

GC-FID analyzed fatty acid composition of honey obtain from stingless bee, *Tetragonula iridipennis* from Nagaland, Northeast India: seasonal variations and human health implications

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Abstract

Background

North-eastern states of India are the hub of varieties of stingless bee species. Honey produced by these have great medicinal and nutritive values. Local people utilized it as wound healer and immunity enhancer by their traditional knowledge. But due to lack of proper scientific documentations, it cannot be picked up for commercialization inspite of huge potentialities. Studies on fatty acid composition of honey that attributed to its medicinal and nutritional values were scares. Present investigations were performed with stingless bee honey produced by *Tetragonula iridipennis* and analysed its fatty acid composition, seasonal variations and human health implications.

Results

Fatty acid compositions of winter (SBHw) and summer (SBHs) samples of honey obtained from stingless bee, *T. iridipennis* in terms of derivatives, Fatty Acid Methyl Esters (FAMES) were analyzed using Gas Chromatography - Flame Ionization Detection (CG-FID) method. Total 23 fatty acids from SBHw and 22 from SBHs including Mono Unsaturated (MUFA), Poly Unsaturated (PUFA) and Saturated Fatty Acids (SFA) were detected. Distinct differentiation in respect of identification and quantifications of fatty acids in winter and summer samples indicated the effect of seasonal variations. Of total nine varieties of MUFA detected, percentage composition of Cis-10-heptadecenoic acid and Palmitoleic acid (Omega-7) found to be higher, indicating antioxidant and anti-inflammatory properties of honey. Out of seven varieties of PUFA, two essential fatty acids, Linoleic acid (Omega-6) and Alpha- linolenic acid (Omega-3), that human body unable to synthesize were detected in both the samples studied. Detection of various forms of omega 3 fatty acid in both the samples were the indicative of brain and cardiovascular supportive functions of honey. Both long, medium and short chain SFA were identified, of these, Stearic acid, Palmatic acid compositions were found to be higher to support anti-inflammatory and neuroprotective actions of honey. Study provides promising insights to the assessment of therapeutic and nutritive values of honey.

Conclusions

Fatty acid compositions of honey harvested in winter and summer seasons from stingless bee, *T. iridipennis* were analyzed. Three major types of fatty acids, MUFA, PUFA and SFA were detected in both the samples tested. Impact of evaluated fatty acids on human health were illustrated. Seasonal variations brought significant alternations on fatty acid compositions of honey were detected.

Introduction

Stingless bees are small eusocial Hymenopteran insect belongs to the family Apidae, subfamily Apinae and tribe Meliponini. Since time immemorial, honey obtain from stingless bees were widely utilized as traditional medicine in tropical and subtropical regions including India. Available literatures suggested that honey from stingless bees though less in quantities (600gm/year) were found to be rich in various nutritive components. But, due to shortage of knowledge on these aspects, this valuable product of stingless bees was yet to be systematically evaluated. Literatures related to fatty acid contents of stingless bee honey were scares till date, although they have immense health benefits to human. Fatty acids present in the honey not only affect on growth and learning processes of the bee colony (Arien et al., 2015), it has great nutritional and therapeutic value to the human health (Jarukas et al., 2021; Valverde et al., 2022; Mititelu et al., 2024). As a structural component of lipid, it is crucial for cell regulation, hormone production, transportation and absorption of fat-soluble antioxidants and vitamins (Meijaard et al., 2022). Fatty acids present in stingless bee honey has important role on those biochemical processes that are related to antimicrobial and antitumor activities of living body (Zhu & Feng, 2020). Bees collect these bioactive compounds from pollen, nectars, and plant exudates, converted to honey by their enzymatic actions. Santos et al., 2021; Ngaini et al., 2021; Mduda et al., 2023 claimed that botanical discriminations related to geographical

locations and environmental conditions have great impact on honey composition. This indicates that fatty acid compositions of honey are also directly influenced by seasonal variations.

Northeastern part of India is known for its unique climatic conditions and rich floral diversity to support wide range of stingless bee species. Out of 25 stingless bee species documented so far in India (Meena et al., 2025), majority were recorded in Northeastern states. Of these, Nagaland underscores maximum of 8 stingless bee species, where *Tetragonula iridipennis* is most prevalent. Since remote past, local tribes of Nagaland do practice Meliponiculture by their traditional knowledge.

In the present study, fatty acids present in the honey of stingless bee, *T. iridipennis* were analyzed and their human health benefits were explored. To the best of our knowledge, this will be the first report of this kind to analyze fatty acids of honey from *T. iridipennis* in terms of its volatile derivatives, Fatty Acid Methyl Esters (FAMES) using Gas Chromatography – Flame Ionization Detection (CG-FID) method. Our attention was also imposed on seasonal variations of fatty acid compositions of honey. For fulfillment of these criteria, honey harvested at winter and summer seasons were collected, and quantified separately after derivatizations to obtain FAMES. Certainly, this identification and quantification of individual fatty acids of stingless bee honey and evaluation of their seasonal variations immensely help for understanding of honey's authenticity apart from nutritional status related to human health.

Materials and Methods

Honey samples were collected from three different locations of study area (latitude of 25.40°N 94.07°E and longitude of 25.67°N 94.12°E) twice in a year during extreme of winter (Temp. $14 \pm 2^\circ\text{C}$) and summer ($26 \pm 2^\circ\text{C}$). They were labeled as SBHw for winter honey, SBHs for summer honey and processed separately to assess seasonal variations of fatty acid composition. Quantified fatty acids were taken for exploration of human health benefits.

1. Determination of FAMES by GC-FID: Analysis of FAMES from samples was performed by the GC 2400 System equipped with an integrated liquid autosampler, capillary injector and Flame Ionization Detector (FID). Analyzed samples were chromatographed on a PerkinElmer Clarus 690 GC (100m by 0.25 mm ID by 0.20 μm ; N9311570), meant for high performance, high-throughput laboratory analysis.

1:1 Analytical conditions: Temperature for autosampler: 250–300°C, while that of oven with modified Ramp: 220–240°C with total run time 47.6 min. Injection volume was 1 μL and split ratio 100:1.

1:2 Extractions of FAMES

Sample extract (10 mg) was dissolved in hexane and methylated with 14% methanolic boron trifluoride at 95°C for 1 hour. The reaction was quenched with the addition of double distilled water. Fatty acid methyl esters were extracted into hexane, evaporated to dryness under a gentle nitrogen stream, then resuspended in hexane prior to transfer to a GC insert and vial for analysis.

1:3 Quantification of FAMES by GC-FID:

Individual FAMES were identified by comparing retention time to FAME reference standard (GLC 37, Nu-Check Prep, Inc., Elysian, MN), consisting of 37 FAMES. The reference standard was prepared in hexane at 20 mg of total FAME per mL of solvent. All FAME samples were injected at approximately 10–15 mg of total FAME per mL of solvent.

2 Calculations

Percentage composition of each fatty acid was calculated from peak area of the total area obtained from chromatograph.

Target compound= Peak area / Total area X100

Results and Discussion

Analytical workflows of Fatty Acid Methyl Esters (FAMES) using GC-FID method were performed and results expressed in the Chromatogram 1: A and B for winter honey (SBHw) and summer honey (SBHs) respectively. Individual FAME was depicted by their existing numbers of carbon atom against time and spike height. On the basis of saturation, Fatty acids were categorised as Mono Unsaturated Fatty Acid (MUFA), Poly Unsaturated Fatty Acid (PUFA), Saturated Fatty Acid (SFA) and Trans fat. They were categorically organized in the Table 1 and Fig. 1: A,B,C respectively.

Table 1
Percentage Fatty acid composition of winter (SBHw) and summer (SBHs) honey identified as FAMES (Fatty Acid Methyl Esters)

Monounsaturated Fatty Acids (MUFA)							
Sl. No.	Time (Min)	Abbreviation	Compound name	Common name	Sample name	Composition (%)	
1	39.93	C16:1	Methyl palmitoleate	Palmitoleic acid- Omega 7 fatty acid	SBHw, SBHs	1.774, 4.415	
2	42.47	C17:1	Methyl cis-10-heptadecenoate	Cis-10-heptadecenoic acid	SBHw, SBHs	13.107 , 1.077	
3	44.22	C18:1	Methyl cis-9 oleate	Oleic acid- omega 9 fatty acid	SBHw, SBHs	1.196, 2.203	
4	55.63	C24:1	Methyl nervonate	Nervonic acid	SBHw, SBHs	1.559, 1.984	
5	47.98	C20:1	Methyl cis-11-eicosenoate	Cis-11-eicosenoic acid- omega 9 fatty acid	SBHs	0.427	
6	43.72	C18:1	Methyl trans-9 eladiate	Elaidic acid-trans isomer of Oleic acid	SBHw	1.144	
7	51.64	C22:1	Methyl erucate	Erucic acid- omega 9 fatty acid	SBHw	2.233	
8	35.48	C14:1	Methyl myristoleate	Myristoleic acid- omega 5 fatty acid	SBHw	0.530	
9	48.63	C21:0	Methyl Heneicosanoate	Heneicosanoic acid	SBHw	0.978	
Polyunsaturated Fatty Acids (PUFA)							
1.	46.04	C18:2	Methyl linoleate	Linoleic acid - omega-6 fatty acid , EFA	SBHw, SBHs	2.614, 3.035	
2.	48.31	C18:3	Methyl linolenate	α -linolenic acid (ALA) - omega-3 fatty acid , EFA	SBHw, SBHs	11.557 , 2.692	
Sl. No.	Time (Min)	Abbreviation	Compound name	Common name	Sample name	Composition (%)	Composition (%)
3.	49.84	C20:2	Methyl cis-11,14-eicosadienoate	Cis-11,14-eicosadienoic acid	SBHw, SBHs	4.100, 1.458	
4	54.74	C20:5	Methyl cis-5,8,11,14,17-eicosapentaenoate	Methyl eicosapentaenoate (EPA)- omega- 3 fatty acid	SBHw, SBHs	5.713, 9.746	
5	60.71	C22:6	Methyl cis-4,7,10,13,16,19-docosahexaenoate	Docosahexaenoic acid (DHA)- omega-3 fatty acid	SBHw, SBHs	3.479, 9.005	
Each value of the table represents the mean of three replicates. EFA-Essential Fatty Acid, LCFA-Long Chain Fatty Acid, MCFA- Medium Chain Fatty Acid, SCFA- Short Chain Fatty Acid. For the discussion in the text, maximum amount of percentage composition were taken into considerations. Highest quantities of MUFA, PUFA and SFA were highlighted. No Trans fat were detected in the honey samples, except Elaidic acid (trans isomer of Oleic acid).							

Monounsaturated Fatty Acids (MUFA)						
6	51.24	C20:3	Methyl cis-8,11,14-eicosatrienoate	Dihomo-gamma-linolenic acid – omega 6 fatty acid	SBHs	0.393
7	53.7	C22:2	Methyl cis-13,16-docosadienoate	cis-13,16-docosadienoic acid- omega 6 fatty acid	SBHs	1.931
Saturated Fatty Acids (SFA)						
1	42.7	C18:0	Methyl stearate	Stearic acid (LCFA)	SBHw, SBHs	15.104, 24.119
2	50.44	C22:0	Methyl behenate	Behenic acid (LCFA)	SBHw, SBHs	1.960, 1.164
3	52.33	C23:0	Methyl tricosanoate	Tricosanoic acid (LCFA)	SBHw, SBHs	4.114, 9.218
4	46.72	C20:0	Methyl arachidate	Arachidic acid (LCFA)	SBHs	1.570
5	48.63	C21:0	Methyl heneicosanoate	Heneicosanoic acid (LCFA)	SBHw	0.978
6	25.84	C11:0	Methyl undecanoate	Undecanoic acid (MCFA)	SBHw, SBHs	0.064, 0.070
7	33.52	C14:0	Methyl myristate	Myristic acid (MCFA)	SBHw, SBHs	0.947, 0.793
8	35.96	C15:0	Methyl pentadecanoate	Pentadecanoic acid (MCFA)	SBHw, SBHs	0.522, 1.774
9	38.33	C16:0	Methyl palmitate	Palmitic acid (MCFA)	SBHw, SBHs	21.010, 20.411
10	40.54	C17:0	Methyl heptadecanoate	Heptadecanoic acid (MCFA)	SBHw, SBHs	1.900, 3.170
11	12.65	C6:0	Methyl hexanoate	Hexanoic acid (SCFA)	SBHs	0.085
Each value of the table represents the mean of three replicates. EFA-Essential Fatty Acid, LCFA-Long Chain Fatty Acid, MCFA- Medium Chain Fatty Acid, SCFA- Short Chain Fatty Acid. For the discussion in the text, maximum amount of percentage composition were taken into considerations. Highest quantities of MUFA, PUFA and SFA were highlighted. No Trans fat were detected in the honey samples, except Elaidic acid (trans isomer of Oleic acid).						

Effect of seasonal variations on fatty acids composition

Identification and quantification of fatty acid composition of stingless bee honey were done separately for winter and summer samples to observe the influence of seasonality. Total 23 compounds were detected in SBHw, while that of 22 for SBHs. Of these, Myristoleic acid, Elaidic acid, Heneicosanoic acid, Erucic acid, 11-14-17-eicosatrienoic acid and Arachidonic acid were detected only in SBHw, while Hexanoic acid, Arachidic acid, Cis-11 eicosanoic acid, Dihomo-gamma-linolenic acid, Cis-13, 16-docosadienoic acid were shown as unique components of SBHs, indicating a clear differentiation of fatty acid composition of honey harvested in winter and summer seasons. Quantitative variations were also prominent in two sets of honey samples focused on the Table 1. And Fig. 1: A,B,C. Santos et al., (2021) and Lavinas et al., (2025) opined that climatic factors have great effects on the chemical composition of honey, making it a unique product. Working on honey composition of *A. mellifera*, Oyedokun et al. (2025) and Kenneth et al., (2026) reported that volatile organic compounds of honey acted as

an indicator of surrounding ecosystem structure and vegetation. In this context, derivatized forms (FAMES) of fatty acid content has claimed its position as a bioindicator for assessment of honey.

Composition of Mono Unsaturated Fatty Acids (MUFA) in honey samples

MUFAs are considered as healthy fat that reduce the risk of cardiovascular disease (Wang & Guo 2025), regulate cholesterol (Verruck et al., 2019), having anti-inflammatory activities. MUFAs found to be common in both SBHw and SBHs were Palmitoleic acid (Omega-7, 4.415%), Cis-10-heptadecenoic acid (13.107%), Oleic acid (Omega-9, 2.203%) and Nervonic acid (1.984%). Presence of Palmitoleic, Cis-10-heptadecenoic and Oleic acids was the indicative of anti-oxidant, anti-inflammatory and anti-tumor effects of honey (Hu et al., 2019; Atar et al., 2021; Wang & Guo 2025). Nervonic acid found to be crucial for brain development, forming myelin sheath in nerve fibers (Phung et al., 2024). Furthermore, Elaidic acid (1.144%), Erucic acid (Omega-9, 2.233%) and Myristoleic acid (Omega-5, 0.530%) were detected in SBHw. Elaidic acid, the trans isomer of Oleic acid, was the only trans fat detected in stingless bee honey implicated in heart disease (Tardy et al., 2011). Erucic acid and Myristoleic acid were cardio protective, able to rise good cholesterol (HDL) level (Matsumoto et al., 2013; Verruck et al., 2019). SBHs alone contained Cis-11-eicosenoic acid (Omega-9, 0.427%) having antioxidant and anti-inflammatory properties. However, all the Omega-7 and Omega-9 fatty acids present in honey claimed to be responsible for anti-inflammatory and antioxidant activities of honey (Hu et al., 2019; Fun et al., 2022; Wang & Guo, 2025). Highest quantity of MUFA detected was Cis-10-heptadecenoic acid, shown in the Table 1 and Fig. 1A.

Composition of Poly Unsaturated Fatty Acids (PUFA) in honey samples

PUFAs were vital dietary elements that play a significant role in human nutrition (Mititelu et al., 2024). These were said to be 'healthy' fat, crucial for brain and cardiac functions, cell growth and lowering triglyceride level. Table 1 and Fig. 1:B highlighted the composition of PUFA in honey samples where, Alpha- linolenic acid found to be highest. Both the samples contain Linolenic acid (Omega-6, 3.035%) and Alpha - linolenic acid (Omega-3, 11.557% ALA) that are considered as essential fatty acids as human body has no any mechanism to produce these. Linoleic acid involved in maintaining cholesterol level and support immune system (Basak & Duttaroy 2020), while Alpha- linolenic acid effect on brain cells and heart health (Bertoni et al., 2023). Other PUFAs detected in both SBHw and SBHs were Cis-11,14-eicosadienoic acid (Omega-6, 4.100%), Methyl eicosapentaenoate (EPA, Omega-3, 9.746%), Docosahexaenoic acid (DHA, Omega-3, 9.005%). Swanson et al., (2012) considered ALA, EPA and DHA as the most important omega-3 fatty acids that can reduce inflammation. Both EPA as well as DHA reported to be cardio-vascular and brain health supportive, maintain cholesterol level (Nassar et al., 2023; Richard and Monk 2023). Unique component of SBHs were Dihomo-gamma-linolenic acid (Omega-6, 0.393%) also known for anti-inflammatory and cardiovascular supportive activity (Abu-Ghose et al., 2021), Cis-13, 16-docosadienoic acid (Omega-6, 1.931%), reported to be a metabolite in biological system (Tang et al., 2024).

Composition of Saturated Fatty Acids (SFA) in honey samples

Total 11 varieties of SFA having long chain (LCFA), Medium (MCFA) and short chain (SCFA) were detected in stingless bee honey (Table 1 and Fig. 1:C). Stearic acid (24.119%), Behenic acid (1.960%) and Tricosanoic acid (9.218%) were the LCFA detected in both SBHw and SBHs. Other LCFA, Heneicosanoic acid (0.978%) in SBHw and Arachidic acid (1.570%) in SBHs were detected. Satisfactory amount of Stearic acid and Tricosanoic acid along with Behenic acid in both types of honey might be responsible for honey's anti-inflammatory, neuroprotective and cardiovascular supportive activities (Chen et al., 2020; Liu et al., 2024; Yang et al., 2025). Working on several honey samples from Turkey, Karlidag et al., (2021) reported the presence of high concentration of Stearic acid in *Apis* honey also. Undecanoic acids (0.070%), Myristic acid (0.947%), Pentadecanoic acid (1.774%), Palmitic acid (21.010%), Heptadecanoic acid (3.170%) were MCFA found in both the honey samples tested. Presence of Pentadecanoic and Heptadecanoic acids were the indicative of honey's anti-inflammatory properties, lower the risk of cardiometabolic diseases linked with human longevity pathways (Venn-Watson et al., 2020;

Venn-Watson et al., 2023). Being a key structural component of cell membranes, Palmitic acid present in the honey may be crucial for maintaining membrane phospholipids balance and skin barrier integrity (Carta et al., 2017). Hexanoic acid, commonly known as flavouring agent, being the only SCFA detected in SBHs (0.085%) that support glucose metabolism (Ikeda et al., 2025). Present study on identification and quantification of total fatty acid composition and their seasonal variations definitely shedding light on nutritional and therapeutic importance of stingless bee honey.

N.B. For Discussion, highest percentage composition of fatty acids were mentioned in the text regardless of seasonality.

Conclusion

Percentage composition of total fatty acids of honey obtain from stingless bee, *T. iridipennis* were analyzed in terms of FAMES. Fatty acids belongs to MUFA, PUFA and SFA were separated out for winter and summer samples. Effect of seasonal variations on existence and composition of fatty acids were distinct. This findings expected to help in standardization of honey’s commercial characters. Impact of evaluated fatty acids on human health were assessed with existing literatures. The results reflected that the fatty acids detected in the stingless bee honey has key role on enhancement of antioxidant, anti-inflammatory, neuroprotective and cardio- protective properties of honey. Trans fat that is detrimental to human health were not detected in honey samples except less amount of Elaidic acid (trans isomer of Oleic acid) in winter sample.

Abbreviations

GC-FID Gas Chromatography - Flame Ionization Detection

FAMES Fatty Acid Methyl Esters

SBHW Stingless Bee Honey winter sample

SBHs Stingless Bee Honey summer sample

MUFA Mono Unsaturated Fatty Acid

PUFA Poly Unsaturated Fatty Acid

SFA Saturated Fatty Acid

LCFA Long Chain Fatty Acid

MCFA Medium Chain Fatty Acid

SCFA Short Chain Fatty Acid

EFA Essential Fatty Acid

Declarations

-Ethics approval and consent to participate

Not applicable

-Consent for publication

Not applicable

Availability of data and material

All datasets generated during the current study are available from the corresponding author on request

-Competing interests

Authors declare no competing interest

-Funding

Not applicable

-Author's contributions

Kepevitso Pier¹ : Performed laboratory experiments, formal analyses and investigations

Rashmimala Kakoti^{2*} : Conceptualization, supervision and manuscript preparation

Both the authors approved the final manuscript.

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-Ethics approval and consent to participate

Not applicable

-Consent for publication

Not applicable

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Figures

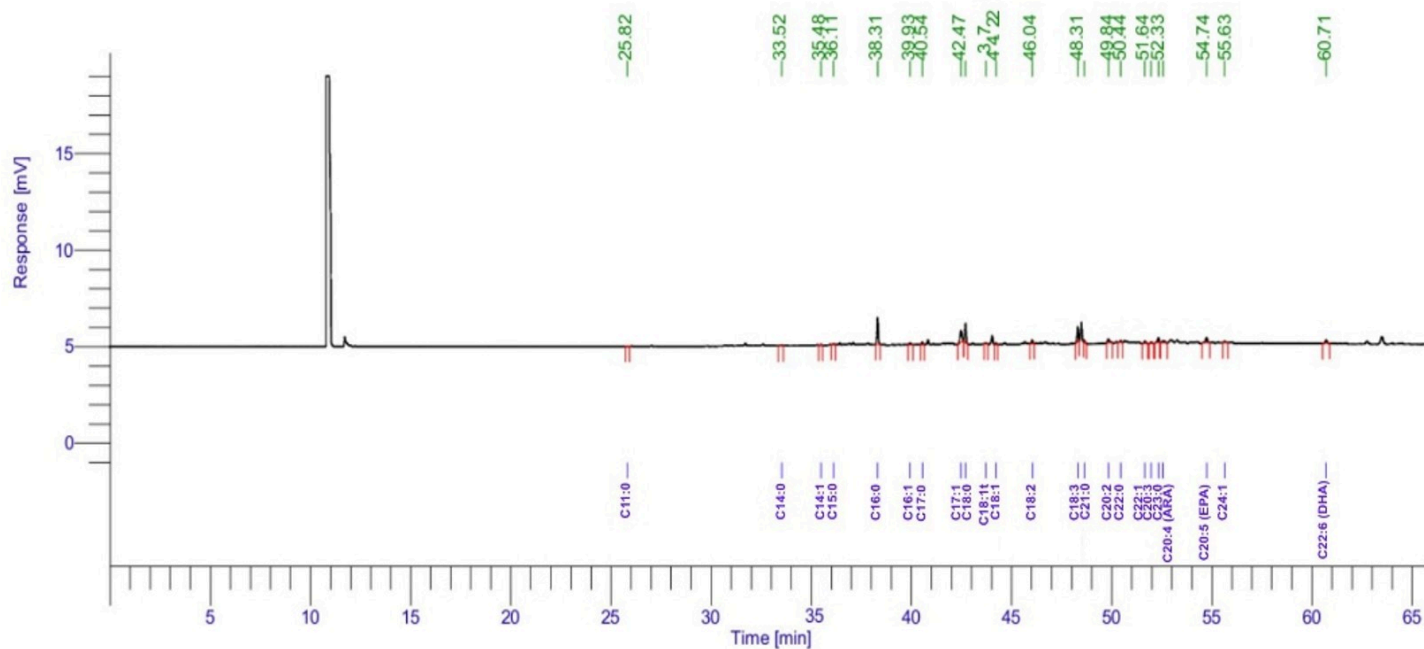


Figure 1

Chromatogram 1A: Showing Fatty Acid Methyl Esters (FAMES) present in winter honey (SBHw) samples of *T. iridipennis* (The obtained FAMES were demarcated by their existing number of carbon atoms)

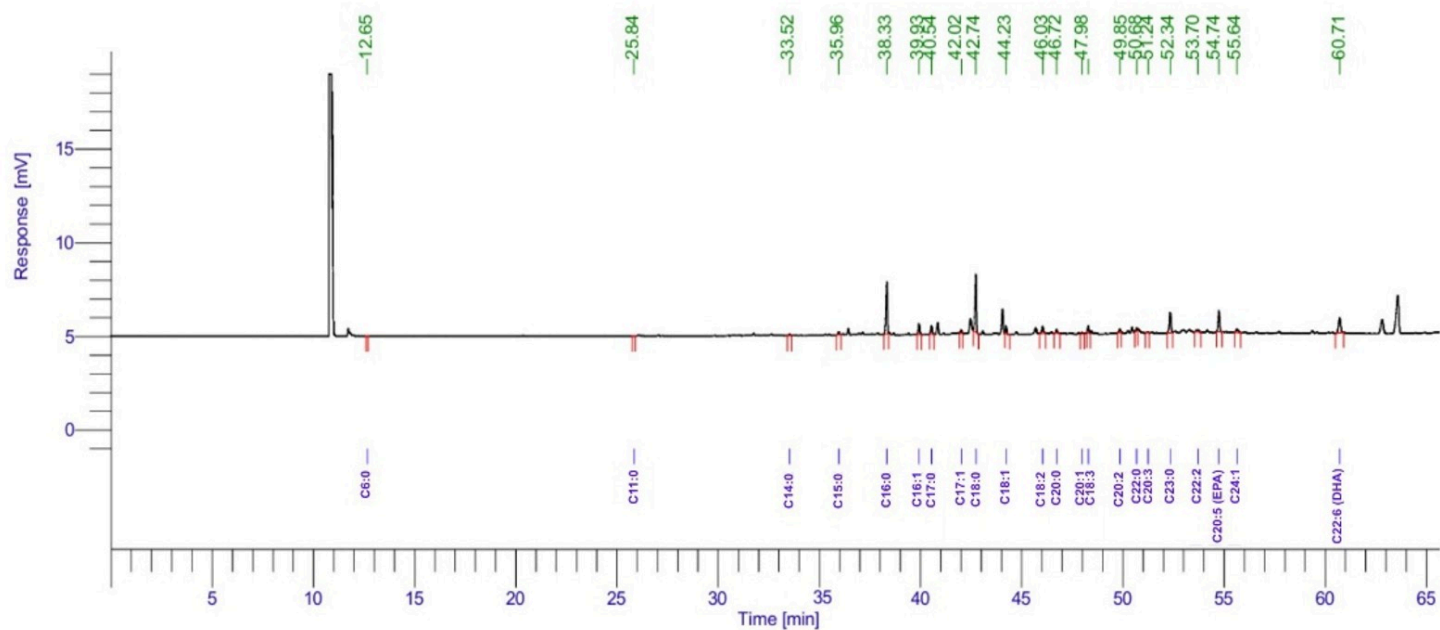


Figure 2

Chromatogram 1B: Showing Fatty Acid Methyl Esters (FAMES) present in summer honey (SBHs) samples of *T. iridipennis* (The obtained FAMES were demarcated by their existing number of carbon atoms)

MUFA

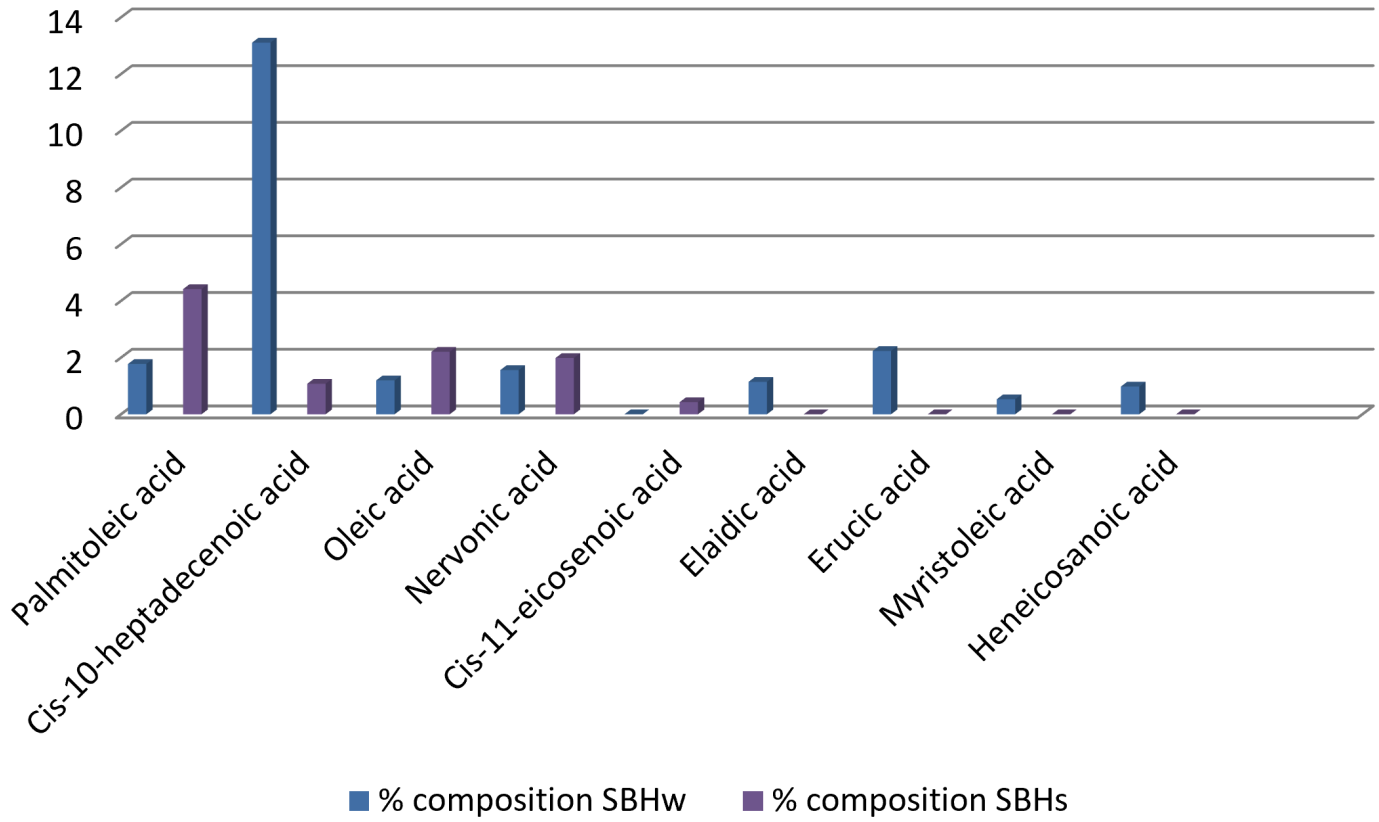


Figure 3

Fig1 A: Percentage composition of Mono- Unsaturated Fatty Acid (MUFA) in winter (SBHw) and summer honey (SBHs) samples of *T. iridipennis*

PUFA

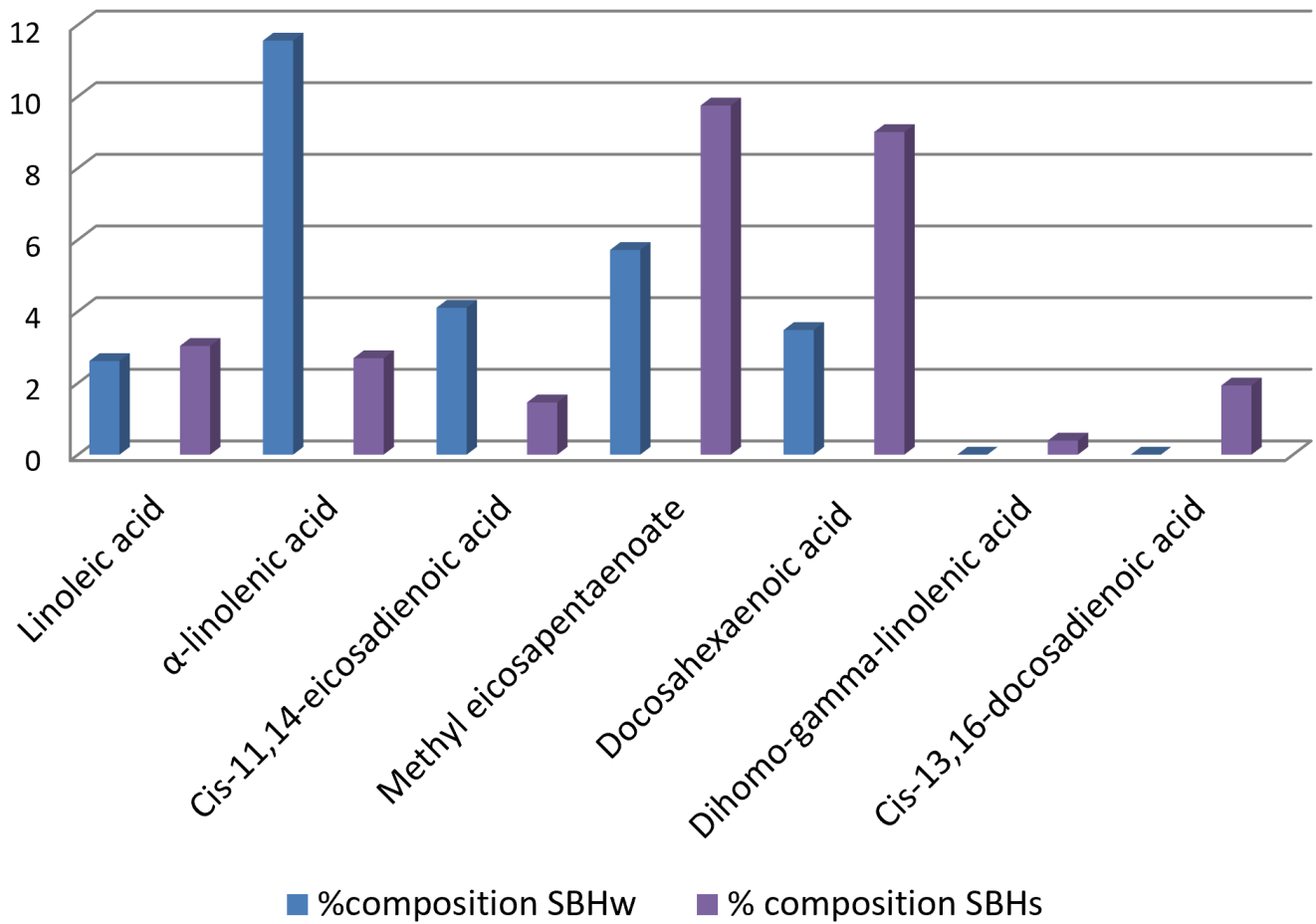


Figure 4

Fig1B: Percentage composition of Poly- Unsaturated Fatty Acid (PUFA) in winter (SBHw) and summer honey (SBHs) samples of *T. iridipennis*

SFA

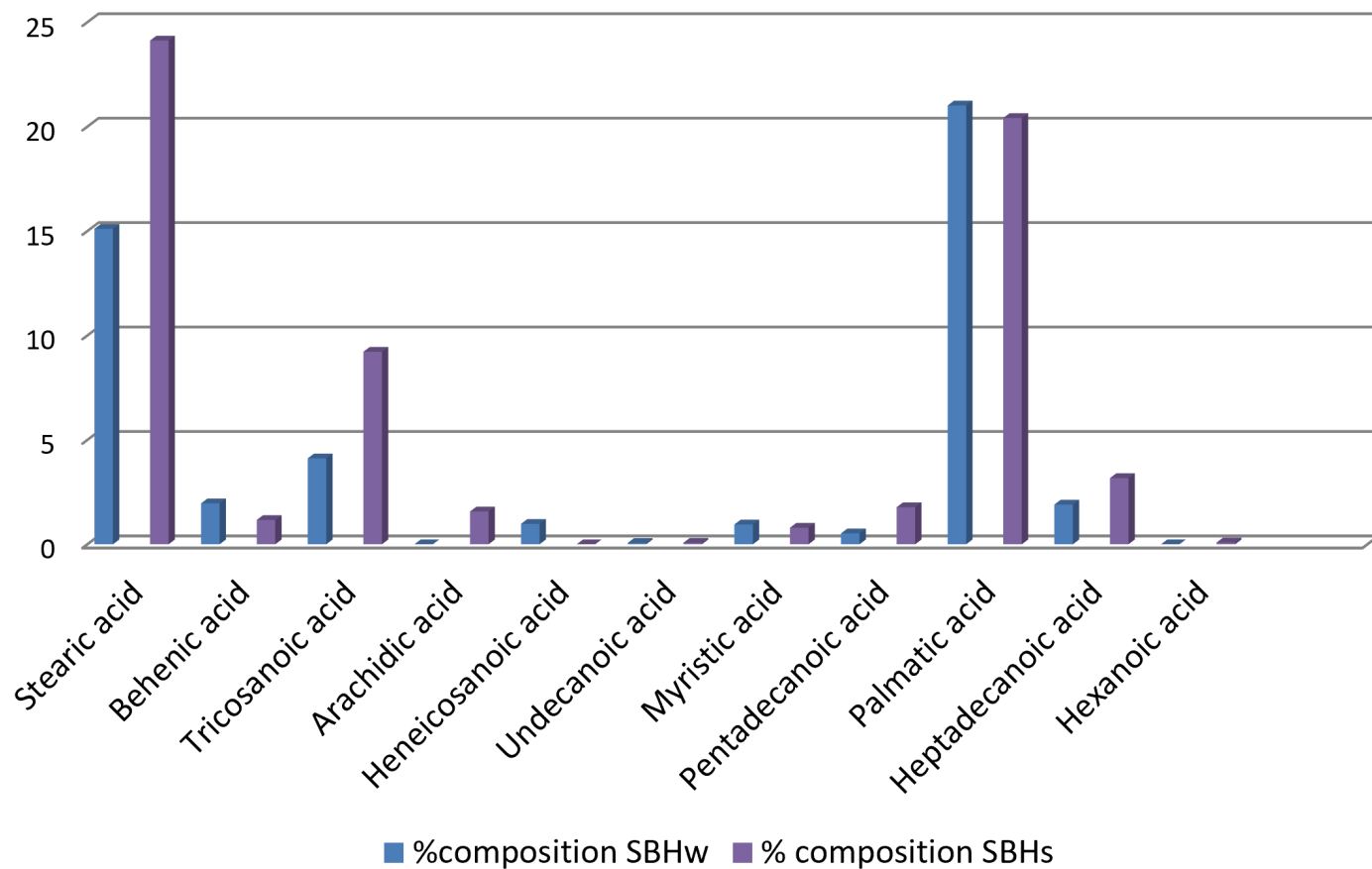


Figure 5

Fig1C: Percentage composition of Saturated Fatty Acid (SFA) in winter (SBHw) and summer honey (SBHs) samples of *T. iridipennis*