Food Sci. Biotechnol. Vol. 18, No. 5, pp. 1071 ~ 1075 (2009)

Food Science and Biotechnology

Microencapsulation Effects of Allyl Isothiocyanate with Modified Starch Using Fluidized Bed Processing

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Abstract Allyl isothiocyanate (AI), a volatile compound of mustard, has excellent antimicrobial effects, but its volatility hinders its wide usage as an ingredient of food products. Microencapsulation technique, therefore, was applied for delaying the release time of AI. For delaying the release time of AI, the mustard powder, which contained AI, was microencapsulated with 5% modified starch by using fluidized bed processing. The efficiency of the controlled release of AI at various pH was analyzed by the head space (HS) analysis and solid phase microextraction (SPME) method using gas chromatography (GC). Also, modified starch encapsulated powder was added into *kimchi* for applying in food industry. As the result, the release time of AI was delayed by microencapsulation with modified starch and the higher pH could be the faster release of AI. Also, the period until the pH values and total acidity of *kimchi* reached up to 4.5 and 0.6%, which give its malsour taste, was extended by microencapsulation. These results showed that modified starch encapsulated powder could prolong the preservation in food system.

Keywords: microencapsulation, ally isothiocyanate (AI), controlled release, modified starch, fluidized bed processing

Introduction

The glucosinolate in mustard powder release the allyl isothiocyanate (AI) by enzymatic reaction such as myrosinase at aqueous status (1). The released AI has antimicrobial activities at several microorganisms such as *Lactobacillus plantarum*, *Lactobacillus brevis*, *Pediococcus cerevisiae*, *Leuconostoc mesenteroides*, and Vibrio (2-4). Among them, the *Leuc. mesenteroides* and *L. plantarum* are the major microorganisms during *kimchi* fermentation. The *Leuc. mesenteroides* grew at early fermentation stage and gave delicious sour taste. However, *L. plantarum* grew at latter fermentation stage and gave malsour taste (5). Therefore, the fermentation by *L. plantarum* has to be delayed. At the latter fermentation, AI can be used for antimicrobial substance. However, because the AI is very volatile, a delaying of the release time of AI is needed until start of the latter fermentation. Microencapsulation of volatile components in solid matrixes allows them to be handled as free flowing powders with limited evaporation of volatile components prior to their usage (6).

For encapsulation, the coating may be selected from among cellulose derivatives, dextrins, emulsifiers, lipids, protein derivatives, and starch derivatives. Especially, the carbohydrates such as hydrolyzed starches, emulsifying starches, and gums are the most common coating materials (7). However these carbohydrate materials are hydrophilic and then they have little affinity for hydrophobic materials such as oils and aromas. Therefore, for altering the hydrophilic nature, the starch was modified with *n*-octenyl succinic anhydride (8). The hydrophobic octenyl side chains also impart emulsifying capability to the starches. These modified starches have been successfully optimized for flavor encapsulation (9) and encapsulation of nutraceutical monoterpenes (10).

Though the coating may be done well, the efficacy of encapsulated volatile compounds should be detected. Detection of the volatile component from encapsulated powder is not easy because of their soluble characteristics. Therefore, the selection of media for releasing volatile compounds is considered. After selection of media for releasing volatile compounds, the volatile compound is separated from matrixes. One of the separation methods is head space analysis (HSA). HSA is widely used because of its relatively low cost per analysis, simple sample preparation, and the elimination of reagents. However, for very low levels of analyte concentration in the original sample material, HAS techniques may lack the sensitivity required for the determination (11). For overcoming the disadvantages of HSA, solid phase microextraction (SPME) was developed by Pawliszyn's group in the early 1990's. Jelen *et al*. (12) reported that a good agreement between HSA and SPME determination of alcohols and esters in beer. Also they mentioned that SPME could be improvement of sensitivity and exposure time due to a variety of fiber coating developed when relatively compared with HSA. SPME is a relatively novel, solventless method of volatile extraction from gaseous, solid, or liquid phase (13-16). Although it was developed for the analysis of pollutants in water, it has been successfully utilized for analyzing many food substances and flavors (17-19).

In this study, for delaying the volatility of AI, the mustard powder, which contained AI, was microencapsulated with modified starch using fluidized bed processing. And the release times of AI of the mustard powders (MP) and microencapsulated mustard powders (MSEP) were analyzed by using HSA. And the pH stability of MSEP was analyzed by using SPME. Also, the MP and MSEP were added into *kimchi*, then, the pH, acidity, and the sensory

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Received November 28, 2008; Revised February 24, 2009;
Accepted April 29, 2009

characteristics such as sour taste and mustard flavor were analyzed.

Materials and Methods

Microencapsulation of allyl isothiocyanate (AI) The mustard powder was encapsulated with 5% modified starch using Fluidized Bed Processor (Model GRE Lab 1; GR Eng. Co., Gyeonggi, Korea). The modified starch (sodium octenyl succinate modified starch) was obtained from Samyang Genex (Daejeon, Korea). The operating conditions by using fluidized bed processor were followed as feeding rate, 0.6 mL/min; inlet air temperature, 65°C and outlet air temperature, 40°C.

Sample preparation To equilibrate the AI, each 0.1 g of mustard powder (MP) and microencapsulated mustard powder (MSEP) was put in 5-mL vial capped with septum. The volume was up to 1 mL with pH adjusted solution, which prepared to pH 4, 5, and 6 with NaOH citrate buffers. Then, the vial capped with Teflon lined cap equipped septum. And the prepared samples were stirred for 1, 2, 3, 5, 7, 10, 15, 30, and 60 min, respectively.

Head space analysis (HAS) The headspace gas of each sample, which was stirred for 1, 2, 3, 5, 7, 10, 15, 30, and 60 min, were analyzed on gas chromatograph (MD606; Youngin Co. Ltd., Gyeonggi, Korea) equipped with splitless mode injector and flame ionized detector (FID). AI was resolved on a Supelcowax (Supelco, Bellefonte, PA, USA) capillary column (30 m×320 μm i.d.×1 μm) in the following parameters: initial oven temperature was 40°C kept for 1 min, then raised at 10°C/min to 210°C and kept for 10 min at 210°C. Injection port temperature was kept at 220°C, and carrier gas (nitrogen) flow rate was 2.0 mL/min. Detector temperature was 250°C. Samples were injected 1 mL of equilibrium head space gas by means of the 5-mL gas tight syringe (20). The results were represented as the mean value of release rate. The release rate was calculated as the following equation.

Release rate=(Area of released AI/Area of total AI in mustard powder)×100 (When the released AI in mustard powder was retained the constant amount, it was represented as 100% release rate)

SPME headspace analysis Supelco SPME fiber holder (manual) and 65-μm polydimethyl siloxane divinylbenzene coated fiber (Supelco) were used for the SPME analysis. Before using, the fiber was preconditioned in the gas chromatography (GC) injection port at 250°C for 30 min. When samples were analyzed using the SPME method, a 0.9-mL single taper liner used in the headspace method was replaced with a narrow 1-mm i.d. direct liner. The same chromatographic parameters were used as in the HSA method except injection port temperature 240°C. The AI released from each sample was absorbed into SPME fiber for 10 min at room temperature and the SPME fiber was desorbed at injection port for 5 min. The results were represented as the mean value of release rate.

Preparation of *kimchi* The nappa cabbage purchased from a market place was sliced into a 3×4 cm size piece, and then marinated for 4 hr in 10% salt water. After that, the salted nappa cabbage were mixed with spices such as garlic paste, dried capsicum powder, ginger paste, fish sauce, thin sliced radish, and sugar. Three types of *kimchi* were made as follows: no mustard powder (control), 0.5 g MP added and 0.525 g MSEP added/100 g nappa cabbage during the mixing time. Then, the 3 types of *kimchi* were packed in a vacuum with polyvinyl bag and stored at 10°C.

pH and acidity Two-hundred g of each type of *kimchi* were homogenized and filtered through gauze. Twenty mL of each filtrate was measured the pH using pH meter (DP215M; Dongwoo Medical System, Gyeonggi, Korea). Twenty mL of each filtrate was used for evaluating the total acidity. The acidity was represented as lactic acid concentration (%).

Sensory evaluation

***Panel selection and training*:** For sensory evaluating the *kimchi*, the quantitative descriptive analysis (QDA™) was used (21). During the study, panelists evaluated *kimchi* samples/session, with the typical session being 60 min. Also, the panelists were asked to quantify differences among the samples in descriptive terms on a scale of 0 to 15. Twelve trained panelists completed 18 hr the training for identifying and rating the sour taste and mustard flavor in *kimchi*.

***Sample presentation and experimental design*:** Sour taste and mustard flavor of *kimchi* stored at 10°C were evaluated using 15 cm line scale, which anchored 'not at all' to 'extremely'. Samples were served in 3 random code numbers. Mustard flavor was evaluated first by requesting panelists to open glass vials (25-mL filled containers), sniffing twice, recapping, and then marking the mustard odor intensity on the appropriate score card. After flavor evaluation was completed, panelists were served *kimchi* samples (5 pieces of *kimchi* served in plain glass dishes) in a random order and panelists rated the sour taste. Panelists were instructed to spit out the samples, rinse mouth with deionized water, and eat unsalted crackers between each sample. References were provided for all the sensory attributes. Values were reported in centimeters from the 'not at all' end. Triplicates of all samples were presented, evaluated, and averaged.

Statistic analysis The data were analyzed by analysis of variance (ANOVA) and least significant difference (LSD) using SPSS program (ver. 10; SPSS Com, Chicago, IL, USA) at $p<0.05$.

Results and Discussion

Microencapsulation efficiency In this study, mustard powder, which contains AI, was microencapsulated with modified starch. The modified starch has hydrophobic part such as octenyl group and hydrophilic part such as sodium and hydroxyl group. The AI might be encapsulated in hydrophobic part during fluidized bed processing. For evaluating the microencapsulation efficiency, the AI has to release from microencapsulation structure. For releasing the AI, the microencapsulated modified starch structure

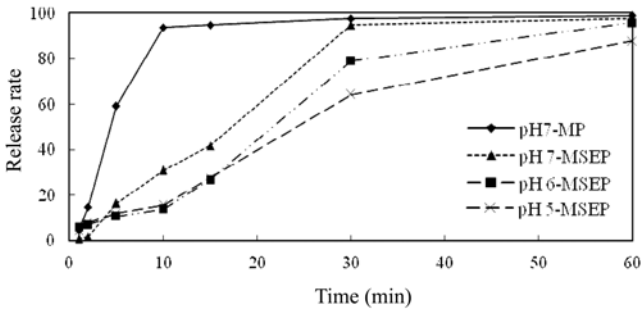

Fig. 1. Relative comparison of the release rate of AI in mustard powder (MP) and microencapsulated mustard powder (MSEP) using HSA analysis.

was broken by physical method. So the microencapsulated mustard powder was mixed with water, and stirred for more releasing the AI. After that the released headspace AI was analyzed using GC.

***HSA*:** HSA is used worldwide, being both reliable and predisposed to automatization. Gunning *et al*. (6) was analyzed the volatile components from microencapsulated flavor in carbohydrate matrixes by using headspace analysis. In this study, HSA was performed for deciding the stirring time in water. And the results were presented in Fig. 1. The AI from MP at pH 7 was almost released after 10 min stirring. That of MSEP at pH 7 was almost released after 30 min stirring. When it was stirred for 5 min, the release of AI was not enough to compare between MP and MSEP. When it was stirred for 10 min, the release of AI was enough to compare. Therefore, 10 min stirring time was decided to compare the release rate of AI among samples.

***SPME analysis*:** In this study, HAS was just used for determining the release rate of AI. And the measurement of pH stability was performed using SPME. During 10 min of stirring, the head space gas was absorbed into polydimethyl siloxane divinylbenzene coated fiber and then the absorbed material was released in high temperature at injection port (22). The release rate of AI was relatively compared to total AI content in headspace for 10 min of stirring. The results were presented in Table 1. In Table 1, the release rate of MSEP was shown 12.2, 12.6, 15.6, and 36.1% at pH value 4, 5, 6, and 7, respectively. That of MP was shown 61.0, 67.4, 77.9, and 98.0%, respectively. The

Table 1. Release rate of AI in mustard powder (MP) and microencapsulated mustard powder (MSEP) at various pH values using SPME analysis

	pH 4	pH 5	pH 6	pH 7
MP	[1)a2)]61.0±5.4[c3)]	[a]67.4±5.1[bc]	[a]77.9±1.9[b]	[a]98.0±3.1[a]
MSEP	[b]12.2±5.1[b]	[b]12.6±4.5[b]	[b]15.6±7.0[b]	[b]36.1±2.1[a]

[1)]Means with the different letter are significantly different at the same column (n=3, p<0.05).
[2)]Mean±SD of release rate.
[3)]Means with the different letter are significantly different at the same row (n=3, p<0.05).

release rate of AI in mustard powder (MP), even though not microencapsulated, showed that the lower pH values were the lower the release rate. The MP itself might delay the release time because the mustard powder was enzymatically decomposed by myrosinase (1). Furthermore, the microencapsulation may delay the release rate of AI more. These results supposed that microencapsulation could delay the release rate of AI and the lower pH could reduce the release of AI. Therefore, the AI microencapsulated with modified starch could be used more effectively at the lower pH value foods for delaying the release time.

Application results of MSEP in *kimchi* The AI, which is the main volatile component in mustard, is an effective antimicrobial material. However, its usage in food industry is limited because of its high volatility. For overcoming the volatility, Chacon *et al*. (2) encapsulated the AI with gum acasia and added it to chopped beef. They mentioned that AI microencapsulated with gum acasia could be used in chopped beef to reduce or eliminate large number of *Escherichia coli* O157:H7. In this study, the mustard was microencapsulated with modified starch. And the MSEP was applied at *kimchi* for elongation of storage time. And the elongation effects of the *kimchi* storage time were analyzed based on the pH, acidity, and sensory characteristics.

***pH and titrable acidity*:** Three types of *kimchi*, with no mustard powder (control), mustard powder (MP), and modified starch encapsulated mustard powder (MSEP), were made for evaluating the extension of the storage time. The results were presented in Fig. 2. The pH values and total acidity for the adequate delicious sourness was 4.2-4.5

Table 2. Sensory comparison of the *kimchi* mixed with no mustard powder (control), mustard powder (MP), and microencapsulated mustard powder (MSEP) stored at 10±1°C

		Storage period (day)									
Terms	Treatment	0	2	5	8	10	11	12	14	20	22
Sour taste	Control	[1)ab2)]1.88±0.12[d3)]	[a]2.00±0.43[d]	[b]2.33±0.39[d]	[a]6.66±0.89[c]	[a]7.33±0.76[bc]	[a]7.55±0.99[bc]	[a]8.77±0.79[b]	[a]11.11±0.89[a]	[a]11.21±0.86[a]	[a]11.7±0.78[a]
	MP	[a]2.22±0.43[f]	[a]2.44±0.12[f]	[a]3.22±0.54[ef]	[b]4.66±0.49[e]	[ab]6.33±0.76[d]	[b]6.77±0.94[d]	[b]7.55±1.13[c]	[b]9.55±1.37[b]	[b]9.88±0.98[b]	[a]11.44±1.02[a]
	MSEP	[b]1.55±0.32[h]	[a]2.55±0.14[g]	[b]2.22±0.64[g]	[b]4.77±0.34[f]	[b]5.55±1.21[e]	[b]6.44±0.98[d]	[b]7.55±1.28[c]	[c]7.77±0.87[c]	[c]8.88±1.34[b]	[b]9.94±0.87[a]
Mustard taste	Control	[c]2.66±0.23[b]	[c]3.44±0.34[a]	[b]2.66±0.54[b]	[a]2.66±0.45[b]	[a]1.88±0.32[b]	[a]1.73±0.12[b]	-	-	-	-
	MP	[a]4.66±1.21[a]	[b]4.2±0.98[a]	[a]3.44±0.65[ab]	[a]2.88±0.55[b]	[a]2.22±0.65[bc]	[a]1.44±0.32[c]	-	-	-	-
	MSEP	[b]3.33±0.89[ab]	[a]5.00±1.11[a]	[a]3.11±0.76[b]	[a]2.88±0.76[b]	[a]2.44±0.87[bc]	[a]1.77±0.26[c]	-	-	-	-

[1)]Means with the different letter are significantly different at same column (n=12, p<0.05).
[2)]Means±SD of values of sensory strength.
[3)]Means with the different letter are significantly different at same row (n=12, p<0.05).

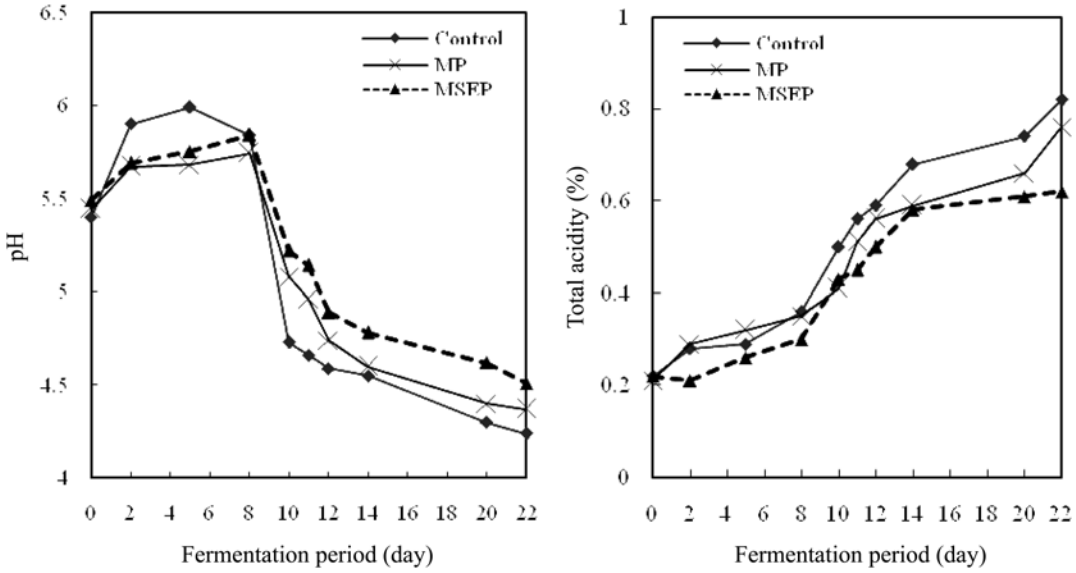

Fig. 2. The change of pH and total acidity of *kimchi* mixed with no mustard powder (control), mustard powder (MP) and encapsulated mustard powder (MSEP) stored at 10±1°C.

and 0.5-0.6%, respectively (5). In our study, the pH values of control and MP were decreased to 4.5 after 10 and 14 day fermentation, respectively. However, that of MSEP was retained to 4.5 after 20 day. The acidity of control and MP were increased to 0.6% after 12 and 14 day fermentation, respectively. However, that of MSEP was retained to 0.6% after 20 day. These results mean that microencapsulation could delay the fermentation of *kimchi* more.

***Sensory evaluation*:** The trained panelists evaluated the sour taste and mustard flavor. When the average sensory value of sour taste of *kimchi* is over 10, the panelists evaluate that the *kimchi* is highly acceptable. In this study, the average sensory value of sour taste of *kimchi* as control was over 10 after 14 day. Those of the *kimch* treated with MP and MSEP were over 10 after 20 and 22 day, respectively. These results could explain that the mustard powder itself could delay the *kimchi* fermentation; however, microencapsulation could delay the fermentation of *kimchi* more.

Pechacek *et al*. (23) mentioned that AI is an exceptionally organolepic compound, a strong lachrymator, and a vesicant. Nevertheless, this compound is used as flavoring agent for imitation of mayonnaise and a flavor fortifier for mustard and horseradish. Especially, in *kimchi*, the AI is not totally negative in consumer acceptance, but too strong AI flavor can negatively affect. Therefore, the mustard flavor was evaluated. In this study, the panelists evaluated the mustard flavor as the low recognition. Furthermore, after 8 days, the sensory values were presented as below 2. This value could explain recognizing threshold terms, which could be referred to sensory evaluation sheet. Therefore, using the mustard in *kimchi* may not affect overall consumer acceptance.

As the results, microencapsulation with modified starch could delay the release rate of AI and the higher pH could fasten the release of AI. These results supposed that the AI microencapsulated with modified starch could be used more effectively at the lower pH value foods for delaying the release time. And MSEP could delay the fermentation of *kimchi* and using the mustard in *kimchi* may not affect overall consumer acceptance. As a conclusion, microencapsulation could delay the release of AI; however, the MSEP is more adjustable when it is used in lower pH foods.

References

1. Cole RA. AIs, nitriles, and thiocyanates as products of autolysis of glucosinolates in Cruciferae. Phytochemistry 15: 759-762 (1976)
2. Chacon PA, Buffo RA, Holly RA. Inhibitory effects of microencapsulated allylisothiocyanate (AIT) against *Escherichia coli* O157:H7 in refrigerated, nitrogen packed, finely chopped beef. Int. J. Food Microbiol. 107: 231-237 (2006)
3. Hong WS, Yoon S. The effects of low temperature heating and mustard oil on the *kimchi* fermentation. Korean J. Food Sci. Technol. 21: 331-337 (1989)
4. Seo KI, Park SK, Park JR, Kim HC, Choi JS, Shim KH. Changes in antimicrobial activity of hydrolyzate from mustard seed (*Brassica juncea*). J. Korean Soc. Food Nutr. 25: 129-134 (1996)
5. Kim KK, Kim SY, Lee JK, Noh BS. Effects of xylose and xylitol on the organic acid fermentation of *kimchi*. Korean J. Food Sci. Technol. 32: 889-895 (2000)
6. Gunning YM, Gunning PA, Kemsley EK, Parker R, Riing SG, Wilson RH, Blake A. Factors affecting the release of flavor encapsulated in carbohydrate matrixes. J. Agr. Food Chem. 47: 5198-5205 (1999)
7. Reineccius GA. Carbohydrates for flavor encapsulation. Food Technol. -Chicago 45: 144-149 (1989)
8. Qi ZH, Xu A. Starch based ingredients for flow encapsulation. Cereal Food World 44: 460-465 (1999)
9. Buffo R, Reineccius GA. Optimization of gum acacia/modified starch/maltodextrin blends for the spray drying of flavors. Perfume Flavor. 25: 45-54 (2000)
10. Mourtzinos I, Kalogeropoulos N, Paradakis SE, Konstantinou K, Karathanos VT. Encapsulation of nutaraceutical monoterpenes in β-cyclodextrin and modified starch. J. Food Sci. 73: 89-94 (2008)
11. Wampler T, Thomas P. Analysis of food volatiles using headspace-gas chromatographic techniques. pp 27-58. In: Techniques for Analyzing Food Aroma. Marsili R (ed). Marcel Dekker Inc., New

York, NY, USA (1997)
12. Jelen HH, Wlazly K, Wasowicz E, Kaminski E. Solid phase microextraction for the analysis of some alcohols and esters in beer: Comparison with static headspace method. J. Agr. Food. Chem. 46: 1469-1473 (1998)
13. Pawliszyn J. Solid-phase Microextraction: Theory and Practice. Wiley-VCH: New York, NY, USA (1997)
14. Arthur CL, Pawliszyn J. Solid-phase microextraction with thermal desorption using fused silica optical fibers. Anal. Chem. 62: 2145-2148 (1990)
15. Zhang Z, Pawliszyn J. Headspace solid-phase microextraction. Anal. Chem. 65: 1843-1852 (1993)
16. Ibeanez E, Bernhard R. Solid-phase microextraction of pyrazines in model reaction system. J. Sci. Food Agr. 72: 91-96 (1996)
17. Mills GA, Walker W. Headspace solid-phase microextraction procedures for gas chromatographic analysis of aromatic and medicinal plants. J. Chromatogr. A 902: 267-287 (2000)
18. Lecanu L, Ducruet V, Jouquand C, Gratadoux JJ, Feigenbaum A. Optimization of head space solid phase microextraction (SPME) for the odor analysis of surface-ripened cheese. J. Agr. Food Chem. 50: 3810-3817 (2002)
19. Baek HH, Kim HJ. Solid phase microextraction-gas chromatography-olfactometry of soy sauce based on sample dilution analysis. Food Sci. Biotechnol. 13: 90-95 (2004)
20. Arvisenet G, Voilley A, Cayot N. Retention of aroma compounds in starch matrices: Competitions between aroma compounds toward amylase and amylopectin. J. Agr. Food Chem. 50: 7345-7349 (2002)
21. Stone H, Sidel JL. Descriptive analysis. Chap. 6, pp. 202-242. In: Sensory Evaluation Practices. 2nd. Tayler SL (ed). Academic Press, Inc., New York, NY, USA (1997)
22. Dauria M, Mauriello G, Racioppi R. SPME-GC-MS analysis of horseradish (*Armoracia rusticana*). Ital. J. Food Sci. 4: 487-490 (2004)
23. Pechacek R, Velisek J, Hrabcova H. Decomposition products of allyl isothiocyanate in aqueous solutions. J. Agr. Food Chem. 45: 4584-4588 (1997)