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The Change of Thiocyanate (Goitrogen) Amount, Indolylmethyl Glucosinolate Content and Myrosinase Activity in Redish Kimchi during Fermentation

Mee Ree Kim and Hei Soo Rhee*

Department of Food and Nutrition, Chungnam National University
**Department of Food and Nutrition, Seoul National University*

무우김치 숙성 중 thiocyanate (gitrogen) 함량, 기질(indolylmethyl glucosinolate) 함량 및 myrosinase 활성도 변화에 관한 연구

김 미 리 · 이 혜 수*

충남대학교 식품영양학과 · *서울대학교 식품영양학과

요 약

깍두기 숙성 중 thiocyanate 함량 및 thiocyanate 생성에 영향을 주는 인자들의 변화를 측정하였다. 관능검사 결과, 숙성 적기는 3일로 판정되었으며, 이 시기의 pH는 3.8, 산도는 16.1 mEg NaOH/100 g이었다. 무우중의 thiocyanate 함량은 0.44~2.28 mg/100 g이었으며 깍두기중의 thiocyanate 함량은 숙성 1일에 73%, 숙성 3일에 32%로 감소하였다. Thiocyanate 생성에 관여하는 indolylmethyl glucosinolate 함량 및 myrosinase 활성도는 깍두기 숙성중 점차 감소하여 각각 25% 및 4%에 달하였으나, myrosinase의 조효소로 작용하는 아스코르브산의 함량은 숙성기간중 약 1 mM 정도를 유지하였다. 따라서, 깍두기 숙성중 thiocyanate 생성감소 현상은 김치 숙성중 pH 감소에 의한 indolylmethyl glucosinolate의 분해와 myrosinase의 불활성화에 기인되었다.

INTRODUCTION

Kimchi is a favorite kind of fermented foods and makes a significiant contribution to the diet for the Korean. Major material of Kimchi is Chinese cabbage or radish. According to the report from the National Nutrition Survey[1], the consumption of radish, which is second to Chinese cabbage averaged 48.2 g/person/day.

Radish, belonging to Cruciferae family, posesses pungent flavor and taste which was by enzymatic products of glucosinolate, such as nitrile and isothiocyanate[2]. Isothiocyanate and its secondary products, thiocyanate[3] and thiooxazolidone are known to posess goitrogenic properties[4~7]. These

compounds act by either lowering the iodine concentration of the thyroid or by possibly inhibiting the organic binding of iodine[8].

Many studies have related goiterogenic toxicity of cruciferous plants to thier thiocyanate contents[7]. Paxman and Hill[9] indicated that thiocyanate in kale leaves is largely responsible for thieir goitrogenicity in rats. Especially, 3-indolylmethyl glucosinolate (glucobrassicin) and N-methoxy-3-indolylmethyl glucosinolate (neoglucobrassicin) have been identified as the thiocyanate-yielding glucosinolates in cabbage with 3-indolylmethyl glucosinolate making the major contribution (up to 68%) of glucosinolates in this species[10]. Carlson[11] reported that thiocyanate ion is derived from myrosinase-catalyzed hydrolysis of indolylmethyl glucosinolate in radishes.

Recently, Park[12] measured the content of thiocyanate ion in the homogenate of fresh radishes from Korean origin. However, there is no information about the change of factors responsible for the formation of thiocyanate in Korean radish Kimchi.

Accordingly, the objective of this investigation was to evaluate the pattern of change in thiocyanate content, indolylmethyl glucosinolate content and myrosinase activity during fermentation of "Kagdugi" (a flavorite kind of Korean radish Kimchi).

MATERIALS AND METHODS

Preparation of radish Kimchi ("Kagdugi")

Freshly harvested radishes (Raphanus sativus L.) of Taebeck type (weight about 1.5 kg) were obtained from the Daejeon vegetable market. Samples were washed, and slices of lower part of radish root (2.5×2.5×2.5 cm) were weighed (300 g), placed into 500 ml glass jar and admixed with salt (5 g), leek (10 g), ginger (1.5 g), garlic (5 g), sugar (7 g) and water (50 g), and the glass jars were tightly closed.

Fermentation was contrinued for 7 days at 20° ± 2℃ and analyzed 1, 2, 3, 4, 5, 6, and 7, days after initial brining, respectively.

pH, acidity and saltiness

The pH, titratable acidity and saltiness of radish Kimchi were measured periodically during fermentation according to the AOAC (13), respectively. Samples (50 g) were analyzed after homogenization in the distilled water (100 ml) using Waring blender, and filtration.

Thiocyanate determination

Thiocyanate was determined acccording to the colorimetric method of Johbnston & Jones[14].

Each sample (150 g) was homogenized in distilled water (450 ml) using a Waring blender. The crude extract (12 ml aliquots) was clarified weth lead acetate ($Pb[CH_3(COO)]_2.3H_2O$, 0.1 g), and centrifuged. The aliqutos (0.5~2.5 ml) were diluted with distilled water to make up a 2.5 ml mixture which was mixed with 2.5 ml of 0.4 M $Fe(NO_3)_2$ in 1 N HNO_3 The optical density of the final mixture was read at 460 nm. A reference blank was obtained by destroying the red ferric thiocyanate complex weth one drop of 5% HgCl. Readings of the thiocyanate content are expressed as mg KSCN per g fresh weight of radish tissue.

Separetly, the sample was homogenized in the buffers of pHs betwwen 3.9 and 8.7, and the aliquot was measured as described previously.

Measurement of indolylmethyl glucosinolate

Preparation of indolylmethyl glucosinolate was carried out as described[15]. Brussels sprout (50 g) was extracted with boling methanol (80 ml). The extract evaporated to dryness was taken up in water (20 ml), and then filtered through Celite. The clarified extract was applied to a column (3×15 cm) of acidic alumina, which was washed with water, and the glucosinolates was eluted with 100 ml of 1% aqueous potassium sulfate solution. The salt solution was evaporated to dryness in vacuo, and the dried residue

was extracted with hot methanol. The methanolic extract was evaporated to dryness, and the residue was taken up in 20 ml of water. The desalted glucosinolate solution was applied to a column of DEAE Sephadex A-25 (3×20 cm), pre-swollen in ammoinium bicarbonate (2.0 M) and subsequently washed with distilled water. The column was eluted with aqugous ammonium acetate solution (0.1 M, 0. 5 , 1.0 M and 2.0 , 40 ml of each) at a flow rate of 1 ml/ min. The elution of the indolemethyl glucosinolates with 2.0 M salt solution was monitored by thin layer chromatography (TLC, n-butanol: ethanol: water=4: 1:2, using p-dimethyl aminocinnamaldehyde spray reagent (16). The spot (Rf=0.48) was scraped off and used as standard indolylmethyl glucosinlate.

Reverse phase-high performance liquid chromatography (RP-HPLC) of glucosinolate was carried out as described by Bjorkqvist (17). Methanolic extract from 20 g of fresh or fermented radishes was dried and dissolved in the distilled water. The aqueous sample was passed though Ecteola cellulose column (2×5 cm) which was washed with 30 ml of 1 M $NaHCO_3$ (pH 9.0), and collected into the flask containing 60 ml of ethanol. The eluate in ethanol was evaporated to dryness. The dried sample was dissolved in hot methanol (7 ml). The aliquot (30−l) was injected into the C_{18}column (3.9×300 mm), which was eluted with the mobile phase (0.1 M ammonium acetate: acetonitrile=9:1) at a flow rate of 1 ml/min. The peaks were monitored at 280 nm. The relative amount of indolylmethyl glucosinolate was determined by measurement of the height of peak corresponding to the standard indolylmethyl glucosinolate.

Assay of myrosinase activity

Myrosinase activity was determined as described by Palimieri et al[18]. The standard assay mixture contained 60 mM ascorbic acid and 34 mM sinigrin in 3 mlof 50 mM phosphate buffer, pH 6.5. The enzyme reaction was started by adding 30−1 of enzyme preparation from radish homogenate (20 g). The rate of absorbance decrease at 227.5 nM (ε max=6,950 M^{-1} cm^{-1}) was continuously monitored, using the M 250 spectrophotometer (Gilford). A unit of enzyme was defined as the amount of the enzyme which can hydrolyze 1 nmole of sinigrin per 1 min under the assay conditions.

Assay of ascorbic acid content

The content of ascorbic acid in of fermented radish homogenate was measured with fluorometer (KOTAKI) as described in AOAC microfluorometric method[13].

Sensory evaluation

Radish Kimchies fermented at 20℃ were evaluated by a 8-member trained sensory panel as described previously[19]. Each member independently evaluated each sample for ripeness and overall eating quality

Table 1. Mean scoring values for ripeness, overall eating quality of Korean Radish Kimchi (Kagdugi) during fermentation time

Characteristics	Fermentation time (days)							
	0	1	2	3	4	5	6	7
Ripeness	1.00[a]	1.50[b]	3.25	4.50	2.63	1.38[b]	1.13[a]	1.00[a]
Overall eating quality	1.50[b]	1.75	3.75	4.00	2.63	1.38[b]	1;13[a]	1.00[a]

a : Any two means in the same row followed by the same superscript letters are not sifnificantly different ($p < 0.05$)
Based on the following scales : 1=very poor, 5=very good,

(5=very good, 1=very poorf)

RESULTS AND DISCUSSION

The optimum fermentation time for good flavor of radish Kimchi, "Kagdugi" was found to be around three days, based on the sensory evaluation (Table 1). On the third day, the saltiness was 0.22±0.24 NaCl %. The pH decreased gradually and below pH 4 on 3rd day of fermentation, as shown in Fig. 1. Meanwhile, the titratable acidity increased gradually and on 3rd day to 16.1 mEq NaOH/100 g.

The content of thiocyanate was determined according to the colorimetric method described by Johnston et al[14]. The recovery of potassium thiocyanate added to the radish homogenate turned out to be greater than 90%. In the peeled lower part of fresh radish root, the thiocyanate content ranged between 0.44 and 2.28 mg/100 g fresh radish, and varied greatly among variety, season and origin. The thiocyanate content in the peelings approximately tripled that in the peeled part of the radish root, as decribed in Table 2. These results are similar to Park's report[12] that the content of thiocyanate in various cultivars of Korean origin ranged from 0.67 to 1.4 mg/100 g fresh radish. When the content of

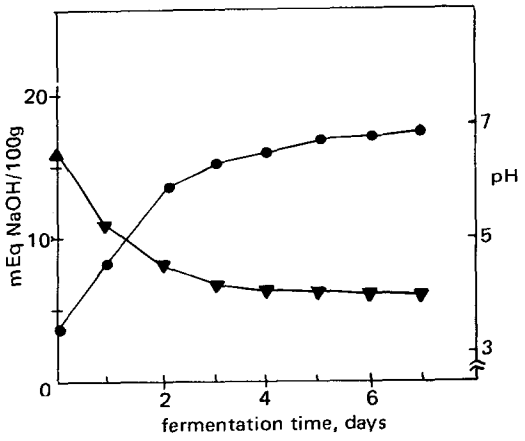

Fig. 1. The change of pH and acidity in radish Kimchi during fermentation at 20°C.
△——△ : pH ; ○——○ : acidity

Table 2. The amount of thiocyanate in peelings and peeled lowe and higher part of radish roots

	SCN^-(mg/100g)	SCN^-(Mean ± S.D.)
Peelings	1.7800 1.7631 1.7946	1.779 ± 0.0158
Peeled lower radish roots	0.7102 0.7008 0.6937	0.7016 ± 0.0083
Peeled higher radish roots	0.7001 0.6913 0.6901	0.6838 ± 0.0055

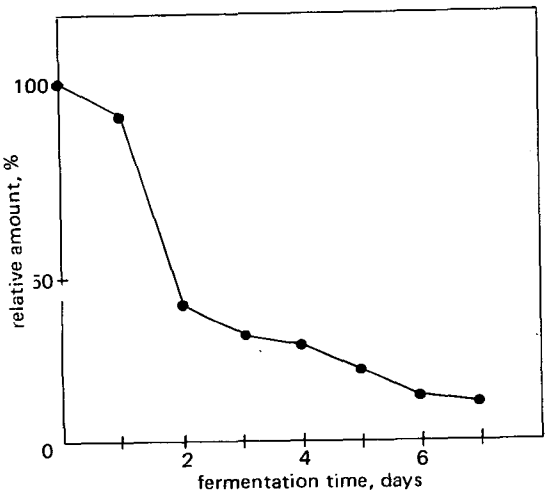

Fig. 2. The change of relative amount of thiocyanate in radish Kimchi during fermentation at 20°C.

thiocyanate formed in the homogenates of the fermented radishes was quantitatively determined. the fermentation time-demented radishes was quantitatively determined, the fermentation time-dependent decrease of thiocyanate formation was observed, as demonstrated in Fig. 2. On the 3rd day the content of thiocyanate in the homogenate of fermented radish decline to 32% of control.

These results might be explained by the observation (Table 3) that while the thiocyanate content of the homogenization of fresh radish in the acidic buffers of pH 3.9 and 5.0 is 44.7% and 87.1%, re-

Table 3. The thiocyanate amount in fresh radish homogenate adjusted with several buffers

Control (pH 6.5)	Acetate buffer (pH 3.9)	Phosphate buffer (pH 5.0)	Borate buffer (pH 8.7)
2.052	0.855	1.840	2.011
2.162	0.950	1.840	2.243
2.131	1.026	1.840	1.905
*2.116 ±0.057	0.944 ± 0.086	1.840 ± 0.000	2.053 ± 0.173
100%	44.7%	87.1%	97.1%

* Mean ± S.D.

spectively, as compared to that in the control (pH 6.5), and no significiant change was observed with homogenization in weakly alkaline buffer of pH 8.7. Therefore, it is suggested that the decrease of the thiocyante formation in the homogenate of radish Kicmchi was caused by the decrease of pH during fermentation.

Although goitrogenicity was reported to be induced in rats fed with cabbage[7], it is less likely that fermented radish is related to the goitrogenicity, because the amount of thiocyanate in the homogenate of 3 day fermented radishes is less than a tenth of that in the cabbage homogenate. Concerned with the above observations, there can be two explanations for the decrease of thiocyanate formation; one way of the gradual decrease of thiocyanate formation may be derived from the gradual reduction of indolyl methyl glucosinolate content and the other resulting from the decrease of myrosinase activity, because thiocyanate is known to be generated from the enzymatic hydrolysis of indolylmethyl glucosinolate by myrosinase, which is ascoric acid-dependent[20].

Fig. 3A shows HPLC profile of indolylmethyl glucosinolate (retention time, 12.1 min) prepared from fresh radish. When standard indolylmethyl glucosinolate isolated from ion exchange resin column chromatograpy and TLC (Rf value, 0.52, purple) was injected onto RP-HPLC, one major peak with

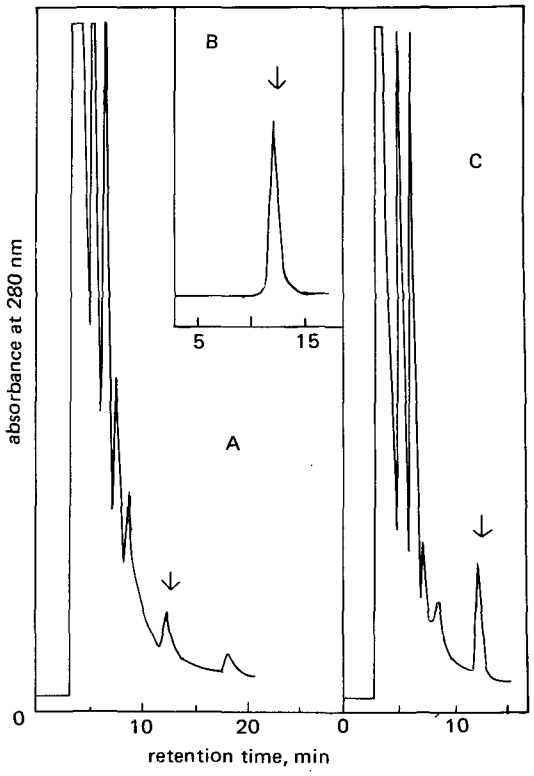

Fig. 3. HPLC profile of indolymethyl glucosinolate (↓). (C_{18} column (3.9 x 300mm), eluated with 0.1M ammonium acetate : acetonitrile = 9 : 1, at a flow rate of 1ml/min)
A : sample prepared from fresh radish.
B : standard prepared from Brussels sprout.
C : sample (A) coinjected with standard (B).

retention time of 12.1 min was observed (Fig. 3B). And when the extract from fresh radish was cotime of 12.1 min. was observed again (Fig. 3C). These results confirmed that the peak (retention time, 12.1 min) corresponds to the indolylmethyl glucosinolate. Fig. 4 shows the profile for the change of indolylmethyl glucosinolate content in the tissue of fermented radish, based on RP-HPLC measurement. The change of indolylmethyl glucosinolate declined to 73% after 1 day, after 3 days to 25% of control showing the profile similar to that observed with the change of thiocyanate as depicted in Fig. 2. These observations demonstrate that indolylmethyl glucosinolate content decreases gradually in accor-

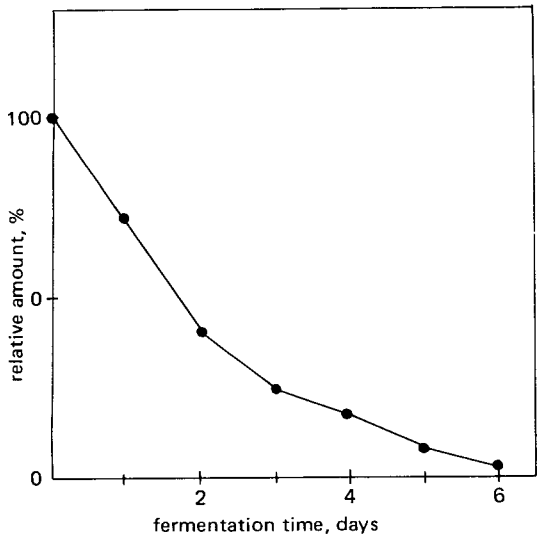

Fig. 4. The change of relative amount of indolylmethyl glucosinolate in radish Kimchi during fermentation at 20°C.

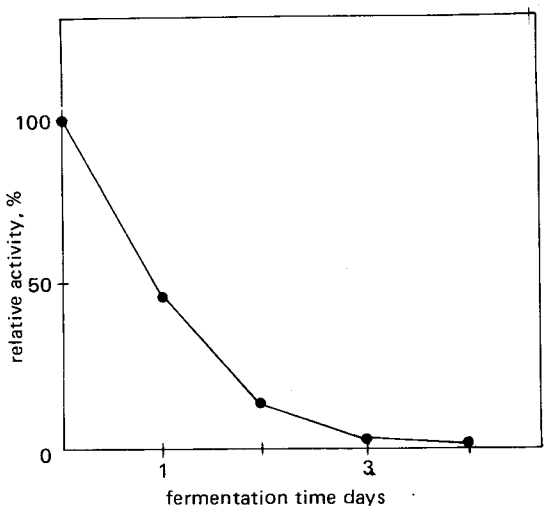

Fig. 5. The change of relative myrosinase activity in radish Kimchi during fermentation at 20°C.

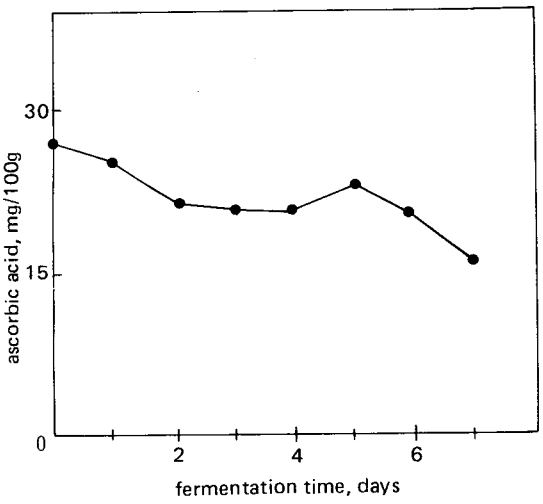

Fig. 6. The change of ascorbic acid content in radich Kimchi during fermentation at 20°C.

dance with the decrease of pH in the tissue of radish Kimchi during fermentation.

In earlier study, it was reported that glucosinolate was unstable in the acidic buffer[19]. Accordingly, it can be concluded that the gradual decrease of indolylmethyl glucosinolate content in the radish Kimchi during fermentation might be caused by the gradual decrease of pH during fermentation.

On the other hand, another cause for the reduction of the thiocyanate formation was suspected to be the decrease of the myrosinase activity during fermentation. Fig. 5 demonstrates that the myrosinase activity in the radish tissue decreases gradually, based on the hydrolysis of sinigrin, suggensting the gradual loss of myrosinase activity in the radish tissue during fermentation. On 3rd day, total enzyme activity in the radish tissue decreased to less than 4 % of control. To see whether the myrosinase activity is affected by the ascorbic acid content in the fermented radish, the content of ascorbic acid in the radish tissue during fermentation was measured by fluorometric method. Fig. 6 demonstrates that the content of the ascorbic acid ranges beween 18 mg/100 g and 28 mg/100 g. Because the content of ascorbic acid was around 1.3 mM, which is the concentration enough to saturate the radish myrosinase[22], the relationship if ascorbic acid content to the decrease of myrosinase activity was less likely. These results support the assumption that the gradual inactivation of myrosinase in the radish tissue is another factor causing the decrease in the formation of thiocyanate

in the homogenate of the fermented radish. Concerned with the inactivation of myrosinase the decline of pH in the fermented radish tissue was supposed to be responsible for the decrease of the myrosinase activity. When the myrosinase purified from radish roots was incubated in the phosphate buffer, pH 6.5, and significant inactivation of myrosinase was not observed until 4 days of incubation. Meanwhile, in the acetate buffer, pH 4 the gradual inactivation of myrosinase was observed[22]. These results suggest that the decrease of pH during fermentation causes the loss of the myrosinase activity.

Based on these observation, it is concluded that the decrease of thiocyanate formation in the homogenate of the fermented radish is caused by both the decomposition of indolylmethyl glucosinolate and the loss of myrosinase activity which are accelerated by lowered pH during fermentation.

SUMMARY

The study on the change of general properties of Kagdugi during dfermentation reveals that around the third day of fermentation, optimum for good flavor, the pH decreased to around or below 4 while the acidity increased gradually. The relative amount of thiocyanate in the radish Kimchi homogenate decreased to 73% after 1 day and after 3 days to 32 % of control. And the content of indolylmethyl glucosinolate and total myrosinase activity in the tissue of radish Kimchi decreased gradually and on 3rd day to 25% and 4% of control, respectively. On the other hand the concentration of ascorbic acid in the radish Kimchi was found to vary around 1 mM.

Based on these results, the gradual decline of thiocyanate formation in the radish Kimchi homogenate is concluded to be caused by the gradual decomposition of indolylmethyl glucosinolate and the decline of myrosinase activity, which are directly affected by the change of pH during fermentation.

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