Chapter 8
Effect of Temperature on Antibacterial Activity and Fatty Acid Methyl Esters of Carica Papaya Seed Extract

Muhamad Shirwan Abdullah Sani, Jamilah Bakar, Russly Abdul Rahman, and Faridah Abas

Abstract This chapter addresses the antibacterial activity of *Carica papaya* seeds due to their abundance in bioactive compounds and these seeds contain high levels of fatty acid methyl esters (FAMEs). However, no report is available to indicate (1) which FAMEs are potent against pathogens and (2) the effect of temperature on the distribution of FAMEs. Therefore, this study aims to evaluate the effect of temperature against the antibacterial activity of *Carica papaya* seed extract (CPSE) and its FAME profile via extraction of the seeds using methanol and the extract was subjected to test of antibacterial activity against *Salmonella enteritidis, Bacillus cereus, Vibrio vulnificus,* and *Proteus mirabilis.* FAME profiling was done using GC/MS incorporated with principal component analysis (PCA). The CPSE at 5.63 mg/mL was potent against these pathogens at < 40 °C. Although the CPSE was rich with FAMEs, the PCA result had identified individual FAMEs that inhibited the pathogen growth. Palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1n9c), and cis-vaccenic acid (C18:1n11c) had strongly inhibited *V. vulnificus* and *P. mirabilis* growths and moderately inhibit *S. enteritidis* growth. To avoid the formation of trans FAMEs, this study also suggested that the CPSE temperature should be held at < 150 °C.

Keywords *Carica papaya* seed · GC/MS · Antibacterial activity · Toxicity · Stability

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A. Amid (ed.), *Multifaceted Protocols in Biotechnology, Volume 2*,
https://doi.org/10.1007/978-3-030-75579-9_8

8.1 Introduction

Papaya or *Carica papaya* is a highly commercialized tropical fruit. Ripe *Carica papaya* is mainly eaten as a dessert and papain from the plant's latex is commercialized as a meat tenderizer and is also been used as an enzyme in several enzymatic extraction works (Vasu et al., 2012). Not much attention has been paid to the fruit as a potential source of phytochemicals other than the flesh of the fruit itself. Out of 28 million metric tons of produced papaya worldwide, 5 million metric tons of the seeds were discarded in 2017 (FAOSTAT, 2019). Although *Carica papaya* seeds are discarded from other parts of the world, they are popularly consumed in India, Central Asia, and the Middle Eastern countries. They have been used to marinate meat, substitute for black pepper, and are added to salad dressings due to their spicy flavor (Lim, 2012). Several reports on antibacterial (Sani, 2018), antifungal (Chávez-Quintal et al., 2011) and anticancer (Nakamura et al., 2007) activities of *Carica papaya* seeds obtained from several solvent extracts could be found. Moreover, Sani et al. (2017b) found that the crude *Carica papaya* seed extract (CPSE) was active against *S. enteritidis, V. vulnificus, P. mirabilis, and B. cereus*, but reported potent antibacterial compounds. Since the yield and consumption of *Carica papaya* seeds are very high, it is worth investigating their compositions and potent antibacterial components that potentially inhibit these pathogenic bacteria.

The composition of CPSE was dominated by fatty acid methyl esters (FAMEs) (Sani et al., 2017b). However, there was limited information on which FAMEs had rendered bacterial inhibition. Also, trans FAMEs are of high concern because they have been linked to nutritional health issues (Tao, 2007), especially C18:1n9t (Preedy et al., 2011) while subjecting heat treatment. Cis and trans FAMEs have been stated to be hardly separated due to the same molecular weight except in the use of HP-88 column and GC/MS detection (Albuquerque et al., 2011). Thus, this study was performed in order to (1) investigate the efficacy of antibacterial CPSE and its distribution of FAMEs as heat-affected and (2) identify potential FAMEs that render antibacterial activity in CPSE.

8.2 Methodology

8.2.1 Plant Material

Carica papaya cv. Sekaki fruits were bought from D'Lonek Sdn. Bhd. Organic Farm, Rembau, Negri Sembilan, Malaysia. *Carica papaya* plant, flower, and fruit from this farm were deposited to Herbarium of the Institute of Bioscience, Universiti Putra Malaysia, and a plant voucher, numbered as SK 2368/14, was issued. The seeds of *Carica papaya* were removed from the fruit and treated as described by Sani et al. (2017a). The seeds were thoroughly washed in distilled water, oven-dried at 40 °C for three days, kept in airtight amber bottles, and stored at −20 °C until further analysis.

8.2.2 Extraction of Phytochemicals

Methanol (MeOH) was the solvent used for extraction. Dried seeds were ground to fineness for 5 min in a 240 W electrical blender (Panasonic MX-337, Malaysia) before extraction. A solvent-to-solid ratio of 10:1 was used in this study. Briefly, 50 g of dried ground *Carica papaya* cv. Sekaki seeds were weighed in a conical flask, and 500 mL of MeOH was added. The extraction was carried out at room temperature (27 °C) for 8 h in a shaker (100 rpm) followed by filtration through Whatman No.1 filter paper (GE Healthcare, UK). The filtrate was transferred to pre-weighed flat bottom flasks and concentrated using a rotary vacuum evaporator (Eyela N-1001, Japan) at 40 °C. The concentrated CPSE was kept at 4 °C until further use (Sani et al., 2017a).

8.2.3 Effect of Temperature on the Extract

About 29.5 mg of nitrogen-blown extract was heated at 60, 80, 100, 150, and 200 °C for 15 min and mixed with 1% Tween 80 and TSB for a final volume of 5 mL. The final extract concentration used in this study was 5.63 mg/mL, which was the MIC value determined by Sani et al. (2017b). Heated-extract solutions were subjected to the test of percentage growth inhibitions of *S. enteritidis, V. vulnificus*, *P. mirabilis,* and *B. cereus.*

8.2.4 Percentage of Growth Inhibition

The growth of *Salmonella enteritidis* (ATCC 13076), *Vibrio vulnificus* (ATCC 27562), *Proteus mirabilis* (ATCC 12453), and *Bacillus cereus* (ATCC 10875) was monitored using 96 well-microplates. A volume of 10 μL of TSB containing 10^6 CFU/mL of the tested pathogen was mixed with 190 μL of the TSB solutions in 96 well-microplates and assessed in a microplate spectrophotometer. Positive controls containing the tested solution was inoculated with the respective pathogens. Negative controls contained a mixture of crude extract, Tween 80, and TSB. The 96 well-microplate was incubated at 37 °C for 24 h on a Heidolph Inkubator and Titrama × 1000 (Germany) at 210 rpm to prevent adherence and clumping, after which the optical density was measured at 600 nm in Tecan Infinite® 200 Microplate Reader (Switzerland) before (T_0) and after (T_{24}) incubation.

The percentage growth inhibition (Patton et al., 2006) for TSB solutions was determined using Eq. (8.1)

$$\text{Growth Inhibition (PI)}\% = (1 - (\text{OD test well}/\text{OD of positive control well})) \times 100 \quad (8.1)$$

8.2.5 Effect of Temperature on Fatty Acid Profile in Carica Papaya Seed Extract

The CPSE was heated at 40, 60, 80, 100, 150, and 200 °C for 15 min and subjected to pretreatment before GC/MS analysis.

8.2.6 Profiling of Fatty Acid Methyl Esters by GC/MS Analysis

8.2.6.1 Sample Preparation

An amount of 0.01 g/mL of the heated extract at 40, 60, 80, 100, 150, and 200°C for 15 min was re-dissolved in 0.6 mL of hexane and added with 0.4 mL of 1 M sodium methoxide and vortexed for 30 s. The top hexane layer (0.6 mL) was subjected to FAMEs quantification by gas chromatography-mass spectrometry (GC/MS) analysis.

8.2.6.2 Preparation of Calibration Curve

The linearity of the methods was evaluated using different concentrations of FAME standards, ranging from 0.0005 – 3 mg/mL and cis-vaccenic acid (0.0001 – 0.5 mg/mL). Linearity was assessed using the linear regression equation, where the correlation coefficient $r > 0.98$, indicated an acceptable identification (Fagundes & Caldas, 2012). The prepared standards were analyzed using GC/MS.

8.2.6.3 Quantification of Fatty Acids Methyl Esters

Characterization of FAMEs was performed using the Agilent-Technologies 7890A GC system equipped with the Agilent-Technologies 5975 mass selective detector (Agilent Technologies, USA). The compound separation was achieved using an HP-88 capillary column (100 m × 0.25 mm, film thickness 0.20 μm) with an oven temperature program at 150 °C for 5 min, heated to 240 °C at the rate of 4 °C/min and held for 15 min. Samples were injected in split mode with the injector temperature at 260 °C with helium as a carrier gas at a constant flow rate (1 mL/min). For MS detection, the electron ionization mode with ionization energy of 70 eV was used with a mass range of m/z 20–700 units. The MS transfer line and MS quadrupole temperature were set at 230 °C and 150 °C. The mass spectrometer was operated in both the scan and selected ion monitoring (SIM) modes for compound identification and quantification, respectively. In order to avoid the need to modify the retention times in the calibration tables due to column maintenance or column change, the

calibration of standards was performed in the retention-time-lock mode (Caven-Quantrill & Buglass, 2007) where palmitic acid (C16:0) was chosen as the locking standard due to its stability. Compounds were identified by their retention times and mass fragmentation patterns of standards using the National Institute of Standard (NIST) Mass Spectral 11 offline library.

8.2.7 Statistical Analysis

8.2.7.1 ANOVA

Data were expressed as mean ± standard deviations of triplicate R_f and LC_{50} and residual methanol. One way analysis of variance (ANOVA) with Tukey's test was conducted using XLSTAT-Pro (2014) statistical software (Addinsoft, Paris, France) to determine the significant difference between the means at 95% confidence level ($p < 0.05$) for bioautography, toxicity assay, and residual methanol.

8.2.7.2 Principle Component Analysis (PCA)

Principle component analysis (PCA) is the most commonly unsupervised pattern recognition technique used in the distribution of compounds in the food sample (Dorta et al., 2014). The PCA was employed to elucidate the data variance of inter-correlated variables and transform them into independent variables called a principle component (PC). PCA also excludes the less significant parameters.

In this study, PCA was applied to FAME's contribution to the PI of tested pathogens as affected by temperature. From the calculation of the eigenvalue, a new set of groups called PCs was generated for each eigenvalue > 1. The PC was influenced by factor loading > 0.75, 0.74 - 0.50, and 0.49 – 0.30, which were considered as strong, moderate, and weak (Retnam et al., 2013). The profile of factor loadings and specific indicative FAMEs were used to deduce the FAMEs contribution on PI of tested pathogens as affected by temperature. PCA was conducted using XLSTAT-Pro (2014) statistical software (Addinsoft, Paris, France).

8.3 Discussion

8.3.1 Effect of Temperature on Antibacterial Activity and Fatty Acids Profile of Carica Papaya *Seed Extract*

The effect of various heating temperatures on the antibacterial activity of CPSE indicated the stability of the extract as shown in Fig. 8.1. The potency of the extract

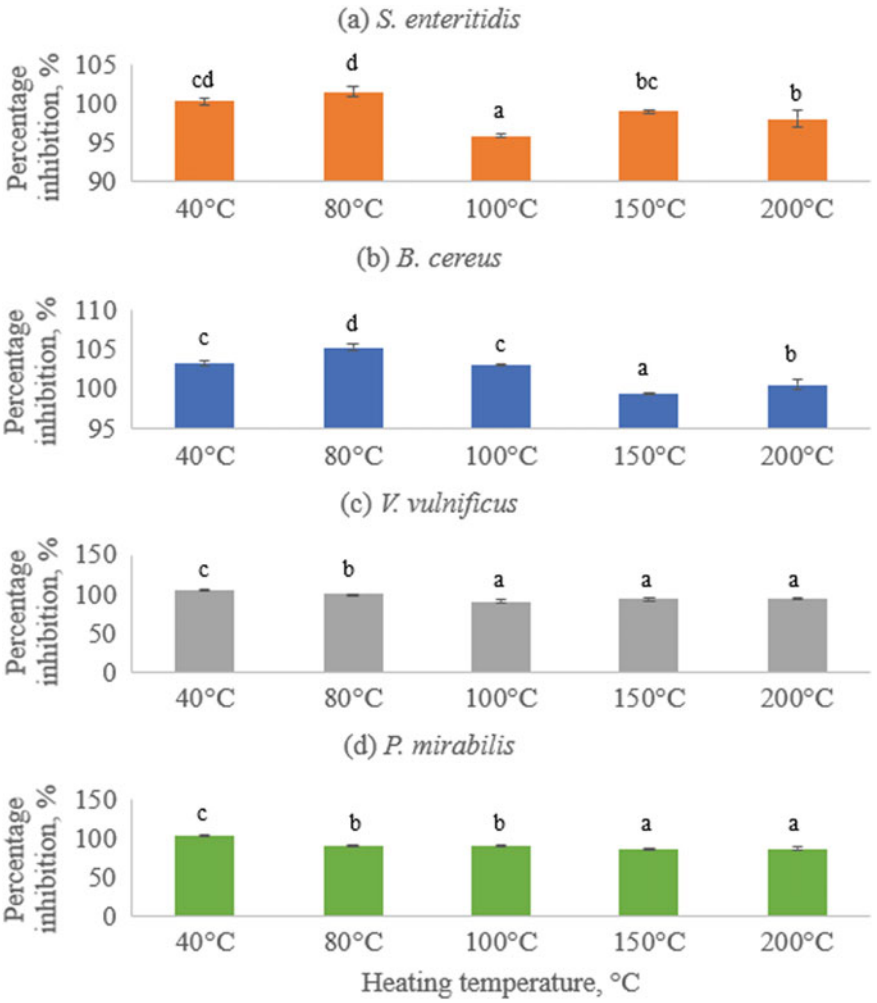

Fig. 8.1 Effect of heated extract on antibacterial activity of *Carica papaya* seed extract (a) *S. enteritidis*, (b) *B. cereus,* (c) *V. vulnificus* and (d) *P. mirabilis* growths

against *S. enteritidis* and *V. vulnificus* had the same characteristics where the extract heated > 100 °C had a percentage inhibition of < 100%. Only the heated extract at 150 °C indicated a percentage inhibition < 100% (99.45%) against *B. cereus*. For *P. mirabilis*, only the heated extract at 40 °C was potent to the pathogen because the percentage inhibition > 100%, even though Nychas et al. (2003) reported that low temperatures reduced antibacterial activities. However, He et al. (2010) found that most antibacterial agents had lost their inhibitory efficiency at high temperatures. In general, all tested pathogens were sensitive to the extract in TSB at < 40 °C and this

finding could be proposed for food incorporated with the extract to be handled below this temperature before consumption.

8.3.2 *Effect of Temperature on Fatty Acid Methyl Esters Profile of* Carica Papaya *Seed Extract*

Linear relationships between the ratios of the peak area signals and the corresponding concentrations of FAMEs content were observed in the analytical curves Fig. 8.2 when using different concentrations. The parameters of the analytical curves with the correlation of determination (R^2) are shown in Table 8.1. The values of the analytical curve led to the conclusion that the linear regression model was adequate for the analytical determinations in this study, as R^2 was higher than 0.98 (Fagundes & Caldas, 2012). In this study, we did not calculate trans vaccenic acid, and thus only cis-vaccenic acid (C18:1n11c) was recorded. The chromatogram of the FAMEs is shown in Fig. 8.3.

The PCA was used to establish the relationship between the FAMEs identified by GC/MS and PI of *S. enteritidis*, *B. cereus*, *V. vulnificus,* and *P. mirabilis* as affected by heat. The four main principal components (PCs) characterized were having an eigenvalue > 1 (Saiful et al., 2019) which was considered as significant factor loadings (FL) ($p < 0.05$). The four PCs also had a cumulative explained total variance of 100% which consisted of PC1 (47.99%), PC2 (22.01%), PC3 (20.23%), and PC4 (9.77%) (Table 8.2). The data variances were explained at 70% for PC1 versus PC2 and 68.22% for PC1 versus PC3.

The FL table showed the loading values between the FAMEs and the PI of tested pathogens (Table 8.2). Based on the strong FL limit (> 0.75), PC1 was mainly characterized for a higher content of C16:0, C16:1, C18:0, C18:1n9c, C18:1n11c, and

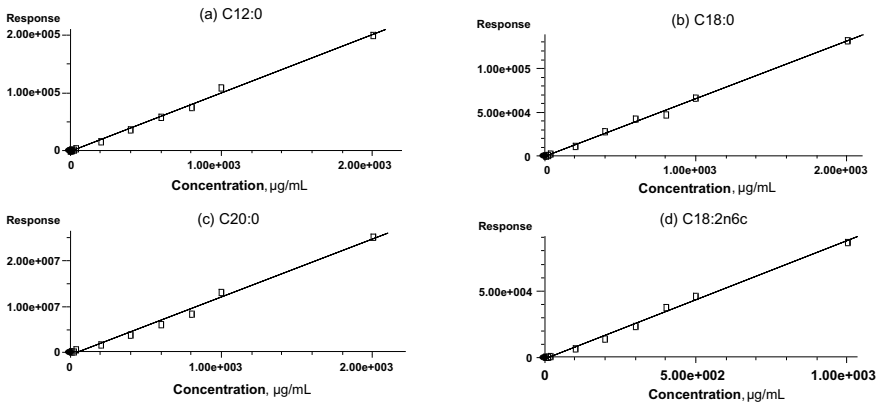

Fig. 8.2 Calibration curve of (a) C12:0, (b) C18:0, (c) C20:0 and (d) C18:2n6c

Table 8.1 Calibration information of fatty acid methyl esters

No	Compound[1]	Assignment	Mass	Rt[2]	$(R^2)^3$	Linearity equation
1.	Butyric acid	C4:0	102	9.690	0.9974	y = 0.3406x + 14.727
2.	Hexanoic acid	C6:0	130	10.144	0.9966	y = 0.0923x − 15.233
3.	Octanoic acid	C8:0	158	10.940	0.9952	y = 0.3662x + 59.885
4.	Decanoic acid	C10:0	186	12.304	0.9969	y = 2.0091x − 437.51
5.	Undecanoic acid	C11:0	200	13.253	0.9945	y = 1.5095x − 323.16
6.	Dodecanic acid	C12:0	214	14.399	0.9966	y = 4.0352x − 1227.9
7.	Tridecanoic acid	C13:0	228	15.689	0.9962	y = 1.481x + 26.513
8.	Myristic acid	C14:0	242	17.148	0.9964	y = 4.2624x − 1108
9.	Myristoleic acid	C14:1	240	18.327	0.9962	y = 0.4265x + 230.14
10.	Pentadecanic acid	C15:0	256	18.670	0.9961	y = 2.5957x − 136.37
11.	Cis-10-pentadecenoic	C15:1	254	19.912	0.9946	y = 0.4751x + 182.81
12.	Palmitic acid	C16:0	270	20.285	0.9964	y = 13.148x − 3445.9
13.	Palmitoleic acid	C16:1	268	21.295	0.9885	y = 0.4777x + 666.03
14.	Heptadecanic acid	C17:0	284	21.822	0.9960	y = 2.8421x + 105.16

(continued)

Table 8.1 (continued)

No	Compound[1]	Assignment	Mass	Rt[2]	$(R^2)^3$	Linearity equation
15.	Cis-10-heptadecenic acid	C17:1	282	22.877	0.9863	y = 0.5708x + 420.79
16.	Stearic acid	C18:0	299	23.418	0.9972	y = 2.6297x − 466.87
17.	Elaidic acid	C18:1n9t	296	24.007	0.9903	y = 0.7292x + 523.17
18.	Oleic acid	C18:1n9c	296	24.329	0.9908	y = 1.3187x + 894.16
19.	Linolelaidic acid	C18:2n6t	294	24.947	0.9916	y = 1.7255x + 495.27
20.	Linoleic acid	C18:2n6c	294	25.631	0.9947	y = 1.7667x − 902.56
21.	Arachidic acid	C20:0	327	26.430	0.9911	y = 504.82x − 542642
22.	γ-linolenic acid	C18:3n6	292	26.580	0.9923	y = 155.49x + 166488
23.	Linolenic acid	C18:3n3	292	27.142	0.9959	y = 163.93x − 36714
24.	Cis-11-eicosenoic acid	C20:1	325	27.259	0.9975	y = 210.9x − 23947
25.	Cis-vaccenic acid	C18:1n11c	282	27.613	0.9979	y = 0.1722x + 7.134
26.	Heneicosanoic acid	C21:0	341	27.835	0.9833	y = 278.58x − 425782
27.	Cis-11,14-eicosadienoic acid	C20:2	323	28.547	0.9950	y = 177.5x − 51134
28.	Behenic acid	C22:0	355	29.305	0.9904	y = 670.73x − 984863

(continued)

Table 8.1 (continued)

No	Compound[1]	Assignment	Mass	Rt[2]	$(R^2)^3$	Linearity equation
29.	Cis-8,11,14-eicosatrienoic acid	C20:3n6	321	29.505	0.9973	y = 164.72x − 23659
30.	Cis-11,14,17-eicosatrienoic acid	C20:3n3	321	30.146	0.9918	y = 261.24x − 230243
31.	Erucic acid	C22:1n9	353	30.147	0.9965	y = 187.08x + 42244
32.	Arachidoic acid	C20:4n6	318	30.245	0.9926	y = 152.73x + 32586
33.	Tricosanic acid	C23:0	369	30.713	0.9900	y = 2.1268x − 1870.7
34.	Cis-13,16-docosadienoic acid	C22:2n6	351	31.517	0.9970	y = 1.2291x − 1040.4
35.	Cis-5,8,11,14,17-eicosapentaenoic acid (EPA)	C20:5n3	316	31.901	0.9917	y = 122.77x + 6381.4
36.	Tetracosanoic acid	C24:0	383	32.281	0.9842	y = 8.0799x − 11217
37.	Cis-15-tetracosenic acid	C24:1n9	381	33.234	0.9947	y = 232.97x − 73520
38.	Cis-4,7,10,13,16,19-docosahexaenoic acid (DHA)	C22:6n3	343	36.354	0.9908	y = 101.36x + 62011

[1]Fatty acids detected were in their esters (FAMEs) form
[2]Rt = retention time
[3]R^2 = coefficient determination

lower content of C23:0, also had strongly contributed to the PI of *V. vulnificus* and P.*mirabilis* and moderate contribution to *S. enteritidis.* The PC2 was related principally to the higher content of C13:0 and C20:2. PC3, on the other hand, was dominated by a higher content of C10:0 and a lower content of C20:0 and moderately contributed to the PI of *B. cereus.*

The observation plot allowed exploring the correlations between the PCs and the extract as affected by temperature (Fig. 8.4). The extract heated at 40 °C clearly showed a significant antagonistic correlation in PC1 against 100 °C, 150 °C, and 200

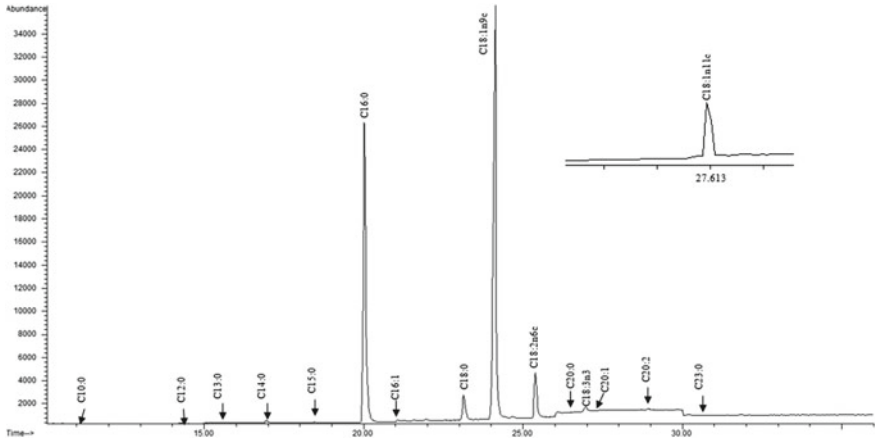

Fig. 8.3 Chromatogram of fatty acid methyl esters in *Carica papaya* seed extract separated by using HP88 column

°C, and significant antagonistic correlation against 80 °C in PC3 (Fig. 8.4b). In PC3 also, a significant antagonistic correlation was exhibited by 80 °C and 150 °C.

The variable plot in Fig. 8.5 helped to establish which FAMEs and PI of the tested pathogens discriminated against the heated extracts. The extract heated at 40 °C, as seen in Fig. 8.4a, had a strong PC1 score that has been related to higher content in C16:0, C16:1, C18:0, C18:1n9c, C18:1n11c (Fig. 8.5a). Nevertheless, the heated extract at 80 °C had a high PC3 score, as seen in Fig. 8.4b; therefore, had higher contents of C10:0 (Fig. 8.5b). The heated extract at 100 °C (Fig. 8.4a) had a higher content of C13:0 in Fig. 8.5a, whereas the extract heated at 150 °C had a lower content of C12:0 and C18:1n9t. However, the C23:0 was lower in 200 °C heated extracts.

These results suggest that the PI of the tested pathogens was addictively and antagonistically facilitated by different compositions of FAMEs. Besides, the individual FAMEs had different stability against different temperatures. In summary, from PC1 alone, we found that C16:0, C16:1, C18:0, C18:1n9c, and C18:1n11c from CPSE had strongly inhibited *V. vulnificus* and *P. mirabilis* growths and moderately inhibit *S. enteritidis* growth.

8.3.3 Profile of Cis and Trans Fatty Acid Methyl Esters as Affected by Temperature

FAMEs were dominant in CPSE (Sani et al., 2017b). Among the FAMEs, their trans form has received high concern due to its negative health impact, especially when the extract was used in the food and undergoes heat treatment, such as cooking and deep-frying. Thus, this study was done to identify the profile of the trans-FAMEs when

Table 8.2 Factor loadings of fatty acid methyl esters and percentage inhibition of tested pathogens as affected by temperature

Fatty acid methyl esters and percentage inhibition[1] of tested pathogens	Factor loadings (FL)[2,3,4]			
	PC1	PC2	PC3	PC4
C10:0	0.200	−0.253	**0.937**	−0.132
C12:0	−0.258	−0.733	−0.493	−0.391
C13:0	−0.611	**0.755**	−0.008	0.238
C14:0	0.137	−0.549	0.470	0.677
C15:0	−0.515	0.432	0.027	0.740
C16:0	**0.940**	−0.162	−0.274	0.122
C16:1	**0.898**	0.158	−0.383	0.148
C18:0	**0.989**	0.088	−0.068	0.101
C18:1n9c	**0.979**	0.016	0.026	0.201
C18:1n9t	−0.552	−0.667	−0.461	0.194
C18:2n6c	0.670	−0.620	0.043	0.406
C20:0	0.409	−0.092	**-0.890**	0.182
C18:3n3	−0.396	−0.720	0.404	−0.401
C20:1	−0.494	0.467	0.703	0.208
C18:1n11c	**0.877**	0.264	0.350	−0.194
C20:2	0.438	**0.776**	−0.359	−0.278
C23:0	**−0.952**	−0.011	−0.076	0.295
PI of *V. vulnificus*	**0.962**	−0.159	0.114	0.192
PI of *S. enteritidis*	***0.662***	−0.603	0.434	0.096
PI of *B. cereus*	0.539	0.375	***0.727***	−0.201
PI of *P. mirabilis*	**0.919**	0.356	−0.163	−0.051
Eigen value	10.078	4.623	4.248	2.051
Variability, %	47.99	22.01	20.23	9.77
Cumulative, %	47.99	70.01	90.23	100.00
Significance level, α	< 0.05	< 0.05	< 0.05	< 0.05

[1]PI = percentage inhibition
[2]The FL were considered strong (> 0.75), moderate (0.74 - 0.50) and weak (0.49 – 0.30)
[3]Strong FL correlation > 0.75 was shown in bold
[4]Moderate FL correlation (0.74 - 0.50) for PI of *S. enteritidis* and *B. cereus* was shown in italic

the extract is heated. Also, the study of other FAMEs profile in non-heated CPSE (unheated) *Carica papaya* seed was done due to the capability of the HP-88 column to separate cis and trans-FAMEs, unlike the HP-5 ms column used in common plant metabolites analysis using GC/MS.

The profile of cis and trans-FAMEs affected by temperature is shown in Fig. 8.6. The highest concentration of oleic acid (C18:1n9c) was recorded at low temperatures

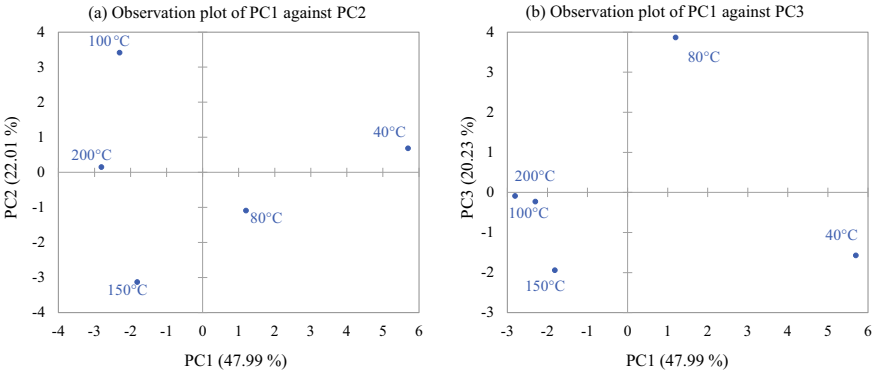

Fig. 8.4 Observation plot of (a) PC1 and PC2 and (b) PC1 and PC3 of extract distribution as affected by temperature

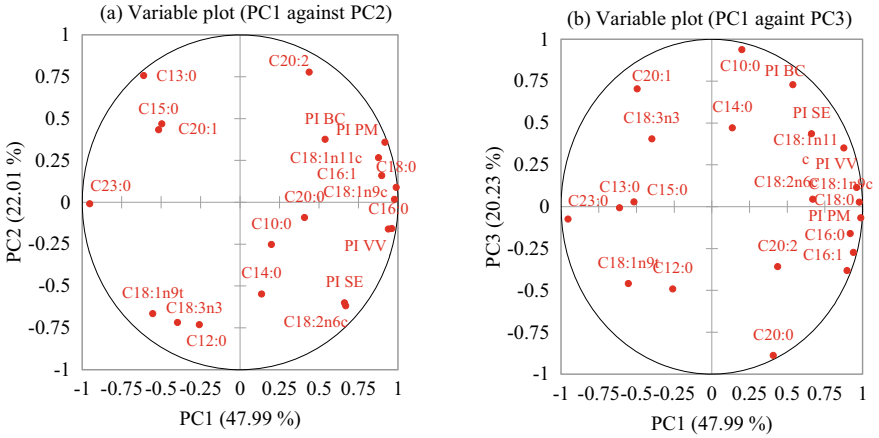

Fig. 8.5 Variable plot resulting from (a) PC1 against PC2 and (b) PC1 against PC3 of fatty acid methyl esters and PI of tested pathogens present in extract as affected by temperature

(40 °C and 60 °C), whereas cis-vaccenic acid (C18:1n11c) was detected at each heating treatment and exhibited a reducing trend; C18:1n11c was the most stable FAMEs at 100 °C whereas other FAMEs were undetected.

C18:2n6t was not detected in each sample treatment, indicating that C18:2n6c was stable against heat and did not convert to trans FAMEs. Meanwhile, C18:1n9c showed a drastic reduction when heated at higher temperatures and producing its trans form (C18:1n9t) at 150 °C and 200 °C, thereby supports the finding of Gürdeniz et al. (2013) since all naturally occurring FAMEs of plant origin are in the cis form, and the trans form is generally generated when oils and fats are hydrogenated or heated at a high temperature (Tao, 2007). Thus, it can be proposed that the food incorporated with the CPSE could be handled at a temperature < 150 °C. However, C18:1n9c was

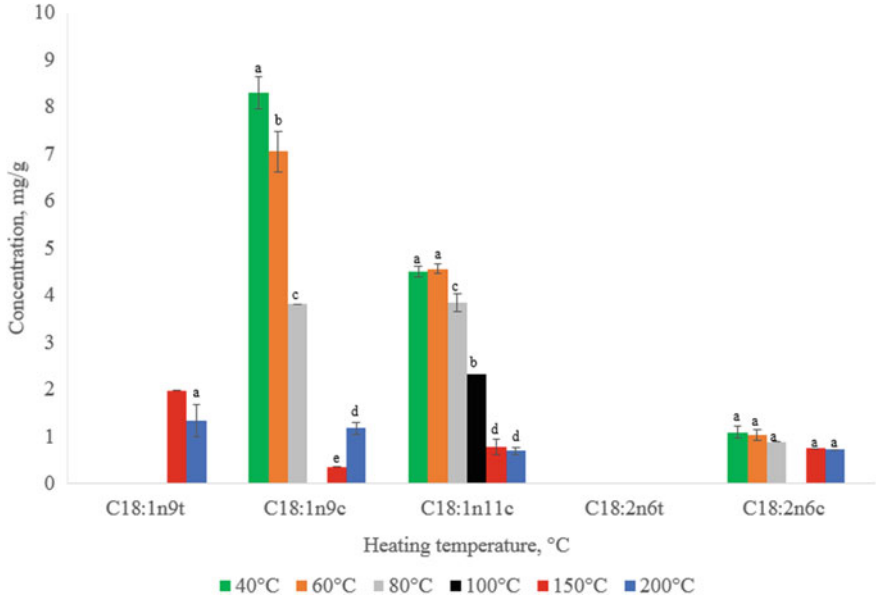

Fig. 8.6 Cis and trans fatty acids profile; (a) C18:1n9t, (b) C18:1n9c, (c) C18:1n11c, (d) C18:2n6t, (d) C18:2n6c

still detected at 150 °C and 200 °C because of its high oxidative stability (Preedy et al., 2011) Fig. (8.6).

8.4 Conclusion

In summary, the crude of CPSE cv. Sekaki/Hong Kong had demonstrated antibacterial activity against *S. enteritidis, V. vulnificus, P. mirabilis*, and *B. cereus.* These tested pathogens were sensitive to the extract in TSB at < 40 °C. From the PCA, C16:0, C16:1, C18:0, C18:1n9c, and C18:1n11c from CPSE had strongly inhibited *V. vulnificus* and *P. mirabilis* growths and moderately inhibit *S. enteritidis* growth. The treatment of CPSE against heat had also caused the generation of the trans-FAMEs at 150 °C and 200 °C, thus indicating that CPSE should be handled at < 150 °C in food applications.

Acknowledgements This work was supported by the Fundamental Research Grant Scheme (FRGS19-041-0649) of the Ministry of Higher Education Malaysia and Research University Grant (vot no. 9328200) of Universiti Putra Malaysia.

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