Effect of Oxidation Product of Cod Fish Oil and Adsorbed Water on Oil Tannage

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Abstract

This study was concerned with influence of the oxidation products of cod fish oil on oil tannage. Hide powder was tanned with cod fish oil under the different amount of adsorbed water and tanning period. The oil tanned hide powder was evaluated by the denaturation temperature (T_{DSC}), the browning degree, the combined aldehyde and the peroxide value (POV) of extracted cod fish oil.

The combined aldehydes were obtained as each 2,4-dinitrophenyl hydrazone by the steam distillation of acid hydrolyzate. The low molecular aldehydes from oxidized cod fish oil and degreased tanned hide powder were separated and determined by TLC, GLC and IR. As the browning discoloration of protein involved in the aldehyde with the unsaturated group, the browning degree is given as the indication of chemical reaction with the oxidation products. The amount of low molecular aldehydes combined with the hide powder increased during early stage of the oil tanning reaction. A major low molecular aldehyde from oxidized cod fish oil was acetaldehyde and acrolein, as other aldehydes were formaldehyde, butanal, pentanal and hexanal. As time goes on, the quantity of released acetaldehyde and acrolein from oil-tanned leather decreased. It is suggested that those aldehydes changed to other compounds. Adsorbed water of 25% or above in hide powder accelerated an oxidation of the cod fish oil and increased the T_{DSC} and the browning degree.

Keywords
Oil tannage, Aldehyde, Browning discoloration, Adsorbed water

1. Introduction

The oil tanning has been used for a long-standing production of a soft leather. Large portion of this oil tanning mechanism is unknown, because between collagen and oxidation products of the fish oil is complex one. The research of oil tanning process has been studied from for a long time[1)]. Conventional tanning theory is as follows, aldehydes and oxidated oil compounds formed by the oxidation of fish oil react with amino groups in collagen and the oil tanning effect is expressed by those chemical reaction. On the other hand, Zhou et al[2)] pointed out that oil tanning is a physical process, not a chemical process.

In this paper, the variation with time of the bound aldehyde to hide powder and the aldehyde composition produced by the oxidation of fish oil were revealed by the analysis of TLC, IR and GLC methods. The effect of adsorbed water on the oil tanning process was also examined by the measurement with time of the oxidation of fish oil, the amount of bound aldehyde, the browning discoloration degree and the thermal denaturation temperature (T_{DSC}) using hide powder and deaminated hide powder.

1. Material and Methods

2.1. Fish oil, hide powder and deaminated hide powder

Raw cod fish oil (Iodine value; 151.8, Acid value; 0.51) was provided from Nippon fine chemical Co. LTD. Hide powder was prepared our laboratory as following method. Fresh hide after the slaughter was rinsed in water and the hide was divided into three layers. The middle layer cut into 2～3mm square and the middle layer was refined according to the Veis's methods [3)]. The refined hide piece was dehydrated with acetone and crushed with a Wiley mill. Furthermore, the hide powder obtained was degreased by the chloroform/methanol (2/1,V/V) solution.

The deaminated hide powder was prepared as follows; 20g of the hide powder was immersed in 200ml of water solution including 10g of sodium sulphate and 9g of sulphuric acid for 24 hours with occasional shaking. After having removed the solution, the hide powder immersed in 100ml of 5% nitrous acid solution and stirring for 30min. Furthermore, 20% of sulfuric acid solution was dropped to the hide powder after having added 20ml of 25% nitrous acid solution. The immersed solution was stirred sometimes for 24 hours in 5 ℃ or less more. After the deaminated hide powder was washed with distilled water repeatedly, the hide powder was dehydrated with acetone. The decrease in nitrogen of deaminated hide powder was 0.9m mole/g (Untreated, total nitrogen:17.9%, treated：16.5%).

2.2. Oil tannage under different water activity

The hide powder was equilibrated over saturated salt solutions and water for at least 2 weeks at 25℃ in desiccators. The water activity (Aw) was adjusted with saturated salts which were LiCl(Aw=0.11), $MgCl_2$(0.33), K_2CO_3(0.43), $Mg(NO_3)_2$ (0.53), NH_4NO_3(0.62), NaCl (0.75), KCl (0.84), K_2SO_4(0.97) and water (1.00). Total water content of each samples were determined by measuring the loss in weight on drying in an oven at 105℃ until a constant weight was reached.

The oil tannage carried out by mixing homogenously 10g of cod fish oil (50% hexane solution) to 10g hide powder or fine glass beads and incubated at 40℃ under the different Aw for 1~8 days. Extraction method of the oil from the tanned hide powder was as follow. By the soxhlet method using dichloromethane, the sample carried out the extraction processing over

8 hours after vacuum drying at room temperature over 7 hours. Oxidation of oil also carries out with fine glass beads in substitution for the hide powder.

2.3. Determination of combined aldehydes

The degreased tanned hide powder (2g) was decomposed with 500ml of 15% phosphoric acid solution and the released aldehydes were steam distillate and collected as corresponding to the mixture of aldehyde 2,4-dinitro phenyl hydrazone (2,4-DNPH). The mixture of 2,4-DNPHs was analyzed by a gas-liquid chromatography (GLC). The recovery of the combined formaldehyde by this method was 98%[4)]. Thin layer chromatography (TLC) has been applied to separation and determination of 2,4-DNPHs. The isolated 2,4-DNPH of each aldehyde was decided the structure by comparing the retention time of the standard material and IR.

2.4. Determination of denaturation temperature (T_{DSC}), browning discoloration degree and peroxide value (POV)

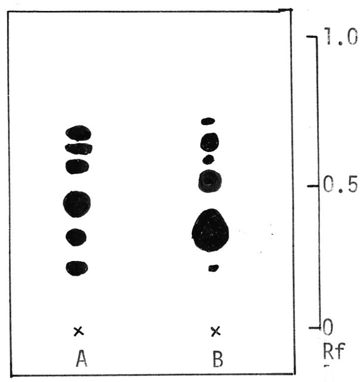
The denaturation temperature of the oil tanned hide powder was measured with a differential scanning calorimetry (DSC) (Shimadzu model DT-30L calorimeter) inside hermetically sealed aluminum sample pans and alumina was used, in the same type of pan, as reference material. The equilibrated samples in the DSC pans were sealed in the hermetic aluminum pans.

It is known that the aldehyde having an unsaturated group and ethanal are closely associated in the browning discoloration of protein by the oxidation of fish oil. The browning degree of oil tanned hide powder was determined periodically by measuring the absorbance at 490nm. The mixture of 8% KOH ethanol solution (20ml) and 1g of the tanned hide powder was refluxed for 3 hours. The transparency solution obtained was filled up to 100ml with water and measured the absorbance of 490nm.

POV was measured periodically according to the standard method of Japan Oil Chemists' Society. Hydroperoxide was reduced by adding a potassium iodide aqueous solution to the oxidized oil solutions and the iodine thus produced was titrated by an aqueous solution of sodium thiosulfate to measure the POV. The oxidized oil was extracted with methanol and diethyl ether, the oil solution was dried over anhydrous sodium sulphate. The solvent was evaporated reduced pressure at less than 30℃. The extracted oil used as POV measurement.

2. Results and Discussion

3.1. Low molecular weight aldehydes from oxidized fish oil and oil tanned hide powder



A : Standard aldehyde DNPHs $C_1 \sim C_6$
B : from Oxidized fish oil
Solvent:Trichloromethane:Hexane: Ethyl Acetate=77:15:8
Layer : Silicagel-G

Fig. 1 TLC of 2,4-DNPHs

Figure 1 shows TLC of 2,4-DNPHs released from the oxidized cod fish oil and the standard aldehydes. Six spots were detected from the oxidized cod fish oil. Each 2,4-DNPH scraped were extracted with hexane. The 2,4-DNPHs obtained were determined the structure by GLC and IR. It was decided that each spots were corresponded to low molecular weight aldehyde having a carbon number of 1~6.

Figure 2 shows that the GLC of released low molecular aldehydes from the oxidized cod fish oil and the raw cod fish oil. From the Figure 2, 10 peaks were detected from the oxidized fish oil. As the results of qualitative analysis by using the standard aldehydes for determining the constituents of the released aldehydes, each peak number of the GLC corresponded to;

1: Formaldehyde, 2: Ethanal (acetaldehyde), 3:2-propenal (acrolein) and propanal, 4: Unknown, 5: 2-butenal and butanal, 6: Unknown, 7: Pentanal, 8: Unknown, 9: Hexanal, 10: Heptanal.

Figure 3 shows the time course of quantitative changes in the total aldehyde content, those aldehydes were released from the oil tanned hide powder and the oil tanned deaminated hide powder by the acid decomposition. The amount of aldehyde from the oil tanned hide powder during the first 4 days has increased, and then decreased. In the case of the deaminated hide powder, the amount of released aldehyde was less than that of oil tanned hide powder. Time course of the released aldehyde from deaminated hide powder was also different from that of the hide powder, the amount of aldehyde was gradually increased within the range of the experiment.

Low molecular weight aldehydes released from oil tanned hide powder by acid decomposition were shown in Figure 4. Total peak area was decreased as the tanning period is longer. Ethanal (acetaldehyde), 2-propenal (acrolein) decrease was particularly pronounced.

From the above experimental results, the low molecular weight aldehydes were produced by the oxidation of cod fish oil. It has been suggested that the lower aldehydes bound to the amino groups of collagen, and then the bound aldehydes were not released by subsequent chemical reaction. It is distinct that ethanal and acrolein are involved in the reaction.

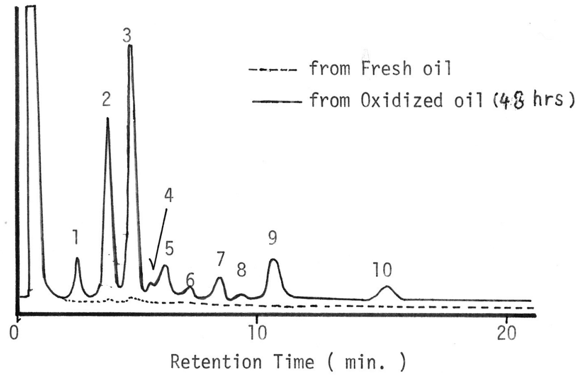


Fig.2 Gas chromatograms of 2,4-DNPH of low molecular weight aldehydes isolated from oxidized and raw cod fish oil

1: Formaldehyde, 2: Ethanal, 3: -propenal (acrolein) and propanal, 4: Unknown, 5: 2-butenal (croton aldehyde) and butanal, 6: Unknown, 7: Pentanal, 8: unknown, 9: Hexanal, 10: Heptanal

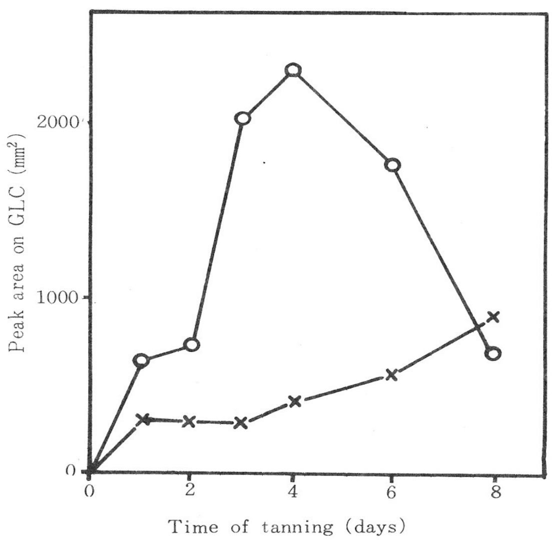


Fig.3 Variation of total amount of released aldehydes by acid decomposed on tanning period.
—○— : Hide powder,
—×— : Deaminated hide powder

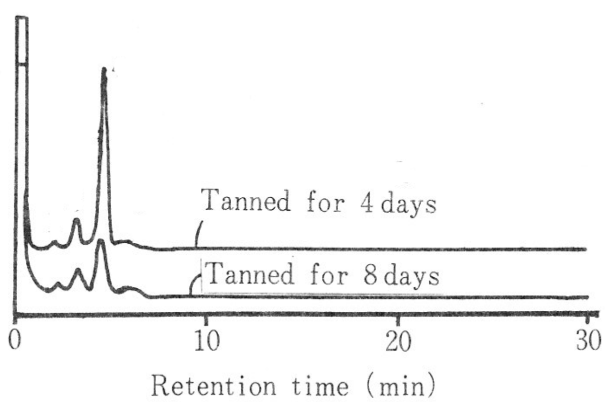


Fig.4 Gas chromatograms of 2,4-DNPH of low molecular weight aldehydes isolated from tanned hide powder with cod fish oil.

2.2. Effect of oil tanning on browning discoloration degree and T_{DSC}

Figure 5 shows that the variation with time of the POV, T_{DSC} and the browning discoloration degree of hide powder and deaminated hide powder.

POV value is increased from the second day and showed a maximum at the third day. It is suggested that an auto-oxidation of the cod fish oil was the start of the second day. The T_{DSC}

of tanned hide powder was increased as the longer tanning period, after 4 days was constant at 67~69 ℃. The browning discoloration degree of oil tanned hide powder after 2 days was gradually increased within the range of the experiment. The behavior with time of the T_{DSC} and the browning degree were different, that is, the browning reaction was slower than the cross linking reaction.

In the case of deaminated hide powder, the browning degree and T_{DSC} did not indicate a significant change during this oil tanning period. It is clear that amino groups such as guanidino group of arginine and lysine ε-amino group are participated in both of the T_{DSC} and the browning reactions. It can be considered that both the cross-linking reaction and the browning reaction is essentially an amino-carbonyl reaction.

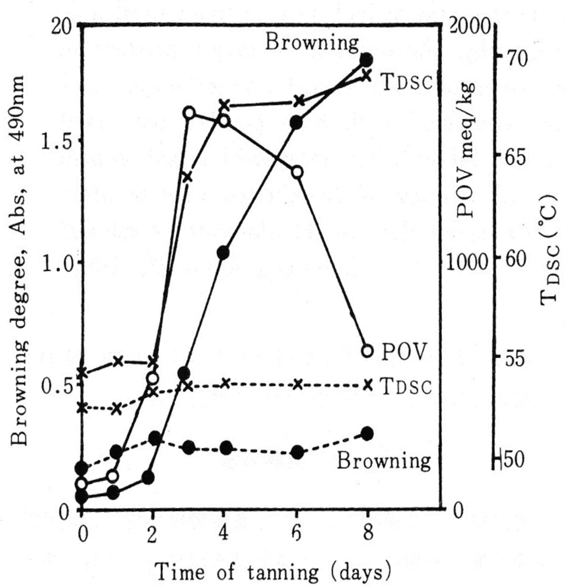


Fig.5 Change in parameters with oil tanning periods

—◍— Browning degree of hide powder

---◍--- Browning degree of deaminated hide powder

—×— T_{DSC} of hide powder

---×--- T_{DSC} of deaminated hide powder

—○— POV of oxidized cod fish oil separated from the oil tanning system

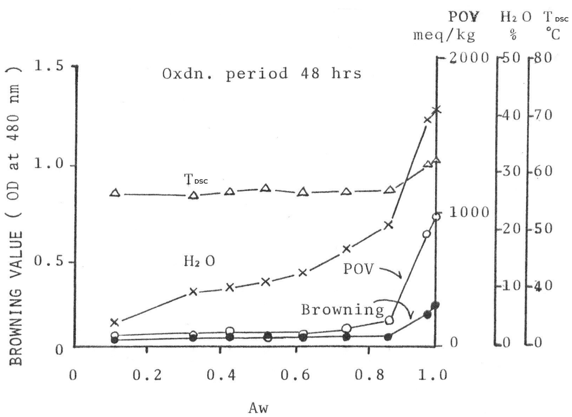
2.3. Effect of adsorbed water on oil tanning

As an oil tanning factor, POV, T_{DSC} and the browning degree were measured. Figure 6~8 shows the results were obtained under a different tanning period and a different water content.

If the water content is more than 40% after 48 hours and more than 25% after 96 hours and 144 hours, the POV was increased with the increase in moisture content. However, if the water content was less than 25%, the POV and the browning degree were increased regardless of the water content. However, an increase in the T_{DSC} was not observed. From the above results, it was found that oxidation of the oil is enhanced with high water content. Both of the T_{DSC} and the browning degree of oil tanned hide powder were also increased in the water content of 25% or more. In the case of the water content is less than 25%, the oil tanning was difficult to proceed.

It was clear that an oil tannage was greatly affected by the higher amount of adsorbed water.

This adsorbed water has the following properties. Above 0.75 of Aw (water content of 23% or more), the water condensed in capillary between collagen fibers and the movement of the water molecule is weakly restricted by physical force, while the adsorbed water under low humidity is strongly held by interaction force of the side chain of the collagen [5,6]. It would consider that this adsorbed water presents as a medium for the chemical reaction and accelerate the chemical reaction for oil tanning.



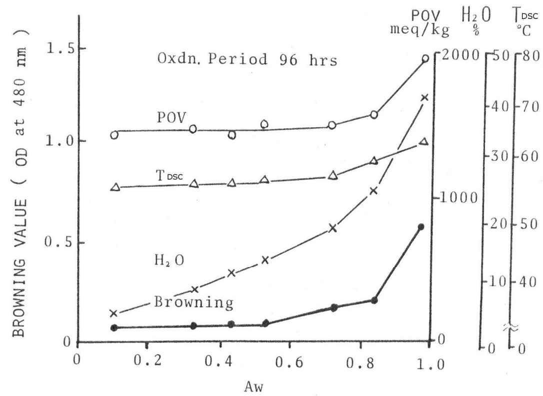


Fig.6 48 hours after of oil tanning period

Fig.7 96 hours after of oil tanning period

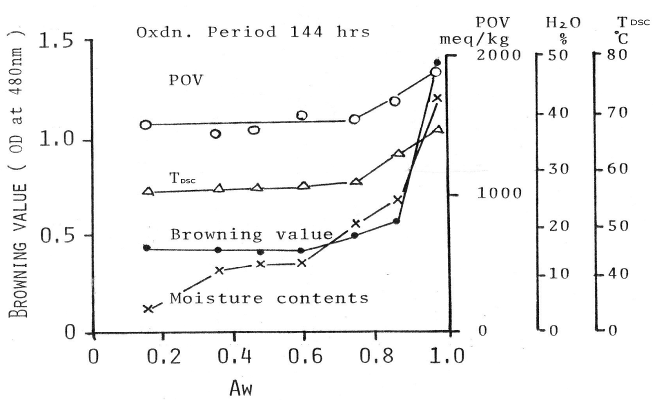


Fig.8 144 hours after of oil tanning period

3. Conclusion

1) The low molecular weight aldehydes which were produced by the oxidation of fish oil bound to the amino groups of collagen, and then the bound aldehydes were not released by subsequent complex chemical reaction. It is distinct that ethanal and acrolein are involved in the reaction.

2) The amino groups such as guanidino group of arginine and lysine ε-amino group are participated in both of the T_{DSC} and the browning reactions. Both of the cross-linking reaction and the browning reaction is essentially the amino-carbonyl reaction.

3) Oil tannage was greatly affected by the higher amount of adsorbed water (water content of 25% or more). This water present in the collagen fibers as a medium for the chemical reactions and the water is accelerate the oil tanning reaction. When the water content is less than 25%, the oil tanning was difficult to proceed.

4. References

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