Preparation of Single Cell Protein (SCP) for Food and Improving of Its Spinnability

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Experiments were carried out to establish a new method for the preparation of protein from single cell microbes combining an Impact-Cell-Mill (ICM) method with a technics of wet spun fiber formation, and also were studied on the relationship among viscoelastic properties, preparations and the spinnabilities.

The obtained results were as follows:

- 1) ICM method was superior to alkaline extraction method both in separation and yield respectively.
- 2) Yeast off-flavour of SCP prepared by ICM method was markedly decreased by employing a hot ethanol treatment. Dynamic modulus (G') of SCP caused an increase and a shift to high frequency side (ω) by employing a hot ethanol treatment, and the purity of SCP was improved by removing lipid and carbohydrate with present method.
- 3) SCP prepared by ICM method was found inferior to casein phase separation and spinnability. But its rheological properties and spinnabilities could be changed with the hot ethanol treatment. Present SCP showed a higher value in recovery and good coloring as a spun-fiber, but a decrease in nucleic acid contents by the spinning process. The dope in which nucleic acid content was markedly decreased showed good spinnability.

Necessities of researching on utilization of protein from single cell microbes have been pointed out in these days.^{1~4)}

The possible use of microbial single cell protein as a new protein resources for human food has been receiving much attention nowadays. However, some adverse responses occur when large amounts of products are incorporated into human food. A large limitation to the use of SCP as a food source is controlled by its high nucleic acid content.^{5,6)}

Experiments are being carried out to establish a new method for the production of protein from Baker's yeast employing an Impact-Cell-Mill (ICM) method^{15,16)} and a technics of wet spinning. Spun fibrous yeast protein is suitable for food. Because the nucleic acid content was below 2%.

Kelley *et al.*⁷⁾ Naismith,⁸⁾ and Balmaceda *et al.*¹³⁾ studied the spinnability of soya protein and peanut protein. Ultracentrifugal examination of some proteins treated with various bases had been made. It has been shown that

only bases with dissociation constants higher than 10^{-4} cause an appreciable reduction in the molecular weight of protein and are suitable for fiber formation with the protein.

Héden *et al.*⁹⁾ and Huang and Rha¹⁰⁾ studied on the spinnability of SCP from hot melt, and found that it could not be used for food directly, because it contains a lot of nucleic acid and some basic salts.

Authors studied on the relations among viscoelastic properties and spinnabilities of SCP prepared by ICM method, commercial casein and soya protein. Conventional SCP and soya protein were inferior to commercial casein in the spinning condition. Accordingly, for the improvement of its spinnability a great importance was suggested to increase strength of fiber in the spinning.

MATERIALS AND METHODS

Material and preparations. a) Baker's yeast was prepared from *Saccharomyces cerevisiae* (produced by

FIG. 1. Production Scheme of Yeast Protein.

Kanegafuchi Chemical Industrial Co., Ltd.) as shown in Fig. 1.

b) Soya protein was prepared from defatted soybean meal (produced by Showa Sangyo Co., Ltd.) as shown in Fig. 2.

c) A commercial casein was obtained from Ishizu Pharmaceutical Co., Ltd.

d) Spinning dopes were prepared by taking maturing time (5~120 min) after adding NaOH (final concentration 0.9~2.4%) to 10~25% protein suspension and heating 50°~80°C.

Viscoelasticity measurement. Dynamic modulus (G') and loss modulus (G'') were calculated with Markovitz's equation.¹¹⁾

Spinnability test. The spinnability of dope was

measured by a trial spinning machine which was developed in our laboratory as shown in Fig. 3, with the following equipments, (1) hopper: volume 250 ml, thermoregulated, (2) nozzle: diameter 0.1~0.3 m/m, 1~15 holes, (3) coagulating vat: volume 8 liters, length 1500 m/m, made from a transparent vinyl chloride, (4) reel: velocity 10~100 m/min, in variable.

A commercial casein had been used as a standard material for spinnability test. The spinning condition was classified with a fiber formation in spinning.

Classification "A" indicated the best fiber formation when the fiber could be reeled more than 35 m/min with commercial casein. Alkali extracted SCP showed "a," ICM treated SCP employing acetone drying showed "a," and ICM treated SCP employing a hot ethanol treatment showed "1."

Classification "B" indicated a condition that fiber

FIG. 2. Preparation of Soybean Protein.

FIG. 3. Schematic Diagram of the Trial Spinning Machine.

① dope vat, ② control valve, ③ spinneret, ④ coagulating vat, ⑤, ⑥ rolls, ⑦ reel, ⑧ rpm counter disk, ⑨ motor, ⑩ pump, ⑪ drain.

could be reeled with velocity slower than 35 m/min with casein. Also “ β ” in alkaline extraction method, “b” in ICM treated SCP employing acetone drying, “2” in ICM treated SCP employing the hot ethanol treatment.

Classification “C” indicated a condition that a fiber

could be made like a yarn, but could not be reeled with casein. Also “ γ ” in alkaline extraction method, “c” in ICM treated SCP employing acetone drying, “3” in ICM treated SCP employing the hot ethanol treatment.

Classification “D” indicated a gel formation as a dope did not resolve with casein, also “ δ ” in alkaline extraction method, “d” in ICM treated SCP employing acetone drying, “4” in ICM treated SCP employing the hot ethanol treatment.

Classification “E” indicated a sol that the dope dissolved into a smaller molecularity, as this dope went to dispersion in the coagulation solution then flowed from a spinneret with casein, also “ θ ” in alkali extracted SCP, “e” in ICM treated SCP employing acetone drying “5” in ICM treated SCP employing a hot ethanol treatment.

Gel filtration of spinning dope. Sepharose 6B was used to obtain the gel filtration chromatogram of these spinning dopes. A column of the Sepharose (1.5 × 90 cm) was equilibrated with 0.076 M Tris-citrate buffer (pH 8.6) containing 0.01 M 2 mercapto ethanol and 0.4 M NaCl. A flow rate 5 ml/hr was maintained by restricting a screw cock. Every 2 ml aliquot was collected with a fraction collector and absorbance at 280 nm was measured with a Hitachi-Perkin Elmar 139 UV Spectrophotometer using 0.5 cm light path.

RESULTS AND DISCUSSION

SCP production methods and compositions

ICM method is superior to an alkaline extraction method (hot or cold extraction) both in extraction and yield respectively. SCP that was extracted by a hot alkaline extraction method indicated about 94% and that was extracted by ICM method employing a hot ethanol treatment indicated 89.4% purity. Also it was about 81% when SCP was lyophilized. These values are shown in Table I.

However, SCP has a high nucleic acid con-

TABLE I. ANALYTICAL DATA OF BAKER'S YEAST PROTEIN

	ICM extraction				Alkaline extraction	
	Lyophilization	Acetone drying	Hot ethanol treatment (1 hr) (2 hr)		(0.5 N, 20°C, 2 hr)	(0.125 N, 80°C, 1 hr)
Crude protein ^{a)}	80.4	87.0	86.8	89.4	81.7	94.2
Lipid ^{b)}	12.3	6.4	6.31	4.86	6.84	1.90
Carbohydrate ^{c)}	2.81	2.68	2.89	2.57	6.46	2.70
Ash	4.17	4.00	3.91	3.25	5.00	1.20
Organic phosphorus ^{d)}	1.13	1.09	1.43	1.36	0.933	0.978

^{a)} Kjeldahl N × 6.25, ^{b)} chloroform-methanol mixture, ^{c)} phenol-sulfuric method,

^{d)} the proposed modification of Allen's method: (total phosphorus) — (non organic phosphorus).

TABLE II. INFLUENCE OF PREPARATIONS ON THE COLORING AND RECOVERY

	ICM extraction				Alkaline extraction	
	Lyophili- zation	Acetone drying	Hot ethanol treatment (1 hr) (2 hr)		(0.5 N, 20°C, 2 hr)	(0.125 N, 80°C, 1 hr)
O.D. ₄₀₀	0.320	0.196	0.150	0.120	0.326	0.328
Recovery		62%			17%	32%

tent notwithstanding protein extraction method from single cell microbes in general. Especially, ICM method showed high value of nucleic acid content 20~30% than alkaline extraction method. Also the recovery in alkaline extraction method was small, an amount of SCP and nucleic acid extracted from single cell microbe showed a low value than ICM method.

Generally, by observing the degree of coloring of the obtained protein with five preparations obtained by combined methods of extraction and drying, it was seen that the hot

ethanol defatted and dried preparation was the best one, giving light colored (O.D.₄₀₀=0.120) material, the value was small as shown in Table II. Conventional SCP was colored in all alkaline extractions notwithstanding extraction temperature.

SCP prepared by ICM method has a strong off-flavour in general, but it showed less off-flavour with the hot ethanol treatment.

On the other hand, the off-flavour is obscured by alkaline extraction in general.

Accordingly, ICM method shows about

TABLE III. EFFECT OF DOPE COMPONENTS ON THE SPINNABILITY

Protein concentration (%)														
	25	D	B	B	B	γ	γ	γ	γ	β	β	γ	γ	
24	D	B	B	A	A	B	B	β	β	α	α	α	β	γ
23	B	B	A	A	A	B	B	β	β	β	β	β	β	γ
22	A	A	A	A	A	B	C	β	β	β	β	θ	θ	
21	A	A	A	A	A	B	B	θ	dθ	dθ	c	c	c	b
20	A	A	A	A	B	B	C	θ	d	d	c	c	b	b
19	A	A	A	A	B	B		d	c	b	b	b	c	e
18	A	A	A	B	B	B		d	b	b	b	b	c	c
17	A	A	A	B	C	C		c	b	b	c	c	1	2
16	A	A	A	B	C		c	c	c	b	b	b	c	3
15		B	B	C	3	2	1	1	1	2	2	2	3	
14		B	B	E	3	2	2	1	1	2	2	3		
13		B	C	3	2	1	1	2	3					
12		C	3	2	1	1	2	3	3					
11	3	2	1	1	2	3	5							
10	2	1	1	2	3	5								
	0.7	1.0		1.5		2.0		2.5		NaOH concentration (%)				

Commercial casein: classification mark "A," "B," "C," "D," "E."

Alkali extracted SCP: classification mark "α," "β," "γ," "δ," "θ."

ICM treated SCP employing acetone drying: classification mark "a," "b," "c," "d," "e."

ICM treated SCP employing a hot ethanol treatment: classification mark "1," "2," "3," "4," "5."

62% of protein recovery with two times alkali extracted.

Spinnability and dope composition

The spinnability of SCP was measured with a trial spinning machine. The results are shown in Table III. Casein showed good results in many field in comparison with other protein as shown in Table III. Spinnability of the dope which was prepared by the hot alkaline extraction method resembled with casein. But the fiber could not be recovered as they were dissolved almost completely in water through washing process after spinning.

SCP prepared by ICM method employing a hot ethanol treatment could be spun like casein in a range from 10% to 13% protein concentration. Present spun fiber has a good elasticity, tenacity, and shows good fiber formation.

Distribution of molecular weight of SCP and casein

SCP showed some differences in spinnability according to the preparing and extracting processes of SCP. In order to know the differences in the spinnability of SCP, gel filtration (Sephacrose 6 B) was used to compared SCP with casein. The result is shown in

FIG. 4. Column Chromatograms of an ICM Treated SCP, an Alkali Extracted SCP, ICM and Hot Ethanol Treated SCP, and Casein with Sepharose 6B.

◇—◇, casein; ○—○, ICM, acetone dried SCP; ●—●, ICM, hot ethanol treated SCP; ▲—▲, hot alkali extracted SCP.

Fig. 4, in this case, a 0.5% concentration of SCP was used. From the result in Fig. 4, SCP extracted by ICM method employing a hot ethanol treatment showed a peak at effluent volume of 52~56 ml. The dimension of the chromatogram behind the peak was comparatively larger, similar to that of SCP which was extracted by ICM method.

This SCP showed about $M_w > 600,000$ ($K_{av} = 0.095$), the molecular weight indicates a little increase after a hot ethanol treatment. On the other hand, a chromatogram of SCP that was extracted by hot alkaline solution for one hour showed a peak at effluent volume of 135~145 ml, the dimension of the chromatogram before the peak was comparatively larger, its molecular weight was indicated to be about $M_w = 36,000$ ($K_{av} = 0.722$) a chromatogram of casein showed a peak between SCP from ICM method and SCP from the alkaline extraction, its molecular weight was estimated to be about $M_w = 45,000$ ($K_{av} = 0.636$), and it will be seen that a casein has a limited distribution of molecular weight from a effluent pattern.

Relation of $\log G'$ and $\log \omega \cdot a_T$

After measuring the viscoelastic properties of spinning dopes, authors studied a hypothesis of "time-temperature reducibility" of spinning dopes, the criterion, the form of $\log a_T$ as a function of temperature was applied first by noting that it was a smooth function with no gross fluctuations or irregularities and then by fitting the empirical values to an expression which has proved to be widely applicable.

$$\log a_T = -C_1(T-T_s)/(C_2 + T - T_s)^{14)}$$

For this purpose, $(T-T_s)/\log a_T$ listed in previous paper¹²⁾ were plotted against $(T-T_s)$ and reduced to $T = 30^\circ\text{C}$ as Fig. 6. These values were actually used in constructing Fig. 5. From these results, there were some fundamental difference between the dope of casein and the dope of SCP. From the slope S of strage modulus of spinning dopes against frequency reduced to 30°C .

When a slope S showed a change in a limita-

FIG. 5. Storage Modulus of Spinning Dopes against Frequency Reduced to 30°C.

①, casein, protein concentration 20%, 0.9% NaOH; ②, hot ethanol treated SCP, protein concentration 16%, 1.6% NaOH; ③, hot alkali extracted SCP, protein concentration 24%, 1.7% NaOH, ④, acetone dried SCP, protein concentration 20%, 2.0% NaOH.

tion from 1/2 to 2 against frequency, the dope showed a “rubber-like flow” by the spectrum of dynamic modulus. Thus, this behavior was like an uncross-linked polymers. It is superior to spin as casein takes a single dispersion in this rubber-like flow, but a slope S of SCP showed a small value than slope S of casein. There were no fundamental difference among the dopes of SCP that were prepared from ICM method combined with a hot ethanol treatment or alkaline extraction, *etc.*

As the spectrum of dynamic modulus of SCP shifted to high frequency side with the hot ethanol treatment. So the molecule of SCP appeared to be combined as subunits or subchains by the hot ethanol treatment.

Relation of $\tan \delta$ and $\log \omega \cdot a_T$

The relation of preparation methods of SCP and $\tan \delta$ are shown in Fig. 7. The spinning dope made from casein shows the transition zone that a rubber-like flow changes into rubber-like elasticity against increasing the frequency (ω) in the limitation from -1 to 1 , but a dope of SCP which was prepared by ICM method employing acetone drying had no transition zone from a rubber-like elasticity

FIG. 6. Temperature Dependence of Shift Factor a_T Used in Previous Paper, Chosen Empirically; Curve, from WLF Equation.¹⁴⁾

No.	Sample	Protein	NaOH	Mark	Ts(°C)
1	Casein	20.0	1.0	●	-45.0
2	Casein	10.0	1.0	△	8.0
	M. yeast P.	10.0			
3	Casein	26.0	1.0	◇	21.0
4	Casein	24.0	1.0	○	24.0
5	Soy bean protein	18.0	2.1	●	43.0
6	Soy bean protein	18.0	1.9	○	41.5
7	Soybean protein	18.0	1.8	◇	37.8
8	Soybean protein	20.0	2.0	◆	-6.5
9	Soybean protein	18.0	2.2	△	-102.0
10	M. yeast protein	18.0	1.8	○	-12.0
11	M. yeast protein	18.0	1.4	●	-14.0
12	M. yeast protein	18.0	1.2	◇	-18.0
13	M. yeast protein	20.0	1.5	△	27.5

to rubber-like flow. These phenomena are unsuitable to spin a fiber formation. On the other hand, a dope of SCP which was prepared by employing the hot ethanol treatment showed a viscoelastic properties as well as alkaline extraction protein, the dynamic modulus increased along with frequency (ω) but less than casein.

FIG. 7. Relation between $\tan \delta$ and Kinds of Protein Reduced to 30°C.

①, ICM treated SCP employing a hot ethanol treatment; ②, alkali extracted SCP; ③, ICM treated SCP employing acetone drying; ④, commercial casein.

FIG. 8. Relaxation Spectrum of Spinning Dopes. ①, dope "A," "1"; ②, dope "B," "b," "2"; ③, dope "C," "c," "3"; ④, dope "D," "d," "δ," "4"; ⑤, dope "β," "γ."

Relaxation spectrum of spinning dope

The relaxation spectrum of spinning dope shows a typical box type where the dopes have a good spinnability as shown in Fig. 8. Especially, a dope that has good spinnability shows a spectrum like type "A" in Fig. 8. The dope of SCP that was prepared by ICM method shows a spectrum like the type "B" usually, but these spectra change from type "B" to type "A" by employing the hot ethanol treatment in protein concentration about 10~13%. It is consider single dispersion to show the spectrum like type "A."

In the hot alkaline extraction protein, the spectrum usually shows a spectrum like type "E," from these phenomena, this molecular distribution is considered to be wide and multi dispersion. Thus, ICM method is superior to prepare SCP for spinning than alkaline extraction.

SCP that was prepared by ICM method was inferior to casein in the phase separation and spinnability. But SCP could be changed in rheological properties and spinnabilities by employing the hot ethanol treatment. SCP prepared by this method showed a high recovery and good coloring as a spun fiber, and nucleic acid content was decreased below 2% by the spinning process. The analytical data are shown in Table IV. The dope that nucleic acid content is largely on the decrease shows good spinnability in general.

TABLE IV. ANALYTICAL DATA OF SPUN-FIBER YEAST PROTEIN

	Classification "1" (good formation)	Classification "3" (nonphase separation)
Crude protein ^{a)}	92.0	89.1
Lipid ^{b)}	4.38	4.12
Carbohydrate ^{c)}	0.45	1.07
RNA ^{d)}	1.12	4.34
Ash	1.31	1.30

^{a)} Lowry's method.

^{b)} Chloroform-methanol mixture (1:1).

^{c)} Phenol-sulfuric method.

^{d)} The proposed modification of Allen's method: (total phosphorus) - (non organic phosphorus).

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