Purification and Concentration of Antioxidative Dipeptides Obtained from Chicken Extract and Their Application as Functional Food

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ABSTRACT

In Japan, about 150,000 tons of egg-laying hens are discarded annually because quality of their meet is low. In order to add further value to carcasses of the spent egg-laying hens and utilize them, an efficient purification and concentration process for functional dipeptides in chicken extract obtained from these carcasses was developed in this study by combining ion-exchange chromatography and nanofiltration (NF) treatment. The functional dipeptides, Anserine and Carnosine, consisting of β-alanine and L-histidine (ACmix), showed a basic property and could be adsorbed directly onto a cation exchange resin. The ACmix were completely separated from other chicken extract elements when the chicken extract was passed through the column packed with cation exchanger and the dipeptides were eluted from the column by using diluted alkaline solution. Major contaminated substances in the ACmix after ion-exchange chromatography were Na^+, K^+ and creatinine. A NF membrane could remove these contaminants and concentrate ACmix without any heat treatment. Based on the results of pilot scale experiments, a mathematical model which could express efficiency of a NF process was newly proposed. By using the mathematical model, an industrial scale NF process which could process 3.6 tons of carcasses in a day were designed, where minimum purify and yield of ACmix in the final product were set at 90% and 95%, respectively. The ACmix had an antioxidant activity specifically to hypochlorite radicals and markedly reduced an oxidative stress in normal human volunteers in combination with vitamin C and ferulic acid.

Key words: Anserine, Carnosine, cation-exchanger, chicken extract, Nanofiltration (NF)

INTRODUCTION

Membrane separation technology is a fine filtration technology which can separate molecules according to their molecular size. It requires low initial cost and small energy while can keep the quality of products high because it is a simple process which requires no heat treatment[(1)].

On the other hand, approximately 200,000 tons of egg-laying hens are discarded annually in Japan because quality of their meat is low. However, chicken meat is rich in anserine and carnosine which are dipeptides having unique and strong antioxidant functionality.

Anserine and carnosine consisting of β-alanine and L-histidine (Figure 1) are strong antioxidants against hypochlorite radical (ClO・) and they are antioxidants that reduce lipid oxidation which affects on flavor, aroma, texture, color and nutritional compositions[(2,3)]. Also anserine and carnosine have immuno-response modulation, blood fat reduction and enhanced wound healing functions *in vivo*[(4-6)]. Anserine and carnosine remarkably reduced an oxidative stress in normal human volunteers in combination with vitamin C and ferulic acid[(6)].

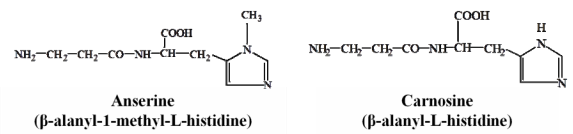

Figure 1. Rational formula of Anserine and Carnosine (AC).

If anserine and carnosine contained in the chicken meat can be purified with low cost using membrane separation technology, they can be promising components of a wide variety of functional foods. In this study, in order to add extra value to the

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discarded hen-meat and utilize them, a membrane separation process which can purify anserine and carnosine contained in extract from the chicken meat was developed and its efficiency was demonstrated.

MATERIALS AND METHODS

Chicken extract was obtained from whole chicken carcasses by heating the carcasses with water (Figure 2). Then the extract was treated with ion exchange resin in order to remove acidic and neutral amino acids and proteins. The chicken extract after the treatment with the ion exchange resin contained 6.79 g/L of anserine and carnosine and their purity was 60 - 70%. Impurities contained in the extract after the treatment with the ion exchange resin were creatinine and sodium chloride. Concentrations of these impurities were 2.30 g/L and 0.85 g/L, respectively. The extract after the treatment with the ion exchange resin was used as materials for membrane separation experiments.

A bench scale membrane separation unit supplied by DSS (Danish Separation System) was used in this study.

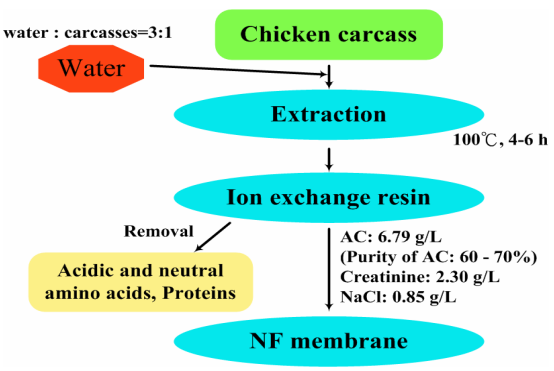

Figure 2. Procedure for preparation of chicken extract.

In order to purify anserine and carnosine contained in the chicken extract, impurities which are mainly creatinine and sodium chloride need to be removed. Molecular weights of these impurities are 113 and 58, respectively, while average molecular weight of anserine and carnosine is 234. Therefore, thirteen different kinds of nanofiltration membranes which had NaCl rejection values of 10 to 60%, or molecular weight cut-off values of 700 to 2500 were chosen. Membranes tested in this study are listed in Table 1.

At first, total circulation experiments were conducted with the membrane unit. In this experiment, composition of the feed solution was kept constant by returning the permeate from the membranes to the feed tank, and effect of operating conditions on separation efficiency of each membrane was evaluated under different operating conditions. Based on the experimental results obtained in the total circulation experiment, suitable membranes and operating conditions for purification of anserine and carnosine were selected.

Table 1. Nanofiltration membranes tested in this study

Membrane Type	NaCl rejection (*MW cut-off)	Manufacturer	Material
NFT50	55	DSS	Polypiperazine / polyamide/
DRA4510	45	DAISEN	Polyamide
Desal DL	15	Desalination	Polyamide (aromatic)
Desal DK	50	Desalination	Polyamide (aromatic)
NTR7430	30	Nitto Denko	Sulfonated polyether sulfone
NTR7450	50	Nitto Denko	Sulfonated polyether sulfone
NTR7250	60	Nitto Denko	Polyvinyl alcohol
MPF34	35	Abcor	Polysulfone
MPF36	10	Abcor	Polysulfone
MPF44	25	Abcor	Polyacrylonitrile (PAN)
MPF50	700*	Abcor	Polyacrylonitrile (PAN)
G-5	1000*	Desalination	Polyamide
G-10	2500*	Desalination	Polyamide

Then, batch-wise concentration experiments were performed with the selected membranes and conditions. In this experiment, impurities such as creatinine and sodium chloride were taken out from the system together with permeate, and anserine and carnosine were purified and concentrated.

During all the membrane separation experiments, feed and permeate were sampled periodically. Concentration of anserine and carnosine was analyzed with HPLC. Concentration of creatinine and sodium ion was determined with HPLC and ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectroscopy), respectively. Then, rejection ability of a membrane against each component was calculated with Eq. 1,

$$R_i = 1 - C_{p,i}/C_{f,i} \qquad (1)$$

where R_i, $C_{p,i}$, and $C_{f,i}$ are observed rejection against component i, concentrations of component i in permeate and in feed, respectively.

RESULTS AND DISCUSSION

I. *Total Circulation Experiment*

Effects of operating pressure on permeate flux value and rejection value during the total circulation experiments are shown in Figure 3. Operating pressure

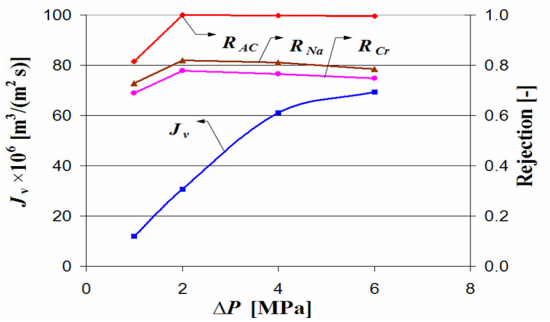

Figure 3. Effect of operating pressure (ΔP) on permeate flux (J_v) and observed rejection for anserine-carnosine (R_{AC}), creatinine (R_{Cr}) and sodium ion (R_{Na}) with NFT50 membrane.

had little effect on the rejection value. Permeate flux value increased linearly with operating pressure up to 4 MPa. Once the operating pressure exceeded 4 MPa, permeate flux did not increase linearly. Therefore, suitable operating pressure for separation of anserine and carnosine with the nanofiltration membranes was determined to be 4 MPa. Similar results were obtained with other membranes.

Effects of feed flow rate on permeate flux value and rejection value during the total circulation experiments are shown in Figure 4. Feed flow rate showed almost no effect on rejection value. In the case of membranes which showed low permeate flux value, feed flow rate had almost no effect on permeate flux value. However, in the case of membranes with high permeate flux value such as NFT-50, permeate flux increased with feed flow rate up to 10 L/min as shown in Fugure 4. Therefore, 10 L/min was chosen as suitable feed flow rate for separation of anserine and carnosine with the nanofiltration membranes.

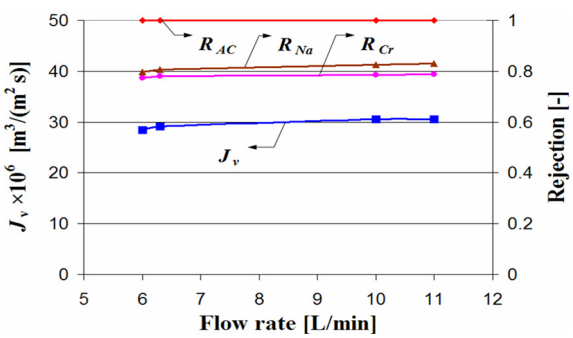

Figure 4. Effect of flow rate on permeate flux (J_v) and observed rejection for anserine-carnosine (R_{AC}), creatinine (R_{Cr}) and sodium ion (R_{Na}) with NFT50 membrane.

Summary of results obtained in the total circulation experiments with the thirteen different nanofiltration membranes are listed in Table 2. The purpose of the nanofiltration treatment is to improve the purity of anserine and carnosine (AC) contained in the feed solution by removing impurities such as creatinine (Cr) and sodium chloride (NaCl) into the permeate. Therefore, a membrane which shows higher rejection ability against anserine and carnosine and low rejection ability against creatinine and sodium ion is preferred for this purpose. Furthermore, higher permeate flux value implies that a process with the membrane will require smaller membrane area and lower initial cost. Based on these criteria, four membranes (NFT-50, DRA-4510, Desal DK and Desal DL) were chosen as suitable membranes for purification and concentration of anserine and carnosine from chicken extract.

Table 2. Summary of results of the total circulation experiments

Membrane	J_v x10^6 [m^3/(m^2 s)]	R_{AC} [-]	R_{Cr} [-]	R_{NaCl} [-]
NFT50	**61.1**	**0.998**	**0.765**	**0.811**
DRA4510	**54.9**	**0.994**	**0.813**	**0.835**
Desal DK	**42.4**	**0.992**	**0.713**	**0.733**
Desal DL	**36.8**	**0.997**	**0.439**	**0.446**
MPF36	**34.7**	**0.751**	**0.490**	**0.257**
NTR7250	**29.2**	**0.888**	**0.564**	**0.234**
MPF50	**28.5**	**0.017**	**0.035**	**-**
NTR7430	**27.8**	**0.925**	**0.600**	**0.719**
NTR7450	**13.9**	**0.941**	**0.704**	**0.842**
MPF34	**11.8**	**1.000**	**0.990**	**0.980**
G-10	**8.8**	**0.453**	**0.214**	**0.588**
MPF44	**6.3**	**0.940**	**0.886**	**0.757**
G-5	**4.6**	**0.406**	**0.070**	**0.593**

II. *Batch-Wise Concentration Experiment*

Batch-wise concentration experiments were conducted with the four kinds of membranes under the selected operating conditions. Experimental results obtained in the batch-wise concentration experiments are shown in Figure 5, where feed flow rate and operating pressure were 10 L/min and 4 MPa, respectively. Figure 5 show changes in yield of each component with concentration factor, which is defined as ratio of initial feed volume to feed volume. Yield of creatinine and sodium decreased with increase in volume reduction factor while that of anserine and carnosine was almost constant at unity. These results imply that anserine and carnosine were concentrated and their purity was increased during the batch-wise concentration treatments.

III. *Proposal of a Mathematical Model*

Based on the experimental results obtained in the batch-wise concentration experiments, a mathematical

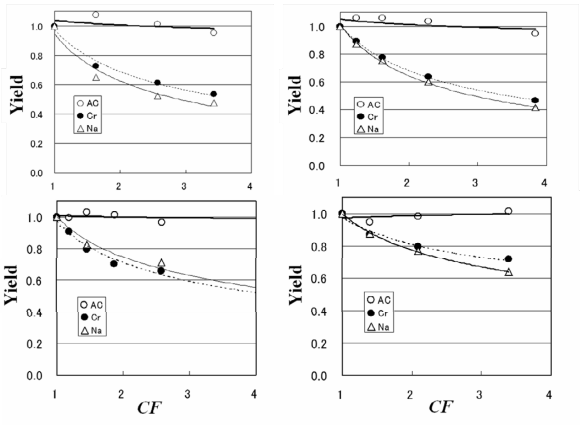

Figure 5. Changes in yield of each component with concentration factor (*CF*) during batch-wise concentration experiments, where *CF* is defined as ratio of initial feed volume ($V_{f,0}$) to feed volume (V_f). (flow rate: 10 L/min. pressure: 4 MPa.)

model which can express efficiency of the purification and concentration process with nanofiltration membranes was proposed. Figure 6 shows the proposed model. By solving this model, change in concentration of each component with time can be calculated.

Changes in purity and yield of anserine-carnosine, concentrations of each component, and permeate flux value with processing time calculated with the mathematical model are compared with experimental results. The calculated values are in good agreement with experimental values, and it was confirmed that the efficiency of the membrane purification process could be predicted with this model precisely.

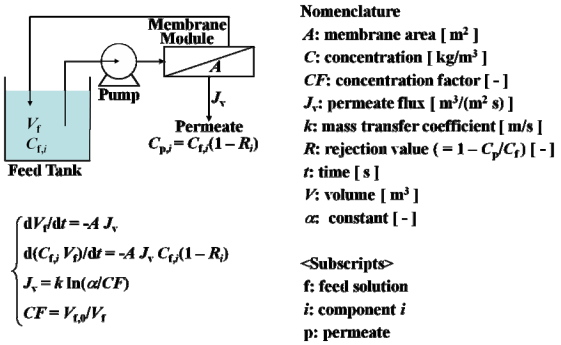

Figure 6. A mathematical model to describe efficiency of a purification and concentration process with nanofiltration membrane.

IV. *Process Design*

Then, by applying the mathematical model, a practical size membrane process was designed. The minimum purity and yield of anserine and carnosine required in the final product were set at 90% and 95%, respectively. NFT-50 membrane was chosen as the nanofiltration membrane. Flow diagram of the designed process is shown in Figure 7. The simulation result showed that 5.3 m^2 of nanofiltration membrane was needed for the process which could process 3.6 t of chicken carcasses in a day, and 7.4 kg of purified anserine and carnosine could be obtained with this process.

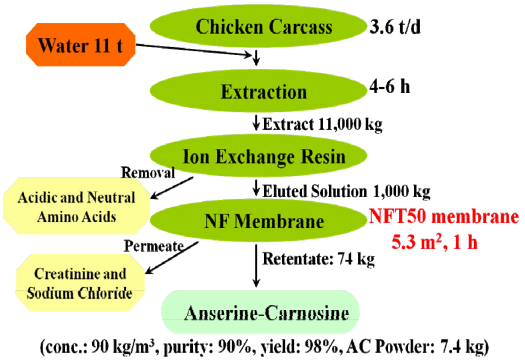

Figure 7. Schematic flow diagram of the designed process which can process 3.6 t of chicken carcasses in a day.

Applicability of this purification and concentration process with nanofiltration membrane might be high because it is a simple process which requires low cost and low energy consumption. We are planning to apply this process to purification and concentration of value added components contained in wide variety of natural resources.

CONCLUSIONS

In order to add extra value to discarded chicken carcasses and utilize them, efficiency of a membrane process for purification and concentration of antioxidative dipeptides which were antioxidation dipeptides contained in chicken extract was investigated.

1. Thirteen different kinds of nanofiltration membranes were tested and suitable membranes and operating conditions for purification and concentration of anserine and carnosine were selected.
2. By applying the selected membranes and conditions, anserine and carnosine was purified and concentrated with a pilot scale unit.
3. Based on the experimental results, a mathematical model which can express efficiency of a nanofiltration process was proposed, and an industrial scale nanofiltration process which could process 3.6 t of chicken carcasses in a day was designed by applying the model.

ACKNOWLEDGMENTS

This work was supported in part by a grant-in-aid (Development of evaluation and management methods for supply of safe, reliable and functional food and farm produce) from the Ministry of Agriculture, Forestry and Fisheries of Japan.

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