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Effect of Extrusion-Cooking on the Molecular Structure and Alcohol Yield of Wheat Starch

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Abstract

Wheat flour was extruded by a single-screw extruder, and used for the ethanol production of *takju*. The molecular structure and enzymic susceptability of extruded starch were compared to those of steam cooked one. The gel permeation chromatographic pattern of wheat flour extrudates was not significantly different from those of raw and steam cooked starches. However, the conversion rate of extruded starch into maltose by α-amylase hydrolysis was significantly faster than those of raw ad steamed starch. The molecular weight of starch estimated from GPC pattern and the intrinsic viscosity were remarkably reduced by extrusion cooking followed by the enzymic hydrolysis for 30 min, while steam cooking and enzymic hydrolysis for 30 min did not change them significantly. Extrusion-cooked flour produced alcohol 26% higher than that of steamed flour in the laboratory *takju* fermentation, and 10% more alcohol in the pilot plant scale *takju* production.

Key words : extrusion-cooking of flour, starch molecular structure, enzymic susceptability, alcohol yield, *takju* fermentation

Introduction

Extrusion-cooker has been studied for the use of chemical reactor of biological materials. Boulle(1) used a twin-screw extruder for the manufacture of caseinate in a few second of reaction time and compared to the conventional reaction tank and drum drier, which required minimum 30-40 min of mixing time. Linko *et al.*(2) and Hakulin *et al.*(3) demonstrated the possible use of extruder as a bioreactor. The most widely studied area of extrusion technology as bioreactor is the enzymatic hydrolysis of starchy materials into glucose syrup. The ethanol production rate of the extrudate of cereal substrates was compared to those of untreated or conventionally gelatinized starchy(4,5). The bioconversion rate was influenced by screw dimension, barel temperature, residence time and the feed moisture content(3,6,7). Lee *et al.*(8) used extruder for the pretreatment of substrates for lactic acid fermentation of rice and defatted soybean meal mixture. They used it for the further breakdown of plant tissue, sterilization of the prefermented substrates for the subsequent lactic acid fermentation and elimination of undesirable volatiles.

The accelerated enzymatic reaction during or after extrusion-cooking of starchy materials would be mainly caused by the effective disruption of starch-protein matrix of plant tissue subjected to the high-shear and sudden pressure drop during extrusion cooking. The breakdown of starch molecules during extrusion-cooking has been studied by gel permeation chromatography(7,9). Davidson *et al.*(10) emonstrated the reduction of molecular size during extrusion-cooking of starch by both GPC and intrinsic viscosity determined by a capillary viscometer.

In the present study, wheat flour was extruded by a single-screw extruder, and subjected to the ethanol production of *takju*, a traditional Korean alcoholic beverage which is unfiltered turbid beer. The effect of extrusion-cooking on the alcohol yield was explained by the enzymatic susceptability of extruded materials. The molecular size reduction during extrusion-cooking and further enzymatic hydrolysis of starch was demonstrated by the GPC pattern and intricsic viscosity.

Materials and Methods

Sample preparation

Patent grade wheat flour containing 8.7% protein was obtained from Daesun Flour Milling Co. in Seoul. The moisture content of flour was adjusted to 25% by adding water and equilibrated in a plastic bag at

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4℃ for 24 hrs before extrusion-cooking.

A laboratory-made pilot plant scale single screw extruder(D=59.4 mm, L/D=11) was used(11). The screw rotational speed was 300 rpm and feed rate was 250 g/min. The median residence time, determined by Kao and Allison's color distribution method(12), was 35 sec.

Measurements of extrudate properties

The bulk density of extrudate was determined by measuring the volume of known mass a sample in 1 *l*-mess cylinder filled with millet. The water absorption indes(WAI) and water solubility index(WSI) were measured by Anderson's method(13). The amylogram viscosity of flour and extrudate was measured by Brabender Visco-Amylograph. The differential scanning calorimetry pattern was measured with Perkin-Elmer DSC-4 according to Donovan's method(14).

Enzyme susceptability measurement

500 mg of starch sample was dispersed in 19.5 m*l* of acetate buffer(20 mM, pH 5.0) by shaking at 55℃. 0.5 m*l* of dilute α-amylase(Fungamyl, Novo A/S) solution(1 FAU) was added and kept shaking for 2.5~60 min. One FAU wa sdefined as the α-amylase activity of break-down 5.26 g starch per an hour. The hydrolized sample was taken periodically, and the enzymic action was terminated by pH adjustment with NaOH solution combined with heating in boiling water for 5 min. It was immediately cooled in ice water and added with 19.5 m*l* of 2 N KOH, and centrifuged at 2000 rpm for 20 min. The supernatant was subjected to the measurements of reducing sugar and gel permeation chromatography pattern.

Gel chromatography

The molecular size of starch in raw, steamed(160 ℃, 40 min) and extruded flours was compared by gel permeation chromatography. Sepharose CL-2B(Pharmacia Fine Chemicals) was filled in a column (D=2 cm, Height: 100 cm). 4 m*l* of sample solution was applied and eluted with 0.1 N-KOH solution at a flow rate of 20 m*l*/hr by using peristalic pump. The eluent was received in a tube of 4 m*l* each, and the total sugar content was measured by phenol-sulfuric acid method(15). The same method was also used for the determination of molecular size change after enzymic hydrolysis of starch samples. Standard dextrans(MW 5×10^5~5×10^6) were eluted for the estimation of molecular weight.

Measurement of intrinsic viscosity

The changes in molecular size of starch was demonstrated by intrinsic viscosity measurements as an indirect estimtion of molecular size. According to Greenwood method(6), samples were disolved in 2.0 N KOH solution and diluted with 0.2 N KOH to the concentration of 0.65-0.3%(w/v). It was filtered through glass filter(ASTM 10-15) and subjected to viscosity measurement by using Ubbelohde capillary viscometer at 25℃ water bath. The specific viscosity($\eta_{\sigma\pi}$) was calculated from the flow times of solution (t) and solvent (t_0).

$$\eta_{sp} = (\eta - \eta_0)/\eta_0 = (t - t_0)/t_0$$

$$[\eta] = \lim \ \eta_{sp}c$$

Where η and η_0 are the viscosities of solution and solvent respectively, and $[\eta]$ is intrinsic visccosity.

Tests of *takju* fermentation

Test fermentation of *takju* was carried out in the laboratory as well as in pilot plant scale. The alcohol yields of steamed flour and extrusion-cooked flour were compared. The extrudate of flour was used for the second fermentation(75% of total amount of substrate), as shown in Fig. 1.

The exact recipe and fermentation conditions are described in a previous report(17).

Results

Physicochemical properties of extrudates

Table 1 shows that the expansion ratio increased and bulk density decreased as the feed rate increased. WAI increased as the feed rate increased but WSI reached to the maximum at the feed rate of 250 g/min. All the extrudates did not have paste viscosity maximum in amylograph and enthalpy peak in DSC thermogram but showed 32-45% of degree of gelatinization.

Enzyme susceptability

Fig. 2 shows the changes in maltose content during the enzymic hydrolysis of starch in the flours treated in different methods. Raw starch did not hydrolize into maltose in any significant amount by the action of α-amylase for 60 min. Steamed flour showed a moderate rate of hydrolysis, while extruded flour was hydrolized 3-4 times faster than steamed one. After one hour of hydrolysis the maltose equivalent of dif-

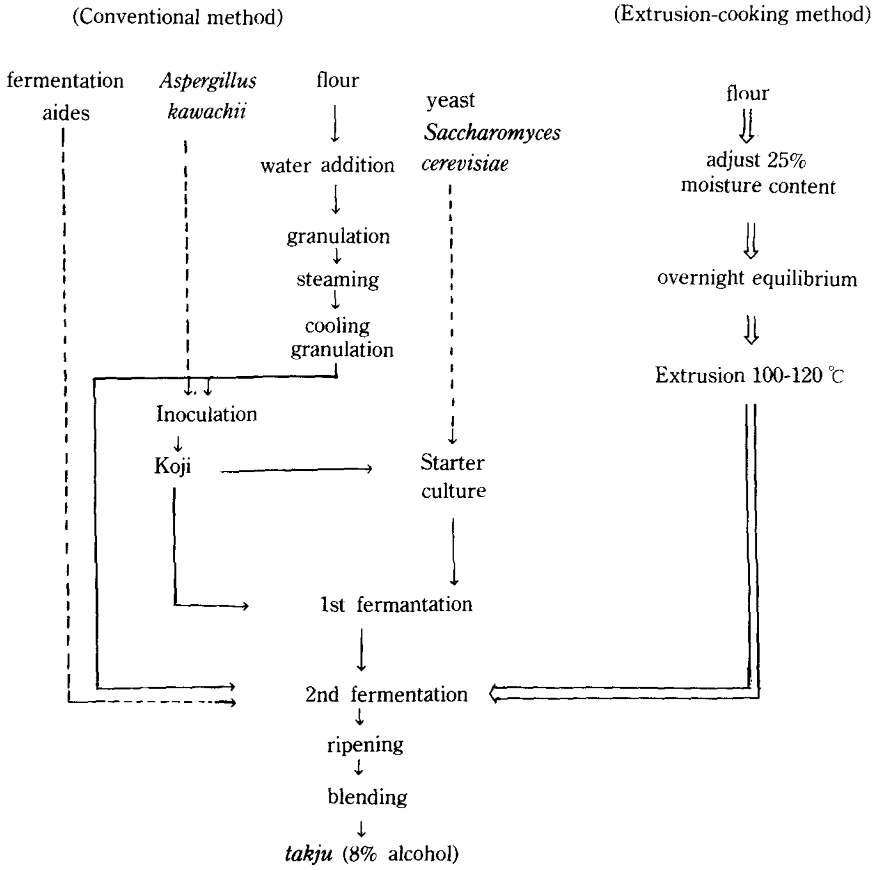

Fig. 1. Flow chart for *takju* fermentation

Table 1. Physicochemical properties of wheat flour and extrudates

Sample	E.R	B.D	WSI	WAI	P.V	D.G	ΔH
Raw	–	–	0.38	1.50	420	0.0	0.92
E200	1.86	0.90	3.63	5.73	–	39.1	–
E250	2.39	0.42	4.20	6.03	–	45.9	–
E300	2.88	0.42	3.62	6.43	–	32.3	–
E350	2.97	0.34	3.29	6.32	–	37.9	–

E; extrudate; Subnumbers, feed rate(g/min); E.R, expansion ratio; B.D, bulk density(g/cm^3); WSI, water soluble index(%) per 1 m*l*; WAI, water absorption index(g/g); P.V, paste viscosity at maximum peak(B.U); D.G, degree of gelatinization(%); ΔH, enthalpy at the first peak of the DSC thermograms(cal/g)

ferent flours, raw, steamed and extruded, were 0.4, 2.0 ad 5-7 g maltose/100 g solid, respectively. It indicated that extrusion-cookig in a single screw extrder enhanced the enzyme suseptability of wheat starch significantly, compared to the conventional steam cooking.

The GPC patterns of the three differently treated starch samples were not noticeably different one another, as shwon in Fig. 3. However, when they were treated with α-amylase for 30 min the GPC patterns varied remarkably one another, as shwon in Fig. 4. Raw starch was not changed significantly in their GPC patterns by α-amylase treatment. The high molecular weight fraction(5×10^6 dalton) of steamed starch was reduced by enzymic hydrolysis. In case of extruded starch it was apparent that most of high molecular weight fraction was converted into smaller molecular weight(5×10^5) polysaccharides.

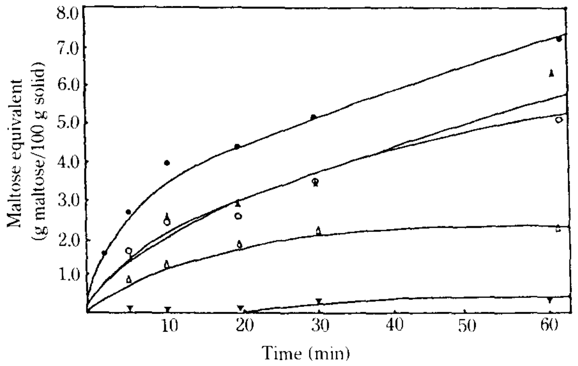

Fig. 2. Changes in maltose equivalent of flour by the treatment with α-amylase (E/S; 1FAS/500 mg, temp.; 55 °C, pH;5.0) after different cooking treatments
▼; wheat flour, △; steaned wheat flour, ○; extrudate (feed rate 200 g/min.) ●; extrudate (feed rate 250 g/min.) ▲; extrudate (feed rate 300 g/min.)

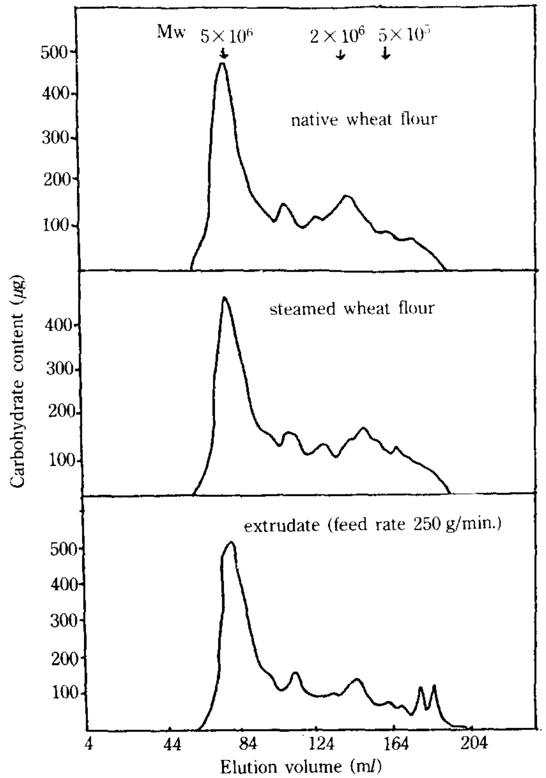

Fig. 3. Elution pattern on Sepharose CL-2B of wheat flour, steamed flour and extruded flour

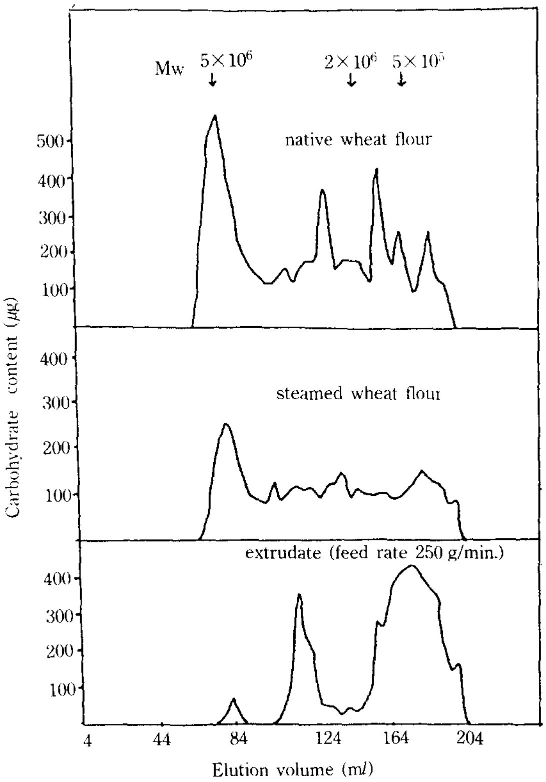

Fig. 4. Elution pattern of α-amylase hydrolyzed raw flour, steamed flour and extruded flour on Sepharose CL-2B gel chromatography

Table 2 shows the molecular size distribution estimated from the GPC pattern. In raw wheat flour, 73% of starch had molecular weight over 2×10^6 dalton, and 14% was smaller than 5×10^5. By α-amylase treatment for 30 min, the fraction of high molecular weight decreased to 61% and the low molecular weight fraction increased to 28%. The effect of α-amylase on steamed wheat flour was similar to that of raw wheat flour.

On the other hand, the high molecular fraction of extruded flour decreased rapidly by α-amylase and the low molecular fraction increased to 55% by 30 min of the enzymic hydrolysis.

The intrinsic viscosity measurement confirmed the above GPC results. Table 3 shows that the intrinsic viscosity of raw flour was 1.240 and 1.219 dl/g, respectively, before and after enzymic treatment. It was reduced slightly by steaming, but drastically reduced to 0.389 dl/g by extrusion-cooking followed by enzymicc treatment.

Alcohol yield

Table 4 compares the alcohol yield of extrusion-cooked flour to that of steamed flour. In the laboratory scale fermentation, extrusion-cooked flour pro-

Table 2. Distribution of molecular weight of α-amylase digested sample as determined by Sepharse CL-2B column chromatography

Time of enzymic treatment (min)	Molecular weight			Average
	$Mw \geq 2 \times 10^6$	$5 \times 10^2 \leq Mw \leq 2 \times 10^6$	$Mw \leq 5 \times 10^5$	Mw (10^6)
Raw flour				
0	73.00%	12.80%	13.94%	1.70
10	66.42	13.96	19.3	1.60
30	62.35	10.23	28.42	1.50
Steamed flour				
0	72.06	10.40	15.43	1.65
10	64.11	11.65	22.27	1.69
30	62.27	10.27	25.59	1.50
Extruded flour				
0	77.67	9.16	12.92	1.71
10	60.06	14.28	24.13	1.50
30	32.30	9.28	55.18	1.04

Table 3. Intrinsic viscosity(dlg^{-1}) of flour solutions in 0.2 N KOH before and after α-amylase treatment

Sample	Before enzymic treatment	After enzymic treatment
Raw flour	1.2400	1.2176
Steamed flour	0.9911	0.6575
Extruded flour	0.9585	0.3894

duced alcohol 26% higher than that of steamed flour. In the pilot plant scale fermentation extrusion-cooking resulted in 10% higher alcohol yield than the conventional steam cooking.

Discussion

Linko *et al.*(3) reported that extruded starches could yields higher reducing sugar by α-amylase treatment compared to the conventionally gelatinized starches. Similar result was also demonstrated in this experiment. Wasserman *et al.*(9) could demonstrate the changes in GPC pattern after extrusion-cooking of starch. They used Sephacryl S-100 gel for the separation, and a twin-screw extruder. But the present study could not demonstrate any significant changes in GPC pattern by extrusion-cooking only.

However, the present study could distinguish clearly the molecular changes between conventional steam cooking and extrusion-cooking of starches by using a short-time α-amylase treatment after cooking. The enhanced enzymaticc susceptability of extruded sample may come from the reduction of starch molecules during extrusion process. But it may be caused partly, or more importantly, by the disruption of starch-protein matrix of plant tissue, which will expand the room for enzymic contact to the reaction sites of the substrate.

The expanded enzymic contact to the substrate will increase the reaction rate as well as the amount of reaction product. The increased alcohol yield of extruded wheat flour is appeared to be resulted by the combination of these two effects. Since *takju* is

Table 4. Yields of alcohol during *takju* fermentation of flours by extrusion-cooking(E) and steam-cooking(S)

Flour treatment	Time for 2nd fermentation (hr)	Alcohol concentration (%)	Alcohol yield (ml alcohol/ 100 g solid)	% increase (E/S)
Laboratory test				
wheat grade 1				
steamed	45	8	25	
extruded	45	8.3	31.6	26
Pilot plant scale production				
wheat grade 1				
steamed	72	13.3	40	
extruded	72	12.8	44.1	10.3

made by incomplete alcohol fermentation in reltively short time, 2-3 days of fermentation, the enhanced reaction rate will increase the alcohol yields of the product. The increase in alcohol production by extrusion cooking shown in this experiment was significantly high and warranted for further study on the cost effectiveness.

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압출조리에 의한 밀가루 전분질의 분자구조 변화와 알콜발효 효율에 관한 연구

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단일축 압출조리기를 이용하여 밀가루를 조리한 후 탁주발효용 기질로 사용하여 알코올 수율을 조사하였다. 또한 스팀가열과 비교하여 압출조리에 의한 밀가루 전분질의 분자구조 변화와 효소 민감성에 대하여 gel permeation chromatography(GPC)와 고유점도 측정으로 평가하였다. 밀가루의 GPC patternd은 스팀가열이나 압출조리에 의하여 크게 변화되지 않았다. 그러나 α-amylase를 30분간 처리할 경우 압출성형된 밀가루의 맥아당 생산량이 크게 증가하며 GPC pattern도 크게 변하여 저분자 전분질의 함량이 증가하며 고유점도는 감소하였다. 압출성형된 밀가루로 탁주발효를 한 결과 스팀증자된 밀가루로 탁주발효를 한 경우보다 실험실 규모 실험에서는 26%의 알콜생산 증대가 기록되었으며 공장규모 발효에서는 10% 이상의 알콜생산 증대를 얻을 수 있었다.