REMOVING LIPID FROM CRUDE COLLAGEN OF *PANGASIUS HYPOPHTHALMUS* WITH SUPERCRITICAL CARBON DIOXIDE

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ABSTRACT

Freeze-dried collagen from basa fish *(Pangasius hypophthalmus)* skin has been removed lipid with SC-CO_2 equipment (Thar SFC SN.11419), the efficiency of the lipid extraction reached 90%, corresponding to the decrease of lipid content in collagen from 4.07% to 0.40% at conditions as follows: 200 bar, 42^oC, 60 minutes, ethanol 10% and flow rate 10g/min. The lipid extraction yield increased when pressure, temperature, flow rate and ethanol content increased, respectively. The collagen product has high molecular weight and consists of α_1, α_2, β and γ chain. The composition of collagen was: HP of 93.72 mg/g, organic compounds of 84.02%, moisture of 15.25%, lipid of 0.40% and ashes of 0.291%. The characteristics satisfied the requirements of cosmetic, pharmaceutical and food applications.

Keywords. collagen, supercritical carbon dioxide, pangasius hypophthalmus fish skin

1. INTRODUCTION

Supercritical carbon dioxide (SC-CO_2) fluid extraction has been applied in the commercial production of flavouring cosmetics, pharmaceuticals and food products. Examples are decaffeinated coffee, hop extract, extraction of turmeric essential oils, and ginger flavouring. In the oleo-industry, numerous researchers have tried extracting from seeds and refinement of plant oils with SC-CO_2. There are several advantages in using SC-CO_2 in industrial production. CO_2 has several desirable properties, such as non- corrosion, non- toxicity, non-flammability and non explosibility. Because CO_2 is stable chemically, it never reacts with other materials. Easy separation and removal of CO_2 from products eliminates problems related to toxic residual solvent. It is inexpensive and readily

available. The low critical temperature and pressure (Tc = 31.3^{o}C, Pc = 7.4MPa) can be utilized to establish an energy saving process.

Collagen is a polymeric protein and is composed of triple helix fibers. More than a third of the body's protein is collagen. Collagen is the fibrous protein constituent of skin, cartilage, bone, tooth, muscle and other connective tissue. Collagen acts as a scaffold for our bodies, controlling cell shape and differentiation.

To produce highly qualified collagen from fishery wastes, non-collagen accompanied residues such as: lipid, minerals, colorants, and odorants should be well removed. For a long time, organic solvent was used to removing lipid. Nevertheless, due to the toxic effects of organic solvent, there have been some research on extraction of lipid from fishery raw materials with SC-CO_2 recently such as: from Antarctic Krill [2]; Salmon Roe [7]; Brown Seaweed [4].

In this study, freeze-dried crude collagen from *pangasius hypophthalmus* fish skin was used as raw material to remove lipid by SC-CO_2. Influence of temperature, pressure, content of co-solvent, CO_2 flow rate on lipid extraction yield was investigated; the collagen product after lipid extraction by SC-CO_2 were characterized (molecular weight, hydroxyproline content, lipid content, etc.)

2. EXPERIMENTAL METHODS

2.1. Materials

Freeze-dried crude collagen was obtained from basa fish skin by treatment and purification process of Le Thi Thu Huong et al [3]. Freeze-dried crude collagen contain: moisture of 15.25%, lipid of 4.07%.

2.2. Extraction of lipid by SC-CO_2

Lipid contaminant of freeze-dried crude collagen was removed by SCCO_2 with the CO_2 flow rate of 5, 10, 15g/min; pressure of 100, 200, 300 bar; temperature of 34, 38, 42^{o}C and ethanol content of 5, 10, 15% for 30 minutes. After that, effect of extraction time (30 -120 minutes) to lipid removing yield and characteristic of collagen product was investigated. The lipid content in collagen was determined before and after extraction. The extraction of lipid were perform by SC-CO_2 equipment with model *Thar SFC SN.11419.*

2.3. Gel SDS-polyacrylamide (SDS-PAGE) electrophoresis

SDS-PAGE gel electrophoresis was performed (Laemmli, 1970) using the Buffer System, a mini- PROTEAN Tetra cell manufactured by BIORAD. The resolving gel was 7% and stacking gel was 5%. After electrophoresis, gel was dyed by 0.05% (w/v) Coomassie blue R-250 in 15% methanol and 5% (v/v) acetic acid. Then the gel was dipped in the solution of 30% (v/v) methanol and 10% (v/v) acetic acid to remove the color. The molecular mass of collagen protein was determined using a standard protein scale, ranging from 75 kDa to 250 kDa.

2.4. Quantitative analysis methods

Collagen amount was relatively quantified via the content of hydroxyproline, an amino acid occupied approximately 14% of collagen. This content of the hydroxyproline, in turn, was determined by the method of Switzer (1991). The remained lipid content after SC-CO_2 extraction was determined using the TCVN 4331:2001 norm. TCVN 4326:2001 was used also for moisture measurement, while AOAC 923-03 norm was used for ash determination.

3. RESULTS AND DISCUSSIONS

3.1. Effect of extracting pressure on lipid residue

Fig.1 shows the influence of CO_2 pressure on lipid extraction (other conditions were constant such as: flow rate was 10g/min; temperature - 38^oC; retention time - 30 min).When the pressure of CO_2 increased from 100 to 300 bar, the lipid content decreased from 4.07% to 0.72%. The supercritical fluid pressure has close relation with its solvent ability for lipid. When pressure of CO_2 increases, the solubility and thus the lipid extraction efficiency was increased [5][6]

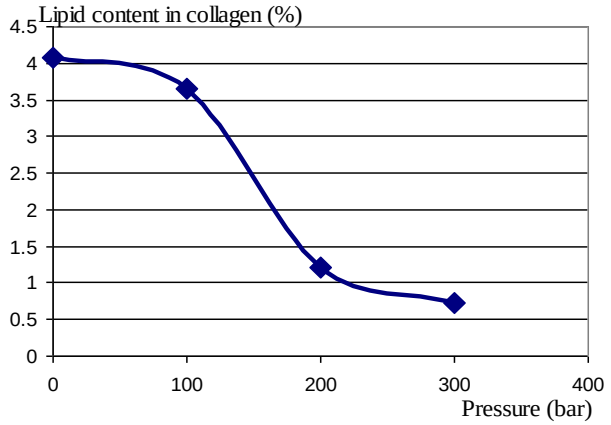

Figure 1. Influence of CO_2 pressure on lipid residue

3.2. Effect of CO_2 flow rate on lipid extraction

Fig. 2 shows the influence of CO_2 flow rate on lipid extraction (other conditions were constant such as: pressure 200bar; temperature 38^oC; retention time 30 min). When the flow rate increased from 0 to 10 (g/min), the lipid content decreased rather quickly, from 4.07% to 1.21%; but when the flow rate changed from 10 to 15 (g/min), the lipid content decreased more slowly, from 1.21% to 0.89%. Therefore, the CO_2 flow rate of 10g/min was chosen for further investigations.

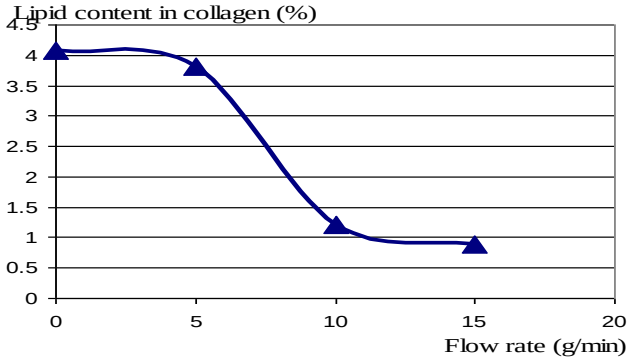

Figure 2. Influence of CO_2 flow rate on lipid residue

3.3. Effect of extracting temperature

Fig.3 shows the influence of temperature on lipid extraction. While the temperature of $SCCO_2$ increased (above the critical point) from 34 to 42^oC with pressure 200bar, retention time 30 min and the flow rate of CO_2 was held constantly (10g/min), the lipid content decreased from 4.07% to 1.21%. The result was consistent with the conclusion of Turner *et al.*(2001) that above the supercritical point, the extraction efficiency increased proportionally with the temperature of CO_2 flow

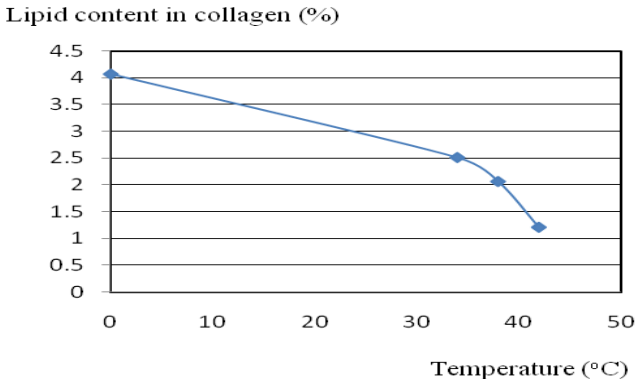

Figure 3. Influence of temperature on lipid residue in collagen

3.4. Effect of ethanol addition

The influence of ethanol on lipid extraction is shown in Fig.4. Ethanol has played a role as a polar co-solvent to enhance extraction efficiency. If the ethanol content increased from 5% to 15% (other conditions were constant such as: flow rate was 10g/min; pressure - 200bar; temperature - 38^oC; retention time - 30 min), the lipid content decreased from 4.07% to 0.39%. Mixing ethanol with CO_2 in the flow the extraction efficiency was rapidly increased. This is due to the fact that carbon dioxide can only extract non-polar lipid materials, whereas mixture of ethanol and carbon

dioxide can extract both polar and non-polar lipid materials.[1][5][6]

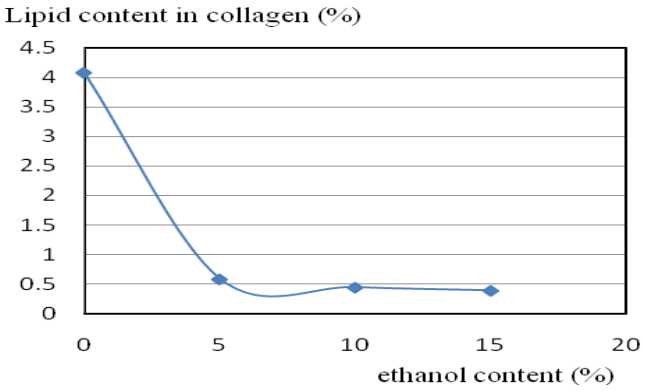

Figure 4. Influence of ethanol added on residual lipid content in collagen

3.5. Effect of retention time on residual lipid content

Fig.5 shows the influence of retention time on lipid extraction. The extraction was performed at 200 bar, flow rate 10g/min and 42^{o}C at 30, 60, 120 minutes. After the first 30 minutes, the extraction rate increased very fast and the lipid content decreased from 4.07% to 0.44%. In the next 90 minutes, the extraction rate slow down, and the lipid content deceased only a little, from 0.44% to 0.32%. Extraction yield of lipid reached 92.14%. The result was consistent with the conclusion of Y. Tanaka *et al.*(2004) that extraction lipid from *Salmon Roe* with SC-CO_2 and ethanol mixtures achieved an extraction yield of more than 80%.

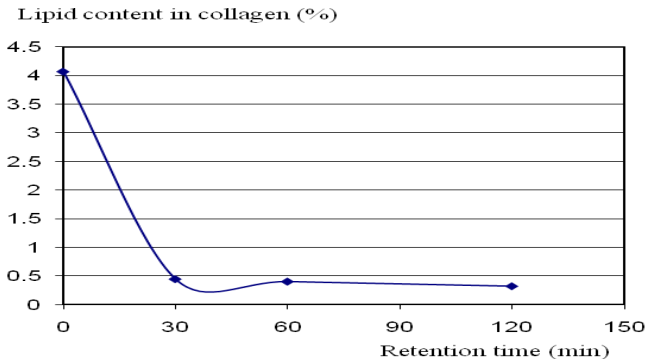

Figure 5. Influence of retention time on residual lipid content in collagen

3.6. SDS-polyacrylamide (SDS-PAGE) gel electrophoresis

Fig.6 shows the influence of SC-CO_2 extraction retention time on collagen molecular mass. Three samples: sample 0; sample 1 and sample 2 consists of α_1, α_2, β and γ chain; the only sample 3 consists of α_1, α_2, β without γ chain. That prove the longer the the retention time, the higher the collagen degradation level. Therefore, the extraction duration of 60 minutes was chosen for ensuring acceptable high-enough molecular weights of the targeted collagen, when extraction yeild of lipid reached 90%.

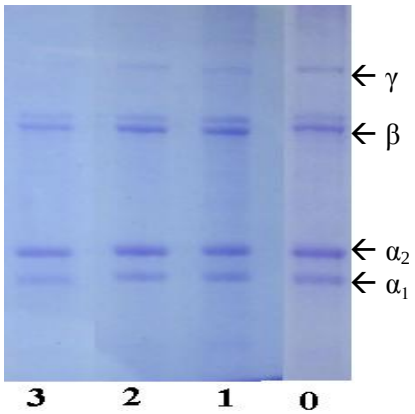

Figure 6. Molecular weight of different samples of collagen. 0- collagen sample before lipid extraction, 1- 3: collagen samples during extraction (1 – after 30 minutes, 2- after 60 minutes, 3- after 120 minutes)

3.7. Analytical result of collagen product

After removing lipid by SC-CO_2 at the chosen conditions above; the product collagen has been analysed chemical components. The result as follow

Table 1. Composition of collagen product

Composition	Content (*)
Hydroxyproline (mg/g)	93,72 ± 8,19
Lipid (%)	0,40 ± 0,02
Moisture (%)	15,25 ± 0,338
Ashes (%)	0,291 ± 0,074
Organic compounds (%)	84,02 ± 0,302
(*) ± SD - each result was repeated three times	

4. CONCLUSION

$SC\text{-}CO_2$ extraction is suitable method for lipid separation conditions as follows: pressure 200 bar, temperature 42^oC, flow rate 10g/min (with 10% ethanol) in 60 min. The quality of resulted collagen was satisfied to applying in food, cosmetic and pharmaceutical industry.

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