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Nutritional Evaluation of Tofu Containing Dried Soymilk Residue(DSR)
1. Evaluation of Protein Quality

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Abstract

The effect of dried soymilk residue (DSR) on protein quality of tofu was studied. The amount of added DSR into soybean water extract was corresponding 10% (dry basis) of soybean used in tofu manufacturing. Proximate composition and *in vitro* protein qualities of soybeans at different stages of the conversion into tofu have also been investigated. Partially substituted tofu with DSR (TDSR) had higher moisture content (80.6%) than that of tofu prepared in traditional manner (TT). TDSR contained lower content of protein (38.9%) and total lipid (26.9%) compared to 45.8% of protein and 34.3% of total lipid for TT. A large amount of trypsin inhibitor (TI) in raw soybeans was diminished and extracted through tofu processing, and only 10~13% of TI in raw soybean remained in both tofu products (TDSR and TT). There was not a considerable difference in amino acid profiles between TT and TDSR, but TDSR had a higher content of lysine than that in TT. *In vitro* studies showed that TDSR and TT were comparable in terms of both *in vitro* digestibilities (90% over for four-enzyme digestibility and predicted digestibility) and discriminant computed protein efficiency ratio (2.07~2.14, DC-PER). Unlike those *in vitro* indices for protein quality, computed protein efficiency ratio (C-PER) of TDSR was much lower (1.4) than that of TT (1.95). It was revealed that C-PERs of tofu products were not in agreement with rat-PERs (1.7~1.9) in previous reports except for TT. However, DC-PER assay was more recommendable for protein quality of tofu products than C-PER assay.

Key words ; dried soymilk residue (DSR), tofu, digestibility, *in vitro* protein quality

INTRODUCTION

Tofu has long been an important source of protein in the diet of oriental countries. Recently, western consumers' interests in nutrition has increased significantly, one result being increased consumption of soy proteins as a primary source of dietary vegetable protein. However, production of tofu is increasing due to an increase in vegetarians and acceptance by the general population. Especially in Korea, tofu has found acceptance as a high protein food, and 10 more kinds of tofu have been manufactured according to traditional recipe[1]. But a tendency toward increased those tofu demand, only 15% of raw soybean had been consumed as foodstuffs in Korea, 1991[2]. One of the major reasons for lower utilization may be a great portion of insoluble residue with a range of 15~25% of soybean used in soymilk or tofu production[3,4]. As a result, utilization of the soymilk residue (called Bi-jee in Korean and Okara in Japanese) obtained as a by-product is a major concern for soymilk or tofu processors, since its nutritive values such as PER[5~9] and sulfur-containing amino acid content[8,9] are higher than those of soy milk. Although protein quality of soymilk residue is excellent, this fraction has difficulty in use for human food due to rapid spoilage and lipid deterioration during storage. In an attempt to improve the usefulness of soymilk residue, dried soymilk residue (DSR) had been prepared by hot-air drying[10] and solvent washing[11,12]. The supplementary effect of DSR on water sorption isotherms with wheat flour[13] and physical characteristics of tofu[14] had also been studied. Sohn and Kim[14] reported that substituting soybean with DSR by 10% was recommendable in overall physical and sensory quality. They proposed the substitution is done by adding DSR corresponding

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10% of soybean used for tofu into water extracts. Neither of the above reports mentioned the protein nutritional quality of tofu containing DSR.

The purpose of the present work is to compare the proximate composition and antinutritional factors such as trypsin inhibitor (TI) in soybeans at different stages of the conversion into tofu. An *in vitro* digestibilities and PERs of partially substituted tofu with DSR at 10% (TDSR) to those of traditional soy-based tofu (TT) were determined in order to confirm the protein nutritional quality of TDSR.

MATERIALS AND METHODS

Materials

Imported fresh soybeans (*Glycine max.* L) from California (USA) and moistured soymilk residue were obtained by the courtesy of Dong-Wha Tofu Co., Ltd.(Pusan, Korea).

Preparation of traditional tofu(TT)

One hundred grams of soybeans washed and then soaked in tap water for 24 hours at room temp. The soak-water was discarded and the swelled beans were ground in Waring blendor with 700ml of distilled water. The resulting mash was heated to 100° C for 15 minutes and filtered through three-layers of cheese cloth to remove residue. The filtrate was reheated to 80° C with continuous stirring, and calcium sulfate solution (5.4g of $CaSO_4 \cdot 1/2H_2O$ in 15ml of distilled water) added to coagulate the protein. When coagulum appeared, the coagulum was poured simultanously into cylindrical plastic molds. A pressure of 1.2kg/cm^2 was then applied for 10 minutes and the resulting products were submerged in cold water (ca. 3° C) for 20 minutes. The cooled tofu was freeze-dried and ground to pass through 60 mesh screens. The prepared samples were sealed in a plastic bottle and stored at 4° C prior to use in analyses.

Preparation of dried soymilk residue(DSR) and partially substituted tofu with DSR(TDSR)

Dried soymilk residue was prepared using the procedure described in Kim et al.[12]. A 30 gram of soymilk residue was mixed with 75ml of acetone and stirred with magnetic stirrer for 30 minutes. The mixture was filtered under reduced pressure over Whatman filter paper (# 42). The residue was rinsed two more times with 75ml of acetone for 2 minutes. The filtrate was refiltered using Büchner funnel with No. 40 Whatman filter paper and dried at 60° C in vacuum drying oven (NAPCO, Model 5831) under 15 inches Hg degree of vacuum. The procedure for production of TDSR is based on modifications which were previously shown statistically to give optimum physical characteristics, such as brittleness and granularity, and sensory quality[14]. Substitution is accomplished by adding 10 grams of DSR into water extracts from 100 grams of soybeans for tofu manufacturing. Sample preparation, treatment of coagulator and coagulum recovery were accomplished by the same method as for traditional tofu (TT). Preservation of the manufactured TDSR was also carried on identically as in TT.

Analyses of proximate composition

The nitrogen content of soybean products was determined by the micro-Kjeldahl method and used nitrogen conversion factor was 5.71[15]. Total lipid, ash and moisture content was determined by AOAC method[16]. Carbohydrates were expressed as the difference between the dry weight and the sum of the values for proteins ($N \times 5.71$), lipids and ash.

In vitro digestibility and trypsin inhibitor(TI) assay

The *in vitro* protein digestibility of the all samples was measured by the AOAC procedure[17] using four enzymes including trypsin (Sigma, 14,600 BAEE unit/mg solid), α-chymotrypsin (Sigma, 41 units/mg solid), peptidase (Sigma, 50units/g solid) and bacterial protease (*Streptomyces griceus*, Sigma, 58-units/mg solid). The reference protein used in digestibility assay was ANRC casein. TI contents were determined using the procedure of Ryu[18] which is modified from Rhinehart's method[19]. Results of TI are expressed in trypsin inhibitor equivalents, which equals the mg of purified soybean trypsin inhibitor per gram sample. The correlation coefficient between pH and TI content was 0.9935 and the equation for calculation is Y=4.0307X − 27.6300, where Y=purified soybean trypsin inhibitor(mg) and X=pH at 10 minutes incubation.

Amino acid analyses

Amino acid composition of the samples were analyzed by amino acid autoanalyzer(LKB, 4150, α type). The samples were hydrolyzed with 6N HCl under vacuum at 110°C for 25 hours to release the acidic, neutral and basic amino acids. Tryptophan was released using an alkaline hydrolysis by Hugli and Moore method[20], and sulfur-containing amino acids were quantitatively oxidized using performic acid[21].

Calculation of computed protein efficiency ratio(C-PER), predicted digestibility(P-dig.) and discriminant computed protein efficiency ratio(DC-PER)

C-PER, P-dig. and DC-PER were calculated using the corrected procedure of AOAC[17].

Protein digestibility was determined via four enzyme procedures and amino acid profiles were used in the calculation of C-PER. Predicted digestibility and DC-PER were calculated solely from amino acid profiles of sample proteins.

RESULTS AND DISCUSSION

Proximate analysis

The chemical composition of soybean at different stages of tofu processing is shown in Table 1. Both soymilk residues(SR1 and SR2) contained about 1~2% more total solids than that reported by others[22,23]. The soymilk residue made in laboratory (SR1) had high protein and lipid contents compared to manufactured residue(SR2). Values for carbohydrate content of SR1 was lower than SR2. The differences in those components were due essentially to the handling of the raw material including water soaking and coagulum treatment. Because of differences in soybean species and different preparation conditions of soymilk residue, previous investigators reported the various protein content ranged from 17.3%[23] to 31.4%[12]. Data in Table 1 indicate that both prepared soymilk residue contained

Table 1. Proximate composition of soybean and their products*

Sample	Total solids	Protein (N×5.71)	Lipid	Ash	Carbohydrate
Raw soybean	86.4	36.0	25.4	2.2	33.4
Soaked soybean[a]	39.6	35.4	23.0	5.0	36.6
Soymilk	7.3	37.1	22.4	5.1	35.4
SR 1[b]	17.6	25.1	13.7	4.5	56.7
SR 2[c]	18.9	19.8	11.4	3.9	64.9
DSR[d]	89.7	23.1	4.8	4.1	68.1
TT[e]	22.5	45.8	34.4	4.6	15.2
TDSR[f]	19.5	38.9	26.9	5.8	28.3

*Values except total solids are means of three determinations expressed on dry matter basis(g/100g)
[a] Soybeans are soaked in tap water for 24 hours
[b] Soymilk residue made in laboratory
[c] Soymilk residue obtained from Dong-Wha Tofu Co. Ltd.
[d] Dried soymilk residue with solvent washing
[e] Traditional tofu
[f] Partially substituted tofu with DSR corresponding 10% of soybean used for tofu preparation

appreciable amounts of protein ranging from 19.8%(SR2) to 25.1%(SR1) with a mean value of 22.5%. Dried soymilk residue(DSR), made by employing solvent washing, had about 60% less lipid than the both reisdues(SR1 and SR2). The DSR preparation procedure adopted in this paper, however, seemed to result in a lower retention of lipids since the defatting method used solvent(acetone)[12]. Addition of DSR resulted in low protein and lipid content(TDSR) compared to traditional tofu(TT). But carbohydrate and ash contents increased by adding DSR corresponding 10% of soybean used for tofu preparation. The values obtained for total solid contents of both tofu products showed that TDSR had higher moisture content than TT, but this did not reflect the high water holding capacity since DSR have appreciable amounts of insoluble carbohydrates[13,24].

Protein digestibility and trypsin inhibitor activity

It is well known that protease inhibitors distributed in plant tissue were inactivated by heating and then the nutritional value of those protein sources were improved. The heating time and temperature, particle size, and moisture content have a direct influence on the trypsin inhibitor activity and protein digestibility of soybean products[24]. Fig. 1 shows the changes in trypsin inhibitor activity and protein digestibility at different stages of TT and TDSR processing. 15% of trypsin inhibitor of raw soybean was diminished during water soaking, but protein digestibility was only slightly changed. Trypsin inhibitor activity was reduced by 65% and 87% in soymilk and soymilk residues respectively, presumably by the heat treatment step[25]. It should be noted, however, that a thermolabile protein is responsible for trypsin inhibitor activity in soybean[26] and that leads to a difference of trypsin inhibitor content between milk and residues. Similar trypsin inhibitor activity was observed in both tofu products and those results of reduced activity were greater than the Kim's[27]. On the other hand, protein digestibility increased from 78% for raw soybean to 87% for the intermediate tofu products including soymilk and residues. Dried soymilk residue containing tofu (TDSR) had high protein digestibility of 91% which is slightly lower than digestibility of 92% for traditional tofu. These results show that it is possible to substitute tofu with dried soymilk residue which is not inferior to traditional tofu in the protein digestibility and trypsin inhibitor activity.

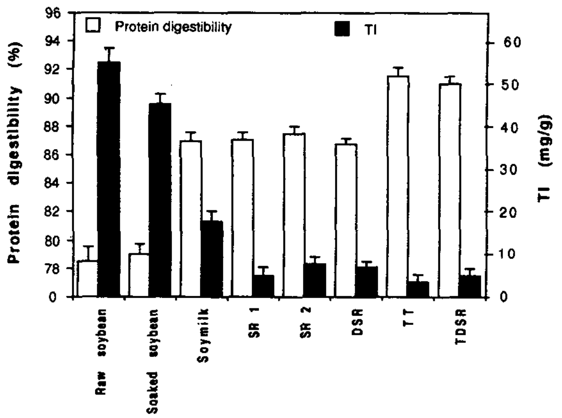

Fig. 1. Variations in protein digestibility and trypsin inhibitor of soybean products.*
*See the abbreviations in Table 1

Amino acid profiles and protein qualities

The amino acid profiles were similar to soybean and tofu products except for lysine which was present in a higher content in soymilk residues, and cystine and tryptophan which were present in lower contents in the same residues compared with other samples (Table 2). Analyses indicated that subst-

Table 2. Amino acid profiles of the soy samples* at different stages of tofu processing

(g amino acid/16 g N)

Amino acid	Raw soybean	Soaked soybean	Soymilk	SR 1	SR 2	DSR	TT	TDSR
Asp	7.83	7.38	8.38	8.57	9.12	9.69	7.31	7.36
Thr	4.36	4.38	4.24	4.43	4.59	7.98	4.37	4.29
Ser	5.06	5.16	5.07	5.34	5.57	5.58	5.09	5.16
Glu	15.17	15.41	15.71	16.19	15.98	11.24	14.69	14.81
Pro	5.77	6.32	5.49	5.68	4.90	5.95	6.14	5.76
Gly	4.36	4.69	4.35	4.78	5.31	5.35	4.37	4.46
Ala	4.40	4.35	4.26	4.43	4.38	4.39	4.37	4.37
Val	4.84	4.83	4.74	5.04	4.75	5.10	5.07	4.89
Met	1.23	1.29	1.49	1.04	1.18	1.23	1.32	1.46
Ile	4.79	4.71	4.57	4.35	4.23	4.73	4.85	4.73
Leu	7.37	7.43	7.10	7.66	7.68	4.49	7.31	7.43
Tyr	3.10	3.22	3.14	2.80	2.78	3.07	3.54	3.73
Phe	5.75	5.67	5.43	5.34	5.21	5.53	6.14	5.92
Lys	5.90	5.85	5.78	6.16	6.45	6.62	5.58	5.64
His	4.15	4.24	5.25	4.39	4.48	4.73	3.72	3.76
NH_3	0.72	0.68	0.68	0.82	0.88	0.76	0.67	0.68
Arg	7.35	6.74	7.36	6.28	6.14	7.27	7.47	7.27
Cys	1.21	1.20	0.86	0.96	1.10	0.93	1.30	1.42
Trp	1.65	1.45	1.11	0.76	0.30	0.36	1.15	0.64
E/T[a](%)	39.3	39.4	38.4	38.7	38.8	40.8	40.4	40.6

*See the abbreviations in Table 1
[a] Essential amino acids/total amino acids (%)

Table 3. Evaluation of the protein nutritive value of soybean products* as determined by *in vitro* experiments

Source of protein	Digestibility (%)			Protein efficiency ratio		
	In vivo	*In vitro*	Predicted	Rat-PER	C-PER	DC-PER
Raw soybean	75[a]	78.02	91.40	1.60[b]	1.94	2.08
Soaked soybean	–	79.18	90.92	–	2.23	2.08
Soymilk	91[c]	86.80	92.38	1.6~2.3 (2.0)[c]	2.37	2.09
SR 1	–	87.02	90.70	2.37[d]	1.00	2.23
SR 2	–	87.30	91.00	2.37[d]	0.47	2.94
DSR	–	86.85	104.65	–	0.53	2.57
TT	96[e]	91.48	91.15	1.92[f]	1.95	2.07
TDSR	–	90.91	90.79	–	1.40	2.14

*See the abbreviations in Table 1
[a]Cited from Kuppuswamy et al.[28] [b]Cited from Jewell et al.[29] [c]Cited from Liener[30] [d]Cited from Rackis et al.[31]
[e]Cited from Pian[5] [f]Cited from Hackler and Stillings[9] and Standal[7]

ituting TT with DSR led to the supplementary effect of lysine on TDSR. Percentages of essential amino acids to total amino acids for both TT and TDSR were 40. 4% and 40.6%, respectively. These values were greater than the proportion reported by Kim[27], presumably due to the different handling conditions and high proportion of essential amino acids for DSR. Table 3 gives the *in vitro* results of protein qualities for soybean products with *in vivo* results of other investigator's[5,7,9,28-31]. The main observation is that soybeans and soybean products may exhibit considerable variations in digestibility and PER values depending on processing conditions and evaluation techniques. When *in vitro* protein digestibility of raw and soaked soybeans which have high levels of trypsin inhibitor activity (Fig. 1) compared with *in vivo* results, digestibility using four enzyme assay was very close to those *in vivo* digestibility, but there was a great discrepancy between predicted digestibility and *in vivo* digestibility. In case of soy products which have low trypsin inhibitor activity and a high protein content, predicted digestibility assay could give favorable result compared to *in vivo* results, rather than enzymatic assay, except for dried soymilk residues. These data also serve to judge that soymilk residues and TDSR were nutritionally equivalent to TT and soymilk. Comparison of the 3 sets of PER values showed that DC-PERs of tofu products were close to rat-PERs in comparison with C-PER, except for soymilk and TT. It appeared that soy products which possess high *in vitro* digestibility and protein content, and low trypsin inhibitor acivity need DC-PER procedure rather than C-PER procedure to predict the protein quality. In addition, soymilk residues also have high protein quality with TT, and those of TT would be improved by adding DSR (TDSR) on the basis of PER values.

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건조비지 첨가 두부의 영양적 품질평가
1. 단백질의 영양가

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요 약

두부 제조공정 중의 부산물인 비지의 효율적인 이용을 위하여, 원료 대두 중량의 10%에 해당 하는 건조비지를 첨가시킨 두부를 제조하여 이의 영양적 품질을 검토하였다. 일반성분은 수분의 경우 건조비지 첨가 두부가 80.6%, 일반 두부가 77.5%로써 비지 첨가 두부가 다소 높았으며 단백질의 함량은 건조비지 첨가 두부와 일반 두부 각각 38.5%, 45.8%였으며, 지질의 함량은 각각 34.3%, 26.9%로써, 단백질과 지질 함량이 낮은 건조비지 첨가로 인해 일반 두부에 비해 건조비지 첨가 두부가 낮은 함량을 보였다. Trypsin inhibitor(TI) 함량은 비지의 경우 잔존량이 6.9(mg/g시료)정도였고, 건조비지의 경우에도 거의 비슷하였다. 건조비지 첨가 두부에는 원료 대두에 있었던 TI의 12% 정도만 남아 있었다. 단백질의 효소소화율(enzyme digestibility)은 비지, 건조비지 각각 87%, 86% 정도로 높았고 건조비지 첨가 두부도 일반 두부와 거의 동일한 91% 정도였다. 효소소화율은 TI함량이 저하함에 따라 역상관관계를 가지면서 상승하였다. 구성 아미노산 조성은 일반 두부와 건조비지 첨가 두부는 거의 차이가 없었으며, 총아미노산에 대한 필수 아미노산의 비율은 일반 두부가 40.4%, 비지첨가 두부가 40.6%였으며, lysine 함량이 높은 건조비지로 인하여 건조비지 첨가 두부는 lysine의 보충 효과가 있었다. 단백품질을 Computed Protein Efficiency Ratio(C-PER)로 계산할 때 일반 두부는 1.95, 비지 첨가 두부는 1.4였으나 Discriminant Computed Protein Efficiency Ratio(DC-PER)로 계산할 때는 오히려 비지 첨가 두부경우가 높았다. 여러 연구자들의 대두가공품에 대한 생체실험 결과와 비교할 때 일반 두부를 제외하고는 두유, 비지 및 비지첨가 두부의 영양평가는 DC-PER 방법이 유리한 것으로 생각되었다.