POV, TBA, and VBN Values, and Proximate, Amino Acid, and Fatty Acid Compositions of Scrap Meals Fermented with Fungi^{*1}

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To find out some suitable fungi which can effectively reduce oxidized lipids from fish waste, the POV, TBA, and VBN values, and the proximate, amino acid, and fatty acid compositions of scrap meals fermented with fungi isolated from smoked-semidried skipjack were compared with those of nonfermented scrap meal (NF) and mackerel waste.

The POV and TBA values were considerably low in the fermented-resteamed scrap meals with 5 fungi being identified *Aspergillus terreus*, *Asp. flavus*, *Asp. oryzae*, *Penicillium citrinum* (PC), *Peni. citrinum* (PG), compared with those of the waste and the NF. On the other hand, the VBN increased by the fermentation, but it decreased by re-steaming after fermentation.

The essential amino acid composition and the ratio of total essential amino acids to total nonessential amino acids (EAA/NEAA) showed scarcely any difference between the NF and the fermented-resteamed scrap meals.

On the other hand, in spite of the similar lipid contents, the percent of ω 3 HUFA in total fatty acids was remarkably higher in the fermented-resteamed scrap meals than the NF.

In the previous study,¹⁾ it was found that the combination of the fermentation with *Aspergillus oryzae*, *Asp. sojae*, *Saccharomyces cerevisiae*, and *Bacillus subtilis* and the re-steaming decreased both toxic oxidized lipids and protein degenerates in fish waste and improved the nutritive value of scrap meal. On the basis of this result and the established fact that the lipid in skipjack decreased by fermentation with *Aspergillus* sp.,²⁾ it is assumed that fungi isolated from smoked-semidried skipjack "Katsuonamabushi" may have similar effects to the combined microbe.¹⁾

In the present study, therefore, the peroxide (POV), thiobarbituric acid (TBA), and volatile basic nitrogen (VBN) values, and the proximate, amino acid, and fatty acid compositions of re-steamed scrap meals fermented with fungi from smoked-semidried skipjack were compared with those of mackerel waste and nonfermented scrap meal prepared from the mackerel waste.

Materials and Methods

Isolation and Identification of Fungi

Smoked-semidried skipjack were obtained from

a factory in Makurazaki city, Kagoshima Prefecture. Powdery substances from the surface of the sample were inoculated on Czapek-Dox agar medium containing 20% sucrose. After the incubation period of 24-74 hours at 30°C, strains from visible colonies were carefully isolated and transferred from 20% sucrose agar medium to 10, 5, and 2% medium by degrees, and fungi with good growth were employed. 5 fungi were identified as *Aspergillus terreus*, *Aspergillus flavus*, *Aspergillus oryzae*, *Penicillium citrinum* (PC) and *Penicillium citrinum* (PG) by morphological observations using an electron microscope.

Microorganism Seeds

Asp. terreus, *Asp. flavus*, *Asp. oryzae*, *Peni. citrinum* (PC) and *Peni. citrinum* (PG) were inoculated into 20 ml MY liquid medium in a 100 ml test tube and cultured at 28-30°C for 48 hours, respectively, with shaking on a reciprocal shaker. Each cultured fungus was inoculated on 50 g sterilized wheat bran and cultured in a 1 l flask at 30°C for 3-4 days. The seeds were prepared by mixing 20 g of fungus cultured on wheat bran and 25 g of sterilized wheat bran. The seeds of

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Table 1. Operating conditions of gas-liquid chromatography (GLC)

Instrument:	JEOL Model JGC-20 KFP (detector: Flame ionization detector)
Stationary phase:	10% Diethylene glycol succinate polyester chromosorb W (AW, 100 mesh)
Column:	3 mm ϕ $\times$ 3 m sus-column (sus-27)
Carrier flow:	N ₂ 45 ml/min.
H ₂ pressure:	0.85 kg/cm ²
Air pressure:	2.5 kg/cm ²
Injection port temp.:	285°C
Column temp.:	185°C

combined microorganisms (*Asp. oryzae*, *Asp. sojae*, *Sac. cerevisiae*, and *Baci. subtilis*) was prepared by the same method as the previous study.¹⁾

Nonfermented and Fermented-Resteamed Scrap Meals

Nonfermented scrap meal (NF) and fermented-resteamed scrap meals with *Asp. terreus* (AT), *Asp. flavus* (AF), *Asp. oryzae* (AO), *Peni. citrinum* (PC), and *Peni. citrinum* (PG), and the combined microorganisms (FS) were prepared from mackerel waste by the same processes as the previous paper.¹⁾

Proximate, Amino Acids, and Fatty Acids Analyses

Moisture, protein, lipid, ash, carbohydrate, and POV, TBA, VBN values were quantified by the same methods described in the previous paper.¹⁾ Amino acids were analysed by an amino acid analyzer (JCL-6AH) after hydrolysis using 6 N HCl. Lipids were extracted from the scrap meals by the method of Folch *et al.*,³⁾ and transesterified by the method of Morrison and Smith.⁴⁾ Methyl

esters were stored at -12°C under nitrogen until analyzed by gas-liquid chromatography (GLC). GLC operating conditions for the determination of fatty acids are summarized in Table 1. The components of methyl esters were identified by Ackman and Burgher method⁵⁾ and equivalent chain length values.⁶⁾ The quantitative fatty acid data were calculated directly as area percent by triangulation method.

Results and Discussion

As shown in Table 2, the lipid content was considerably low in the nonfermented and fermented-resteamed scrap meals, compared with the waste (FW). On the other hand, the lipid, protein, and ash contents of fermented-resteamed scrap meals were similar to those of nonfermented scrap meal. However, the POV and TBA values of lipids from the fermented-resteamed scrap meals were remarkably lower than the waste and the nonfermented scrap meal. This indicates that the fungi from smoked-semidried skipjack can remove the toxic oxidized lipids as the combined microbe. On the other hand, VBN increased during the period of fermentation, but it declined to the lower value than that of nonfermented scrap meal by the resteaming and drying after fermentation.

The amino acid composition and the ratio of total essential amino acids to total nonessential amino acids (EAA/NEAA) have no significant difference between nonfermented and fermented-resteamed scrap meals, as shown in Table 3. Moreover, the EAA index calculated taking nonfermented scrap meal protein as standard showed about 93–98 in the fermented-resteamed scrap meals. Namely, it appears that the fermentation by these microbe don't change the nutritive value of

Table 2. Proximate compositions, and POV, TBA and VBN values of mackerel waste, non-fermented, and fermented-resteamed scrap meals

Meal		FW	NF	AT	AO	AF	PG	PC	FS
Moisture	(%)	68.6	2.1	3.3	2.4	3.7	2.9	3.4	3.6
Protein	(% d.m.)	52.5	53.9	52.6	52.8	52.3	52.1	52.6	52.9
Lipid	(% d.m.)	37.9	19.7	19.0	17.7	18.6	18.3	18.5	17.7
Ash	(% d.m.)		18.5	17.7	17.7	17.4	17.9	17.6	17.4
Carbohydrate* ¹	(% d.m.)		—	3.8	3.9	3.9	4.0	4.0	4.1
POV	(meq/kg)	220.1	165.8	26.0	29.4	32.0	30.1	29.6	28.4
TBA	(Number)	52.6	56.4	23.1	24.1	25.7	23.6	22.8	24.0
VBN	(mg %)* ²	61.1	39.4	33.1	32.9	34.8	34.1	35.6	35.6
				(79.7)	(75.6)	(83.0)	(87.9)	(99.1)	(84.8)

*¹ as glucose. *² VBN of mackerel waste, scrap meal and fermented-resteamed scrap meals, number in parenthesis indicates the values of VBN just after fermentation before resteaming. FW: mackerel waste, NF: nonfermented, AT: fermented with *Asp. oryzae*, AF: *Asp. flavus*, PG & PC: *Penicillium citrinum*, FS: combined microbe.

Table 3. Amino acid compositions of nonfermented and fermented-resteamed scrap meals

Amino acids	NF		AT		AO		AF		PG		PC		FS	
	g/100 g* ¹	%* ²	g/100 g* ¹	%* ²	g/100 g* ¹	%* ²	g/100 g* ¹	%* ²	g/100 g* ¹	%* ²	g/100 g* ¹	%* ²	g/100 g* ¹	%* ²
Lys.	3.90	7.5	4.23	8.3	4.41	8.6	3.83	7.6	4.03	8.0	4.39	8.7	4.07	7.9
His.	1.45	2.8	1.66	3.3	1.50	2.9	1.42	2.8	1.53	3.0	1.46	2.9	1.56	3.0
Arg.	2.94	5.6	2.79	5.5	3.50	6.8	2.76	5.5	2.85	5.6	1.85	3.6	3.35	6.5
Asp.	5.07	9.7	5.15	10.1	5.70	11.1	5.09	10.1	5.14	10.2	5.16	10.2	5.27	10.3
Thr.	2.36	4.5	2.36	4.6	2.55	5.0	2.27	4.5	2.31	4.6	2.32	4.6	2.35	4.6
Ser.	2.35	4.5	2.25	4.4	2.51	4.9	2.34	4.6	2.26	4.5	2.42	4.8	1.95	3.8
Glu.	7.04	13.5	6.88	13.5	7.47	14.5	6.70	13.3	6.81	13.5	6.55	12.9	6.89	13.4
Pro.	1.27	2.4	1.68	3.3	1.77	3.4	1.71	3.4	1.62	3.2	1.68	3.3	1.64	3.2
Gly.	4.85	9.3	4.60	9.0	5.01	9.7	4.41	8.8	4.63	9.2	4.72	9.3	4.55	8.9
Ala.	3.78	7.2	3.55	7.0	4.08	7.9	3.56	7.1	3.68	7.3	3.76	7.4	3.75	7.3
Val.	2.75	5.3	2.99	5.9	3.26	6.3	2.87	5.7	3.03	6.0	3.00	5.9	3.07	6.0
Met.	1.59	3.0	1.07	2.1	1.39	2.7	1.47	2.9	1.48	2.9	1.38	2.7	1.63	3.2
Ileu.	2.16	4.1	2.26	4.4	2.49	4.8	2.18	4.3	2.26	4.5	2.21	4.4	2.22	4.3
Leu.	3.87	7.4	4.04	7.9	4.35	8.4	3.83	7.6	4.01	7.9	3.92	7.7	3.93	7.7
Tyr.	1.63	3.1	1.46	2.9	1.53	3.0	1.48	2.9	1.53	3.0	1.67	3.3	1.42	2.8
Phe.	2.50	4.8	2.25	4.4	2.30	4.5	2.00	4.0	2.42	4.8	2.07	4.1	2.02	3.9
ΣEAA (%)* ²	45.0		46.1		50.0		44.9		47.3		44.6		47.1	
ΣNEAA (%)* ²	49.7		50.5		54.5		50.2		50.9		50.6		49.7	
EAA/NEAA	0.91		0.92		0.92		0.89		0.93		0.87		0.95	
EAA index* ³			94.7		98.0		97.0		97.1		93.2		97.0	

*¹ sample. *² % of crude protein. *³ calculated taking NF as standard.

Table 4. Fatty acid compositions of nonfermented and fermented-resteamed scrap meals

Fatty acids	NF	AT	AO	AF	PG	PC	FS
12:0	1.3	0.9	1.1	1.0	1.6	1.2	0.9
13:0	0.7	0.6	0.9	1.0	0.8	1.1	0.5
14:0	6.5	6.4	5.9	5.7	5.4	6.5	7.0
15:0	1.0	1.2	1.7	1.3	1.0	1.8	1.3
16:0	15.7	11.3	12.2	14.4	20.8		
16:1 ω 7	6.9	6.1	7.0	7.1	6.8	8.2	6.4
18:0	5.9	5.0	5.8	6.0	4.7	5.6	5.8
18:1 ω 9	23.3	19.8	19.7	17.7	14.8	20.4	17.3
18:2 ω 9	2.4	3.4	3.2	3.6	3.4	3.3	3.4
18:2 ω 6	1.1	1.0	1.4	1.3	1.3	1.5	0.7
18:3 ω 3	1.6	1.8	1.4	1.4	1.4	1.6	1.4
20:1 ω 9	7.4	7.1	6.5	7.4	6.4	6.8	7.2
20:1 ω 7	2.2	1.8	1.2	2.1	2.6	1.6	2.1
20:2 ω 9	1.9	0.8	0.6	1.1	0.9	1.5	0.9
20:4 ω 6	0.8	1.3	1.5	1.1	1.0	1.5	0.6
20:5 ω 3	3.4	6.1	5.1	4.8	5.4	4.6	4.4
22:1	5.6	6.0	5.8	5.6	4.8	5.0	5.8
22:5 ω 6	1.4	1.3	2.6	1.3	0.9	1.9	1.1
22:5 ω 3	0.3	1.6	0.5	0.7	0.3	0.1	0.5
22:6 ω 3	4.3	10.2	8.4	8.0	8.5	6.5	5.5
$\Sigma\omega$ 3 HUFA	8.0	17.9	14.0	13.5	14.2	11.2	10.4

the protein.

The fatty acid compositions of nonfermented and fermented-resteamed scrap meals are shown in Table 4. In spite of the similar lipid contents, the percent of ω 3 highly unsaturated fatty acids with more than 20 carbons (HUFA) in total fatty acids was considerably higher in the fermented-resteamed scrap meals than the nonfermented scrap meal. On the other hand, the POV and TBA values decreased by the fermentation. Therefore, the increase of ω 3 HUFA by the fermentation appears to be related to the decrease of oxidized lipid.

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