CHAPTER V

VOLATILE ORGANIC COMPOUNDS OF LAB-INOCULATED FERMENTED CHILI MASH USING STATIC HEADSPACE GC-MS

5.1 INTRODUCTION

Chili flavors were characterized as a mixture of pyrazines, that is, 3-isopropyl-2-methoxypyrazine, 3-butyl-2-methoxypyrazine and 3-isobutyl-2-methoxypyrazine (Murray & Whitfield, 1975). The pungency contributing principles are the capsaicinoids, which are vanillyl amides of various acids, out of which capsaicin as vanillyl amide of isodecanylic acid is the most important. Presence of volatile organic compound (VOC) interfered directly the sensory quality of the chili fruits as well as sensory quality of fermented chili (Pontes et al., 2009). The carbohydrates, fats and protein can generate numbers of flavor chemical compound by conversion of carbohydrates, fats and protein to more complex flavor molecules as a result of fermentation process (Krings & Berger, 1998). Starter cultures produce primary metabolites in considerable amounts but only traces of more complex aroma chemicals. VOC by lactic acid fermentation produce different type of aroma in food (Braga et al., 2015; Bryant & McClung, 2011).

Inoculation of lactic acid bacteria into fermented vegetables was proven to improve the flavor profile since capability of lactic acid bacteria to influence the biochemistry of vegetables substrate. Research conducted by Abolhassani et al., (2009) found out *L. plantarum* and *L. bulgaricus* generate volatile organic compound in fermented curry paste. Co-inoculation of LAB with yeast modify the flavor of the Merlot wines resulted in different trend production of organic compound such as ester and di-acetyl in wine making (Antalic et al., 2013).

Detection of aromatic component in food can be done by different technique. Flavor is mainly detected by headspace GC-MS. (Ray et al., 2014) The simplest way to assess the chemical composition of the aroma is direct analysis of a portion of the air in contact with the odor source, without any other sample treatment step. The levels of VOC related to the aroma profile in food matrix are largely influenced by the extraction technique of the volatile fraction used in gas chromatograph analysis (Xie et al., 2008). Headspace sampling techniques are widely used for providing volatile profiles near the profiles experienced by humans. This technique has been used to extract VOC from a variety of natural products and is now considered a matured extraction technique. It able to quantitatively quantify numbers of volatile compound in passion fruit juice, rice, chili cultivars, orange and blackcurrant (Braga et al., 2015; Bryant & McClung, 2011; Blaythe et al., 2006).

Volatiles are important flavor compound as it impart aroma to chilies. Fermenting chili mash with different type of LABs resulted to generation of different volatiles components. The analyses of volatile compounds in chili from different countries have been studied using different methods and sample preparations (Garruti et al., 2013; Junior et al., 2012). Static headspace technique applied small samples of the atmosphere around the samples is injected directly onto the GC column without the use of organic solvent. Successful extraction method depends on parameters such as such as temperature, time, ionic strength, headspace volume and the volume of headspace in static headspace method (Mestres et al., 1997). Applying different injection mode resulted to different volatile compound detection. In static headspace GC, the critical part of determining the compounds is depending on injection mode. Splitt injection modes suitable for a concentrated solution enough to achieve the required detection limit of VOC whereas splittless injection is suited for detection analyte concentration which expected to be very low (Hoh & Mastovska, 2008). The knowledge of the composition of these volatiles compounds is an important tool for differentiating between the capabilities of LAB isolates to produce different flavor notes as compared to non-inoculated fermented chili mash. Therefore this study was conducted to evaluate the capability of different LAB strain to modify the flavor compound in fermented chili mash applying two mode GC injection split and splittless mode by GC-MS static headspace.

5.2 MATERIALS AND METHODS

5.2.1 Preparation of Lactic Acid Fermented Mash

Cilibangi fruits were selected to remove their pericarps, free from blemishes, defects, and insect damage. The chilies were washed with portable water to remove any impurities then ground using a food blender (Panasonic) with 6% rock salt added. Fermentation was conducted using 150 ml scotch bottle each containing 100 g of pepper mash inoculated with log 10 cfu/mL 24 h cultures of LAB (1% v/v). Fermentation was carried at 30°C for 14 days.

5.2.2 Identification of Volatile Organic Compounds

Five grams of each fermented chili mash (fermented chili mash) sample was introduced into a 10 mL headspace vial and was thus ready to be analyzed. Volatile compounds in fermented chili mash samples were identified using an Agilent-Technologies 7890A GC system equipped with an Agilent-Technologies 5975C Inert MSD with triple-axis detector and Agilent-Technologies G1888 Network headspace sampler (Agilent-Technologies, Little Falls, CA, USA). GC/MS analysis of volatile compounds was performed using DB-WAX column (30 m × 0.25 mm × 0.25 μm) (J&W Scientific, Folsom, CA, USA). The volatile compounds were identified by comparing and matching mass spectra fragment with the NIST08 MS library (50 % similarity). The extraction protocol by direct injection method was simplified in Table 7.

Table 7: Extraction Protocol for GC and Headspace Condition

GC CONDITION		
10:1 Injection		Splittless Injection
Mass range of m/z	29–450	560-650
Split ratio	10:1	Splittless
Oven temperature	Set at 37 °C for 6 min, Heated to 100 °C at 10°C/min for 5 min Ramp 200 °C at 15°C/min for 10 min Final 230°Cat 20°C/ min for 2 min	Set at 37 °C for 0 min, Heated to 100 °C at 10°C/min for 5 min Ramp240°C at 10°C/min for 0 min Final 230°Cat 20°C/ min for 2 min
GC injector line	250 °C	260 °C
MS transfer line	230 °C	280 °C
Helium flow rate	1 mL/min	1 mL/min
Run time	35.8 min	20.5 min
HEADSPACE CONDITION		
10:1 Injection		Splittless Injection
Oven temperature	80 °C	70 °C
Loop line	90 °C	90 °C
Transfer line temperature	100 °C	120 °C
MS quad	230 °C	280 °C
MS source	150 °C	230 °C
Timing for vial equilibration	15.0 min	15.0 min
Pressurization	0.20 min	0.20 min
Loop fill	0.20 min	0.20 min
Loop equilibrium	0.05 min	0.05 min
Inject	1.00 min	1.00 min

5.3 RESULTS

Table 8 and Table 9 show the number of compound and the respective total peak area corresponding to each chemical class. The components were classed into 7 consisting of ester, alcohol, alkane, acid, ether and nitrogen containing compound. Table 8 shows static GC-MS headspace applying 1:10 split injection detected 4 volatile components consisting of alcohol (28.4%), alkane (22.3%), acid (29.9%) and hydrocarbon (19.23%) with respect to the total peak area in raw chili mash. Nitrogen containing compound was only detected present in spontaneous fermentation and LAB inoculated fermented chili mash. However, hydrocarbon (19.2%) was detected present only in raw chili while ester (0.48%) compound only detected present in fermented chili mash inoculated with *L. plantarum* ALO1. Ether was detected present in spontaneous fermentation (0.68%) and fermented chili mash inoculated with *L. pentosus* ALO2 (4.85%). Three types of acid were detected present in fermented chili mash inoculated with *L. plantarum* AU2 (5.32%) while only one type of acid detected present in raw chili with (29.9%).

Table 8: Volatile Compounds Identified in Spontaneous and LAB Inoculated Chili after Two Weeks of Fermentation Using Headspace GC-MS Applying 1:10 Split Injection Mode

Chemical Class	Raw Chili				Spontaneous				*L. plantarum* ALO1				*L. pentosus* ALO2				*L. plantarum* AU2			
	N	N%	A	%A	N	N%	A	%A	N	N%	A	%A	N	N%	A	%A	N	N%	A	%A
Ester	0	0	0	0	0	0	0	0	1	12.5	0.48	0.48	0	0	0	0	0	0	0	0
Alcohol	1	20	1.98	28.41	3	42.86	34.08	34.30	3	37.5	38.65	38.65	2	25	37.4	77.9	2	28.57	38.02	38.78
Alkane	1	20	1.56	22.38	2	28.57	1.59	1.60	0	0	0	0	2	25	1.2	2.5	1	14.29	0.50	0.51
Acid	2	40	2.09	29.99	0	0	0	0	2	28.57	5.77	5.77	1	12.5	0.50	1.04	3	42.86	5.22	5.32
Hydrocarbon	1	20	1.34	19.23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ether	0	0	0	0	1	14.29	0.68	0.68	0	0	0	0	2	25	2.33	4.85	0	0	0	0
Nitrogen Compound	0	0	0	0	1	14.29	63.01	63.42	2	28.57	55.1	55.1	1	12.5	6.58	13.71	1	14.29	54.32	55.39
Total	5	100	6.97	100.01	7	100.01	99.36	100	8	107.14	100	100	8	100	48.01	100	7	100.01	98.06	100

N= number of compound; A= total peak are

Table 9 shows volatile component detected using splitless corresponding to the chemical class. The major different observed between the mode of injection was number of volatile components detected. Splitless injection detected greater number of organic compound as compared to method 1:10 injection. Raw chili mash consist of seven volatile components that include ester (62.44%), alcohol (5.10%), alkane (21.49%), acid (2.77%), ether (6.76%), and nitrogen containing group (2.91%). Raw chili contains the highest ester (66.67%) component with the lowest alcohol (13.35%) component as compared to the other fermented chili mash. Six types of alcohol (20.545) were detected in all the fermented chili mash inoculated with *L. pentosus* AU2. Besides that, nitrogen containing group components were detected present in all samples. Conversely, hydrocarbon was not detected present in raw chili applying this method as compared to 1:10 split injection. Highest alkane (58.26%) was detected in fermented chili mash with *L. pentosus* ALO2. Acid was detected low in raw chili about (9.75%) but present high in all fermented chili mash (14.35%).

Table 9: Volatile Compounds Identified in Spontaneous and LAB Inoculated Chili after Two Weeks of Fermentation Using Headspace GC-MS Applying Splitless Mode of Injection

Chemical Class	Raw Chili				Spontaneous				*L. plantarum* ALO1				*L. pentosus* ALO2				*L. plantarum* AU2			
	N	N%	A	%A	N	N%	A	%A	N	N%	A	%A	N	N%	A	%A	N	N%	A	%A
Ester	4	26.67	42.1	62.44	3	17.64	39.33	44.01	3	20	22.55	29.96	5	26.32	16.15	18.48	3	18.75	19.34	23.37
Alcohol	2	13.35	3.44	5.10	4	23.52	15.07	16.87	3	20	10.01	13.30	3	15.79	4.48	5.13	6	37.5	17.01	20.54
Alkane	7	46.67	14.49	21.49	5	29.41	21.05	23.56	4	26.67	25.34	33.67	5	26.32	50.9	58.26	3	18.75	32.95	39.79
Acid	1	6.67	1.87	2.774	1	5.89	2.30	2.57	1	6.67	10.80	14.35	1	5.26	8.53	9.76	1	6.25	8.85	10.69
Hydrocarbon	0	0	0	0	2	11.76	6.04	6.77	2	13.34	3.22	4.28	3	15.79	3.31	3.79	1	6.25	1.90	2.29
Ether	1	6.67	4.56	6.76	1	5.89	4.54	5.08	1	6.67	2.72	3.61	1	5.26	1.54	1.76	1	6.25	2.04	2.46
Nitrogen Compound	1	6.67	0.96	2.91	1	5.89	1.02	1.14	1	6.67	0.63	0.84	1	5.26	2.46	2.82	1	6.25	0.71	0.86
Total	15	106.7	67.42	101.474	17	100	89.35	100	15	100.	75.27	100.	19	100	87.37	100	16	100	82.8	100

N= number of compound; A= total peak area

Table 10 shows the respective volatile organic compound detected by GC-MS headspace applying 1:10 split mode injection (APPENDIX E). The volatile component consists of different types of alcohol (5), acids (6), ether (1), ester (1), hydrocarbon (1), alkane (3) and nitrogen (1) containing compound. The volatile profiles show diverse qualitative patterns. All the samples did not show any similarities of volatile compounds produced

Major alcohol which was dominated by ethanol was detected present all fermented chili mash samples with the exception to the raw chili sample. Few types of alcohol such as 1-butanol, 3-methyl- and 1-propanol, 2-methyl- present in fermented chili mash inoculated with *L. plantarum* ALO1. A compound of à-terpineol was only detected present in spontaneous fermentation with 0.82% total peak area. The compounds of 1-(+)-ascorbic acid and cyclopropanetetradecanoic acid, 2-octyl, methyl ester were only detected present in ALO1-fermented chili mash with 5.16% and 0.48% total peak area respectively. On the other hand 1,1'-bicyclopropyl-2-octanoic acid was only detected present in fermented chili mash inoculated with *L. plantarum* ALO1 and *L. plantarum* AU2 with 0.50% and 0.54% total peak area. The trans-13-octadecenoic acid only present fermented chili mash inoculated with and *L. plantarum* AU2. 4-chloro-3-n-hexyltetrahyropyran was detected present only in raw chili mash with total peak area 1.34%.

Table 10: Volatiles Compounds Identified in The Spontaneous and LAB Inoculated Chili After Two Weeks of Fermentation Using Headspace GC-MS Applying 1:10 Split Injection

Volatile Compounds	Group	Raw Chili		Spontaneous		*L. plantarum* ALO1		*L. pentosus* ALO2		*L. plantarum* AU2	
		RT	Area (%)	RT	Area (%)	RT	Area (%)	RT	Area (%)	RT	Area (%)
Methyl alcohol	Alcohol	3.24	1.98	3.24	0.67	ND	ND	ND	ND	3.24	0.49
Ethanol	Alcohol	ND	ND	3.84	32.59	3.84	37.04	3.85	36.92	3.84	37.53
1-propanol, 2-methyl-	Alcohol	ND	ND	ND	ND	8.37	0.48	ND	ND	ND	ND
1-butanol, 3-methyl-	Alcohol	ND	ND	ND	ND	10.83	1.13	10.83	0.48	ND	ND
à-terpineol	Alcohol	ND	ND	21.14	0.82	ND	ND	ND	ND	ND	ND
Hydroxyacetic acid, hydrazide	Acid	ND	ND	ND	ND	3.24	0.61	ND	ND	ND	ND
Pterin-6-carboxylic acid	Acid	ND	ND	ND	ND	ND	ND	7.97	0.50	7.95	0.54
[1,1'-bicyclopropyl]-2-octanoic acid,	Acid	24.04	2.09	ND	ND	ND	ND	ND	ND	24.42	0.66
l-(+)-ascorbic acid	Acid	ND	ND	ND	ND	24.88	5.16	ND	ND	ND	ND
Trans-13-octadecenoic acid	Acid	ND	ND	ND	ND	ND	ND	ND	ND	28.40	4.02
Z-(13,14-Epoxy)tetradec-11-en-1-ol Acetate	Acid	21.61	1.99	ND	ND	ND	ND	ND	ND	ND	ND
Octaethylene glycol monododecy ether	Ether	ND	ND	27.91	0.68	ND	ND	26.70	1.74	ND	ND
Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester	Ester	ND	ND	ND	ND	24.38	0.48	ND	ND	ND	ND
4-Chloro-3-n-hexyltetrahydropyran	Hydrocarbon	20.09	1.34	ND	ND	ND	ND	ND	ND	ND	ND
(2S,2'S)-2,2'-Bis[1,4,7,10,13-pentaoXacyclopentadecane	Alkane	ND	ND	25.72	0.84	ND	ND	ND	ND	ND	ND
15,15'-Bi-1,4,7,10,13-pentaoxacyclohexadecane	Alkane	29.32	1.56	ND	ND	ND	ND	31.49	0.48	29.98	0.50
(2S,2'S)-2,2'-Bis[1,4,7,10,13-pentaoXacyclopentadecane	Alkane	ND	ND	29.82	0.75	ND	ND	32.66	0.72	ND	ND
Benzeneethanamine,	Nitrogen Compound	1.53	88.32	1.53	63.01	1.53	48.52	1.54	58.58	1.53	54.32
Benzenesulfonamide, N-butyl	Nitrogen Compound	ND	ND	ND	ND	26.71	6.58	ND	ND	ND	ND

Table 11 shows qualitative result of volatile components detected by GC-MS applying splittless injection. There were alcohol (9), acid (3), ether (1), ester (6), hydrocarbon (5), alkane (13) and nitrogen containing group (2) (APPENDIX F).

It was observed that 1-pentanol and hydrocarbon named as 1,6-octadie-3-ol,3,7-di methyl present in all LAB inoculated fermented chili mash and spontaneous fermented chili. The most abundant ester present in raw chili was 12,15-octadecanoic acid methyl ester (23.97%). However, this volatile compound's concentration decreased in fermented chili mash inoculated with *L. plantarum* AU2. n-hexadecanoic acid was only detected in LAB inoculated fermented chili mash but not in raw chili or spontaneous fermentation since the absent of this compound was detected. However, 3-trifluoroacetoxypentadecane (0.84%) was only detected in raw chili mash sample.

Table 11: Volatiles Compounds Identified in The Spontaneous and LAB Inoculated Chili after Two Weeks of Fermentation using Headspace GC-MS Applying Splitless Injection

Volatile Compounds	Group	Raw Chili		Spontaneous		*L. plantarum* ALO1		*L. pentosus* ALO2		*L. plantarum* AU2	
		RT	Area (%)	RT	Area (%)	RT	Area (%)	RT	Area (%)	RT	Area (%)
1-hexadecanol, 2-methyl	Alcohol	ND	ND	ND	ND	ND	ND	2.95	0.90	ND	ND
9,10-secocholesta-5,7,10(19)-triene -3,24,25-triol, (3á,5Z,7E)-	Alcohol	ND	ND	ND	ND	ND	ND	ND	ND	4.61	8.03
1-pentanol	Alcohol	ND	ND	5.79	3.32	5.78	7.75	5.78	2.46	5.78	2.81
1-pentanol, 4-methyl-	Alcohol	7.11	1.31	7.12	1.05	7.11	0.89	ND	ND	7.11	0.65
1-hexadecanol, 2-methyl-	Alcohol	ND	ND	ND	ND	ND	ND	ND	ND	8.13	0.59
1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol	Alcohol	ND	ND	11.59	0.79	ND	ND	11.88	1.12	ND	ND
à-acorenol	Alcohol	11.87	2.13	ND	ND	ND	ND	ND	ND	ND	ND
4,5-di-epi-aristolochene	Alcohol	ND	ND	ND	ND	11.88	1.37	ND	ND	ND	ND
à-terpineol	Alcohol	ND	ND	11.92	9.91	ND	ND	ND	ND	11.92	4.34
2-propenoic acid, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester, exo-	Acid	12.05	1.87	ND	ND	ND	ND	ND	ND	ND	ND
n-hexadecanoic acid	Acid	ND	ND	ND	ND	14.59	10.80	14.61	8.53	14.62	8.85
Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester	Acid	ND	ND	14.61	2.30	ND	ND	ND	ND	ND	ND
Octaethylene glycol monododecyl Ether	Ether	12.27	2.31	19.85	3.28	19.86	1.82	19.87	1.54	19.87	1.48
[1,1'-bicyclopropyl]-2-octanoic acid,2'-hexyl-, methyl ester	Ester	0.56	0.56	0.56	0.56	0.56	0.56	12.11	0.56	ND	ND
Methoxyacetic acid, 4-tetradecylEster	Ester	8.85	1.16	ND	ND	ND	ND	ND	ND	8.85	0.68
Methoxyacetic acid, 2-tridecyl ester	Ester	ND	ND	ND	ND	ND	ND	8.85	0.60	ND	ND
12,15-Octadecadiynoic acid, methyl ester	Ester	4.59	23.97	4.59	19.13	4.59	8.15	2.46	2.46	ND	ND
Cyclopropanedodecanoic acid, 2-octyl-, methyl ester	Ester	ND	ND	ND	ND	ND	ND	ND	ND	4.37	5.22
9,12,15-octadecatrienoic acid,2-[(trimethylsilyl)oxy]-1-[(trimethylsilyl)oxy]methyl]ethyl ester,(Z,Z,Z)	Ester	1.73	10.15	1.72	13.71	1.72	12.51	1.72	10.19	1.72	10.34

Compound	Group	Raw Chili		Spontaneous		*L. plantarum* ALO1		*L. pentosus* ALO2		*L. plantarum* AU2	
		RT	Area (%)	RT	Area (%)	RT	Area (%)	RT	Area (%)	RT	Area (%)
l-gala-l-ido-octose	Hydrocarbon	ND	ND	ND	ND	ND	ND	9.45	0.62	ND	ND
1,6-octadien-3-ol, 3,7-dimethyl-	hydrocarbon	ND	ND	10.06	2.57	10.04	2.36	10.04	1.92	10.04	1.90
Bicyclo[3.1.0]hexan-3-ol, 4-methyl-1-(1-methylethyl)	Hydrocarbon	ND	ND	ND	ND	12.05	0.86	ND	ND	ND	ND
Isobornyl formate	Hydrocarbon	ND	ND	ND	ND	ND	ND	12.05	0.77	ND	ND
Cyclohexyl-15-crown-5	Hydrocarbon	ND	ND	17.08	3.47	ND	ND	ND	ND	ND	ND
Tridecane, 2-methyl-	Alkane	7.55	1.84	ND	ND	ND	ND	ND	ND	ND	ND
1-iodo-2-methylundecane	Alkane	ND	ND	7.57	1.52	7.57	1.18	ND	ND	7.5	1.16
10-Methylnonadecane	Alkane	ND	ND	ND	ND	ND	ND	7.57	0.94	ND	ND
Octadecane, 6-methyl	Alkane	ND	ND	8.12	0.88	ND	ND	ND	ND	ND	ND
3-trifluoroacetoxydodecane	Alkane	8.13	0.84	ND	ND	ND	ND	ND	ND	ND	ND
15,15'-Bi-1,4,7,10,13-pentaoxacyclohexadecane	Alkanes	ND	ND	ND	ND	ND	ND	4.62	12.85	ND	ND
3-(prop-2-enoyloxy)dodecane	Alkane	5.78	0.87	ND	ND	ND	ND	2.46	2.46	ND	ND
3-trifluoroacetoxypentadecane	Alkane	9.03	0.76	ND	ND	ND	ND	ND	ND	ND	ND
15,15'-Bi-1,4,7,10,13-pentaoxacyclohexadecane	Alkane	14.61	1.02	ND	ND	ND	ND	ND	ND	ND	ND
Octasiloxane1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15	Alkane	18.95	12.27	17.66	3.36	17.66	3.21	ND	ND	ND	ND
Cyclodecasiloxane, eicosamethyl	Alkane	17.	8.34	ND	ND	ND	ND	17.66	2.98	17.64	1.95
(2S,2'S)-2,2'-Bis[1,4,7,10,13-pentao xacyclopentadecane]	Alkane	ND	ND	19.32	2.91	19.32	1.66	19.59	0.64	ND	ND
Cyclodecasiloxane, eicosamethyl-	Alkane	ND	ND	20.17	12.38	20.17	19.29	20.17	31.03	20.17	29.84
2-myristynoyl pantetheine	Nitrogen compound	ND	ND	2.95	1.02	2.94	0.63	2.95	2.46	2.95	0.71
Tetraacetyl-d-xylonic nitrile	Nitrogen compound	13.45	0.96	ND	ND	ND	ND	ND	ND	ND	ND

5.4 DISCUSSION

Levels of individual volatile compounds related to the aroma profile are largely influenced by the extraction technique of the volatile fraction used in gas chromatograph analysis (Bichhi et al., 2008; Schomburg et al., 1995). Using either split injection or splitless injection mode of the headspace showed changes in volatile flavor profile during fermentation. Qualitative data on flavor profile of fermented chili mash indicated the absence or presence of volatile compounds in concentration below detection limits (Table 10 and 11). Components not found on raw chili were detected in fermented chili mash and it was dependent on the type of LAB strains inoculated.

The different injection methods resulted in different types of volatile compounds detected. Split injection mode applying 1:10 ratio detected 18 compounds (Table 8). This mode required split exit which allows a number of small gas fraction being introduced into the column. Split injection mode discriminated against high boiling sample components in proportion to their masses or boiling point passing through the injector into the column which caused loss of compound due to thermal degradation (Grob, 2003). Thus, low boiling point component is more favorable to be detected compared to high boiling point component. For quantitative transfer, the compounds that can be detected have approximately the boiling point of n-C_{20} (Hawthorn et al., 1989)

On the other hand, splitlless injection detected 39 different volatile compounds. This injection mode did not discriminate the volatile component as the entire volatile gas is being introduced from injector into the column. This mode is suited for thermally unstable components and suited for trace components detection (Hawthorn et al., 1989). Splittless injection mode is fast and suitable for highly volatile as the compound can be in injected efficiently from injector into the column (Grob, 2003) This injection mode avoids thermal discriminative effect and heat degeneration of compound (Yang, 2007).

The changes of flavor component are probably attributed to the significant role of microorganisms during fermentation mainly lactic acid bacteria in vegetables during fermentation (Zhou & Mc.Feeters, 1998) and also the indigenous enzymes present in the vegetables that were still active (Mc.Feeters, 2004). The presence of LAB either naturally present or being inoculated into fermented vegetables contributed significant effect to fermentation process mainly by improving the stability of fermented products as well as improving the odour characteristic of fermented vegetables (Mugula et al., 2003). Lactic acid bacteria influenced the flavor of fermented foods in variety of ways, for example by production of volatile components, reducing the activity or completely inactive enzymes which responsible for flavor components in the plant (McFeeters, 2004).

Ethanol was detected as the most abundant compound present in all fermented chili mash and absent in raw chili as detected by the 1:10 split injection mode (Table 10). However, applying splittless mode injection 1-pentanol was detected to be major compound and methyl alcohol such as 1-propanol, 2-methyl, 1-butanol, 3-methyl, methyl-alcohol, 1-hexadecanol-2-methyl (Table 11) present in all fermented chili mash. Nam et al., 2009; Gómez et al. (2006) observed that the presence of alcohols during fermentation resulted from carbohydrate metabolism by the yeast in the presence lactic acid bacteria. Additionally, methyl alcohols could be originated from activities of endogenous enzymes and residual enzymes from microorganisms (Rottsatchakul et al., 2009). The compounds could be generated from reduction of propanal, 2-methyl-1-propanal, pentanal, 4-methyl-1-pentanal (Frankel, 1991)

This study has detected a methyl ester compound in fermented chili mash such as cyclopropane tetradecanoic acid 2-octyl-methyl ester, methoxyaceticacid, 2-tridecyl ester, 2,15-octadecadiynoic acid, methyl ester, cyclopropanedodecenic acid, 2-octyl-methyl ester, 9,12,15-octadecatrienoic acid, 2-(trimethylysily)doxyl-1 [(trimethylsilyl)oxy) methyl)ethyl ester (ZZZ) (Table 10 and 11). Ester can be derived from the chili and from the chemical esterification of alcohols and acid during fermentation process (Apichartsrangkoon et al., 2013). Aging of fermented chili mash during 14 days fermentation resulted in plant tissue breakdown. Pectin esterase is present in plant tissue which specifically affects methyl groups on linear chain of pectin producing the methyl ester compound group (Nancy et al., 2007). LAB is believed to play important role to synthesize ester via esterification reaction in which fatty acyl groups from glycerides were transferred to alcohols (Holland et al., 2005). LAB esterase producer such as *L. plantarum*, *L. pentosus*, *L. fermentum* have the ability to hydrolyze and synthesize ester (Angela et al., 2004). Esterolytic activity liberated by the esterases contributed to wine aroma resulted into increase in wine quality (Etievant, 1991).

It was observed that both lactic acid bacteria and yeast were present during chilli mash fermentation as discussed in CHAPTER IV. This mixed fermentation of inoculated LAB and yeast produced volatile n-hexadecanoic but not detected in raw and spontaneous fermentation of chili mash (Table 4). Hexadecanoic acid was found to be the major constituent of Habanero chili but found to be to be decreased or disappeared once the habanero chilis achieve maturation Rodríguez -Burruezo et al., (2010). However, it was reported that hexadecanoic acid was found to be the common secondary metabolites produced by LAB in malolactic wine fermentation (Lee et al., 2009), similar to as observed in this study. This fatty acid is suggested to be synthesized by yeast during carbohydrate metabolism, since glucose is the main source of its precursor, acetyl-CoA (Lambrechts & Pretorius, 2000).

The acids detected present in LAB-inoculated fermented chili mash were 1-(+)-ascorbic acid, trans-13-octadecenoic acid, pterin-6-carboxylic acid, hydroxy acetic acid (Table 10). Ascorbic acid is a common volatile detected present in different variety of chili cultivars detected by GC-MS using solid-phase micro extraction (Toontom et al., 2012; Howard et al. (2000). However, this study did not detect any ascorbic acid in raw chili and spontaneous fermentation (Table 10 and Table 11). Only fermented chili mash inoculated with *L. plantarum* ALO1 contain 1-(+)-ascorbic acid utilizing 1:10 injection method by static headspace GC-MS. The absent of the ascorbic acid in raw chili might be due to incapability of static headspace GC to detect the compound as ascorbic acid have converted to benzene under certain condition through decarboxylation of benzoic acid (Christof et al., 2008).

Hydrocarbon 1,6-octadie-3-ol,3,7-di methyl or commonly known as β-linalool detected present in all fermented chili (Table 11). This compound is naturally present in different type of spices such as coriander, chili and ginger (Jalal et al., 2014; Mazida et al., 2005). It contributes to flowery and spicy odor in herbs. The present of this compound in all fermented chili was assumed to be metabolically synthesized by yeast. Carrau et al., (2005) studied that 1,6-octadie-3-ol,3,7-di methyl is a yeast metabolite in wine after alcoholic fermentation.

In contrast, it was detected that only 4-chloro-3-n-hexyltetrahyropyran and 3-trifluoroacetoxypentadecane were present in raw chili (Table 10 and 11). This compound seems to be deteriorated after fermentation process. 4-chloro-3-n-hexyltetrahyropyran is a metabolite that present naturally in *pyrus* L (pear). It has potential on anti-amylase and anti-urease activities (Anam et al., 2012). 3-trifluoroacetoxypentadecane is fluro compound has antimicrobial activity. It present naturally in tropical Indian medicinal plant, *Jatropha maheswarii* has the ability to cure rheumatism, eczema and ringworms and insecticide (Maria & Uthayakumari, 2014).

5.5 CONCLUSIONS

Different mode of injection in headspace GC–MS resulted to diverse qualitative detection of volatile organic compound. Discriminative injection by 1:10 injection detected 18 volatile organic compounds whereas non-discriminative splitless injection detected 39 different volatile organic compounds. A few compounds that were detected present in raw chili mash were deteriorated after fermentation process. Spontaneous and LAB inoculated fermentation process resulted to detection volatile organic compound that different than the raw chili. Ethanol was the most abundant compound detected in spontaneous and LAB inoculated fermented chili mash. This result also demonstrated that each LAB isolate was unique in modifying the flavor of fermented chili mash as there are different trend of qualitative volatile organic compound in LAB inoculated fermented chili mash.