CHANGES IN THE LIPID AND FLAVOR OF "KATSUOBUSHI" FLESH THROUGH THE SMOKING PROCESS OF THE TRADITIONAL MANUFACTURING METHOD

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1. Katsuobushi processing

- Skipjack tuna meat
- Boiling
- Drying and Smoking
  - first smoking
  - second smoking
  - third smoking
  - fourth smoking

Molding

Katsuobushi

- smoking and molding
- the most important steps for the formation of the specific and unique flavor
2. Background and purpose

**Molding process**
- Much research
- To be clarified about the change of flavor and lipids

**Smoking process**
- Little research
- Not to be clarified about the change of flavor and lipids

**Purpose of this study**
To clarify the change in the flavor and lipid during the smoking at different stages (the first to fourth stages)
3. Materials

- flesh finished first smoking
- flesh finished second smoking
- flesh finished third smoking
- flesh finished fourth smoking

Whittled 3～5mm from surface step by step
4. Experimental procedure

Whittled flesh

Lipid analysis

Extraction of lipids by Bligh & Dyer method

lipid content
lipid classes
fatty acid composition
triglyceride molecular species

Flavor analysis

volatile flavor components
Whole flavor
Lipid analysis

【Lipid classes by TLC】
- TLC plate: 5 × 20cm (Merck)
- The mobile phase:
  petroleum ether / isopropyl ether/ acetic acid (80/20/1, v/v/v)
- Analytical equipment: Image Capture 1D (lyponics)

【Fatty acid composition by GC】
- Analytical equipment: GC-14A (SHIMADZU)
- Detector: flame ionization detector (FID)
- Column: SUPERCOWAXTM10 (SUPELCO)
  length 30m × inner diameter 0.25nm
  film thickness 0.25µm
- The carrier gas: Helium (1mL/min)
- Injection port temperature: 250℃
- FID temperature: 270℃
Lipid analysis

【triglyceride molecular species by HPLC-ELSD】

Pump : LC-10AD(SHIMADZU)
Detector : ELSD  PL-ELS1000(Polymer Laboratories)
Evaporator temperature : 90 ℃
Nebrizer temperature : 40 ℃
Gas Flow : 1.0 mL/min
Column : DEVELOSIL C30-UG-5×2(TAS :Nomura chemistry)
        250 mm × 4.6 mm i.d., particle size 5 µm, fine pores80 Å
Column temperature  20 ℃
The mobile phase : Acetonitrile / Acetone for the elution solvent in gradient system.

【MS】

MS : Waters Alliance ZMD LC/MS system
Capillary voltage : 4.0 kV
Cone voltage : 40 V
Heater temperature : 400 ℃
Source block temperature : 135 ℃
APCI solvent delay temperature : 400 ℃
Method of flavor analysis

【Analysis by GC-MS】

bottle

Whittled flesh 2g

Saturation of flavor
Room temperature 10min

Adsorption of flavor to fiber
40 °C 10min

Desorption of flavor
250 °C 4min

GC-MS

【Fragrance & flavor analyzer】

Sample bag

Whittled flesh 2g

Saturation of flavor
Room temperature 1~2h

fragrance & flavor analyzer

N₂
Flavor analysis

Volatile flavor components by GCMS-QP5000

SPME fiber: 65µm PDMS/DVB (SUPELCO)
GC: GC-17A
MS: GCMS-QP5050A (SHIMADZU)
Column: HP5 (Hewlette Packard) 60m × 0.25mm i.d. film thickness 0.25µm
The temperature program: 40℃ for 5min, 40-110℃ at 1.5℃/min,
110-160℃ at 3.5℃/min, 160-230℃ at 7.0℃/min,
230℃ for 10min
Ionization: EI
The carrier gas: Helium (1mL/min)
Injection: splitless
Library: NIST107, NIST21
Injection port temperature: 250℃
Detector temperature: 280℃

Whole flavor by fragrance & flavor analyzer

Analytical equipment: fragrance & flavor analyzer FF-2A (SHIMADZU)
Sensor: oxide semiconductor sensor (10 sensors of different characteristic)
The carrier gas: N2 (G1 grade > 99.9999 vol. %)
Gas of aspiration time: 6s, 18s, 60s
Collection tube temperature: 40-220℃
Data analysis: absolute-value expression analysis
principal component analysis (PCA)
5. Result and Discussion

5-1. Lipid content

**Fig.1 Lipid content**

- **First smoking**
- **Second smoking**
- **Third smoking**
- **Fourth smoking**

S : surface
5-2. Lipid classes

Fig. 2 Lipid classes

TG was mainly decreased
5-3. Fatty acid composition

Fig.3-1  Fatty acid composition

※ S : surface
5-3. Fatty acid composition

Fig.3-2  Fatty acid composition

- The lipid content remained in outer part of the flesh > in the inner part of the flesh.

the diffusion and decomposition of the lipid occurred during the fourth smoking process
Change of total fatty acid at surface

- **Antioxidant action by phenol adherent derivative from smoke**
- **Physical structure**

Fig. 4 Change of total fatty acid

- **SFA and MUFA**
  - decrease
- **PUFA**
  - increase
5-4. Triglyceride molecular species

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\begin{align*}
\text{PDD} & : \text{SFA-PUFA-PUFA (palmitic acid-DHA-DHA)} \\
\text{PSD} & : \text{SFA-SFA-PUFA (palmitic acid-stearic acid-DHA)}
\end{align*}
\]

![Graph showing the change of PDD or PSD](image)

**Fig.5 Change of PDD or PSD**

*SFA-rich TG might be decreased rather than PUFA-rich TG*
5-5. Volatile flavor components

Fig. 6 Volatile flavor components
Phenols

Comparison of phenols

Fig.7  Comparison of phenols

- Phenols: much presence in smoke
  - Derivation from smoke
- Furans, Pyrazines: less presence in smoke
  - Generation by Maillard reaction

Total Area of phenols

Fourth smoking flesh
Third smoking flesh
Second smoking flesh
First smoking flesh
5-6. Whole flavor

Fig. 8 flavor contribution

- Flavor intensity
- The outer part > The inner part (Only fourth smoking)
Decrease raw fishy flavor such as amines through each stage of the smoking process.
Fig. 10  principal component analysis

*First smoking*

*Second smoking*

*Third smoking*

*Fourth smoking*

The outer part > The inner part (Only fourth smoking)
6. Conclusion

【Lipid analysis】

● The lipid and TG contents decreased

● TG was mainly decreased by saturated and monounsaturated fatty acids.
  
  * Antioxidant action by phenol related compounds and derivatives from smoke
  * Physical structure

● The lipid content remained in outer part of the flesh rather than in the inner part of the flesh.

  the diffusion and decomposition of the lipid occurred during the fourth smoking process.
6. Conclusion

【Flavor analysis】

- Several phenols, furans, and pyrazines through each stage of the smoking process.

- The final high quality flavor was produced in the inside of the flesh after the fourth stage of the smoking.

  the raw fishy flavor such as amines diminished in the dried flesh during the fourth stage of the smoking process compared to the first to third ones.

Repeating the process of smoking by the traditional method is very important to establish the high quality of the “Katsuobushi” flavor formation.