Bull. Korean Fish. Soc. 17(2), 109~116, 1984 韓水誌 17(2), 109~116, 1984

Distribution of Trypsin Indigestible Substrate(TI) in Seafoods and Its Changes during Processing

3. Changes in TI and Protein Quality of Salted and Dried Yellow Corvenia (*Pseudosciaena manchurica*) during Processing and Storage

Kang-Ho LEE, Hae-Ran KWON
Department of Food Science and Technology, National Fisheries University of Pusan, Namgu, Pusan, 608 Korea
and
Hong-Soo RYU
Department of Nutrition and Food Science, National Fisheries University of Pusan, Namgu, Pusan, 608 Korea

In order to assess the protein nutritional quality of salted and dried yellow corvenia, which was prepared using the different salting methods like dry and brine salting, the changes of trypsin indigestible substrate (TI) and *in vitro* apparent protein digestibility were studied during storage at room temperature (24±1°C). It was also examined the retention of available lysine and formation of nonenzymatic brown pigments under the same conditions of preparing and storage mentioned above and confirmed the relationship between *in vitro* digestibility and the antinutritional factors, such as nonenzymatic browning, unavailability of lysine and TI content.

TI content was gradually increased during the storage and it showed 1.5~2 times more after 57 days storage than that at the initial stage which ranged from 0.11 to 0.17 *mg/g* solid. Of all the products, 25% brine salting product contained the least TI content in 0.21 *mg/g* solid, while 10% dry salting products was the most abundant in 0.30 *mg/g* solid using Hamerstrand method (1981).

In vitro protein digestibility of all dried products was decreased significantly up to 30 days as the contents of TI increased, except 25% brine salted products. After storing for 57 days, the *in vitro* digestibility was only 4% drop showed in 25% brine salted products.

The nonenzymatic brown pigments were also developed on the level of available lysine reduced as *in vitro* protein digestibility was decreased throughout storage. Of all the various salted and dried products of yellow corvenia, 25% salting product showed the lowest rate of browning development and loss of available lysine. Therefore, it was revealed that unavailability of lysine and development of nonenzymatic brown pigments were major factors influencing the protein quality in salted and dried yellow corvenia.

Introduction

Generally, compared to livestock meat, fish muscle have been considered as a good digestible protein source because of the characteristics of their muscular tissue. But this, on the other hand, causes fish meat to be susceptible tc enzyme hydrolysis and perishable easily by microorganisms and undesirable chemical changes due to the high contents of unsaturated fatty acids and other volatile constituents. Therefore, a great deal of fish are processed traditionally

本 研究는 1982年度 財團法人 峨山社會福祉事業財團 研究費로 수행되었음.

into dried products, involving salted and dried, in order to prevent those deterioration or to prolong their shelf-life.

It is known that the curing of fishery products during salting or procedures which involve salting combined with sun drying, is usually accompanied by protein loss and falling quality of their protein. This opinion on dried fishery products has been proved by the enzyme hydrolysis(Tanikawa and Suno, 1952; Adachi *et al.*, 1958; Sawant and Magar, 1961; Ford and Salter, 1966; Jeong *et al.*, 1978; Ryu, 1983) and/or by chemical procedures(Carpenter *et al.*, 1962; Miller *et al.*, 1965; Lee *et al.*, 1976; Bodwell and Womack, 1978), but it is hard to find the investigations of the effect of salting and salting combined with drying on the protein quality of seafoods(Cutting 1962; Deng and Toaszewski, 1980).

Therefore, it was thought that evaluation of protein quality in salted and dried fishery products during processing and storage is an important work in improving the conditions for dried seafood production. The aims and purpose of this work is to investigate the protein nutritional quality of salted and dried yellow corvenia which was consumed popularly and produced about 500 M/T per year in Korea (Yearbook of Fisheries in Korea, 1981). This study was done on the changes of digestibility using *in vitro* procedure.

Development of trypsin indigestible substrate (TI), loss of available lysine content and fat oxidation, which were assumed as antinutritional factors on protein quality, were also determined and confirmed the relationship between those factors and *in vitro* protein digestibility.

Materials and Methods

1. Materials

Yellow corvenia (*Pseudosciaena manchurica*), 22 ~25 *cm* in mean length, 115~130 *g* in average weight, were purchased from Pusan Cooperative Fish Market and transported on ice to laboratory.

2. Preparation of salted and dried products

1) Dry salting combined with air drying Fresh fishes were opened and eviscerated, and layered in barrels. Solid salt were spreaded to those samples and cured for 3 days with restacking and resalting. After curing, air blast drying was performed in air cross-flow dryer (Shirakawa, velocity: 3 *m*/sec) at 24±1 °C. The moisture content was lowered in between 36% to 42% just after drying.

2) Brine salting and air drying Eviscerated samples were soaked in the salt solution of 10%, 15% and 25% for 3 days. Drying with air blast was conducted through the identical procedure described in above. Final moisture content was ranged from 44% to 51% after drying.

3) Stored sample preparation Both dry and brine salted samples were stored in shade at 26± 1 °C for 57 days with free circulation of air around each sample. Each lot was periodically sampled and ground to pass a 80 mesh screen. Unsalted fishes were also dried and stored under the same conditions for dried products and used as control.

3. Experimental procedure

1) Analysis of approximate composition, volatile basic nitrogen (VBN) and salinity:

Moisture, crude protein, crude ash, crude fat and salinity were determined by the procedures in AOAC(1980). VBN was determined employing the microdiffusion technique that introduced by Pearson(1973).

2) Apparent *in vitro* protein digestibility and TI in all samples were checked by the same procedures as described in previous reports (Lee *et al.*, 1984a; Lee *et al.*, 1984b).

3) Available lysine was measured using the procedure in report of Warmbier *et al.* (1976), and nonenzymatic brown pigments was determined using the procedure introduced by Saltmarch (1976).

Results and Discussion

1. Proximate composition of fresh yellow corvenia

The fresh meat of yellow corvenia, which is a kind of white-fleshed fish, was analyzed for proximate components and the results were summarized in Table 1. The samples contained a higher content of crude fat (4.69%) comparing with that in other white-fleshed fishes reported by previous reports (Chemical Composition of Japanese Foods, 1977; Chemical Composition of Marine Products, 1977). Freshness, expressed as VBN content, could be considered as "good" state comparing with the proposed range of freshness for raw fish (Uchiyama *et al.*, 1970) and there was not noticeable difference between the *in vitro* digestibility of fresh meat and that of other white-fleshed fish meat(Ryu, 1983; Lee *et al.*, 1984a). TI contents showed a slight higher value than that in other white-fleshed fishes(Lee *et al.*, 1984a) using both methods described in Table 1.

Table 1. Approximate composition, *in vitro* protein digestibility and TI content in fresh meat of yellow corvenia

Moisture (%)		74.33
Crude protein (%)		18.17
Crude fat (%)		4.69
Crude ash (%)		1.07
Salinity (%)		0.88
VBN(mg/100 g)		16.00
TI(mg/g solid)	H	0.25
	R	15.51
In vitro digestibility (%)		84.72

H: Hamerstrand method(1981)
R: Rhinehart method(1975)

2. Effect of salting methods and salt concentration on TI forming

As shown in Table 2, the possibility of forming TI in all salted and dried products as well as in the control was always present throughout the period of storage and that was increased gradually as the period of storage was prolonged. More TI could be observed in the control than that in all samples processed under different conditions, such as salt concentration and salting methods. Brine salting and high salt concentration helped retain TI forming during the storage at room temperature, with 25% salt performing

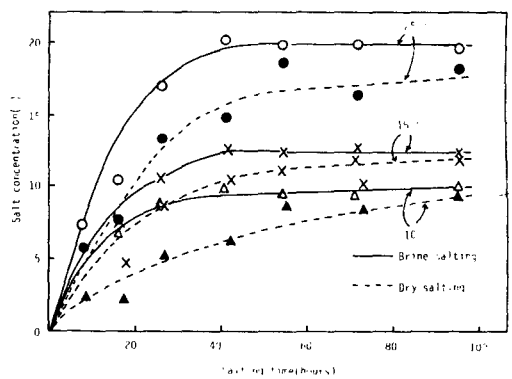


Figure 1. Variations of salt cnocentration in yellow corvenia during salting process.

Table 2. Effect of salting methods and salt concentration on TI forming in salted yellow corvenia (unit: mg/g solid)

Storage period (days)	Control	Brine salting			Dry salting		
		10%	15%	25%	10%	15%	25%
0	0.296[a] (14.01)	0.165 (10.16)	0.148 (7.59)	0.112 (5.02)	0.173 (10.28)	0.154 (8.15)	0.126 (8.05)
15	0.348 (17.08)	0.192 (11.88)	0.160 (11.00)	0.141 (10.81)	0.212 (14.45)	0.212 (14.51)	0.171 (10.89)
32	0.366 (19.99)	0.223 (15.75)	0.230 (13.85)	0.160 (11.05)	0.263 (16.20)	0.263 (15.12)	0.201 (12.12)
57	0.387 (26.21)	0.278 (26.21)	0.271 (17.60)	0.212 (12.44)	0.308 (18.11)	0.308 (18.42)	0.233 (13.30)

a: Determined using Hamerstrand method (1981)
Data in parenthis mean the content of TI determined using Rhinehart method (1975)

Table 3. Influence of salting conditions on the *in vitro* digestibility of salted and dried yellow corvenia during storage at 26±1 °C (unit: %)

Storage period (days)	Control	Brine salting			Dry salting		
		10%	15%	25%	10%	15%	25%
0	84.93	87.97	88.65	90.48	86.71	88.88	89.55
15	80.29	85.04	85.54	88.75	86.01	85.20	88.18
32	77.82	81.21	81.43	87.75	81.15	81.51	86.03
57	78.40	81.63	81.30	85.94	81.69	82.17	85.04

best. It was assumed that the better effect of brine salting was related to a rapid penetration and high concentration of salt maintained at final equilibrium stage as illustrated in Fig. 1. This opinion was supported by the conflicting evidence in the previous literatures(Kunisuke *et al.*, 1978; Florian and Liston, 1981).

3. Influence of salting conditions on *in vitro* digestibility

In an attempt to obtain a salted and dried yellow corvenia with highest protein digestibility during storage, samples were subjected to different salting methods and salt concentrations. The comparative values for the *in vitro* digestibility were given in Table 3. The lower salt concentration led to a decrease in digestibility and there was remarkable difference between control and processed products. It could be suspected that the undesirable chemical reactions for nutritional aspects had been taken place in unsalted(control) meat during air blast drying and those led to drop digestibility severely. Brine salting and high salt concentration in both salting procedures were effective to avoid decreasing digestibility due to the same reason reported above. It was also revealed that the *in vitro* digestibility of all samples fell down gradually within 32 days of storage, but after then, the drop was negligible. Similar result was found in the report of Matsuda (1979) that the soluble nitrogen content and ATP-ase activity in dried carp were decreased until 30 days storage and there was not significant changes of nitrogen solubility after then.

4. Effect of salting on nonenzymatic browning development

Nonenzymatic brown reaction is well known as a major deteriorative reaction in dried foodstuffs (Labuza *et al.*, 1966; Labuza *et al.*, 1972; Kim *et al.*, 1973; Lee *et al.*, 1982). As demonstrated in Table 4, examination of developing nonenzymatic brown pigment in salted products gave a pattern very similar to TI forming in that products throughout the storage. In fact, dry salted product treated with lower salt concentration (10% and 15% NaCl concentration) had the same level of brown pigments with the control at the initial stage of storage, while 25 % dry salted one showed lower degree. But those could gave a slight effect of reducing brown pigment development during storage. It was apparent that the effectiveness of high salt concentration on brown pigment development was

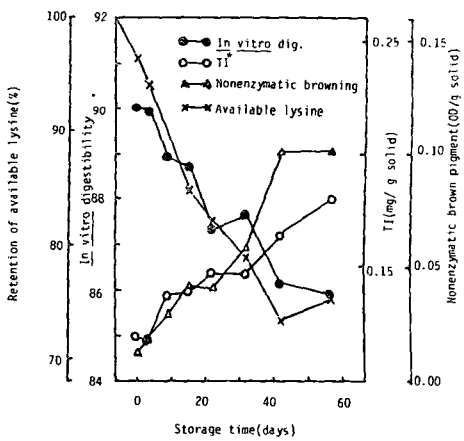
Table 4. Effect of salting on nonenzymatic browning development in salted and dried yellow corvenia during storage at 26±1°C (unit: OD/g solid)

Storage period (days)	Control	Brine salting			Dry salting		
		10%	15%	25%	10%	15%	25%
0	0.0396	0.0260	0.0259	0.0193	0.0335	0.0338	0.0239
15	0.0695	0.0602	0.0596	0.0397	0.0639	0.0554	0.0464
32	0.1248	0.0891	0.0919	0.0447	0.0991	0.0833	0.0604
57	0.1354	0.1080	0.1154	0.0802	0.1266	0.1197	0.0989

due to the dehydration through osmotic action of salt and that led to falling down water activity that able to give a smaller brown pigment (Choi *et al.*, 1973; Kim *et al.*, 1973; Han *et al.*, 1973; Labuza, 1980; Labuza *et al.*, 1981). There was, otherwise, a noticeable effect of retarding brown pigment development in brine salting, and high concentration of brine (25%) had more effect than the lower concentration (10% or 15%). It was suspected that more salt soluble protein was extracted into brine during the period of brine salting and then the contents of substrate, which could take part in browning, was reduced. Those could be proved by the earlier reports that nitrogenous compounds (amino N. and nonproteinous N.) were involved in nonenzymatic browning (Fujimoto *et al.*, 1968; Lee *et al.*, 1982).

5. Effect of salting on available lysine retention

It is known that lysine, contains a pollar ε-amino group, is partially destroyed or inactivated during processing and that the recovery must be determined to attain the desirable conditions for heat treatment (Lea *et al.*, 1958; Carpenter *et al.*, 1962; Warmbier *et al.*, 1976). As shown in Table 5, the lysine contents in stored brine salted products were higher than those in dry salted ones but there was not severe deviation in lysine contents within salting methods practically at initial stage of storage. This result proposed that carbonyl compound, from oxidation occurred severely in dry salted product, could reduced the available lysine as reported by Byun *et al.* (1978). It was also revealed that retention of available lysine for brine salted fell progressively to some degree of 60.0~75.4% while it showed degree 64.3~72.3% of its original value for dry salted and 60.1% for control. And the contents and retention degree of available lysine were directly proportional to the salt concentration in both salting procedures. In conclusion, it might be proposed that there was an inverse relationship between the decreasing of *in vitro* digestibility and TI, and between the development of nonenzymatic brown pigments and available lysine retention as shown in Fig. 2. The-



Figre 2. Relationship between *in vitro* protein digestibility and TI content, nonenzymatic brown pigment and retention of available lysine in 25% brine salted yellow corvenia during storage at 24 ±1°C. TI was determined using Hamerstrand method (1981).

Table 5. Retention of available lysine in dried yellow corvenia as fuction of salt concentration and salting methods during storage at 26±1°C (unit: g/100 g solid)

Storage period (days)	Control	Brine salting			Dry salting		
		10%	15%	25%	10%	15%	25%
0	17.174	15.744	15.318	14.452	15.472	15.331	14.731
15	11.793 (68.67)	12.128 (76.89)	12.721 (83.05)	12.233 (84.65)	11.494 (74.29)	12.024 (78.43)	12.147 (82.41)
32	10.033 (58.42)	10.466 (66.48)	10.125 (66.10)	11.389 (78.81)	9.823 (63.49)	9.736 (63.51)	11.517 (78.18)
57	10.321 (60.10)	10.592 (67.28)	10.096 (65.91)	10.894 (75.38)	10.100 (65.28)	9.855 (64.28)	10.647 (72.28)

Data in parenthis mean the retention (%)

refore, it was suggested that unavilability of lysine and development of nonenzymatic brown pigments were major factors influencing the protein quality, such as *in vitro* digestibility and TI, in salted and dried yellow corvenia during storage and processing.

References

Adachi, R.R., L. Sheffner and H. Spector. 1958. The *in vitro* digestibility and nutritional quality of dehydrated beef, fish and beans. Food Res. 23, 401–406.

AOAC. 1980. "Official Methods of Analysis", 13th edition, Association of Official Analytical Chemists, Washington D.C.

Bodwell, C.E. and M. Womack. 1978. Effects of heating methods on protein nutritional value of five fresh or frozen prepared food products. J. of Food Sci. 43, 1543–1549.

Byun, D.S., Y.O. Song and J.H. Pyeun. 1978. Changes in available lysine and lipid oxidized products during the storage of dried sea eel, *Muraesox cinereus*. Korean J. of Food Sci. and Tech. 10, 387.

Carpenter, K.J., C.B. Morgan, C.H. Lea and L.J. Parr. 1962. Chemical and nutritional changes in stored herring meal. 3. Effect of binding ofamino acids in freeze dried herring cake. Brit. J. Nutrition, 16, 451–465.

Chemical Composition of Marine Products. 1977. Edited by National Fisheries Research and Development Agency in Korea. p.9.

Chemical Composition of Japanese Food. 1977. in "Handbook of Food Analysis, in Japanese", ed. by Mihara, T., I. Suzuki and D. Iwami, Kenpakusa, Tokyo. p.760.

Choi, H.Y., M.N. Kim and K.H. Lee. 1973. Nonenzymatic browning reactions in dried squid stored at different water activities. Bull. Korean Fish. Soc. 6(3, 4), 97.

Cutting, C.L. 1962. Influence of drying, salting and smoking on the nutritive value of fish. in "Fish in Nutrition", Fishing News (Books) Ltd., London. p.161.

Deng, J.C. and F.B. Toaszewski. 1980. The use of response surface methodology to determine the effects of salt, tripolyphosphate and sodium alginate on the quality of fish patties prepared from minced fish, croaker. in "Advances in Fish Science and Technology", edited by Connell, J.J. and staff of Torry Research Station, Fishing News Books Ltd., Farnham, Surrey, England. 218–222.

Florian, M.O. and J. Liston. 1981. Agents of proteolysis and its inhibition in Patis (fish sauce) fermentation. J. of Food Sci. 47, 198–206.

Ford, J.E. and D.N. Salter. 1966. Analysis of enzymatically digested food protein by Sephadex-gel filtration. Brit. J. Nutr. 20, 843–859.

Fujimoto, K., M. Maruyama and T. Kaneda. 1968. Studies on the brown discoloration of fish products. 1. Factors affecting the discoloration. Bull. Japan Soc. Sci. Fish. 34(6), 519–523.

Han, S.B., J.H. Lee and K.H. Lee. 1973. Nonenzymatic browning reaction in dried anchovy when stored at different water activities. Bull. Korean Fish. Soc. 6(1, 2), 37–44.

Jeong, B.Y., D.S. Byun and J.H. Pyeun. 1978. Nutritional evaluation of muscle proteins of flounder, *Limanda herzensteni*, dried at different methods. J. of Korean Soc. of Food and Nutrition 7, 1–6.

Kim, M.N., H.Y. Choi and K.H. Lee. 1973. Nonenzymatic browning reactions in dried Alaska pollock stored at different water activities. J. Korean Soc. Food and Nutrition 2(1), 41–48.

Kunisuke, N., F. Yutako and I. Senji. 1978. Studies on salted and dried sardine. 1. Changes of the chemical components in sardine meat during salting, drying and

storage. Bull. Tokai Reg. Fish. Res. Lab. No. 95, Oct., 75—86.

Labuza, T.P., J.F. Maloney and M. Karel. 1966. Autooxidation of methyl linoleate in freeze-dried model systems. Ⅱ. Effect of water on covalt-catalyzed oxidation. J. Food Sci. 31, 885—894.

Labuza, T.P. and M. Saltmarch. 1981. The nonenzymatic browning reactions as affected by water in foods. in "Water Activity Influence on Food Quality", Ed. L. Rockland, Academic Press. New York.

Labuza, T.P. 1972. Nutrients losses during storage of hydrated food. Critical Rev. Food Tech. 4, 217—224.

Lea, C.H., L.J. Parr and K.J. Carpenter. 1958. Chemical and nutritional changes in stored herring meal. Brit. J. Nutrition, 12, 297—306.

Lee, K.H., H.S. Kim and H.S. Ryu. 1984(a). Distribution of trypsin indigestible substrate (TI) in seafoods and its changes during processing. 1. Distribution and postmortem changes of TI in fish muscle. J. Korean Soc. Food and Nutrition 13, 15—24.

Lee, K.H., J.H. Jo and H.S. Ryu. 1984(b). Distribution of trypsin indigestible substrate (TI) in seafoods and its changes during processing. 2. Changes in TI and *in vitro* apparent protein digestibility of boiled and dried anchovy during processing and storage. Bull. Korean Fish. Soc. 17(1), (in publishing).

Lee, K.H., D.S. Song, B.J. You and M.N. Kim. 1982. Changes in available lysine and extractable nitrogen, extent of browning during the storage of dried fish meat. Bull. Korean Fish. Soc. 15(4), 271—282.

Lee, E.H., N.J. Sung, J.H. Ha and S.Y. Chung. 1976. Changes in free amino acids of yellow corvenia, *Pseudosciaena manchurica*, during Gulbi processing. Korean J. Food Sci. Tech. 8(4), 225—229.

Miller, E.L., A.W. Hartley and D.C. Thomas. 1965. Availability of sulfur amino acids in protein foods. 4. Effect of heat treatment upon the total amino acid content of cod muscle. Brit. J. Nutr. 19, 565—573.

Pearson, D. 1973. in "Laboratory Techniques in Food Analysis", Botter Worth, London. p. 170.

Ryu, H.S. 1983. Nutritional evaluation of protein quality in some seafoods. Ph.D. Thesis of National Fisheries Univ. of Pusan, Korea.

Sawant, P.L. and N.G. Margar. 1961. Studies on dried bombay duck (*Harpoden nehereus*). Ⅱ. Changes in the nutritive value during processing. J. Sci. Food Agric. 12, 302—305.

Saltmarch, M. 1979. The influences of temperature, water activity and physicochemical state of lactose on the kinetics Maillard reaction in spray dried sweet milk whey powder stored under steady and nonsteady storage conditions. Ph.D. Thesis of Univ. of Minnesota.

Tanikawa, E. and M. Suno. 1952. Studies on the complete utilization of squid (*Ommastrephes sloani* pacificus). Ⅴ. Nutritive and digestibility of squid meat. Bull. Fac. Fish. Hokkaido Univ. 3, 75—80.

Uchiyama, H., S. Ehira, H. Kobayashi and W. Shimizu. 1970. Singnificance in measuring volatile base and trimethylamine nitrogen and nucleotides in fish muscle as indices of freshness of fish. Bull. Jap. Soc. Sci. Fish. 36(2), 177—183.

Warmbier, H.C., R.A. Schnickel and T.P. Labuza. 1976. Effect of glycerol on nonenzymatic browning in a solid intermediate moisture model system. J. Food Sci. 41, 981—987.

Matsuda, Y. 1979. Influence of sodium glutamate on the protein denaturation of lyophillized carp myofibrills during storage. Bull. Jap. Soc. Sci. Fish. 45(6), 733—741.

魚貝類의 Trypsin活性 沮害物質의 分布와 加工中의 變化

3. 鹽乾조기 加工貯藏中의 TI 및 蛋白質品質變化

李 康 鎬 · 權 惠 蘭 · 柳 洪 秀

釜山水產大學 食品工學科　　釜山水產大學 食品營養學科

鹽藏방법을 달리하여 만든 鹽乾조기의 蛋白質 品質을 評價하기 위하여, 室溫貯藏 中의 트립신 活性沮害物의 含量과 試驗管的 蛋白質 概算消化率(*in vitro* apparent protein digestibility)의 變化를 測定하였으며, 併行하여 有効性 라이신의 保留量과 非酵素的 褐變度의 變化도 分析比較하여 各 實驗結果에 대한 相關性을 檢討하였다.

트립신 活性沮害物의 含量은 貯藏 中 점차 增加하였으며, 貯藏 57日後에는 加工 直後에 比하여 1.5~2倍에까지 增加하였다. 加工方法別로 比較하였을 때 25% 鹽水에 간하여 乾燥한 것이 TI 含量으로 0.21 *mg/g* solid로써 가장 적었고, 10% 固形食鹽으로 간한 것은 0.30 *mg/g* solid로써 가장 많았다.

試驗管的 蛋白質 消化率에 있어서는, 25% 鹽水로써 간하여 乾燥한 것을 除外하면, 모든 試料가 30日까지는 TI含量의 增加와 더불어 有意的으로 低下하였다. 貯藏 57日 後의 試驗管的 消化率은 25% 鹽水로 간하여 乾燥한 것에서는 不過 4%의 減少를 나타내었다.

全 貯藏期間을 통하여 非酵素的 褐變色素는 有効性 라이신量의 減少 및 試驗管的 蛋白質消化率의 低下와 더불어 增加하였다.

鹽藏方法을 달리한 鹽乾조기 製品 中에서는 25% 鹽水로써 간하여 乾燥한 것이 가장 낮은 褐變化率과 有効性 라이신의 減少를 보였다.

따라서 라이신의 失効化와 非酵素的 褐變色素의 增加는 鹽乾조기의 蛋白質 品質에 가장 主要한 影響因子임을 알 수 있었다.