The effect of spontaneous fermentation on the volatile flavor constituents of durian

[1*]Neti, Y., [2]Erlinda, I. D. and [2]Virgilio, V. G.

[1]*Department of TIP, Faculty of Agriculture, University of Lampung, Sumantri Brojonegoro #1 Bandar Lampung, Indonesia*
[2]*Institute of Food Science and Technology, College of Agriculture, University of the Philippine at Los Baños, Laguna, Philippine*

Abstract: The flavor compounds of spontaneous fermented durian (tempoyak) were analyzed compared to fresh durian using gaschromatography -mass spectrometry (GC-MS). The results of this experiment showed that most of the volatile flavor compounds changed substantially during fermentation. Nevertheless, the major volatile constituents of fermented durian were still sulfur-containing volatiles. Beside the origin flavor constituents, fermented durian generated 5 sulfur compounds and 16 non-sulfur compounds while fresh durian were missing their flavor of 11 sulfur components and 10 non-sulfur components when durian was fermented. Some alcohol, carboxylic acid and ester components not found on the fresh durian were present in fermented durian such as 2,3-butanediol; 1,3-butanediol,octanoic acid and propyl ester acetic acid. The different proportions of volatile components and the presence or absence of sulfur and non sulfur components determine the flavor properties of fermented durian. It is, therefore, there is need in depth research for understanding the mechanisms of volatile compounds metabolism during durian pulp lactic fermentation.

Keywords: *Durio zibethinus*, tempoyak, volatile, flavor, ester, thioester, trithiolane and thiols

Introduction

The exotic fruit durian, originating from tropical regions, has unique and exotic flavor. Stanton (1966) mentioned that durian has a mighty flavor which has been likened to strawberries, cheese, and garlic, while Baldry *et al*. (1972) observed that the odor of durian possesses two distinct notes, one being strong onion like and the other delicate and fruity. Among various durian varieties, sulfur containing volatiles were reported as the major volatile constituents, while esters were the predominant volatile that corresponded to the fruity odor. Organic acids (malic, citric, tartaric and succinic acid) were present in small quantity ranged from 0.76 to 1.78 g/kg in durian (Voon *et al*., 2006); hence they did not distinct enough to the flavor. The sour note was not detected in durian despite the presence of organic acids (Voon *et al*., 2007).

Studies have been carried out to verify the flavor of durian that varies according to the variety and region. Baldry *et al*. (1972) identified a total of 26 volatile constituents of durian fruits from Malaysia and Singapore, which is responsible for the onion like odor and fruity odor. The characteristic odor of fruits from Singapore was a mixture of esters and thioester, whereas fruits from Kuala Lumpur contained thiols rather than thioesters. Moser *et al*. (1980) found that Thailand durian contained eight sulfur compounds where diethyl disulfide and diethyl trisulfide predominate, while the non-sulfur components identified were mostly ethyl 2-methylbutanoate, 1-1-diethoxyethane and ethyl acetate. Wong and Tie (1995) identified 63 volatiles in different clones of durian from Malaysia with the major constituents as 3 hydroxy-2- butanone (32-33%), ethyl propionate (20%), and ethyl 2-methyl butanoate (15-22%). A total of 18 sulfur compounds from Indonesian durians were identified by Weenen *et al*., (1996) with the strongest durian odorant was 3,5 dimethyl-1,2,4-trithiolane and ethyl 2-methyl butanoate. Chin *et al*. (2007) discovered 39 volatile compounds comprising 22 ester, 9 sulphur-containing alkanes, 3 thioacetates, 2 thioesters,2 thiolanes and 1 alcohol on the three Malaysian durian varieties (D2,D24 and D101).

As a popular tropical fruit in South-east Asia countries, durian (*Durio zibethinus*) is usually consumed fresh of its pulp. However, durian fruit has limited shelf life at room temperature and soon turn into overripe that makes its quality become poor to be fresh consumed. In some regions, the poor quality and unconsumed durian pulp is normally processed under spontaneous fermentation. The fermented product is widely known either as tempoyak in both Malaysia

*Corresponding author.
Email: *yuliana_thp@unila.ac.id, netiyuliana@yahoo.com*

and Indonesia (Gandjar, 2000) or sour durian in Thailand. This fermented durian has distinctive durian smell and sour taste. Presence of organic acids in fermented durian such as lactic, malic and acetic acid (Yuliana, 2005; Yuliana and Garcia, 2009) led to decrease of pH of durian pulp from neutral to acidic pH.

Tempoyak is prepared by mixing of durian pulp with salt and placing in a sealed container to allow 4-7 days natural lactic acid fermentation (Battcock and Ali, 1998). Fermentation brings about the texture changes of durian pulp from a solid to a semisolid mass accompanied with a strong acid odor that's contributed to acid organics produced by lactic acid bacteria (LAB) (Yuliana, 2005). Generally, LAB are the bacteria included in the group are gram-positive, nonsporing, nonrespiring cocci or rods, which produce lactic acid as the major end product during the fermentation of carbohydrates (Axelsson, 2004). *Lactobacillus* was reported as predominant lactic acid bacteria (LAB) involved in fermented durian (Ekowati 1998; Leisner *et al*., 2001; Wirawati 2002; Leisner *et al*., 2002). The chemical composition of the durian fruit, with 11–13.55% of total sugar (Brown, 1997) mainly consisted of sucrose, fructose and glucose (Leisner *et al*., 2001; Voon *et al*., 2007) would support the growth of lactic acid bacteria (LAB). These three types of free sugars were used as substrates in durian fermentation (Leisner *et al*., 2001).

Although fermented durian (tempoyak) is recognized as product of lactic acid fermentation, the volatile flavoring constituents of this are largely unknown due to no analysis of the volatile constituents. There is little information regarding the volatile flavors composition of durian product that is processed under fermentation (Yuliana and Garcia, 2009). In this paper, the composition of the volatile flavor components of durian fermented by spontaneous lactic fermentation is described compared to fresh durian

Materials and Methods

Fermented durian preparation

Durian fruit cultivar of Atabrine was obtained from local market at Los Banos Philippines. Fermented durian was prepared in accordance with traditional method of fermentation. Durian pulp (200 g) was mixed with salt (3% by weight) and was placed it in sealed plastic containers. The durian was allowed to spontaneously static ferment during 8 days at room temperature (30^{o} C). Two replications were maintained in this experiment.

Extraction of sample

Selected samples were extracted using a head space solid phase micro extraction (SPME) method. Seventy-five gram of sample was put into an Erlenmeyer flask, sealed with aluminum foil with protective seal and fitted with a space solid phase micro extraction gastight syringe in such a way the syringe would be directed to just above the food sample. The flask was placed in a water bath at 50^{o}C with the syringe still be fitted. The volatiles released from the sample during 30 minutes at 50^{o}C were absorbed onto SPME. The volatile collected from samples were analyzed using a HP 5890A gas chromatograph connected to a HP 5970 mass selective detector (Hewlett Packard).

GC-MS was operated at 70 eV in the EI mode over the range 35-450 amu, column used was SPB-5 column, 30m x 0.25 mm, film thickness = 0.25µm, (Supelco, Sigma-Aldrich Co.). Carrier gas was helium at flow rate 1 ml/minute. The collected volatiles were thermally desorbed at 250^{o}C for 2 minute after desorption, the oven was heated rapidly to 60^{o}C and maintained at this temperature for 2 min before the temperature was increased at 10^{o}C min-1 to 220^{o}C (10 min). The constituents of samples were tentatively identified by matching their mass spectra with those recorded in the computer library (NIST98 and Wiley library). Data were analyzed as mean of two replications.

Results

Table 1 lists out the tentatively volatiles analyzed from fresh and fermented durian using GC-MS. A total of 53 volatile components consisted of 17 volatile sulfur compounds and 36 non-sulfur compounds were extracted from fermented durian, while those were 23 sulfur volatile components and 30 non-sulfur components from fresh durian. A total of 24 sulfur compounds such as thioester, disulfides, trisulfides, trithiolanes, and methylthio compounds were tentatively identified, while another 1 compound was unknown. None of the overall flavor recorded in computer library was met this identified peaks.

A number of 11 sulfur compounds included ethyl (1-methylpropyl) disulfide, N-dimethylthioisophinyl-3-amino; 3,6-dimethyl1,2,4,5 tetrathiaciclohexane, thiazolidine, cyclic octa atomic sulfur, dimethyl tetrasulfide, 1,1-bis(methylthio) ethane, 4,6dimethyl-12,3,5 tetrathiaciclohexane (I), 4-oxo-2 thiazolidin acetonitrile, dipropyl disulfide, dipropyl trisulfide, diethyl trisulfide were not detected in fermented durian.

Table 1. Sulfur compounds in fresh and fermented durian as analyzed by GC-MS

Sulfur Compounds	Fresh	Fermented
	Percent relative peak area	
diethyl trisulfide	21.17	*
unknown sulfur compound	19.50	8.24
N-dimethylthioisophinyl-3-amino	5.67	*
dipropyl trisulfide	4.77	2.39
3,5-dimethyl 1,2,4-trithiolane, (I)	3.91	6.51
ethyl n-propyl disulfide	3.53	3.70
diethyl disulfide	2.17	3.53
3-ethyl-5-methyl-1,2,4-trithiolane (I)	1.48	2.80
3-ethyl-5methyl-1,2,4-trithiolane (II)	1.83	3.27
dipropyl disulfide,	1.80	*
1-1-bis(ethylthio) ethane, I	1.46	4.12
4-oxo-2 thiazolidin acetonitrile	1.39	*
4,6dimethyl-12,3,5tetrathiaciclohexane (I)	1.00	*
1-1-bis(methylthio) ethane,	0.89	0.77
4,6dimethyl-1,2,3,5tetrathiaciclohexane (II)	0.69	0.89
1,1-bis(methylthio) ethane	0.58	*
dimethyl tetrasulfide	0.33	*
cyclic octa atomic sulfur	0.27	*
Thiazolidine	0.26	*
methyl ethyl disulfide	0.25	0.39
methyl propyl disulfide	0.23	0.38
ethyl(1-methylpropyl) disulfide	0.23	*
3,6-dimethyl1,2,4,5tetrathiaciclohexane	0.22	*
3,5-dimethyl 1,2,4-trithiolane, (II)	*	10.96
3-(methylthio)-2-butanone	*	1.60
1-1-bis(ethylthio) ethane, II	*	0.74
ethyl ester propane(dithioic) acid	*	0.51
N-methylthioacetamide	*	0.33

* indicate compounds not detected.

Likewise, 10 more non sulfur volatile compounds of durian fresh were not noticed in fermented durian which included methyl ester glycine, 2-tridecanone, 3,3,6 trimethyl-1,5-heptadien-4-one, oxine methoxy phenyl, 3-hydroxy-2-butanone, isopropyl tiglate, 3-hydroxy ethyl ester pentanoate, ethyl carprate/ethyl decanoate, propyl butyrate, decyl ester formic acid.

Beside disappearance of some flavor, new volatiles were noticed in fermented durian. Some ester, alcohol and carboxylic acid components

Table 2. Non-sulfur compounds in fresh and fermented durian as analyzed by GC-MS

Non-sulfur Compounds	Fresh	Fermented
	Percent relative peak area	
ethyl 2-methyl butanoate	4.14	8.04
propyl 2-methyl butyrate	3.01	6.06
ethyl propionate	2.01	1.80
propyl ester propanoate	1.28	0.50
methyl ester glycine	1.21	*
ethyl ester hexadecanoate	1.20	1.87
ethyl octanoate	1.14	3.16
ethyl tiglate	1.01	2.88
methyl ester hexadecanoate	0.92	1.06
nonyl alcohol	0.91	0.77
prophyl ester hexanoate	0.77	1.16
2-tridecanone	0.78	*
3,3,6 trimethyl-1,5-heptadien-4-one	0.58	*
2 methyl, methyl ester butanoate	0.57	0.89
oxine methoxy phenyl	0.57	*
ethyl ester butanoate	0.48	0.65
propyl caprilate/propyl octanoate	0.42	0.82
3-hydroxy-2-butanone	0.38	*
2- methyl 1-butanol	0.39	0.32
ethyl ester hexanoate	0.39	1.77
ethyl laurate/ethyl dodecanoate	0.24	0.37
methyl laurate	0.35	0.25
Isopropyl tiglate	0.34	*
3- hydroxy, ethyl ester pentanoate	0.32	*
ethyl caproate/ethyl decanoate	0.26	*
3- methyl 1-butanol,	0.24	0.49
propyl butyrate	0.23	*
ethyl 2 hexenoate	0.18	0.48
1-octanol	0.16	0.32
decyl ester formic acid	0.15	*
ethyl sorbate	*	1.90
chlorodiproyl-borane	*	1.59
ethyl ester decanoate	*	1.26
propyl ester acetate	*	0.95
3-hydroxy, ethyl ester hexanotae	*	0.77
methyl ester hepta-2,4-dienoate	*	0.71
3- methyl,prophyl ester butanoate	*	0.70
ethyl ester pentanoate	*	0.69
octanoic acid	*	0.65
azido cyclohexane,	*	0.55
1,3 butanediol	*	0.55
2,3-butanediol	*	0.50
2,8 diagaspirol(4,4)-nonane-1,9-dione	*	0.47
methyl ester octanoate	*	0.38
hexyl ester butanoate	*	0.34
methyl tiglate	*	0.27

* indicate compounds not detected.

not found on the fresh durian were present such as propyl ester acetic acid, ethyl butanoate, methyl tiglate, hexyl ester butanoate, ethyl ester pentanoate, octanoic acid, 3-methyl prophyl ester butanoate, ethyl ester hexanoate, 2,3 butanediol and 1,3 butanediol. Meanwhile, the five sulfur compounds generated only in fermented durian were 3,5-dimethyl 1,2,4-trithiolane (II), 3-(methylthio)-2-butanone, 1-1-bis(ethylthio) ethane (II), ethyl ester propane (dithioic) acid, and N- methylthioacetamide.

Discussion

The results indicated that some of the volatile flavor compounds changed substantially during fermentation (Table 1 and 2). Components not found on the fresh durian were produced on fermented durian, vice versa; the components found in the fresh durian especially the major sulfur compounds such as diethyl trisulfide, N-dimethylthioisophinyl-3-amino and dipropyl disulfide were disappeared. It is supposed that the components were either broken down or conversed to other components. Nevertheless, the non sulfur main flavor compounds responsible to the fruity aroma such as ethyl 2-methyl butanoate and ethyl propanoate were still present in fermented durian.

The changes of those flavor components were probably attributed to the significant role of microorganisms during fermentation mainly lactic acid bacteria and also the indigenous enzymes present in durian fruit that were still active. The presence of LAB as main microorganisms in fermented durian has been previously indicated by some authors (Leisner *et al*., 2001; Yuliana, 2004; Amin *et al*., 2004; Amiza, *et al*., 2006), and in this study (data not shown). Lactic acid bacteria influence the flavor of fermented foods in a variety of ways, for example by production of volatile flavor components, by reducing the activity or completely inactivate enzymes in the plant that generate either flavor components or flavor precursor compounds and by directly metabolize precursor flavor compounds or flavor components themselves (Mcfeeters, 2004).

Two main sugar fermentation pathways can be distinguished among LAB. Firstly, glycolysis (Embden-Meyerhof-Parnas pathway) results almost exclusively in lactic acid as the end product under standard conditions, and the metabolism is referred to as homolactic fermentation. Secondly, the 6-phosphogluconate/phosphoketolase pathway results in significant amounts of other end products such as ethanol, acetate, and CO_2 in addition to lactic acid (Salminen *et al*., 2004) depending on the involved species. Occurrence of lactic acid and acetate in fermented durian (tempoyak) has been reported by previous studies (Yuliana, 2005; Yuliana and Garcia, 2009). These organic acids were either probably further metabolized by other microorganisms or involved in another biochemical pathways which in turn generate the product flavor. It was noticed that even lactate, the end product of normal metabolism, can be fermented to acetate and CO_2 by some LAB (Axelsson, 2004). An examples of this is conversion of lactic acid to acetic acid and 1,2 propanediol by *Lactobacillus buchneri* (Elferink *et al*., 2001). It is, therefore, expected that conversion of sugar to lactic acid by LAB during durian fermentation might lead to lactic acid conversion to 2,3 butanediol and 1,3 butanediol which were only present in fermented durian. Moreover, the pathways leading to acetoin/2,3-butanediol is common among LAB (Axelsson, 2004)

Some LAB are able to synthesize esters via a tranferase reaction in which fatty acyl groups from gycerides were transferred to alcohols (Liu *et al*., 2003). Durian pulp is reported to have 3-5% fat (Brown, 1997) and thus their fatty acid may be considered as the precursors of the volatile esters. These may emerge to put in to some esters volatile compounds generation in fermented durian (Table 2).

LAB such as *Lactobacilli* is believed to play an important role in volatile sulfur compounds, and Lactobacillus was reported as predominant lactic acid bacteria (LAB) involved in fermented durian (Ekowati 1998; Leisner *et al*., 2001; Wirawati 2002; Leisner *et al*., 2002). Ability of LAB to generate the volatile sulfur compounds primarily arise from the biodegradation of the sulfur/carbon bond of amino acids (methionine or cysteine) by a transamination reaction and lyase pathway (Landaud *et al*., 2008) as extensively studied in cheeses, wine and beer flavor compounds production. Brwon (1997) showed that durian is a better source of all the essential amino acids including methionine and cysteine. These could probably play an important role in the volatile sulfur aroma production in fermented durian.

Other than LAB, *Bacillus megaterium* and fungi such as *Aspergillus* and *Penicillium* as well as yeast (genus of *Kluyveromyces*) were also found in some sample of fermented durian (Ekowati, 1998; Yuliana, 2005). Although the role of these fungi and yeast in tempoyak was not known, these microorganisms appear to contribute to the flavor of tempoyak. Non-mainly microorganisms have been reported to play a role in flavor formation in fermented foods. For instance, the 6,7,4 trihydroxyisoflavone (factor 2) is formed from soybean seed isoflavone by bacteria isolated from tempe (Klus *et al*., 1993), bitter taste in cheese is caused by protease activity of *Lactococcus lactis* subsp. *Lactis* (Broadbent *et al*., 2002), and the diversity of lactic acid bacteria influences wine quality (Rodas *et al*., 2003). Even, yeast are also of great importance for their ability to produce volatile sulfur compounds from methionine in the cheeses ripening (Landaud *et al*., 2008).

In some cases, fermentation microorganisms may not directly involved in the formation of flavor. The example of this has been proposed in commercial sauerkraut fermentations (Daxenbichler *et al*., 1980; Viander *et al*., 2003), where the sulfur compounds were generated from hydrolysis of glucosinolates in the raw cabbage by the enzyme that was not found in LAB. Similarly, the disappearance and generation of new sulfur compounds in tempoyak might be not directly microorganism involved but by a role of the enzymes present in the tissues of durian pulp that lead to precursor biochemical reaction. Previous study assumed that propyl-cysteinsulfoxides and ethyl-cystein-sulfoxides would be responsible as the precursors in the biogenesis of volatile sulphur compounds in durian (Chin *et al*., 2007). The formation of sulfur compounds was predominantly attributed to the specific pattern of non-volatile alkylcystein- sulfoxides in plants (Freeman and Whenham, 1975; Yu *et al*., 1989).

Among the non-sulfur compounds existing in fresh and fermented samples, ethyl 2-methyl butanoate was found in the highest amount (Table 2). This is in agreement with Weenen *et al*. (1996), who reported that 3-hydroxy-2- butanone was the most prominent in the case of Cane and Koclak variety of Indonesian durian, while the ethyl 2-methyl butanoate was the second most abundant compound. However, this compound was found to contribute most to the non-sulfur part of the durian flavor as analyzed by GC-Sniff flavor dilution analysis (Weenen *et al*., 1996). Moreover all five previously published studies indicated ethyl 2-methyl butanoate as a major constituent of durian volatile (Baldry *et al*., 1972; Moser *et al*., 1980; Wong and Tie, 1995; Voon *et al*., 2007; Chin *et al*., 2007). The second most abundant compound in Philippines durian (both fermented and fresh durian) was propyl 2-methyl butyrate, followed by ethyl octanoate, and ethyl propionate. Among the alcohols observed in fresh and fermented durian, nonyl alcohol was the predominant component (Table 2). This study indicated that there was no substantially change of the major non-sulfur flavor components during spontaneous fermentation of durian.

With regard to the sulfur compounds, all samples had a large amount of sulfur compounds. Diethyl trisulfide (21.179%) was the sulfur compound present in the highest amount in fresh durian, followed by unknown compounds (19.50% area). This compound is presented here as unknown compound because its mass spectrum had fewer matches with those recorded in computer library. The closer mass spectrum of standard with this compound was propane 1-(methylthio). Diethyl trisulfide as the predominant compound in durian was also reported in Thailand durian as studied in the headspace fraction by Moser *et al*. (1980). Weenen *et al*. (1996) did not find diethyl trisulfide in durian from Indonesia and they identified the S-ethyl thioacetate as the highest sulfur compound. Conversely, this compound was not found in this experiment. The tentatively sulfur compound in fermented durian present in the highest concentration in this experiment was 3,5-dimethyl-1,2,4 trithiolane. Wong and Tie (1995) identified the two 3,5-dimethyl-1,2,4 trithiolane isomers as major sulfur components in durian from Malaysia. In this experiment, the fermented durian was found to have both of components in high concentration. Although 3,5-dimethyl-1,2,4-trithiolane was reported to contribute to a strong durian note, their presence did not correlate well with the intensity of sulfur notes perceived in study reported by Chin *et al*. (2007).

Previous investigations, Baldry *et al*. (1972); Naf and Velluz (1996); Weenen *et al*. (1996) had disclosed that flavor volatiles of durian comprised of 2 major distinct odor characteristics, garlic and onion-like odor and sweet fruity odor. The first odor of durian pulp was contributed by sulfur-containing volatiles (mainly thiols, disulfides and trisulfides), while the second was mainly from esters (mainly ethyl 2-methyl butanoate and propanoic acid ethyl ester as well (Wong and Tie, 1995; Weenen *et al*., 1996). Chin *et al*. (2007) investigated that ethyl propanoate, methyl butanoate, propyl-2-methyl propanoate, ethyl-3-methyl-butanoate, were highly correlated with the sweet and fruity notes of different durian cultivars. In this study, the number of sulfur compounds in fermented durian is lower than those in fresh durian and the number of non sulfur compounds in particularly esters in fermented durian is higher number than those in fresh durian, as well as no qualitatively substantial change of the major non-sulfur flavor components (ethyl 2-methyl butanoate) during spontaneous fermentation of durian. Nevertheless, the general flavor of fermented durian was relatively unpleasant, lack of fruity odor with distinctive aroma. This could be as the concentration of the non sulfur compounds responsible to fruity aroma was probably at a level where it does not result in significant aroma over the sulfur compounds. Although the major sulfur flavor of durian especially trisulfide that responsible to onion odor was disappeared, at the same time, there was generation of new volatile in fermented durian assumed to be responsible for unpleasant fermented durian aroma. For example, hydrolysis of fatty acid ester may produce the fatty acid that has unpleasant flavor. Octanoic acid that was present only in fermented durian has rancid fat aroma (Tabel 2).

Conclusions

Spontaneous lactic fermentation affected the volatile flavor constituents of durian. The flavors composition of fermented durian were fewer sulfur compounds compared to fresh durian due to disappearance of the major sulfur compounds in fresh durian such as diethyl trisulfide, N-dimethylthioisophinyl-3-amino and dipropyl disulfide, and generation of some alcohol, ester and carboxylic acid components in fermented durian. There was also no substantially change of the major non sulfur compounds responsible to the fruity aroma such as ethyl 2-methyl butanoate and ethyl propanoate, however there were absence of 6 flavor volatile ester. Nevertheless, the flavor of fermented durian was relatively not nice and lack of fruity aroma. This is because of the composition of the flavor components in fermented durian may have to possess a not well-balanced fruity and sulfur like aroma. This finding suggests that there is need in depth understanding and controlling the mechanisms of volatile compounds synthesis, in particularly the sulfur compounds, during durian pulp lactic fermentation.

Acknowledgments

The authors would like to thank SEARCA for the financial support in this study, the IRRI for use of GC-MS, and Mrs. Rosario R. Jimenez for her assistance in GC-MS measurements.

Rererences

Axelsson, L. 2004. Lactic Acid Bacteria: Classification and Physiology. In: Salminen, S., von Wright, A and Ouwehand, A (Eds).Lactic Acid Bacteria Microbiological and Functional Aspects. Third Edition, Revised and Expanded. New York: Ed Marcel Dekker Inc.

Amin, M.A., Zakiah, J. and Khim, Ng. L. 2004. Effect of salt on tempoyak fermentation and sensory evaluation. Journal of Biology Science 4(5): 650-653.

Amiza, M.A, Zakiah, J., Khim, N. L. and Lay, K.W. 2006. Fermentation of tempoyak using isolated tempoyak culture. Research Journal of Microbiology 1 (3): 243-254

Baldry, J., Dougan, J. and Howard, G.E. 1972. Volatile flavoring constituents of durian. Phytochemistry 11: 2081-2084

Battcock, M. and Ali, S.A. 1998. Fermented fruits and vegetables, A global perspective. FAO Agric Services Bulletin No 134. Rome, Italy.

Brown, MJ. 1997. *Durio* - A Bibliographic Review. Pusa Campus New Delhi, India: International Plant Genetic Resources Institute.

Broadbent, J.R., Barnes, M., Brennand, C., Strickland, M., Houck, K., Johnson, M.E. and Steele, J. L. 2002. Contribution of *Lactococcus lactis* cell envelope proteinase specificity to peptide accumulation and bitterness in reduced-fat cheddar cheese. Journal of Applied Environmental Microbiology 68:1778-1785.

Chin, S.T., Nazimah,S.A.H., Queck,S.Y.,Che-Man,Y.B., Abdul Rahman, R. and Hashim, D. M. 2007. Analysis of volatile compounds from Malaysian durians (*Durio zibethinus*) using head space SPME coupled to fast GC-MS. Journal of Food Composition and Analysis 20: 31-44

Daxenbichler, M.E., Van Etten, C.H., and Williams, P.H. 1980. Glucosinolate products in commercial sauerkraut. Journal of Agricultural and Food Chemistry 28:809-811.

Ekowati, C.N. 1998. Mikroflora pada fermentasi daging buah durian (tempoyak). Jurnal Sains dan Teknologi Edisi Khusus 136-141.

Elferink, S.J.W.H.O., Krooneman,J., Gottschal, J.C., Spoelstra,S.F., Faber, F and Driehuis, F. 2001. Anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol by *Lactobacillus buchneri*. Journal of Applied and Environmental Microbiology 67:125-132

Freeman, G.G. and Whenham, R.J., 1975. A survey of volatile components of some *Allium* species in terms of S-Alk-(en)-L-cysteine Sulphoxides present as flavour precursors. Journal of the Science of Food and Agriculture 26 (12): 1869–1886

Gandjar, I. 2000. Fermentations of the Far East. In: Robinson, R.K., Batt, C.A., Patel, P.D. (Eds.). Encyclopedia of Food Microbiology,Vol. 2. pp. 767–773, New York, London: Academic Press

Klus, K., Borger-Papendorf, G. and Barz, W. 1993. Formation of 6,7,4 trihydroxyisoflavone (factor 2) from soybean seed isoflavone by bacteria isolated from tempe. Phytochemistry 34(4):979-981.

Leisner, J.J., Vancanneyy, M., Rusul, B., Lefebre, K., Pot, B., Lefebvre, K., Fresi, A. and Tee, L. K. 2001. Identification of lactic acid bacteria constituting the predominating micro flora in an acid-fermented condiment (tempoyak) in Malaysia. International Journal of Food Microbiology 63 (1-2):149-157

Leisner, J. J., Vancanneyt, M., Lefebvre, K., Vandemeulebroecke, K., Hoste, B., Vilalta, N.E., Rusul,G and J. Swings, J. 2002. *Lactobacillus durianis* sp nov, isolated from an acid-fermented condiment (tempoyak) in Malaysia. International Journal of Systematic and Evolutionary Microbiology 52: 927-931.

Liu, S-Q., Holland, R. and Crow, V.L. 2003. Ester synthesis in an aqueous environment by *Streptococcus thermophilus* and other dairy lactic acid bacteria. Journal of Applied Microbiology and Biotechnology 63: 81-88.

Mcfeeters, R.F. 2004. Fermentation microorganisms and flavor changes in fermented foods. Journal of Food Science 69 (1):35-37.

Moser, R., Duvel, D. and Greve, D. 1980. Volatile constituents of durian (*Durio zibethinus Murr*). Phytocemistry 19 (1): 79-81.

Naf, R., and Velluz, A. 1996. Sulfur compounds and some uncommon esters in durian (*Durio zibethinus Murr*.). Flavour and Fragrance Journal 11(5): 295-303

Rodas, A. M., Ferrer, S., Pardo, I. 2003. 16S-ARDRA: a tool for identification of lactic acid bacteria isolated from grape must and wine. Systematic and Applied Microbiology 26 (3):412-422.

Salminen, S., von Wright, A and Ouwehand, A. 2004. Lactic Acid Bacteria Microbiological and Functional Aspects. Third Edition, Revised and Expanded. New York: Ed Marcel Dekker Inc.

Stanton, W.R. 1966. The chemical composition of some tropical food plants: durian. Tropical Science 8: 6-10.

Viander, B., Mäki, M. and Palva, A. 2003. Impact of low salt concentration, salt quality on natural large-scale sauerkraut fermentation. Food Microbiology 20 (4):391-395.

Voon, Y. Y., Abdul Hamid N. Sheikh, Rusul G. and Osman A. 2006. Physicochemical, microbial and sensory changes of minimally processed durian (*Durio zibethinus* cv. D24) during storage at 4^0C and 28^0C. Postharvest Biology and Technology. POSTEC 8493

Voon, Y.Y., Hamid, N.S.A., Rusul, G., Osman, A. and Queck, S.Y. 2007. Characterization of Malaysian durian (*Durio zibethinus Murr.*) cultivars: relationship of physicochemical and flavour properties with sensory properties. Journal of Food Chemistry 103: 1217-1227.

Wirawati, C.U. 2002. Potensi bakteri asam laktat yang diisolasi dari tempoyak sebagai probiotik. Bogor, Indonesia: Institut Pertanian Bogor, MSc Thesis.

Wong, K.C. and Tie, D.Y. 1995. Volatile constituents of durian (*Durio zibethinus Murr*). Flavor and Fragrance Journal 10(2): 79-83.

Weenen, H., Koolhaas, W.E. and Apriyantono, A. 1996. Sulfur-containing volatile of durian fruits (*Durio zibethinus Murr*). Journal of Agricultural and Food Chemistry 44(10): 3291-3293.

Yu, T.H., Wu, C.M., and Liou, Y.C., 1989. Volatile compounds from garlic. Journal of Agricultural and Food Chemistry 37(3):725–730.

Yuliana, N. 2004. Biochemical change in fermented durian (*Durio zibethinus Murr*)., University of the Philippines Los Banos, Laguna, Philippines: Ph.D. Thesis

Yuliana, N. 2005. Organic acids component of tempoyak (fermented durian). Jurnal Teknologi dan Industri Pangan XVI : 90-95.

Yuliana, N. and Garcia, V.V. 2009. Influence of *Pediococcus acidilactici* UP 02 as a starter on the flavor of fermented durian (tempoyak). Indian Journal of Biotechnology 8(3):304-310.