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A Study on the Analysis of Volatile Flavour of Kimchee

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김치 휘발성 향기성분의 분석 방법에 관한 연구

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Abstract : Flavours in kimchee are the result of unique combination of various sugars, organic acids and amino acids as well as various volatile organic compounds including sulfur-containing compounds, terpenes, alcohols, and some volatile organic acids. In the experiment for the flavour extracting methods, dynamic headspace(DHS) is more effective for collection of volatile flavour than simultaneous distillation extraction(SDE). The best polarity available at the moment is 5% phenyl methyl poly-siloxane which will separate non-polar, intermediate and polar components with good resoultion.

요약 : 김치의 향미는 당, 유기산, 유리아미노산 및 휘발성 향기성분들의 독특한 조합에 의한 것이며, 휘발성 향기성분은 함황화합물, 알코올류, 휘발성 유기산 및 터핀유 등 여러 가지 극성 및 비극성 성분들이 매우 복잡하게 혼합되어 있다. 김치 휘발성 향기성분의 분석조건을 검토한 결과 추출 분리에는 연속식 추출장치보다 dynamic headspace concentration법이 효율적이었고 GC에 의한 분석에는 5% phenyl methyl polysiloxane 컬럼에 의한 분리가 우수하였다.

Key words : kimchee, kimchi, flavour, analysis, headspace, column

INTRODUCTION

Kimchee, one of the representative indigenous food in Korea, is the general name given to a group of fermented acid vegetable food with a long tradition in Korea. Kimchee fermentation is a very complex process carried out by the growth of a sequence of heterofermentative and homofermentative lactic acid bacteria. The fermentation of kimchee by a sequence of microflora has been confirmed by many researchers.[1~4] It was found that the earliest stages of kimchee fermentation are dominated by *Leuconostoc mesenteroides* and completed by *Lactobacillus plantarum* and *L. brevis*.[2]

Tressel *et al.*[5] have summarized some of the reactions which microorganisms may accomplish. The reactions catalyzed by microbes are oxidation, reduction, isomerisation, esterification, hydrolysis, acylation, trans-glycosidation, methylation conden-

sation, cleavage of C-C, decarboxylation, dehydration, amination, deamination, halogenation, phosphorylation etc.

However, the research area of kimchee is restricted only on the microbiological growth and a few research has been performed about the changes of chemical components such as organic acids or volatile flavour components. So the research on the flavour components of kimchee is quite difficult to glean from the literature.

Direct evidence of the relative contribution of any components to the kimchee flavour is not clearly demonstrated till now. The analysis of volatile flavour in kimchee was left aside until 1977 by Yoon and Rhee[6] reporting 17 volatile flavour components. However their identification is tentative. First reliable identification of volatile flavour by capillary gas chromatograph/mass spectrometry was carried out by Hawer *et al.*[7] They identified 16 components including dimethyl disulphide, dimethyl trisulphide, dipropyl disulphide, 1-butane-isothiocyanate, methylallylsulphide, diallyl sulphide and diallyl disulphide. The flavours in kimchee are the result of unique combination of various sugars, organic acids and amino acids as well as various volatile organic compounds including sulfur-containing compounds, esters, terpenes, alcohols, and volatile organic acids. But no one has yet attempted to elucidate the mechanism of the formation of flavour by the action of microorganisms nor tried to analyse and identify the flavour components in kimchee thereafter.

This objective of the work set forth in this project is aimed identify the optimum method for isolation and concentration of the volatile kimchee flavour and the best capillary column for analysis with good resolution as well. The rationale for doing the work is to expand knowledge about the formation of the flavour during the fermentation of kimchee so that, hopefully, a more specific and basic knowledge in the pathway of the flavour formation mechanism can be made. Further studies will be, therefore, conducted regarding the formation of flavour during the fermentation of kimchee. This would produce information not now known which should assist in understanding the formation of kimchee flavour during the fermentation.

MATERIALS AND METHODS

Kimchee preparation

All fresh vegetable was purchased from a local wholesale shop and trimmed. Chinese cabbage was cut into about 5cm vertically from the base for salting. The cabbage pieces were put into brine containing 120% of salt. The vegetable which has become properly tender by salting was washed with fresh water for three times and leave for about 10 min for draining. Then, cayenne pepper, garlic, ginger, green onion, fermented fish sauce, sugar etc. are mixed with fine stripes of radish as shown in *Table* 1. The seasoning mixture is mixed with salted vegetable and placed in a glass jar with a little press for anaerobic fermentation at ambient temperature.

Table 1. The ratio of raw materials for preparation of kimchee

cabbage	1000g
radish	100g
cayenne pepper	20g
green onion	20g
ginger	10g
garlic	15g
fish sauce	50g
sugar	10g

Collection of headspace volatiles

The samples were run using a Tekmar LSC-2000 automatic liquid sample concentrator. Sparge was substituted with a 100m*l* glass bottle, 55mm O.D.×

120mm, made by Schott Glasswork, Germany, for small volume and foam. The top containing both inlet and outlet tube, was placed on the bottle and fixed with a PTFE screw-top. The temperature of the bottle was maintained in a thermostatically controlled water bath during the collection.

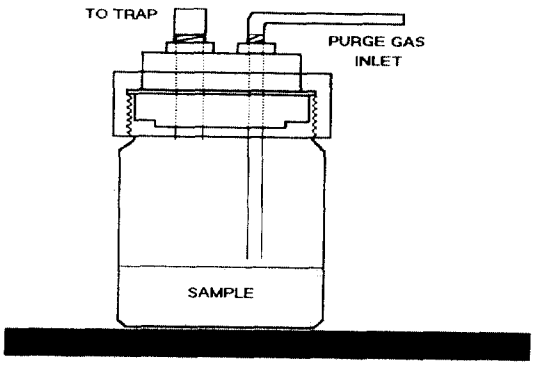

Figure 1. Headspace volatiles collection apparatus.

The outlet was attached with a Teflon tubing to introduce the flavour to the original sparge outlet of the Tekmar Purge and Trap System and the nitrogen supply was connected to the inlet of the bottle which in turn was attached to a tube containing an adsorbent(charcoal) to prevent volatiles from the gas supply reaching the trap.

The purge flow was 50m*l*/min. nitrogen for 30 min. The traps used were 12″×1/8″ stainless steel, packed with 60/80 mesh Tenax GC(a polymer of 2, 6-diphenyl-*p*-phenyl oxide) by the manufacturer. The traps were desorbed for 4 min. at 180℃ with a desorb preheat of 50℃. A tekmar model 1000 capillary interface was not used for capillary column injection of the sample. Instead of the interface, the end of the tube from the trap was connected directly to the injector substituting the glass insert.

Three replicate samples(20g) of kimchee were transferred to a 100m*l* Duran bottle with a PTFE screw-top as shown in *Fig.* 1. The top was placed on the bottle and temperature of the flask was maintained at 30℃ in a thermostatically controlled water bath. The headspace volatiles were swept onto a Tenax trap by purging nitrogen purified with charcoal at 30m*l*/min. During the collection the flow rate was monitored with a bubble flow meter to detect possible leakage. After collecting for 30 min. The trap was replaced with the flask to connect directly to the nitrogen supply for five minutes to remove the moisture. To make sure that all of the chromatograms were originated from the sample, blank runs without sample were executed.

Gas chromatography

A Howlett-Packard, 5890 Ⅱ GC equipped with a flame ionization detector(FID) was used for all analysis and interfaced to a Hewlett-Packard HP 3396A integrator. Helium was supplied at a flow of 20m*l*/min as a carrier gas. The separation of the volatile components was carried out using a 0.32mm I.D.×50m fused silica capillary column DB-5, coated with 5% phenyl methyl polysiloxane made by J & W Scientific, california, U.S.A. Beside this column, 0.32mm I.D.×30m of HP-1, HP-FFAP (Hewlett Packard, U.S.A.), BP-10(S.G.E. Australia) and DB-210(J&W, U.S.A.) capillary column

Table 2. Optimum conditions of gas chromatographic separation for aroma isolates

GC : Hewlett-Packard 5890 Ⅱ Gas Chromatograph
Column : 0.33mm ID×50m Fused Silica Capillary Column DB-5 (J & W, U.S.A.)
Carrier Gas : Helium, 2m*l*/min
Injector Temperature : 150℃
Temperature programme : held at 5℃ for 3 min and programmed to 220℃ at 3℃/min and held at final temperature for 5 min
Detector : Flame Ionisation Detector
Attenuation : 1×10^2
Detector temperature : 250℃
Integrator : Hewlett-Packard 3396A,
Attenuator : 7 Chart speed : 1cm/min
Area reject : 50,000 Threshold : 5
Peak width : 0.04

was used to compare the resolution.

When the Tekmar is ready for purge, the initial temperature of the column oven cooled down to 5℃ to condense the volatiles at the front of the column by cryogenic focusing with liquid nitrogen. The inlet pressure of the carrier gas was controlled at 12 psi and desorption was carried out for 4 min. The oven temperature was cooled at 5℃ with liquid nitrogen for cryofocusing the volatile compounds during the thermal desorption. After desorption, the oven temperature was raised to 220℃ at 3℃/min and kept at this temperature for 5 min. The eluent was detected with a FID at 250℃.

RESULT AND DISCUSSION

So far as kimchee is concerned, volatile component have been somewhat neglected and, in most cases, interest has been restricted to the organic acid, free amino acids or free sugars. It is true that these products are the ones that are most likely to be responsible for the characteristic flavour of kimchee. However, numberous volatile compounds are also produced during the fermentation and these components rather contribute more to the flavour perception of kimchee.

In the analysis of flavour, a particularly convenient and effective procedure is one which combines steam distillation of the food volatiles with simultaneous extraction of the volatiles into a small quantity of a low-boiling and water-immiscible organic solvent. Diethyl ether, pentane, 2-menthyl butane or mixture of these are the solvent commonly used. The features are embodied in an apparatus based on the original design of Likens and Nickerson.[8] Various modifications of which have been extensively used to isolate the flavour volatiles from diverse foods[9~12]

For foods whose flavour is not damaged by heating, or where specific types of cooked flavours are the subject of an investigation, SDE at atmospheric pressure is frequently the method of choice. However, in case of fresh foods, it is emphasized that thermal artefact will be produced during the distillation and it is important to point out that some lossed of the more volatile flavour components will normally occur during the removal of low-boiling solvents.

Equilibrium headspace sampling cas be used normally without the previous problems. However, this technique suffers from both a lack of sensitivity and difficulty in obtaining reproducible quantity. To avoids all these problems, DHS analysis is a method adapted for many types of samples. In DHS analysis, organics are removed from the sample by purging i.e., bubbling through a liquid or sweeping the headspace of a solid with an inert gas. Volatile compounds are effectively removed while leaving behind the nonvolatile matrix without any artefact. The basic procedure of DHS analysis is fairy simple. Flavour and aroma compounds are purged from the sample by sweeping it with an inert gas. The purge gas is passed through a porous polymer adsorbent which retains the organics while allowing the gas and any water vapour to pass through to vent. In this manner a large quantity of flavour compounds can be quickly removed from the sample providing a very effective concentration step and therefore excellent sensitivity. Flavour compounds are purged in direct proportion to their level in the sample, thus insuring an accurate artefact formation has been eliminated. After the purge step, the adsorbent is rapidly heated and backflushed to release the volatiles and sweep them onto the head end of the chromatographic column.

In this experiment, the sparge designed to accommodate sample by the manufacturer, was substituted with a Duran bottle for foam and small volume in DHS analysis of the flavour in kimchee. There are a couple of aspects of the analysis that require special attention for using sparge. Many samples, especially protein or carbohydrates rich

samples tend to foam when purged. This foam must be controlled so that it should not be introduced to the transfer tubing or switching valve. Unlike frit sparger, the purge gas is not finely dispersed in multiple streams of bubbles when using a bottle. The purge gas enters and swept the headspace volatiles only to the trap. The purge efficiency with which volatiles are removed from the sample may be lowered slightly, since the gas/liquid contact area is significantly reduced. However, the resulting loss in sensitivity was not significant between these two sample container for the almost similar chromatogram. Furthermore the sparge is not adequate to accommodate sample such as kimchee for small mouth and the sample cannot be purged for foam.

The characteristic flavours and aroma of food products are the result of often complex mixtures of organic compounds. While gas chromatography is well suited to the separation and detection of these compounds, limitations in injection techniques and sensitivity generally require that some method of sample preparation prior to injection be used. Though purge and trap concentration has been appreciated to be a useful sample preparation tool available in gas chromatography, the broad injections inherent in the system are not well suited for use with capillary columns. To tighten the injection, a focusing technique is necessary. Cryogenics, or cryofocusing has proven to be the most efficient method of choice. For this purpose, the manufacturer recommended a Capillary Interface Model 1000 for the Tekmar Purge and Trap concentrator. It is an automatic cryofocusing device designed to take maximum advantage of the benefits of cryofocusing. Practically, it was not acceptable for using in the analysis of the volatile kimchee flavour. At the outset, this device was applied to the analysis of the volatile flavour of kimchee but only a few peaks appeared on the chromatogram. It was found that the capillary precolumn was stuffed quite often by the concentrated moisture crystallising into ice. This willl prevent the flow of the trapped gas into the capillary column. The Tenax adsorbent used does not trap moisture. It does, however, retard to some extent. The majority of the moisture can be removed from the Tenax by passing dry gas over the trap after the purge step and before desorb. Since the moisture is only weakly retained, it is expected to be eluted from the trap, however, other compounds that are weakly retained may also be eluted. A fact which should not be connive is that this device has not designed to adapt split mode between precolumn and main capillary column. Obviously, in splitless mode, good resolution cannot be expected in capillary GC. Furthermore a GC oven with sub-ambient capabilities does an excellent job and, often, has the added benefit of improving the resolution among early eluting peaks. This is why the Capillary Interface is substituted as previously explained.

As expected, significant differences in flavour profile patterns were shown between these two extracting methods. Typical chromatograms are shown in *Fig.* 2. and 3. In the chromatogram of headspace, peaks are generally high and well separated. The bigger peaks appear in the front of the chromatogram of headspace, while, the bigger peaks do at the end in the chromatogram of SDE. For SDE method, 200g of kimchee was taken into the flask, while for the DHS analysis, 20g of kimchee was taken into the bottle for the analysis. In spite of 1/10 amount of the sample, low molecular components were collected more in headspace concentration while higher molecular component were more in SDE. This fact implies that more volatile components can be analysed by DHS analysis while, the higher volatile components has been removed simultaneously during the concentration of the solution in SDE. Furthermore in the SDE, the sample should be heated up to 90℃. The flavour has been changed by thermal artefact and quite different

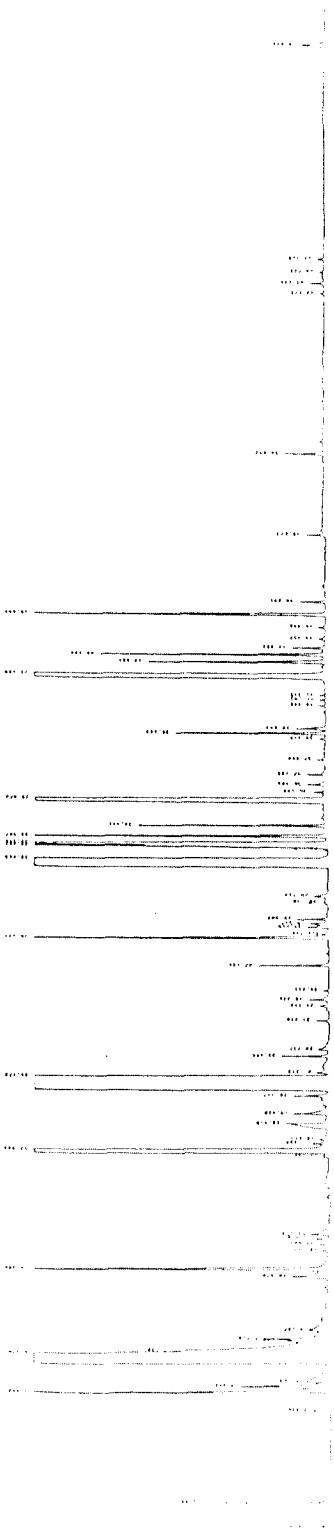

Figure 2. Chromatogram of flavour concentrated by dynamic headspace concentrator.

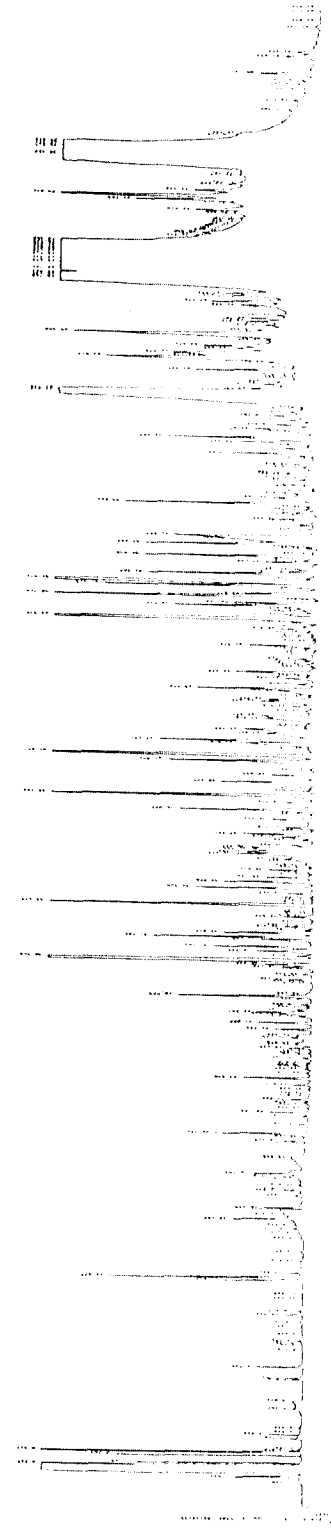

Figure 3. Chromatogram of the flavour extracted by simultaneous distillation extraction.

flavour was smelt in the flask after heating. On the other hand, the flavour components in the kimchee comprise mainly chemical classes of higher polarity. However, the polarity of the solvent used in the SDE is very low, so the polar flavouring components are not moderately miscible to be recovered in the non-polar solvent system.

In DHS, from a relatively small amount of sample as opposed to SDE or other concentrating methods, a good concentration can be obtained. Under these circumstances, the limiting factor in sensitivity is column resolution. By using columns of dissimilar polarities it may be possible to obtain adequate resolution for all compounds of interest. For the selection of the polarity of the capillary column, various columns with different stationary phase were used to determine the resolution. Kimchee flavour isolation and analysis are made difficult also by the fact that flavours comprise a large number of chemical classes. The flavour of kimchee includes not only non-polar component but also extremely polar components such as acids and alcohols. So, the flavours cannot be analyzed completely with only one column of a polarity and thus greatly make the analysis complicated. This attempt was carried out to see which capillary column of polarity can separate as many as possible. HP-1, dimethyl polysiloxane, most non-polar stationary phase capillary column made by Hewlett-Packard, U.S.A., can separate non-polar components eluting later such as terpenes or carbohydrates with good resolution, while it can not separate polar componets such as scids eluting early with the other components simultaneously. However, HP-20M, the most polar column coated with polyethylene glycol, made by Hewlett-Packard in U.S.A., can separate polar acids eluting earlier from the other componets with poor resolution of non-polar components and thus reduced number of peaks with increase of area. Intermediate but slightly polar column, BP-10 made by Scientific Glass Engineering in Australia, 14% cyano propyl phenyl dimethyl siloxane, can separate more peaks than polar column but still showed some unresolved peaks. A moderate polar column, DB-210 made by J&W Scientific in U.S.A., 50% trifluoro propyl, 50% methyl polysiloxane showed similar separation to BP-10. Best polarity available at the moment was BP-5, 5% phenyl methyl poly-siloxane which will separate nonpolar, intermediate and polar components with good resolution. However, a leading peaks are appeared with sharp drop at the end of the peaks. These peaks were considered to be strong acids. By the result, a column with slightly higher polarity such as 6 or 7% phenyl methyl silicone might be considered to be the most optimum column for the analysis of the kimchee flavour. The column with stationary phase of 5% phenyl methyl poly-siloxane was chosen to be the best for the analysis of volatile kimchee flavour.

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