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Fermentable and Nutritional Characteristics of Brewery Meal-Based Fermented Feedstuffs Supplemented with *Aspergillus Oryzae* and *Saccharomyces Cerevisiae*

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Aspergillus Oryzae 및 *Saccharomyces Cerevisiae*를 첨가하여 제조한 맥주박 위주 발효사료의 발효 특성 및 영양학적 특성

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요 약

본 연구는 *Aspergillus oryzae* (AO) 및 *Saccharomyces cerevisiae* (SC)를 첨가하여 제조한 맥주박 위주 발효사료의 발효특성 및 영양학적 특성을 검토하기 위해 실시하였다. 시험구 처리는 시험사료에 AO 1%를 첨가하여 제조한 발효사료 처리구(FFAO), SC 1%를 첨가하여 제조한 발효사료 처리구(FFSC) 및 AO 0.5%와 SC 0.5%를 첨가하여 제조한 발효사료 처리구(FFAS)로 나누었다. 48시간 발효에 따른 조단백질 함량은 처리간에 차이가 없었다. 조지방 함량은 48시간 발효에 의해 유의적으로 증가하였다 ($p<0.05$). FFAO, FFSC 및 FFAS구의 NDF 함량은 48시간 발효로 인해 현저하게 감소하였으나, ADF 및 ADL 함량은 차이가 없었다. FFAO 및 FFAS구의 pH는 FFSC구에 비해 발효시간이 경과함에 따라 신속히 감소하였으나($p<0.05$), 24시간 이후에는 일정 수준을 유지하였다. Alcohol 함량은 FFAO구에서는 발효 18시간까지 증가하였고 FFSC 및 FFAS구에서는 발효 12시간까지 증가하였으며, FFAO, FFSC 및 FFAS구의 alcohol 함량은 발효 24시간 이후에는 일정 수준을 유지하였다. FFAO, FFSC 및 FFAS구의 발효 48시간에 암모니아 함량은 각각 0.022, 0.073 및 0.040%로 나타나 FFSC구가 FFAO 및 FFAS구에 비해 암모니아 함량이 2배 이상 높았다($p<0.05$). Dextrose 함량은 FFAO구에서는 발효 6시간까지 증가하였으나 FFSC 및 FFAS구에서는 발효 6시간까지 급속히 감소하였다($p<0.05$). Lactate 함량은 FFAO 및 FFAS구가 FFSC구에 비해 높았다($p<0.05$). 이상의 결과에서 수분 함량이 높은 맥주박을 이용하여 발효사료 제조시 AO를 첨가하게 되면 조지방, alcohol 및 lactate 함량은 증가하지만, NDF 및 암모니아 함량은 감소하는 것으로 나타났다. 따라서 AO의 첨가는 수분 함량이 높은 발효사료의 사료가치 및 보존성을 증진시키는데 효과적인 것으로 판단된다.

(Key words : Aspergillus oryzae, Saccharomyces cerevisiae, Fermentation, Brewery meal, pH)

I. INTRODUCTION

Various microbial feed additives have been developed and utilized to improve performance of dairy and beef cattle recently(Higginbotham et al., 2004; Yang et al., 2004), and *Aspergillus oryzae*(AO) and *Saccharomyces cerevisiae*(SC), producing various high activated enzymes among these microbial feed additives have been used widely as forms of probiotics and fermentation

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extracts(Garcia et al., 2000; Sullivan and Martin, 1999; Wang et al., 2001).

Generally, SC has been utilized widely as a probiotic for domestic cattle, and SC can't use starch and fiber but can easily utilize monosaccarides and disaccarides as the fermentable substrates.

AO is an aerobic organism which the ranges of the rearing temperature and pH are wide, and AO can utilize organic waste(Garraway and Evans, 1984). In addition, the AO produces various enzymes, including amylase, protease, xylanase, etc. in the multiplication stage(Pettersson et al., 1989). These enzymes increase utilization of substrates by various microbes(Ingold, 1984), and carbohydrates(starch, hemicellulose, etc.) were utilized by AO. Therefore, it is considered that AO have high utilization as feed additive of fermented feedstuff for ruminants.

The SC has been utilized widely as the additive of fermented feedstuffs because SC produces the alcohol in anaerobic conditions and improves feed value of fermented feedstuff in fermentation process of feed(Lin et al., 2001; Yan, 1998). On the other hand, there is no research evidence about the fermented feedstuff supplemented with AO because AO is an aerobic organism, and thus it is not recognized normally as a fermentable microorganism. However, AO consumes and removes rapidly the oxygen intermixed with feed(Wallace, 1994). Therefore, it is expected that AO can have an important role in the fermentation process of feed. If we also add AO at the fermented feedstuff with brewery meal, it is thought that SC should be decreased or not to use SC in formulation of the fermented feedstuff, because some SC exist in the brewery meal.

Generally, the pH of fermented feedstuffs is almost 4.0, and fermentation products(alcohol, lactate, acetate, butyrate, etc.) were produced, and Lin(2001) reported that fermentation products(alcohol, ammonia N, lactate, etc.) were produced in fermentation process of the fermented feedstuff from added SC. Also, Shin et al.(1994) and Lin et al.(2001) added SC to the fermented feedstuff with brewery meal and fed the fermented feedsfuff to Korean native cattle. Performances of Korean native cattle were improved through feeding of the fermented feedstuff with brewery meal. But, researches which fermented feedstuffs added AO alone or mixed AO and SC, in addition, research about a fermentation characteristic and change of nutrients contents of these fermented feedstuffs do not exist.

Consequently, we analysed the nutrient content of brewery meal-based fermented feedstuffs after 48 h of fermentation, and examined the contents of alcohol, ammonia N, dextrose, and lactate, as well as pH changes which was generated during the 48 h fermentation to compare and evaluate fermentation characteristics and changes of nutrient content in the experiment.

Ⅱ. MATERIALS AND METHODS

1. Formulation of experimental diets

Experimental diets were formulated using the procedure described by Lin et al.(2001). Cracked corn and brewery meal was mixed at a ratio of 50:50(as fed basis), and it was remixed after adding 5% rice bran, 5% molasses, and 1% micro mineral solution. Experimental diets were fermented anaerobically in a incubator at 30℃ during 48 h after adding 1% AO, 1% SC, and 0.5% AO and 0.5% SC, respectively. Chemical compositions of brewery meal, cracked corn, rice

Table 1. Chemical composition of experimental diets

	Brewery meal	Cracked corn	Rice bran
Dry matter, %	23.89 ± 0.73	90.75 ± 0.13	89.87 ± 0.15
	..... % of dry matter		
Crude protein, %	26.63 ± 0.31	9.02 ± 0.13	16.87 ± 0.05
Ether extract, %	7.38 ± 0.11	3.09 ± 0.13	19.13 ± 0.41
Crude ash, %	3.85 ± 0.04	1.80 ± 0.13	9.77 ± 0.11
Neutral detergent fiber, %	69.30 ± 0.89	26.62 ± 3.53	29.09 ± 1.61
Acid detergent fiber, %	21.52 ± 0.08	4.52 ± 0.06	10.60 ± 0.70
Acid detergent lignin, %	6.94 ± 0.24	3.05 ± 0.11	5.32 ± 0.19

Means ± standard deviation.

Table 2. Chemical composition of microorganisms

	Aspergillus oryzae	*Saccharomyces cerevisiae*
Dry matter, %	93.05 ± 1.53	32.65 ± 0.10
	..... % of dry matter	
Crude protein, %	29.72 ± 0.75	49.86 ± 0.15
Ether extract, %	25.62 ± 0.30	25.91 ± 0.10
Crude ash, %	5.90 ± 0.02	7.87 ± 0.14

Means ± standard deviation.

Table 3. Components of micro mineral solution

Component	Amount	Unit
$MgSO_4$	0.002	g
Na_2HPO_4	0.05	g
$FeSO_4$	0.002	g
$MnSO_4$	0.002	g
$CaCl_2$	0.05	g
$CaCO_3$	0.05	g
$ZnSO_4$	0.002	g
K_2HPO_4	0.05	g
$(NH_4)_2NO_3$	0.05	g
Distilled water	1.00	ℓ

bran, AO, SC, and micro mineral solution are shown in Tables 1, 2, and 3, respectively.

2. Experimental design

Experiments were divided into three treatment groups; FFAO(fermented feedstuff supplemented with 1% of AO), FFSC(fermented feedstuff supplemented with 1% of SC), and FFAS(fermented feedstuff supplemented with 0.5% of AO and 0.5% of SC). Experiments were accomplished in triplicate. To evaluate fermentable characteristics of FFAO, FFSC, and FFAS, samples were collected every 6 h during the anaerobic fermentation at 30℃ for 48 h, and contents of alcohol, ammonia N, dextrose and lactate, as well as pH change were analysed. Also, change of nutrient content by 48 h fermentation was tracked in this study.

3. Laboratory analyses

The sample of 500 g was collected from each fermented feedstuff at 0 and 48 h, was dried in a forced air oven at 60℃ for 48 h, and then was ground through 1 mm screen in a Wiley mill. The ground samples was analyzed for dry matter, crude protein, ether extract(EE), and crude ash according to the method of AOAC(1995). Neutral detergent fiber(NDF), acid detergent fiber(ADF), and acid detergent lignin(ADL) of the ground samples was determined sequentially according to the method described by Van Soest et al.(1991).

Also, a sample of 1 g was collected from each fermented feedstuff at 6 h intervals during

Table 4. Changes of chemical composition of fermented feedstuffs by 48 h fermentation

Items	FFAO[1]		FFSC[2]		FFAS[3]	
	0 h	48 h	0 h	48 h	0 h	48 h
DM[4] (%)	58.21 ± 1.69[a]	55.74 ± 2.09[b]	55.65 ± 1.94	56.70 ± 2.68	58.03±0.99	59.14±1.29
	..... % of dry matter					
CP[5] (%)	12.27 ± 0.30	12.53 ± 0.12	13.18 ± 0.37	13.27 ± 0.28	12.75 ± 0.10	12.90 ± 0.25
EE[6] (%)	4.53 ± 0.16[b]	5.93 ± 0.27[a]	6.10 ± 0.50	6.33 ± 0.17	5.42 ± 0.11[b]	5.73 ± 0.07[a]
CA[7] (%)	2.92 ± 0.12	2.90 ± 0.17	3.39 ± 0.04	3.45 ± 0.08	3.68 ± 0.40	3.82 ± 0.10
NDF[8] (%)	28.79 ± 0.27[a]	25.36 ± 0.36[b]	33.71 ± 0.95[a]	30.37 ± 0.89[b]	33.15 ± 1.51[a]	28.31 ± 0.59[b]
ADF[9] (%)	7.92 ± 0.21	7.49 ± 0.27	9.39 ± 0.32	8.98 ± 0.08	7.54 ± 0.54	6.69 ± 0.40
ADL[10] (%)	3.00 ± 0.16	2.86 ± 0.26	3.0 ± 0.17	2.88 ± 0.29	3.09 ± 0.25	2.74 ± 0.18

Means ± standard deviation.

[a,b] Means in the same row within same treatment with different superscripts differ ($p<0.05$).

[1] FFAO : fermented feedstuff supplemented with *Aspergillus oryzae*; [2] FFSC: fermented feedstuff supplemented with *Saccharomyces cerevisiae*; [3] FFAS: fermented feedstuff supplemented with *Aspergillus oryzae* and *Saccharomyces cerevisiae*; [4] DM : dry matter; [5] CP : crude protein; [6] EE : Ether extracts; [7] CA : crude ash; [8] NDF : neutral detergent fiber; [9] ADF : acid detergent fiber; [10] ADL : acid detergent lignin.

the 48 h, was diluted by 5 times using the distilled water, and was vortexed for 5 min. The sample was held at 4℃ for 30 min, and then centrifuged at 3,000 × g for 5 min. An aliquot of the supernatant was pipetted into a 2 ml polyethylene tube, and it was used for analysis of alcohol, ammonia N, lactate, and pH.

Also, a sample of 1 g was collected from each fermented feedstuff at 6 h intervals during the 48 h, was diluted by 5 times using a buffer solution(NaH_2PO_4 of 40 g plus Na_2HPO_4 of 10 g/distilled water of 1 ℓ), was vortexed for 5 min, and was retained at 4℃ for 30 min. The samples were centrifuged at 2,000 × g for 5 min. An aliquot of the supernatant was pipetted into a 2 mℓ polyethylene tube, and it was used for analysis of dextrose.

Contents of alcohol, lactate, and dextrose were determined by a glucose analyzer(YSI 2700, USA). pH was measured by pH meter(Corning 445, USA). Analysis of ammonia N was followed by the procedure of Chaney and Marbach(1962).

4. Statistical Analyses

All data were analyzed by Duncan's procedure using the GLM of SAS(1999). The statistical model was as follows;

$Y_{ij} = \mu + T_i + e_{ij}$

Where, Y_{ij} was response variable, μ was overall mean, T_i was effect of treat, and e_{ij} was residual error.

Ⅲ. RESULTS AND DISCUSSIONS

CP content of each fermented feedstuff tended to be higher in samples at 48 h than in samples at 0 h(FFAO; 12.27 vs. 12.53%, FFSC; 13.18 vs. 13.27%, and FFAS; 12.75 vs. 12.90%, respectively). Table. 1. And, the increase of CP content at 48 h compared with 0 h fermentation appeared in the sequence of FFAO>FFAS>FFSC (0.26, 0.015, and 0.09%, respectively).

EE content of FFAO and FFAS was significantly($p<0.05$) increased at 48 h compared with

0 h(FFAO; 4.53 vs. 5.93% and FFAS; 5.42 vs. 5.73%, respectively), and the increase of EE content at 48 h compared with 0 h appeared in the sequence of FFAO>FFAS>FFSC(1.40, 0.23, and 0.31%, respectively).

The NDF content of each fermented feedstuff was significantly($p<0.05$) decreased at 48 h compared with 0 h(FFAO; 28.79 vs. 25.36%, FFSC; 33.71 vs. 30.37%, and FFAS; 33.15 vs. 28.31%, respectively), and the decrease of NDF content at 48 h compared with at 0 h appeared in the sequence of FFAS>FFAO>FFSC(4.84, 3.43, and 3.34%, respectively). The changes of crude ash, ADF, and ADL contents among fermented feedstuffs were similar.

Lin(2001) counted the number of live SC cells of fermented feedstuff with brewery meal at 0 and 48 h fermentation, and he reported that the number of live SC cells was higher at 48 h compared with 0 h, but the number of live SC cells was the range of 10^6 without the relation at different fermentation time; therefore, it was thought that microbial protein could not increase CP content of the feed, although a feed protein was converted to a microbial protein. Also, it has been known that SC converts feed proteins to ammonia N(Hasan and Hall, 1975; Lamanna and Mallette, 1965), but AO makes feed proteins to peptide N(Gomez-Alacon et al., 1987). In addition, there was an increase in ammonia N content in FFSC, a decrease of ammonia N content in FFAO, and a hold of ammonia N content in FFAS(Fig. 1). But, we can not make conclusions about this from our study because we did not examine the number of live SC cells and peptide N content in this experiment.

Lin(2001) reported that fermentable microorganisms use starch and sugar and produce organicacids, lower pH, and control multiplication harmful

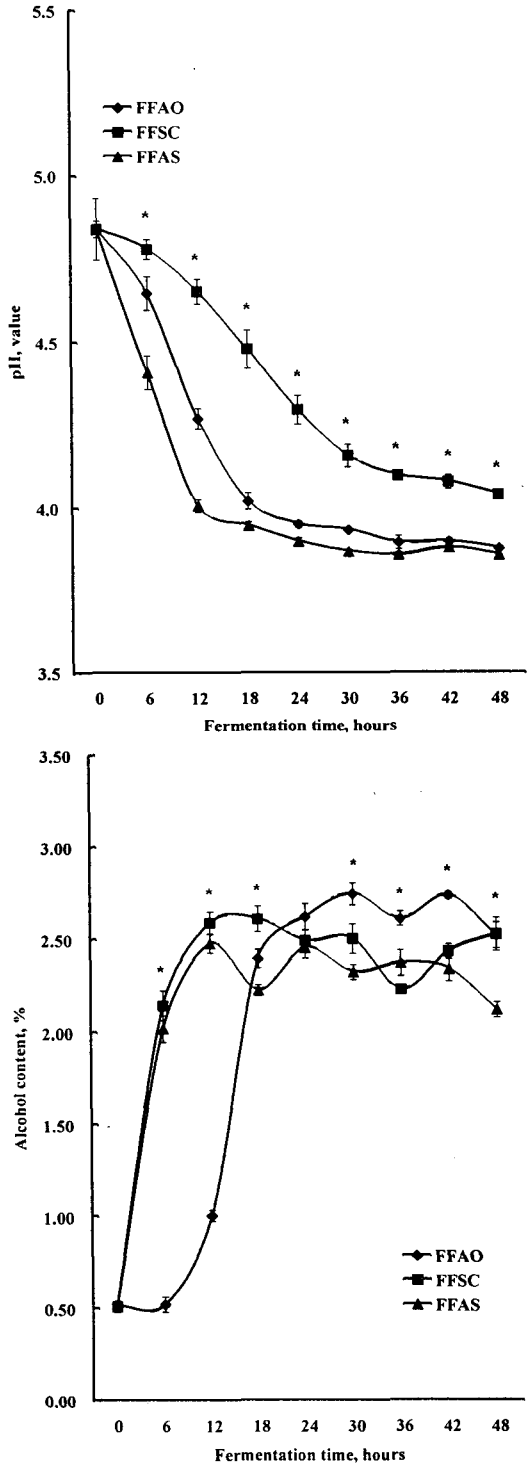

Fig. 1. Changes of pH and alcohol content during 48 h fermentation. *Significant at 5%. Bar: standard deviation.

microbes; therefore, fermentable microorganisms improve preservation and safety of the feed. Also, it has been reported that the fermentation feedstuff which pH is less than 4.5 decrease numbers of coliform bacteria at post-fermentation compared with pre-fermentation; therefore, we examined pH change to express the index of preservation and safety of fermented feedstuff.

The pH of each fermented feedstuffs was lowered until 30 h, and the pH of the feed revealed that it maintained a regular level without a large change as the fermentation time passed after 30 h. The decrease of pH during whole fermentation appeared in the sequence of FFAS>FFAO>FFSC. In addition, the pH of FFAS was the lowest, the pH of FFSC was the highest, and the pH for FFAO and FFAS was similar.

Lin(2001) examined the pH change of alcohol-fermented feedstuff with brewery meal by fermentation, the pH of alcohol-fermented feedstuff was reduced to 5.15 at 27 h, and the change of the pH was small after 27 h. Although the pH of Lin is higher than the pH in our experiment, the results from the two studies support each opinion because the pH was reduced until 24～30 h and the change of the pH was small after 30 h.

Generally, the pH of fermented feedstuffs with by-products is about a-3.5～4.5(Jensen and Mikkelsen, 1998; Mikkelsen and Jensen, 1997; Russell et al., 1996). The average pH at 48 h was 3.98 in our experiment regardless of treatments.

Although there is a little alcohol in the raw material feed and microbes, it has been reported that fermentable microorganisms use feed and produce alcohol(Jensen and Mikkelsen, 1998; Lin et al., 2001; Mikkelsen and Jensen, 1997). In addition, SC generates energy by fermenting sugars to alcohol and carbon deoxidized in anaerobic conditions(Ando et al., 2005). Alcohol is a representative product of the fermented feedstuff; therefore, we investigated changes of alcohol content of each fermented feedstuff in this experiment.

The alcohol content for FFAO, FFSC and FFAS was 2.40, 2.61 and 2.23% at 18 h, respectively, and then the alcohol content was higher in FFSC compared with FFAO and FFAS($p<0.05$). The alcohol content for FFAO, FFSC and FFAS was 2.75, 2.51 and 2.33% at 30 h, respectively, and then the alcohol content was higher in FFAO compared with FFSC and FFAS($p<0.05$). However, it was revealed that alcohol content was reduced in all fermented feedstuffs after 18 h, and alcohol production did not occur in any treatment after 30 h.

These results likely accused in response to low availability of dextrose(Fig. 2) which is used for alcohol production. Alcohol content was higher in FFSC and FFAS compared with FFAO until 18 h, because SC used the substrates of the feed and rapidly produced the alcohol. But alcohol content was higher in FFAO compared with FFSC and FFAS after 18 h because AO quickly consumed the oxygen intermixed with the fermented feedstuffs and improved anaerobic condition of the fermented feedstuffs(Wallace, 1994). Multiplication of fermentable microbes was active in the brewery meal which did not to via a sterilization process and alcohol content was increased.

Lin (2001) added SC at the fermented feed- stuff with brewery meal, and then the alcohol content of the feed increased constantly and was 3.1% at 24 h fermentation. The results of our experiment was similar with the results of Lin, although alcohol content was lower in our experiment.

Nitrogen(approximately 13～20%) is converted to an ammonia N in the formulation of fermented feedstuff and silage(Heron et al., 1986;

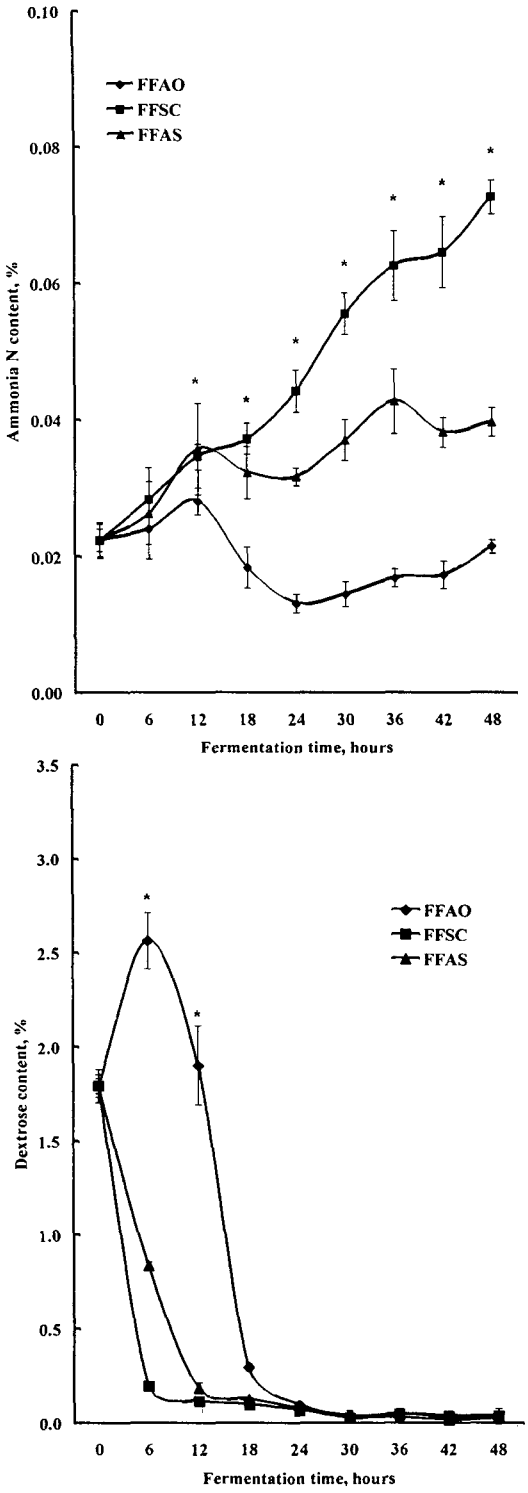

Fig. 2. Changes of ammonia N and dextrose content during 48 h fermentation. *Significant at 5%. Bar: standard deviation.

Ohshima and McDonald, 1978); therefore, change of ammonia N contents of each fermented feedstuff was examined in our experiment.

The ammonia N content of FFAO, FFSC and FFAS was 0.028, 0.035 and 0.036% at 12 h, respectively, and the ammonia N content was higher in FFSC and FFAS compared with FFAO($p<0.05$). Ammonia N content of all fermented feedstuffs was increased continuously until 12 h.

Ammonia N content was decreased in FFAO but was increased in FFSC and FFAS from 6 h to 30 h, and ammonia N content of all fermented feedstuffs was maintained constantly after 30 h. The increase of ammonia N during fermentation appeared in the sequence of FFSC>FFAS>FFAO, and the ammonia N was increased in FFSC but was decreased in FFAO as the fermentation time passed. Therefore, the adding of AO decreased remarkably the content of ammonia N in the fermented feedstuff.

Generally, a protein dissolution ability of SC is limited(Harris, 1958). SC converts nitrate to nitrite and produces ammonia N(Hasan and Hall, 1975; Lamanna and Mallette, 1965). Therefore, SC increases ammonia N content of the feed. It has been reported that protease produced by AO results in peptides release of feed protein but does not result in production of ammonia N(Gomez-Alacon et al., 1987). From these results, if we use AO in the fermented feedstuff, we can prevent the decrease in quality of the feed protein because AO prevents feed protein from being converted to ammonia N.

Fermentable microorganisms, such as yeast, can use dextrose, fructose, and mannose for the fermentable substrates in an alcohol fermentation process. Polysaccharides such as starch and cellulose can be utilized after they are hydrolyzed (Kim et al., 1996); therefore, we investigated a dextrose content to evaluate the fermentation speed

of each fermented feedstuffs and the usage of substrates by fermentable microbes in this experiment.

The dextrose content of FFAO, FFSC and FFAS was 2.57, 0.20 and 0.84% at 6 h, respectively, and then the dextrose content was higher in FFAO compared with FFSC and FFAS($p<0.05$). The dextrose content was decreased rapidly until 6 h and was maintained constantly after 6 h in FFSC and FFAS, but the dextrose content of FFAO was increased dramatically until 6 h and was decreased rapidly until 18 h and was maintained constantly after 18 h. The dextrose content of all fermented feedstuffs was maintained constantly after 18 h; this is because most dextrose was utilized by fermentable microorganisms until after 18 h.

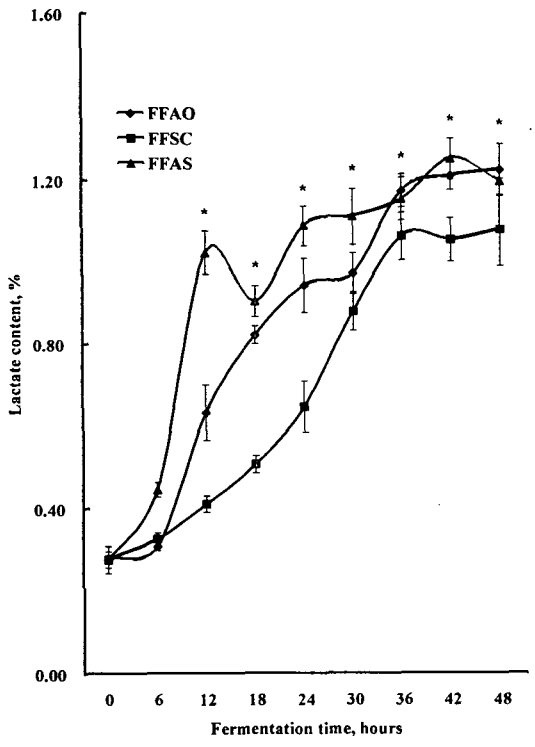

Fig. 3. Changes of lactate content during 48 h fermentation. *Significant at 5%. Bar: standard deviation.

AO produces various enzymes(lipase, cellulase, pectinase, invertase, pytase, etc.), especially amylase and protease(Ingold, 1984). The AO reduces polysaccharides into dextrose which microorganisms can use easily.

The dextrose content of FFAO was increased but that of FFSC and FFAS was decreased until 6 h. This is because microbes(SC, lactate-producing bacteria, etc.) rapidly use the dextrose, thus resulting in a rapid increase of alcohol and lactate contents during initial fermentation.

Therefore, it was considered that the adding of AO on the fermented feedstuff increased dextrose content through degrading of polysaccharides during initial fermentation, and it increased usage of dextrose by microbes.

Generally, fermented feedstuffs have been known to control harmful microbes; this was reported by there being the reason in the antibiotic action of the lactate(Thaela et al., 1998). In addition, it has been reported that fermented feedstuffs contain lactate-producing bacteria similar to the quantity in probiotics and can be utilized instead of the probiotics(Vanbelle et al., 1990). Therefore, we investigated lactate content which is related with the antibiotic action and the preference of each fermented feedstuff.

The lactate content of FFAO, FFSC and FFAS was 0.94, 0.65 and 1.09% at 24 h, respectively. Lactate content of FFAS was highest and that of FFSC was lowest. The lactate content of all fermented feedstuffs was increased continuously until 36 h of fermentation($p<0.05$), but a lactate content stabilized after this time. Lactate content for all treatments was low until 6 h but increased after 6 h. This was because the alcohol produced from the dextrose in the feed until 6 h, and lactate production was increased by a lactate-producing bacteria after 6 h.

Generally, alcohol fermentation and lactate fermentation have been known for the thing by

getting up at the same time by fermentable microbes in anaerobic fermentation(Kim et al., 1996). Furthermore, Lin(2001) reported that some lactate was produced in fermentation of fermented feedstuff with brewery meal, and Lin's results support the result of our experiment.

Ⅳ. ABSTRACT

This study was conducted to compare and evaluate fermentable and nutritional characteristics of brewery meal-based fermented feedstuffs supplemented with *Aspergillus oryzae* (AO) and *Saccharomyces cerevisiae* (SC). Experiments were divided into three treatment groups; fermented feedstuff supplemented with 1% of AO(FFAO), fermented feedstuff supplemented with 1% of SC(FFSC), and fermented feedstuff supplemented with 0.5% of AO and 0.5% of SC(FFAS). For changes of crude protein contents by 48 h fermentation, there were no significant differences among treatments. Ether extract(EE) contents were significantly increased by 48 h fermentation ($p<0.05$). Neutral detergent fiber(NDF) contents of FFAO, FFSC and FFAS were significantly decreased by 48 h fermentation($p<0.05$), but acid detergent fiber(ADF) and acid detergent lignin (ADL) contents were not different. The pH of FFAO and FFAS was decreased more rapidly than that of FFSC($p<0.05$), reaching a plateau after 24 h. Alcohol content was increased rapidly until 18 h in FFAO and was increased rapidly until 12 h in FFSC and FFAS, and alcohol content of FFAO, FFSC and FFAS was maintained constantly after 24 h. The ammonia N content of FFAO, FFSC and FFAS was 0.022, 0.073 and 0.040% at 48 h, respectively, and then ammonia N was over twice higher in FFSC than in FFAO and FFAS($p<0.05$). Dextrose content was increased until 6 h in FFAO but was rapidly decreased in FFSC and FFAS until 6 h($p<0.05$). Lactate content was higher in FFAO and FFAS than in FFSC($p<0.05$). Consequently, when we added AO in formulation of fermented feedstuff with brewery meal which moisture content was high, EE, alcohol, and lactate contents were increased, but NDF and ammonia N contents were reduced. Therefore, it is expected that AO will be effective to increase the feed value and the preservation of fermented feedstuff with a high moisture content.

Ⅴ. ACKNOWLEDGMENTS

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