Pentadecane and heptadecane were major hydrocarbons which accounts (69.7–88.1%) in *A. mellifera* larvae and larval food. The source of pentacosane which was found in all bees waxes we analyzed in this study might be their larvae and larval food.

The gas chromatogram data of the mandibular gland pheromone of *A. florea* foragers revealed that the principal component of the gland pheromones of *A. florea* foragers was 1-eicosanol at (29.98 μg/bee) (Zhang *et al*., 2019). The other main components of its mandibular gland pheromones were nonadecane (5.775 μg/bee), 2-hexyl-1-decanol (4.181 μg/bee), 2-butyl-1-octanol (3.771), eicosane (1.978 μg/bee), heneicosane (1.804 μg/bee), limonene (1.663 μg/bee), 1-butyl-3-methyl acetate (1.646 μg/bee) and 2-heptanol (0.241 μg/bee). *A. andreniformis* foragers use 0.1% (w/v) 1-eicosanol, 10.0% (v/v) eicosane, 1.0%, 5.0% and 10.0% (v/v) heneicosane and 0.1% (v/v) 2-heptanol as attractant substances to mark rich food sources (Zhang *et al*., 2019).

Aichholz and Lorbeer (1999) reported percentage composition and class of compounds identified in beeswax of *A. mellifera* and *A. cerana*. The percentage composition of hydrocarbons in the derivatized solution of our beeswax (Table 3) sample of *A. cerana* is higher compared with those reported for *A. cerana* beeswax

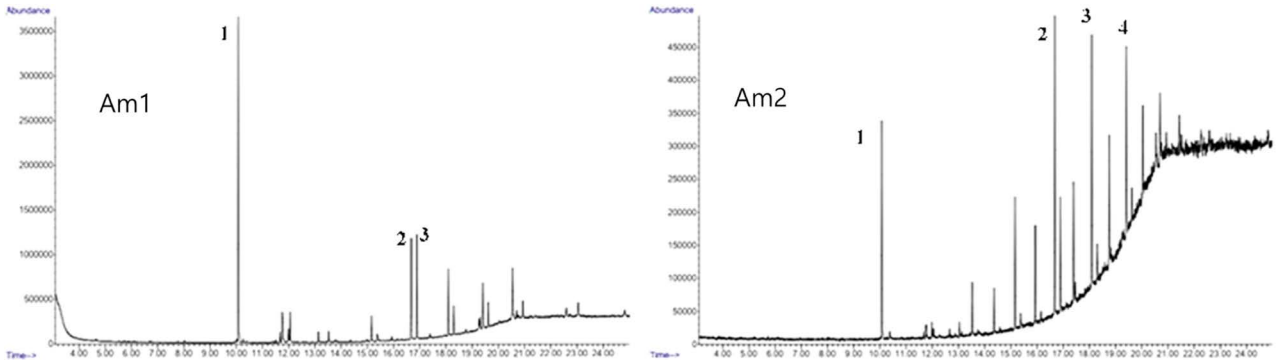

Fig. 5. GC of the derivatized fraction of *Apis mellifera* beeswax sample, Am1 collected from Daegu, South Korea; 1: Methyl palmitate (28.66%); 2: Heptacosane (10.62%); 3: Tetracosanoic acid, methyl ester (9.76%); Am2 from Andong National University bee lab); 1: Methyl palmitate (9.96%); 2: Heptacosane (15.08%); 3: Heptadecane (12.53%); 4: Eicosane, 9-octyl- (10.04%).

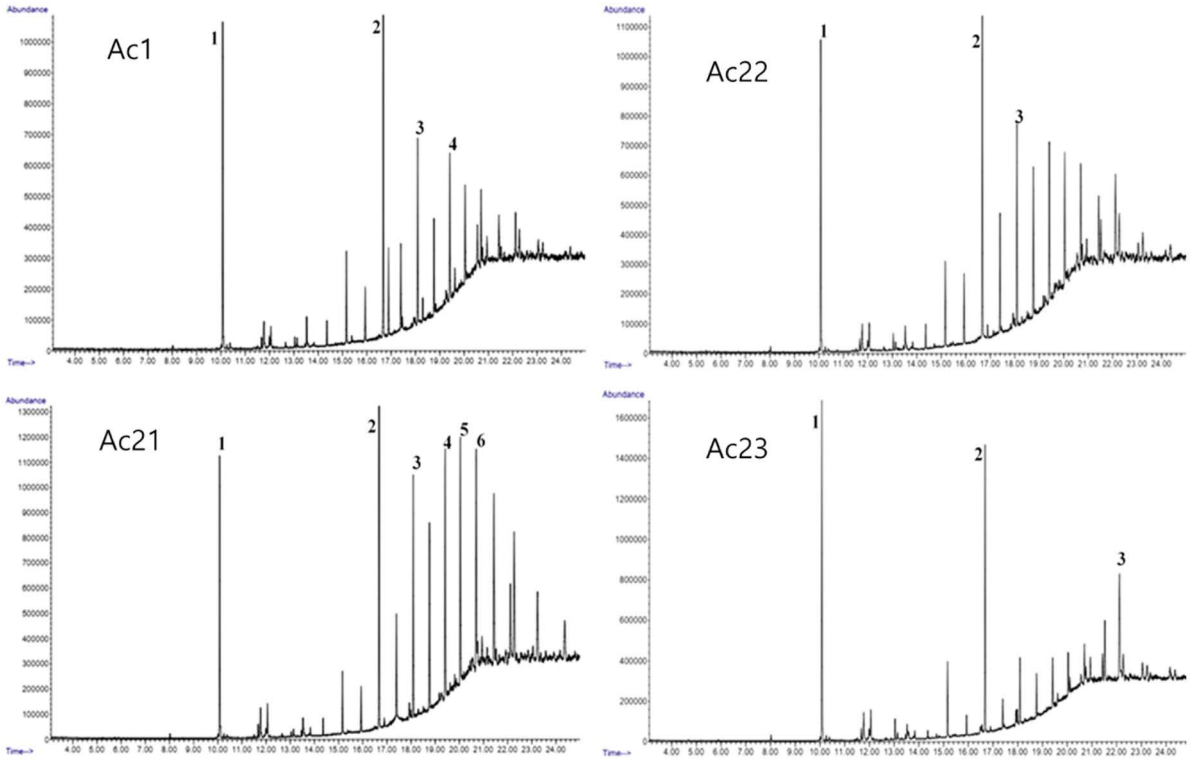

Fig. 6. GC of the derivatized fraction of *Apis cerana* beeswax samples Ac1 from Cheonan, Korea; 1: Methyl palmitate (8.46%); 2: Heptacosane (15.16%); 3: Heptadecane (8.44%); 4: Eicosane, 9-octyl- (7.34%). Ac21 collected from hive 1, Yean, Andong, South Korea; 1: Methyl palmitate (14.87%); 2: Heptacosane (10.21%); 3: Heptadecane (7.72%); 4: Eicosane, 9-octyl- (9.00%); 5: Tetratriacontane, 17-hexadecyl- (8.55%); 6: 2-methylhexacosane (7.22%). Ac22 collected from hive 2, Yean, Andong, South Korea; 1: Methyl palmitate (12.43%); 2: Heptacosane (14.60%); 3: Heptadecane (8.69%). Ac23 collected from hive 3, Yean, Andong, South Korea; 1: Methyl palmitate (18.60%); 2: Heptacosane (17.35%); 3: Cyclobarbital (11.79%).

by Aichholz and Lorbeer (1999) analyzed by high-temperature gas chromatography. We found the derivatized beeswax has monoesters (21.5%) among which hexadecanoic acid, methyl ester (methyl palmitate) (14.1%) was the most abundant monoester in the derivatized solution of beeswax (Table 3, Figs. 5 and 6). The percentage of monoesters identified in this study was relatively lower compared with those reported by Aichholz and Lorbeer (1999).

Jackson and Eller (2006) reported the methanolysis

reaction of beeswax from *A. mellifera* which was catalyzed by an immobilized lipase from *Candida antarctica* in supercritical carbon dioxide and the alcohols. Then the researchers isolated the fatty acid methyl esters by precipitation of the mixture from heptane (Jackson and Eller, 2006). Tulloch (1971) analyzed chemical composition of *A. mellifera* beeswax. The authors identified esters by thin layer chromatography (TLC), gas liquid chromatography (GC) and nuclear magnetic resonance spectroscopy (NMR) and by comparison with synthetic mono-, di- and triesters, hydroxy mono- and diesters and acid esters. Acids, hydroxy acids, alcohols and diols composition from each ester fraction were determined by gas liquid chromatography. Negri *et al.* (2000) alsp reported hydrocarbons and monoesters compounds constituents of beeswax samples. These workers hydrolyzed hydroxide esters fractions first by using alcoholic potassium and then derivatized prior to GC-MS analysis. The beeswax constitutes 15.1−23.5% aliphatic saturated and mono unsaturated hydrocarbons.

Maia and Nunes (2013) analyzed beeswax of *Apis mellifera* using high temperature GC-MS and chemometric. Major components of the beeswax were hydrocarbons, palmitate, oleate and hydroxypalmitate monoesters. Additionally, palmitate and oleate monoesters present in beeswax were obtained from 1-octadecanol and 1-eicosanol (Maia and Nunes, 2013). Blum *et al.* (1988) extracted volatiles oxygenated volatile organic

Table 3. Percentage composition of derivatized beeswax samples collected from two species of honey bees

RT (min)	Compound Name	Am1	Am2	Ac1	Ac21	Ac22	Ac23
10.079	Methyl palmitate	28.66	9.96	14.08	8.46	12.43	18.60
11.67	Methyl 14-methoxyhexadecanoate	1.03					
11.756	11-Octadecenoic acid, methyl ester	4.91					
11.773	Methyl 15-methoxyhexadecanoate			2.33	1.43	1.82	2.67
11.991	Methyl stearate	1.42					
12.059	Methyl tetradecanoate	2.96		1.17	1.30	1.47	1.58
13.038	Methyl 15-acetoxyhexadecanoate						1.17
13.141	3-Allyl-2-methyl-4(3H)-quinazolinethione	1.23					
13.461	8-Azabicyclo[4.3.0]non-3-ene-7,9-dione, 8-phenyl-3-(trimethylsilyl)-						
13.53	Hexadecane			1.32	0.95	1.12	0.91
13.53	Heneicosane	1.25					
13.833	6-Octadecenoic acid, methyl ester, (Z)-						
14.359	Tetracosane		2.12	1.15	0.52	1.03	
15.161	Eicosane	2.41	6.42	4.01	1.97	3.63	4.73
15.161	Pentacosane						
15.384	Docosanoic acid, methyl ester	0.99					
15.933	Octadecane		4.61				1.35
15.939	Hexacosane			2.48	1.52	2.95	
16.66	1-Tetracosanol						
16.677	Heptacosane	10.62	15.08	15.16	10.21	14.60	17.35
16.894	Tetracosanoic acid, methyl ester	9.76	6.21	3.85			1.73
17.398	Octacosane	0.53	6.46	3.46	4.27	5.11	1.51
17.461	Tetracosyl acetate						
17.935	5-Methyl-2-phenylindolizine				0.76		1.28
18.09	Heptadecane	6.25	12.53	8.44	7.72	8.69	4.40
18.828	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-						
18.302	Hexacosanoic acid, methyl ester	2.82	2.11	1.10			
18.76	Docosane		6.25				
18.765	Octadecane, 2-methyl-			4.65	6.10	6.36	2.65
19.263	Benzo[h]quinoline, 2,4-dimethyl-	1.27		0.46			
19.303	1H-Indole, 1-methyl-2-phenyl-	1.25					

Table 3. Continued

RT (min)	Compound Name	Am1	Am2	Ac1	Ac21	Ac22	Ac23
19.412	Eicosane, 9-octyl-	5.02	10.04	7.34	9.00	7.79	3.48
19.618	Octacosanoic acid, methyl ester	2.41	1.86	1.72			
20.041	Octadecane, 3-ethyl-5-(2-ethylbutyl)-		4.22	4.55		5.84	2.69
20.11	1,2-Benzisothiazol-3-amine tbdms						1.10
20.551	17-Pentatriacontene	6.15	3.68	3.44			
20.699	2-methylhexacosane	0.92	3.67	4.24	8.55	4.71	2.56
20.757	3-Phenyl-2H-chromene			1.48	1.21	1.27	1.57
20.94	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-	2.30				1.69	2.13
20.945	2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-	1.58	1.13	4.17			
21.151	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-	0.87			0.87		
21.432	2-methylhexacosane		2.27	2.48	7.22	2.91	2.19
21.529	1H-Indole, 1-methyl-2-phenyl-						5.10
22.118	Cyclobarbital			3.91	4.36	6.68	11.79
22.267	Octadecane, 3-ethyl-5-(2-ethylbutyl)-				6.69	3.27	3.01
22.599	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-	1.81					
23.051	Acetic acid, [4-(1,1-dimethylethyl)phenoxy]-, methyl ester	2.57		1.89			2.36
23.234	Methanol, [4-(1,1-dimethylethyl)phenoxy]-, acetate			1.46			
23.24	Octadecane, 9-ethyl-9-heptyl-				4.17	2.46	2.12
24.361	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene			2.85			
24.831	1,2-Benzisothiazol-3-amine	1.47					
	Total						

*Beeswax samples of *Apis mellifera*; Am1 from Daegu, Am2 from ANU bee lab, and *Apis cerana*; Ac1 from Cheonan, Ac21−23 from yean, Andong, Korea in 2021

compounds by steam distillation, and analyzed it by using GC-MS. The major oxygenated compounds identified were decanal (46%), 1-decanol (10%), nonanal (18%), octanal (6%), heterocyclic aldehyde furfural (10%) and benzaldehyde (10%). Additionally, hydrocarbons such as nonane and C_{15}-C_{27} alkanes were also reported from the steam distillate (Blum *et al*., 1988). The detailed compositional characteristics would be useful for future development into the new biomaterial for functional pharmacological and cosmetic uses.

CONCLUSION

As the beeswax is the honeybee product from wax gland of worker bees, beeswax profiles were more different between species than environmental conditions or surrounding vegetation structures of the honeybee colonies which are their foraging resources. However, there are substantial variation of chemical profiles within the species among the region of the bee hive location or even the bee colonies. Beeswaxes of *A. mellifera* and *A. cerana* are composed of mainly hydrocarbons. The hydrocarbon fraction obtained by extraction using SPE method has higher percentage of hydrocarbon compounds compared with those derivatized beeswax solution.

High percentage of the beeswax of the world market comes from the developing countries which serves as source of income and foreign exchange earnings for those countries. Beeswax is a secondary item obtained from beekeeping next to honey but valuable by-product. Beeswax is used to prepare candles, wrap, polish other products, and also for cosmetics or medical uses. Additionally, beeswax can be used to prepare sheets of waxes which is primarily used to simulate metal or part thickness when making metal molds when a uniform part thickness is desired. The pharmaceutical industries use waxes in various ointments, for coating pills and suppositories and other products, experiencing much higher unit price than honey (Nicholas, 1986; Brown, 1988). In ordinary beekeeping apiaries, much of the beeswax that could be harvested by beekeepers is wasted. The bees-

wax is left or thrown away because beekeepers are not interested to collect them. As a result, only one-third of the world's production of beeswax comes on to the market (Bradbear, 2009). Under local condition the main reasons for the wastage of beeswax would be mainly due to lack of full circular economy for the wax and information on the way of rendering, absence of efficient crude beeswax extraction method, lack of information on their chemical composition for possible application of the material and technologies (Gemeda and Kebebe, 2019). We therefore recommend analysis of chemical composition and physico-chemical properties of honey bee species beeswax originating from different honey bee species and localities to encourage the efforts of biological functionalities and industrialization.

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