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

Hiroshi NABETANI¹, Shoji HAGIWARA¹, Nobuya YANAI¹,², Shigenobu SHIOTANI¹,², Joosh BALJINNYAM¹,³ and Mitsutoshi NAKAJIMA¹

¹ National Food Research Institute, NARO, 2-1-12 Kan-nindai, Tsukuba, Ibaraki, 305-8642 Japan
² Tokai Bussan Co. Ltd., 1-10-5 Iwamoto-cho, Chiyoda-ku, Tokyo, 101-0032 Japan
³ Mongolian University of Science and Technology, Bagatoiruu, Ulaanbaatar, Mongolia

e-mail: nabetani@affrc.go.jp

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Background of the Research Work

Membrane Separation Technology:
- Simple filtration technology which can separate molecules according to their molecular size
- No heat treatment
  → Low Initial Cost, Small Energy, High Quality of Products

Current Status of Poultry Farming in Japan:
- Approx. 200,000 tons of egg-laying hens are discarded annually.
- Chicken meat is rich in anserine-carnosine (AC)
Unique Functionality of Anserine-Carnosine (AC)

- strong antioxidants against hypochlorite radical (ClO·)
- reduce lipid oxidation which affects on flavor, aroma, texture, color and nutritional compositions.
- immuno-response modulation, blood fat reduction and enhanced wound healing functions \textit{in vivo}.

Anserine (\(\beta\)-alanyl-1-methyl-L-histidine) 

\[
\begin{align*}
\text{NH}_2-\text{CH}_2-\text{CH}_2-\text{CO}-\text{NH}-\text{CH}-\text{CH}_2 & \quad \text{CH}_3 \\
\text{COOH} & \quad \text{COOH}
\end{align*}
\]

Carnosine (\(\beta\)-alanyl-L-histidine)

\[
\begin{align*}
\text{NH}_2-\text{CH}_2-\text{CH}_2-\text{CO}-\text{NH}-\text{CH}-\text{CH}_2 & \quad \text{H} \\
\text{COOH} & \quad \text{COOH}
\end{align*}
\]
The reducing effect of AC in combination with vitamin C and ferulic acid on oxidative injury of lymphocyte DNA in normal human volunteers. Symbols indicate mean Comet assay score and vertical bars indicate ±S.D.; *p<0.05, **p<0.01
Expectation for Membrane Separation Technology

If anserine-carnosine (AC) 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.

Objective of the Work

Development of a membrane separation process which can efficiently purify anserine-carnosine (AC) extracted from chicken meat.
Materials
(preparation of chicken extract)

Water: carcasses = 3:1

Chicken carcass

Extraction
100°C, 4-6 h

Ion exchange resin
AC: 6.25 g/L
(Purity of AC: 60 - 70%)
Creatinine: 2.31 g/L
Na⁺: 0.27 g/L

Removal

Acidic and neutral amino acids, Proteins

NF membrane
Apparatus

Lab-Module type 20.(DSS)
Maximum membrane area: 7200 cm² (180 cm² × 40 sheets)
Maximum pressure: 6 MPa
Maximum temperature: 100°C
pH-range: 0-14
Suitable Nanofiltration Membrane for Purification and Concentration of Anserine and Carnosine

Molecular Weight:
- Anserine, Carnosine: 234
- Creatinine: 113
- Sodium Chloride: 58

Feed
(Anserine-Carnosine, Creatinine, Sodium Iron)

Concentrate
(Rich in Anserine-Carnosine)

Permeate
(Creatinine and Sodium Iron)
## Membranes used in this study

<table>
<thead>
<tr>
<th>Membrane Type</th>
<th>NaCl rejection (*MW cut-off)</th>
<th>Manufacturer</th>
<th>Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFT50</td>
<td>55</td>
<td>DSS</td>
<td>Polypiperazine /polyamide/</td>
</tr>
<tr>
<td>DRA4510</td>
<td>45</td>
<td>DAISEN</td>
<td>Polyamide</td>
</tr>
<tr>
<td>Desal DL</td>
<td>15</td>
<td>Desalination</td>
<td>Polyamide (aromatic)</td>
</tr>
<tr>
<td>Desal DK</td>
<td>50</td>
<td>Desalination</td>
<td>Polyamide (aromatic)</td>
</tr>
<tr>
<td>NTR7430</td>
<td>30</td>
<td>NITTO DENKO</td>
<td>Sulfonated polyether sulfone</td>
</tr>
<tr>
<td>NTR7450</td>
<td>50</td>
<td>NITTO DENKO</td>
<td>Sulfonated polyether sulfone</td>
</tr>
<tr>
<td>NTR7250</td>
<td>60</td>
<td>NITTO DENKO</td>
<td>Polyvinyl alcohol</td>
</tr>
<tr>
<td>MPF34</td>
<td>35</td>
<td>Abcor</td>
<td>Polysulfone</td>
</tr>
<tr>
<td>MPF36</td>
<td>10</td>
<td>Abcor</td>
<td>Polysulfone</td>
</tr>
<tr>
<td>MPF44</td>
<td>25</td>
<td>Abcor</td>
<td>Polyacrylonitrile (PAN)</td>
</tr>
<tr>
<td>MPF50</td>
<td>700*</td>
<td>Abcor</td>
<td>Polyacrylonitrile (PAN)</td>
</tr>
<tr>
<td>G-5</td>
<td>1000*</td>
<td>Desalination</td>
<td>Polyamide</td>
</tr>
<tr>
<td>G-10</td>
<td>2500*</td>
<td>Desalination</td>
<td>Polyamide</td>
</tr>
</tbody>
</table>
Total Circulation Experiment

Feed flow rate: 5.8 - 11.3 L/min
Operating Pressure: 1 - 6 MPa
Temperature: 25 °C
Batch-Wise Concentration Experiment

Area of membrane: 360 cm$^2$
Temperature: 25 °C
Initial Volume of Feed: 11.2 L
Final Volume of Feed: 3.0 L
Analyses and Calculation of Rejection Value

• HPLC using column (TSKG2, 500PWXL) with 45% acetonitrile (pH3.0).

• Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) (JICP-PS3000UV; Leeman Lab).

\[ R_{\text{obs},i} = 1 - \frac{C_{\text{p},i}}{C_{\text{r},i}} \]

- \( R_{\text{obs},i} \): observed rejection against component \( i \)
- \( C_{\text{p},i} \): concentration of component \( i \) in permeate
- \( C_{\text{r},i} \): concentration of component \( i \) in retentate
Effect of operating pressure ($\Delta P$) on permeate flux ($J_v$) and observed rejection for anserine-carnosine ($R_{AC}$), creatinine ($R_{Cr}$) and sodium ion ($R_{Na}$) with NFT50 membrane.
Results of Total Circulation Experiment

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
## Results of Total Circulation Experiments

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

$\Delta P$: 4 MPa. Flow rate: 10 L/min.
Changes in yield of each component with volume reduction factor (VRF) during batch-wise concentration experiments, where VRF is defined as ratio of initial feed volume ($V_{f,0}$) to feed volume ($V_f$). (Flow rate: 10 L/min, pressure: 4 MPa)
Proposal of a Mathematical Model

\[
\frac{dV}{dt} = -A \; J_v \tag{1}
\]

\[
\frac{d(C_i \; V_f)}{dt} = -A \; J_v \; C_i \; (1 - R_{obs,i}) \tag{2}
\]

\[
J_v = \alpha - k \ln(VRF) \tag{3}
\]

\[
VRF = \frac{V_{f,0}}{V_f} \tag{4}
\]

\(A\): membrane area

\(J_v\): Permeate flux

\(V_f\): volume of feed solution

\(t\): time

\(C_i\): concentration of component \(i\)

\(R_{obs,i}\): rejection against component \(i\)

\(VRF\): volume reduction factor

\(\alpha\): constant

\(k\): mass transfer coefficient
Changes in purity, yield, concentration ($C_{AC}$) of anserine-carnosine and permeate flux value ($J_v$) with NFT50 membrane (experimental value and calculation line)
**Designed Process**

- **Water 15 t** → **Chicken carcass 5 t/d**
  - Extraction 4-6 h
  - Extract 15,000 kg
  - Ion exchange resin
    - Eluted solution 1,400 kg
    - NF membrane
      - NFT50 membrane
        - 1.2 m², 6 h
      - Permeate
      - Retentate: 150 kg
    - Acidic and neutral amino acids
      - Creatinine and sodium ion
      - Anserine-Carnosine
        - (conc.: 7.5%, purity: 90%, yield: 98%, AC: 11.2 kg)
Conclusions

In order to add extra value to chicken carcasses and utilize them, efficiency of a membrane process for purification and concentration of anserine and carnosine 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 of anserine and carnosine were selected.
2. By applying the selected membranes and conditions, anserine and carnosine was purified 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 5 t of chicken carcasses in a day was designed.
Practical Scale Process for Purification and Concentration of Anserine and Carnosine Extracted from Chicken Meat

Automated Ion Exchange Chromatography Unit

Nanofiltration Membrane Unit
Thank you very much for kind attention!!

謝謝!!
感謝!!

Anserine-Carnosine: 400 mg
Vitamin C: 300 mg
Ferulic Acid: 20 mg

Several products are commercially available!