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STUDIES ON THE EFFECT OF FURYL FURAMIDE (AF-2) ON KOREAN KIMCHI

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Furyl furamide (AF-2)가 김치에 미치는 影響

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要 約

1. Furyl furamide*가 여름철 김치세균에 미치는 영향을 보기 위하여 두가지 여름김치에서 分離한 세균 *Bacillus* group과 Lactic acid bacteria 중 *Bac. megaterium*, *Bac. subtilis* 그리고 *Bac. licheniformis*등이 발효 初期의 젓갈김치에 우세 하였고 국물김치에서는 *Lact. plantarum*과 *Lact. buchneri* 등이 始終 매우 우세하였다.

2. 김치에서 分離된 *Bacillus* group은 10 p.p.m. 以下의 AF—2 低濃度에서 대단히 敏感하게 억제되었으나 Lactic acid bacteria의 group은 50 p.p.m.의 高濃度에서도 低抗하고 있었다.

3. 김치의 AF—2 添加許容濃度는 最高 10 p.p.m.을 넘지 않아야 할 것이다.

4. 김치에 添加된 高濃度(50 p.p.m.)의 AF—2는 김치 발효를 heterofermentation에서 Homofermentation으로 變化시키는 경향이 있었다.

5. AF—2 10 p.p.m. 濃度의 김치에서 bacterial flora는 약간 影響을 받고 있었으나, 50 p.p.m의 高濃度에서도 夏節김치의 酸敗는 억제할수가 없었다.

6. 23°C~25°C 상태의 김치酸酵液中의 AF—2 消長은 酸性에서 比較的 安全하다고 하나 4日後에 約 25%가 減少되고 pH 變化曲線과 거의 平行한 變化를 보였다.

Introduction

Kimchi is one of the most popular and so important side dishies in Korea that Korean adult takes almost 200gm a day.

It is also a considerable fact that intestinal microflora of Korean has many things to do with the kimchi, as it is a bacterial fermentative food. And it changes through four steps, good fermention, acidification, soft deterioration and putrification, in accordance with the microfloral change. As the population of bacteria and acid quantity in kimchi have also a great concerning with the temperature, good kimchi is easily preserved for long time (3 months) in winter, but in summer time it is very difficult to maintain the freshness of kimchi even for two days.

Many biochemical and bacteological studies on kimchi were reported by Jin[1] Kim[11] Chung[10] Kwon[4] and many other Korean, but a few studies on the preservative process from acidification have been known except the auther's report.

It is somewhat reasonable that few studies has been reported on the preservation of kimchi using food preservatives, because Korean takes so much kimchi that it is apt to disorder their intestinal microflora by taking kimchi which contains effective food additives for the bacteria.

The author, employing the new food additive AF-2 which is less poisonous and has broad spectrum activity to the bacteria, tried to investigate the effect of AF-2 on the rapid fermentation of summer kimchies.

In the first place, isolation and identification of

* 2-(2-furyl)-3-(5- nitro-2-furyl) acryl amide

bacteria from summer kimchi were examined, because the conservative strains of summer kimchi bacteria were not found in many laboratories in Seoul and physiological characteristics of bacteria were changeable.

Through the work some new phenomena were observed as shown in the summary.

EXPERIMENTAL

1. Kimchi samples

In summer Juicy kimchi (W) contains mainly sliced raddish and less juicy jokal kimchi (J) contains mainly Chinese cabbage with many kinds of seasonings, were popularly used in Seoul.

For the sample, these two kinds of kimchies were prepared as usually made in Korean home with the following materials. And fresh jokal kimchi (K) made in a certain home in Seoul was also used as the sample for isolation of fermentative bacteria.

materials:

i Juicy kimchi (W)

Sliced raddish and its leaves; about 10kg, garlic; 20gm ginger; 5gm, unripen red pepper slice; 20gm, boiled starch solution; 100ml, salt used; about 400 gm, water; about 5 lit..

ii Jokal kimchi (J)

Sliced Chinese cabbage; 10kg, garlic; 40gm, ginger; 20gm, red pepper powder; 60gm, leak; 50g, *Jokal; 100g. salt used; about 300gm, other vegitable seasonings, water; properly added,

*Jokal:fermented fish with salt.

2. Isolation and identification of fermentative bacteria in summer kimchi

(1) media

medium I: Thioglycolate broth(Difco); 25gm, Agar; 1.5gm, distilled water; 1000ml,

medium II: Yeast ext; 3.5gm, Bactotrypton; 5.0 gm, Glucose; 5gm, Sodiumchloride; 5gm, Agar; 15 gm, distilled water; 1000ml,

medium III: Beef ext; 3gm, Pepton; 5gm, Sodium chloride; 8gm, Agar; 15gm, distilled water; 1000ml,

(2) Procedure

Isolation and identification:

In hot summer kimchi is easily ripen or slightly acidified within 2 days, therefore sampling was done 4 times after 5 hours, 24 hours, 48 hours, and 72 hours from the beginning to isolate the normal strain from bacterialflora during kimchi fermentation.

Media II was used mainly for the plate culture of microbes, because both aerobic and anaerobic microbes in kimchi were esaily cultivated. Media I is used for the stab culture to collect the anaerobic bacteria, and media III is used for the aerobic bacteria.

According to the morphological characteristics of colony, 55 strains were isolated from those media incubated for 30 hours at 37°C.

Techniques of bacteriological identification were taken with the method of Bergey's (8) manual and Christensen's. and Skerman's (7) methods were also applied.

3. Effect of AF-2 on the bacteria isolated from kimchi.

(1) Reagents,

Basal solution of AF-2; 500mg of AF-2 crystal was weighed and dissolved in 250ml of ethanol in a 500ml of brown messflasco, and then diluted with distilled water exactly to 500ml and stored in cool dark place. The basal solution(mg/ml) was diluted with distilled water to a necessary concentration when used.

Medium IV: Yeast ext; 3.5gm, Bactotrypton; 5.0gm, Glucose 5.0gm, Sodium chloride 5gm, distilled water 1000ml, pH adjusted to 7,

(2) Procedure:

To get the inoculating sample of simliar concentration of bacteria, 15 tubes of media IV were inoculated with each strain of identified bacteria, and incubated for 24 hours at 37°C.

In the next, test tubes containing 5ml of media IV were inoculated with the broths of cultivated strain and added AF-2 solution to get standard concentration planned, and incubated.

The turbidity was determined with electrophotometric method after 20 hours incubation for the Bacillus group and 30 hours for the group of lactic acid bacteria.

Electropotometic method: Type of electrophotometer; Atago PC-61, filter; 660mu, transparence of controlled cell; 100% over 80%;over 40%+Less than 40%;+

4. Effect of AF-2 on the bacterial flora of summer kimchi during fermentation.

Procedure:

Kimchi (W) and kimchi (J) were taken up as the sample and the test plots were set as following table I.

1,500gm of sample for each plot was taken in two glass bottles sterized with chloralk solution, and fermented in high room temperature of 30°C —35°C. This examination was repeated again in temperature of 28°C—33°C. To investigate the microfloral change during its fermentation, the sample was examined five times 5 hours, 15 hourse, 30 hours, 48 horus and 72 hours later.

In counting the bacterial number dilution method was applied. 1ml of sample juice was diluted to 10,000 times and 0.1ml of the diluted sample was plate cultured in media II. And number of characteristic colony of the strain was counted through the colony counter, after 20 hours for detecting Bacillus and 30 hours for lactic acid bacteria.

Data obtained were the total mean value both two bottles and two conditions.

Table 1. Examination plots

conc. of AF-2 / kimchi	controlled	10 p.p.m.	50 p.p.m.
J	J-a	J-b	J-c
W	W-a	W-b	W-c

5. Variation of organic acid in kimchi during fermentation when AF-2 is added.

Procedure:

All the kimchi samples were prepared as the case of microfloral examination before mentioned and only red peper was controlled in kimchi(J). The sample was examined at six different times, eg. 5 hours, 15 hours, 30 hours, 48 hours, 72 hours and 96 hours after fermentation started. To prepare the sample for titration, kimchi(J) was completely crushed in a crusher and vacume filtered, and kimchi (W) was filtered through the vacume Seitz filte. The filtrates were used as the samples.

Determination of non volatilic acid:

In total acid titration, 10ml of the filtrate was titrated rapidly with the standard solution of 0.02-N NaOH and with phenolphthalein as an indicator 10ml of the same filtrate was taken in 100ml beaker and concentrated to a sticky state in boiling water bath, and then placed it in dryoven at 100°C for 30 minutes.

After evaporating the volatilc acid 10ml of distilled water was added in the sample and titrated with 0.02-N NaOH solution and with phenolphthalein as an indicator.

Titer of non volatilic acid in kimchi was obtained by multiplying the factor* 1.052 by the test titer, and it was calculated as lactic acid percentage.

Volatilic acid:

Titer of the volatilic acid was obtained by subtracting the titer of non volatilic acid from the titer of total acid, and calculated as acetic acid percentage.

*factor: 10ml of 0.1-N acetic acid and 10ml of 0.1-N lactic acid were combined together in a beaker and condensed and evaporated as above manner. Titer obtained in titration with 0.1-N NaOH solution was 9.5ml. This test was repeated 5 times and mean value was taken.

$$\text{factor} = \frac{10.0}{9.5} = 1.052$$

5. Variation of AF-2 in kimchi during Fermentation.

Kanno's[23] colorimetric method was applied as the analytical technique of AF-2 Materials:

1. sample; kimchi(J) and (W) were made as usual and only red pepper was taken off, and fermented at room temperature of 20°C—25°C.

2. Sulfanilamide solution; 2gm. of sulfanilamide was dissolved in 100ml of 20% hydrochloric acid.

Procedure

Two kinds of kimchi (J) and (W) containing 10 p.p.m. of AF-2 were taken up as the sample and its detail procedure was as following.

1) 10gm of kimchi sample was taken and added 20ml of mixed solution of toluene and butylacetate (1 : 1), and then centrifuged for 5 minutes after fully homogenizing.

2) 10ml of the clear solution (toluene and butylacetate layer) was poured on almina colume

(1.5cm×6cm). Washed with 20ml of n-hexane and 20ml of ethyl ether.

3) Eluted with 40ml of methanol. 4ml of the first elute was taken off and about 35ml of the next elute was collected in the 50ml brown volumetric messflask.

4) 20ml of standard AF-2 solution (5μg/m) and 15ml of methanol were taken in 15ml brown volumetric messflasco.

5) Added 1ml of 20% sodium hydroxide solution in each standard and sample messflasco, and warmed in water bath at 55°C for 50minutes, then cooled in ice water.

6) 2ml of sulfanilamide solution was added in each messflasco and stood for 15 minutes in ice water, and then add 1ml of 0.1% N--naphthyl ethylenediamine dihydrochloride solution and stood for 30 minutes at room temperature.

7) Diluted the colored solution exactly to 50ml with methanol and determined the absorbance at 530mμ(Electrophotometer Atago type pc-61, filter 53). Concentration of AF-2 in the sample was calculated through the following formula.

$$\text{Concentration(ppm)} = 200 \times \frac{A}{B} \times \frac{1}{\text{weight of sample(g)}}$$

A: Absorbance obtained from the sample.

B: Absorbance obtained from 5ppm AF-2 standard solution.

RESULTS AND DISCUSSION

1. Isolation and idenfication of fermentative bacteria in summer kimchi.

55 strains of bacteria were isolated from kimchi (J), (W) and (K), and 15 strains(w-1 w-3 w-6 w-11 w-21 isolated from kimchi(W), J-2 J-3 J-7 J-10 J-16 isolated from kimchi(J) and k-3 k-4 k-8 k-11 k-17 isolated from kimchi (K)) were selected for identification among them.

After some physiological test 15 strains were divided into two groups. Strains J-2, J-7, J-10, W-6, K-3, K-4, K-11 were detected as gram positive, catalase positive, aerobic and spore forming *Bacillus* and J-3, J-16, W-1, W-3, W-11, W-21, K-8, K-7 were gram positive, catalase negative, anaerobic and not spore forming lactic acid bacteria.

Results of morphological and physiological exam ination were as shown in Table II and Table III.

Strains J-2, K-3, W-3 were determined as *Bacillus megaterium*[11] and J-7, K-4, as *Bacillus subtilis*, however some different reactions were observed in carbohydrate test.

Strains J-10, K-11 showed every physiological characteristics almost same as *Bacillus licheniformis*. But nothing was reported about those strains in kimchi study.

In the group of anaerobic bacteria, W-1, W-11, J-3 were identified as *Lactobacillus phantarum*, and K-8, W-21, were determined as *Lactobacillus buchneri*. But W-21 was somewhat similar to *Lactobacillus leichmanni* in fermentability of lactose.

Table II. Physiological characteristics of the strain isolated from summer kimchi (Bacillus group)

Examination \ strains	J—2	K—3	W—6	J—7	K—4	J—10	K—11
Agar colony	L.C.W.	C.W.	C.W.Y.	R.O.	R.O	R.W.	R.W.
Sporangia	No S	No S	No S	No S	No S	No S	No S
Vacuolation on glucose	+	+	+	−	−	−	−
Catalase	+	+	+	+	+	+	+
Urease	−	−	−	−	−	−	−
Rod	1.2×4.0	1.5×5.0	1.2×4.0	0.7×3.5	0.7×3.5	0.8×3.0	0.8×4.0
Gram's stain	+	+	+	+	+	+	+
Salt tolerence	7%	7%	7%	7%	6%	6%	7%
pH of culture	MR+	MR+	MR+	MR−	MR−	MR+	MR+
Opt, temperature	30	〃	〃	〃	〃	〃	〃
Max, temp(°C)	50	50	55	55	55	50	50
Motliity	−	+	−	+	+	−	−

Citrate	+	+	+	+	+	+	+
Nitrate	−	(+)	−	+	+	+	+
V.P	−	−	−	+	+	+	+
Gelatin liq.	H	H	(−)	H	(−)	H	H
Gas from glucose	−	−	−	−	−	−	−
Acid from starch	+	+	+	+	+	+	+
Acid mannit	(−)	+	(−)	+	(−)	(−)	+
Acid sucrose	+	+	+	+	+	+	+
Acid lactose	−	(+)	−	−	−	−	−
Acid glucose	+	+	+	+	+	+	+
Acid xylose	+	+	+	+	+	+	+
Acid alabinose	+	+	+	+	+	+	+
Acid glycerol	+	+	+	+	+	+	+

*L: large C: Creamy W: white Y: yellow R: rough O: opaque No.S: notdefiniteyl swollen
H: hydrolized MR: methyl red ( ): not accorded with manual.

Table. III. Physiological characteristics of the strains isolated from summer kimchi (Group of Lactic acid bacteria)

Examination \ strain	W—1	W—11	J—3	K—8	W—21	K—17	J—16	W—3
Agar colony	P.F.	P.F.	P.F.	P.W.	P.Y	F.W.	S.W.	S.W.
Gram's stain	+	+	+	+	+	+	+	+
Catalase	−	−	−	−	−	−	−	−
Nitrate	−	−	−	−	−	−	−	−
Urease	−	−	−	−	−	−	−	−
Motility	−	+	−	−	−	−	+	−
Rod(micron)	1.0×4.0	0.8×4.0	1.0×4.0	0.8×3.0	0.6×3.0	sphere 1.0	// 1.2	oboid 1.0
Opt temperature	37	//	//	//	//	//	//	//
Max temp(°C)	50	50	55	55	55	50	55	60
Salt tolerence(%)	6%	6.0	6.5	6.0	6.0	7.0	6.0	7.0
Galatin liq.	−	−	−	−	−	−	−	−
Sucrose broth	T	T	T	T	T	T.G	T.G	T
Acid from glucose	+	+	+	+	+	+	+	+
Acid fructose	+	+	+	+	+	+	+	+
Acid mannose	+	+	+	+	+	+	+	+
Acid galactose	+	+	+	+	+	+	+	+
Acid arabinose	+	(−)	+	(−)	(−)	+	+	+
Acid xylose	+	+	+	+	+	+	+	+
Acid maltose	+	+	+	+	+	−	−	+
Acid sucrose	+	+	+	+	(−)	−	+	+
Acid lactose	+	+	+	+	+	+	(−)	+
Acid raffinose	+	+	(−)	+	+	(−)	+	−
Acid rhamnose	−	−	−	−	−	−	−	−
Acid Dextrin	+	(+)	+	−	−	−	−	−
Acid starch	(+)	(+)	−	−	(+)	−	−	−
Acid inulin	−	−	−	−	−	−	−	−
Acid salicin	(−)	+	+	−	−	+	+	+
Acid mannitol	+	+	+	(−)	+	(−)	+	(−)
Acid glycerol	+	+	+	+	+	−	−	+

P: pin point F: faint S: small T: turbid G: Gelatinous membraned cell.

Strains K-17, J-16 were identified as *Leuconostoc mesentroides* with specificity in sucrose broth. And W-3 was as *Streptococcus faecalis*.

Kim[11] reported this strain was aboundantly observed in winter kimchi but it was rarely checked in the sample.

Generally speaking group of *Bacillus* were aboundantly found in kimchi (J) and (K) containing jokal, and group of Lactic acid bacteria were more aboundant in kimchi(J) and kimch(W) containing no jokal.

W-1, W-2, K-8 which belong to *Lactobacillus* showed the fermentability of starch. It was considered that the variety was derived from frequent use of starch summer kimchi.

2. Effect of AF-2 on the isolated bacteria from kimchi.

The result of examination obtained were shown in the following Table IV.

Table. IV. Effect of AF -2 on the isolated bacteria from kimchi

Test Organism	L. plan.		L. buch.		Leu. mes		B. meg		B. sub		B. lich		str. f.
conc. of AF-2	N—1	J—3	W—21	K—8	K—17	J—16	J—2	K—3	J—7	K—4	J—10	K—11	W—3
100 ppm.	−	+	−	−	−	−	−	−	−	−	−	−	−
50 ppm.	+	+	+	−	−	−	−	−	−	−	−	−	−
25 ppm.	+	+	+	+	−	−	−	−	−	−	−	−	−
12.5 ppm.	+	+	+	+	−	−	−	−	−	−	−	−	+
6.25 ppm.	++	+	+	+	+	+	−	−	−	−	−	−	+
3.12 ppm.	++	++	++	++	+	+	−	+	−	−	−	−	+
1.56 ppm.	++	++	++	++	+	++	+	+	−	−	+	+	+
0.78 ppm.	++	++	++	++	++	++	+	+	+	+	+	+	++

Lact. plantarum(W-1, J-3) and *Lact. buchneri* (W-21, K-8) which was dominant and produced a high rate of acid in kimchi fermentation were resistant to high concentration over 50 p.p.m. of AF-2, and *Strept. faecalis* which was comparatively rare in the fermentation was somewhat resistant.

Baci. megaterium (J-2, K-3) and *Baci. lichenifo-rmis*(J-10, K-11) were checked in a low concentration less than 3.12 p.p.m., and *Baci.*[15,19]*subtilis* (J-7, K-4) aboundantly found in the fermentation of kimchi(J) was most sensitive among the strains as expected.

Leuc. mesentroides (J-16, K-17) was more sensitive than homofermentative lactic acid bacteria as *Lact. plantarum* and *Lact. buchneri*.

Generally speaking aerobic strains isolated from kimchi were much sensitive contrary to the fact that anaerobic strains were resistant to high concentration of AF-2.

3. Calculation of maximum amount of AF-2 to be added in Korean kimchi.

In summer time a normal Korean takes almost 200gm of kimchi a day. Allowable daily intake of AF-2 calculated from animal experiment is 468mg, and therefore the allowable amount of AF-2 in kimchi is easily calculated as following.

468mg÷safety coefficient÷taste coefficient
468mg÷100÷2=2.34mg···allowable amount in 200gm of kimchi.
2.34mg×5=11.70mg···allowable amount in 1kg of kimchi.(P.P.M)

Considering the fact a Korean, adult posibly takes other food containing AF-2, allowable concentration of AF-2 in Korean kimchi should not be over 10 p.p.m. as the following example(Table V) explains.

Table. V. Example calculation of AF-2 daily intaken.

Food	Daily consumption of food×taste coefficient	Concentration of AF-2	Intake of AF-2 (mg/man/day)
Kimchi	200g×2=400g	10p.p.m.	4mg
bean curd (Tube)	33.3g×3=100g	5 〃	0.5mg

fish paste	10g×3=30g	2.5//	0.075mg
Total			4.575mg

4.575mg×100<468mg(Allowable amount of AF-2 daily intaken)

4. Effect of AF-2 on the bacterial flora of summer kimchi during fermentation.

Bacterial flora of the kimchi when AF-2 was added were observed as shown in the following table VI and table VII.

Table VI Microfloral population in kimchi (J)

J-a (Controlled) 32°C—35°C

Time (hr's)	Total No. of Baci.	B. meg	B. sub	B. lich	Total No. of Lact	L. plant	L.buch	Leuc. mes.	Str. fae.	Yeast	PH
5	26	2	21	3	—	—	—	—	—	—	6.05
15	54	28	25	1	82	3	3	75	1	—	4.42
30	34	16	18	—	182	45	4	131	2	—	4.01
48	25	5	20	—	1088	1070	18	—	—	1	3.80
72	24	1	12	11	1397	1265	121	3	8	16	3.62

J-b (AF-2 10 p.p.m.) 32°C—35°C

Time (hr's)	Total No. of Baci.	B.meg.	B.sub.	B.lich.	Total No. of Lact.	L.plan	L.buch	Leuc mes.	Str.fae.	Yeast	PH
5	5	2	1	2	2	2	—	—	—	—	5.98
15	14	11	—	3	33	26	5	2	—		4.45
30	4	4	—	—	229	192	35	—	2		4.07
48	27	24	1	2	1375	1256	82	—	7	2	3.69
72	16	16	—	—	1099	878	216	—	6	28	3.62

J-c (AF-2 50 p.p.m.) 32°C—35°C

Time (hr's)	Total No. of Baci.	B. meg.	B. sub.	B. lich	Total No. of Lact	L. plan	L. buch	Leuc. mes.	Str. fae.	Yeast	PH
5	—	—	—	—	2	2	—	—	—	—	5.98
15	—	—	—	—	19	17	2	—	1	—	4.61
30	—	—	—	—	171	145	26	—	—	—	4.03
48	2	2	—	—	921	826	93	—	2	—	3.82
72	—	—	—	—	1134	938	196	—	—	4	3.76

Table VII Microfloral population in kimchi (W)

W-a (Controlled) 32°C—35°C

Time (hr's)	Total No. of Baci.	B. meg.	B. sub.	B. lich.	Total Lact.	L. plan.	L. buch	Leuc. mes.	str. fae.	Yeast	PH
5	6	2	4	—	3	—	—	3	—	—	5.93
15	26	8	18	—	132	10	4	112	6	—	4.10
30	23	21	2	—	461	186	26	217	32	—	3.92
48	11	2	9	—	1504	1362	130	41	61	3	3.81
72	—	—	—	—	1377	1132	217	—	18	23	3.66

W-b (AF-2 10. p.p.m.) 32°C—35°C

Time (hr's)	Total No. of Baci	B. meg.	B. sub.	B. lich.	Total No. of Lact	L. plan.	L. buch.	Leuc. mes.	Str. fae.	Yeast	PH
5	4	4	—	—	15	—	—	15	—	—	6.06
15	1	1	—	—	25	8	6	8	3	—	4.18

30	4	4	—	—	278	167	42	31	38	—	4.03
48	—	—	—	—	1524	1281	166	51	26	4	3.86
72	—	—	—	—	1566	1209	289	—	68	28	3.68

W-c (AF-2 50 p.p.m. 32°C—35°C)

Time (hr's)	Total No. of Baci.	B. meg.	B. sub.	B. lich	Total No. of Lact.	L. plan.	L. buch.	Leuc. mes.	Str. fae.	Yeast	PH
5	—	—	—	—	2	2	—	—	—	—	6.07
15	—	—	—	—	27	16	11	—	—	—	4.26
30	—	—	—	—	237	182	44	—	11	—	4.07
48	—	—	—	—	966	836	121	1	8	—	3.88
72	—	—	—	—	1306	1085	195	—	26	4	3.80

In the controlled plot of J-a, *Bacillus* were somewhat dominant through out the whole experiment, but in the plot of W-a they disappeared in the latter term of the fermenation as *Leuconostoc mesentroides.* did. In all plots, *Lact. buchneri* became gradually dominant in the latter term, and *Lact. plantarum* was most dominant, 15hr's later, while *Strept. faecilis* was increasing very slowly.

Baci. megaterium and *Leuc. mesentroides* were contnually observed but *Baci. subtilis* almost disappeared in the plot of J-b and W-b contained 10p.p.m. of AF-2. And it was an important fact that when 10 p.p.m. of AF-2 was added to kimchi, the color of kimchi had changed into red orange. Especially in the juicy kimchi (W), it was more noticeable.

In the plot of J-c and W-c contained 50 p.p.m. of AF-2, all the *Bacillus* group and *Leuc. mesentroides* were inhibited and pH valve was somewhat higher than the other 4 plots.

But taste of all the kimchi samples was much acidic, equally and without example, after 3 day.

It was easily observed through the examination that the population of fermentative bacteria had inflenced the pH value but had nothing to do with the concentration of AF-2 less than 50 p.p.m.

The sensitive bacterial strains in the sample kichi were more resistant to AF-2 than the same strains in the tube test.

5. Variation of organic acid in kimchi during fermentation when AF-2 is added.

The important problem in acidification of kimchi is the increase of volatilic acid during its fermentation.

Therefore variation of acetic acid and lactic acid in kimchi fermention when AF-2 was added was shown as in the following Tables, VIII, IX, Fig. 1 and 2

Table VIII Variation of acid quantity in kimchi (J) during fermentation at 30°C

Time (hr's)	J-a(controlled)				J-b(10 p.p.m.)				J-c(50 p.p.m.)			
	Acet%	Lact%	A/L%	PH	Acet%	Lact%	A/L%	PH	Acet%	Lact%	A/L%	PH
5	0.01	0.08	12.5	6.05	0.01	0.09	11.1	5.98	0.01	0.08	12.5	5.98
15	0.03	0.14	17.2	4.42	0.03	0.16	18.8	4.45	0.02	0.14	14.3	4.52
30	0.08	0.31	19.6	4.01	0.05	0.29	17.0	4.06	0.07	0.46	15.2	4.04
48	0.18	0.69	24.2	3.80	0.14	0.62	22.5	3.84	0.13	0.61	21.3	3.80
72	0.21	0.67	31.3	3.62	0.20	0.71	28.2	3.76	0.15	0.69	21.7	3.76
96	0.20	0.69	29.0	3.60	0.21	0.73	28.8	3.72	0.16	0.71	22.5	3.78

Table IX Variation of acid quanity in kimchi (W) fermentation at 30°C

Time (hr's)	W-a(controlled)				W-b 10 p.p.m.				W-c 50 p.p.m.			
	Acet%	Lact%	A/L%	PH	Acet%	Lact%	A/L%	PH	Acet%	Lact%	A/L%	PH
5	0.01	0.09	11.1	5.92	0.01	0.08	12.5	6.04	0.01	0.09	11.1	6.04

15	0.04	0.24	16.7	4.10	0.03	0.26	11.5	4.18	0.03	0.20	15.0	4.26
30	0.09	0.46	19.5	3.92	0.06	0.41	14.6	4.04	0.08	0.40	16.0	4.07
48	0.16	0.62	25.8	3.72	0.16	0.70	22.9	3.80	0.15	0.71	21.1	3.88
72	0.22	0.71	31.0	3.60	0.22	0.78	28.2	3.72	0.16	0.70	22.9	3.80
96	0.29	0.77	35.3	3.54	0.25	0.75	33.3	3.70	0.18	0.74	24.3	3.74

Acet: acetic acid. Lact: lactic acid.
AL: acetic acid/lactic acid

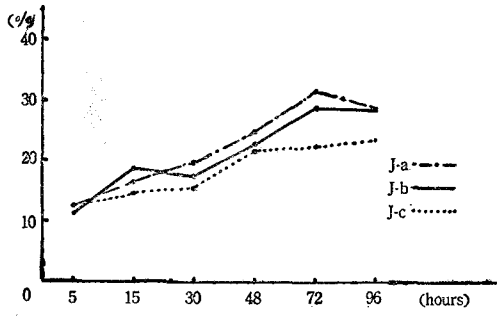

Fig. 1. Curves of acetic/lactic acid ratio in Kimchi (J)

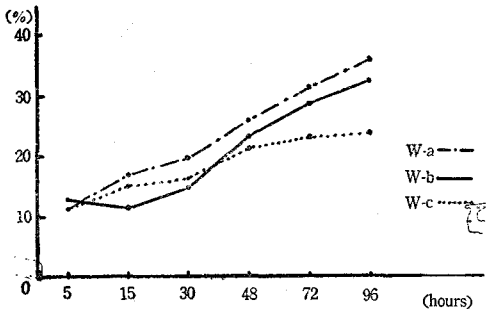

Fig 2. Curves of acetic/lactic acid ratio in Kimchi (W)

The increase of total acid in kimchi (J) according to the fermentation process was somewhat slower than that of kimchi (W) in all cases. But in the third day when kimchi was completely acidified the total acid of kimchi(J) was almost as equal as that of kimchi(W).

In the case of J-b and W-b (added 10 pp.m. of AF-2), differences of acetic acid percentage compared with the controlled were trivially small.

In the case of J-c and W-c (added 50 p.p.m. of AF-2), some differences of acetic acid percentage were observed, and increase of lactic acid percentage was as normal as the others.

In the Fig. 1 and 2 it is easily found that A/L percentage curve of J-c and W-C indicated a slight increasing tendency compared with the controlled, but those of J-c and W-b indicated almost the same tendency as the controlled. This fact means that high concentration of AF-2 leads the heterofermentation of kimchi bacteria to the homofermentation of lactic acid bacteria. But in summer it was difficult to keep the freshness of kimchi from acidification for even 3 days with the concentration of AF-2 less than 50 p.p.m.

6. Variation of AF-2 in kimchi during fermentation.

The result obtained in the examination at 23°C —25°C was shown as in the following Fig. 3

In the normally fermented kimchi low concentration of nitrite was detected, but in the blank test with the kimchi nitrite was not identified. Stability of AF-2 in kimchi was expected as it was stable in acidic solution, (23) but it was observed that AF-2 decreased with a similar tendency of pH value according to the fermentation process. And when the sample kimchi(J) and (W). was fermented for 4 days at 23°C—25°C and still suitable for eating, almost 20% of AF-2 added was consumed.

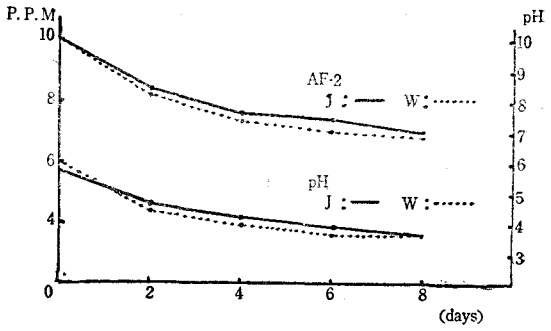

Fig 3. Variation curves of AF-2 in kimchi during fermentation

But in the latter period of the fermention, consumption of AF-2 was very small. It is thought that those properties of AF-2 are ideal as the kimchi preservative in order to avoid the poison of AF-2 accumulation because Koreans eat a lot of Kimchi every day.

SUMMARY

1) Many bacterial strains identified as *Bacillus megeterium*, *Bacillus subtilis* and *Bacillus licheniformis* were aboundantly found in summer jokal kimchi, but the most dominant strains in summer kimchi were *Lactobacillus plantarum* and *Loctobacllus buchneri*.

2) Bacillus groups found in kimchi were sensitive in a low concentration of AF-2, but groups of lactic acid bacteria were resistant to a high concentration of AF-2.

3) Allowable concentration of AF-2 in Korean kimchi is less than 10 p.p.m.

4) AF-2 was not suitable for the juicy kimchi as a preservative because the color of juicy kimchi was somewhat changed into orange red when 10 p.p.m. of AF-2 was added.

5) High concentration of AF-2 leads the heterofermentation of kimchi bacteria to the homofermentation.

6) Microflora of kimchi was influenced even in the concentration of 10 p.p.m. but it was impossible to check the acidification of kimchi in summer with 50 p.p.m. concentration of AF-2.

7) About 25% of AF-2 was consumed in kimchi fermentation for day at 23°C—25°C.

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