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Lactones in Heated Fish Lipid

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Abstract

In the present study gamma C_7 and C_8, and delta C_6 and C_{10} lactones were demonstrated to produce as major components with decreasing of lecithin, cephalin and triglyceride in rainbow trout lipid by heating for 24 hrs at 125°±5°C under bubbling with air.

The lactones in the heated lipid were extracted with methanol, and separated by column chromatography on alumina and subsequently on silicic acid.

The investigation of lactones was carried out by means of thin-layer chromatography and infrared spectrophotometry.

After reducing the lactones with hydriodic acid, the resulting lower fatty acids were analyzed by gas chromatography for the determination of the carbon numbers of the lactones.

Introduction

A number of studies have hitherto been carried out on the flavor compounds produced in several heated lipids.

Watanabe and Sato[1,2] identified gamma and delta lactones in the volatile fraction of heated meat fat and demonstrated that lactone is produced from the corresponding hydroxy acid in glycerides,[2] and is converted from lower saturated fatty acids, aldehydes and alcohols, which are major products of the oxidation of lipids.[3] They[4] also observed the presence of lactones in the products of thermal oxidation of higher fatty acids, which are the major components of meat fats.

Nakanishi and Watanabe[5] performed a detailed examination on the method for the isolation and identification of lactones, and Fioriti et al.[6] reported on lactones in autoxidized vegetable oils.

Direct heating of fish lipids is scarcely seen in our daily life as compared with that of meat fats or vegetable oils, but the degradation products of heated fish lipids such as lactones seem to be also contained in the roasted flavor compounds.

This report deals with an examination on the lactones produced by the heating of fish lipid.

Experiments

Preparation of heated lipid

The lipid was prepared by the method of Folch et al.[7] from the flesh of rainbow trout, *Salmo gairdnerii irideus*, which was cultured at Nanae Fish Pond,

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Fac. Fish., Hokkaido Univ. The lipid (148 g) was transferred into a round bottom flask and heated for 24 hrs at 125°±5°C under bubbling with air (ca. 50 ml/min). The heated lipid was extracted eleven times repeatedly with 4.5 volumes of methanol. The combined methanol solution was concentrated *in vacuo*, and the residue was taken up in ether. A brown-colored material (26.6 g) was obtained by the evaporation of the ether solution.

Isolation of lactones

The brown-colored material was chromatographed on a 4.5×90 cm alumina column. The column was packed with 500 g of alumina (Merck) activated at 400°C for 2 hrs. The material dissolved in hexane was added to the column and eluted successively with each 1000 ml of hexane and ether, 1900 ml of 20% methanol-ether and 2700 ml of 20% ether-methanol. The lactone-rich fraction obtained from the alumina column was concentrated and further fractionated on silicic acid column chromatography. The 2.7×28 cm column was packed with 90 g of silicic acid (Mallinckrodt) activated at 110°C for 24 hrs. The column was successively eluted at 5°C with each 600 ml of hexane, 10% ether-hexane and 20% ether-hexane and 700 ml of 40% ether-hexane.

Analyses of isolated lactones

Thin-layer chromatography (TLC) was carried out by using silica gel, Wako gel B-O (Wako Pure Chemical Ind. Ltd.) plates. For the identification of lipid components before and after heating, a plate was developed with hexane-ether-acetic acid (90:10:1) or chloroform-methanol-acetic acid-water (75:20:1:2). For visualization the plate was sprayed with 50% sulfric acid and subsequently heated on a hot-plate.

The lactone fraction obtained from the silicic acid column chromatography was brought onto a silica gel plate and developed with *iso*-octane-ether (50:50).[8] The plate was sprayed with 50% sulfric acid and treated with the same procedure as mentioned above.

The lipids before and after heating were converted into methyl esters by a treatment with 10% hydrochloric acid-methanol in sealed tubes. The fatty acid methyl esters were analyzed by a Hitachi F6–D gas chromatograph on a 3 mm× 1 m glass column packed with 10% DEGS on 80–100 mesh chromosorb WAW. The column temperature was maintained at 200°C.

The separated lactones were analyzed by a Hitachi 063 gas chromatograph on a 3 mm×1 m glass column packed with 20% PEG–20 M on 80–100 mesh chromosorb WAW-DMCS. The column temperature was programmed from 70° to 220°C at 3°C/min.

The lactones were converted into the corresponding lower fatty acids by reducing with hydriodic acid and red phosphorus as catalyst.[9] The resulting lower fatty acids were also analyzed by gas chromatography (GLC) in the same conditions as described above (in the analysis of fatty acids), but the column temperature was kept at 150°C.

An infrared (IR) spectrum of lactone was obtained in chloroform solution from a Kōken DS-301 (Japan Spectroscopic Co. Ltd.).

Results and Discussion

When fish was roasted with a household electric roaster, the surface temperature was maintained at 120°–130°C. Accordingly the sample lipid prepared from the flesh of rainbow trout was heated at 125°±5°C.

At the termination of the heating for 24 hrs under bubbling with air, the properties of the lipid changed as shown in Table 1.

Fig. 1 and Table 2 show the decrease of lecithin, caphalin and triglyceride and a noticeable increase of monoglyceride and polymers.

The difference in the composition of major fatty acids is scarcely recognizable, suggesting uniformly changes of each component (Table 3).

Table 1. *Properties of lipids before and after heating for 24 hrs at 125°±5°C.*

	Before	After
Iodine value	109.3	105.8
Acid value	5.0	15.8
Refractive index, n_D^{20}	1.4746	1.4750
Phosphorus (%)	0.49	0.22
Nitrogen (%)	0.30	0.15

Table 2. *Compositions of lipids before and after heating for 24 hrs at 125°±5°C.* (%)

Lipid	Before	After
Lecithin	7.9	4.4
Cephalin	4.8	0
Monoglyceride	0.2	13.6
Diglyceride	0.2	0.3
Triglyceride	81.0	64.9
Sterol	5.8	3.8
Polymers	0	13.0

Table 3. *Compositions of major fatty acids before and after heating for 24 hrs at 125°±5°C.* (%)

Fatty acid	Before	After
16:0	23.0	24.1
16:1	9.8	11.8
18:0	6.3	4.8
18:1	33.6	32.6
18:2	11.0	12.0
20:1	4.1	4.1
22:6	3.7	2.5

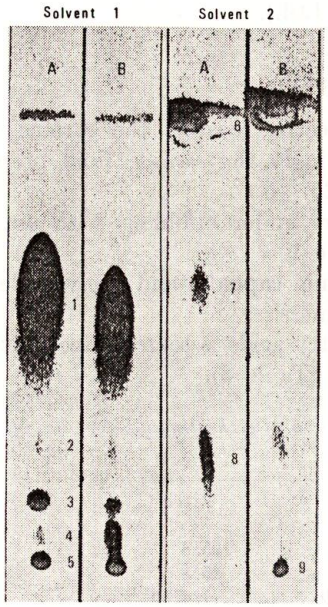

Fig. 1. Thin-layer chromatograms of lipids before (A) and after (B) heating for 24 hrs at 125°±5°C.
1: Triglyceride, 2: Diglyceride, 3: Sterol, 4: Monoglyceride, 5: Conjugated lipids and Polymers, 6: Single lipids, 7: Cephalin, 8: Lecithin, 9: Polymers
Solvent 1: Hexane-Ether-Acetic acid, 90: 10: 1
Solvent 2: Chloroform-Methanol-Acetic acid-Water, 75: 20: 1: 2

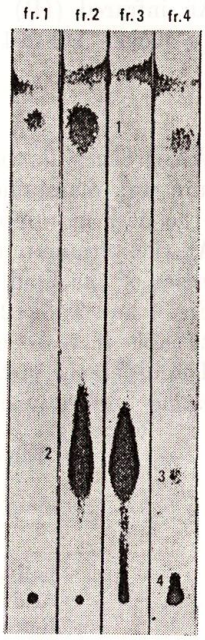

Fig. 2. Thin-layer chromatograms of fractions separated on an alumina column.
1: Fatty acid methyl ester, 2: Sterol, 3: Lactone, 4: Hydroxy-, Keto-acids
Solvent system: *iso*-Octane-Ether, 50: 50

The 26.6 g of brown-colored material was obtained from 148 g of the sample lipid by heating. The material was then chromatographed on an alumina column and separated into four fractions. As shown in Fig. 2, fr. 4 eluting from the column with 20% ether-methanol consisted of three components. Spot 3 seemed to be lactones by comparing with the results obtained by Jurriens and Oele.[8] Spot 1 was ascertained as fatty acid methyl esters by the qualitative analyses with GLC and IR spectrophotometry. Spot 2 in frs. 2 and 3 was identified as sterols by means of color reactions.

The fr. 4 was then chromatographed on a silicic acid column in order to separate the lactones. The material (73 mg) eluted with 40% ether-hexane showed a semi-solid state and had a peach-like odour. As shown in Fig. 3, a strong absorption at 1735 cm^{-1} showed the carbonyl stretching vibration of delta

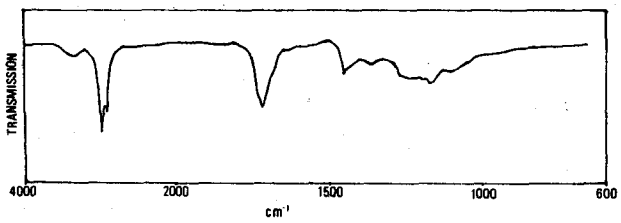

Fig. 3. Infrared spectrum of lactone fraction.

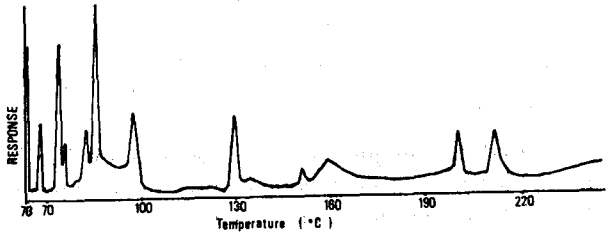

Fig. 4. Gas chromatogram of lactone fraction.
Temperature was maintained at 70°C for 2 min, and then programmed up to 220°C at 3°C/min.

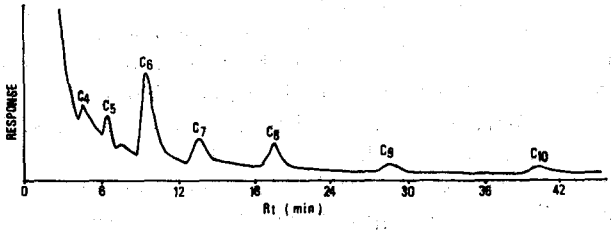

Fig. 5. Gas chromatogram of lower fatty acids obtained by reducing of lactones with hydriodic acid.

lactones.[3,5,6] Hence, the delta lactones were considered to be present in much larger quantity than the gamma lactones.

The gas chromatogram (Fig. 4) indicates seven groups of peaks of lactones based on their carbon numbers. Comparing with the results of Watanabe et al.[4] and Fioriti et al.,[6] the former and the latter peaks of each group seemed to be gamma and delta lactones, respectively. The carbon numbers of the lactones were determined by GLC after reducing them into corresponding lower fatty acids with hydriodic acid. As shown in Fig. 5, C_4–C_{10} saturated fatty acids were identified. From the results shown in Figs. 4 and 5, the lactones produced by the heating of rainbow trout lipid can be tabulated in Table 4. C_7 and C_8 lactones were major components of the gamma type, while C_6 and C_{10} lactones were found to be major components of the delta type. Watanabe et al.[4] have identified gamma C_7, C_8 and C_9 lactones as major products in heated higher fatty acids. Furthermore,

Table 4. *Lactones produced by heating of the flesh lipid of rainbow trout for 24 hrs at 125°±5°C.*

Carbon number	%	Type	
		gamma	delta
4	2.7	+	?
5	8.9	++	+
6	39.5	+	++
7	13.4	++	+
8	19.9	++	+
9	6.4	+	++
10	9.4	+	++

The symboles, ++ and +, stand for large and small, respectively.

they[3] have reported that the major lactones produced by the heating of lower fatty acids and aldehydes have been predominantly delta types of the same carbon numbers as those of starting materials. The production of delta C_6 lactone in this sample seemed to concern with the results reported previously,[10] in which a caproic acid had been one of the major components in the autoxidized products of fish lipids.

Fioriti et al.[6] have suggested that no lactones have been detectable in fresh, refined soybean oil, but that considerable amounts of both gamma and delta lactones have been found to be present in highly peroxidized oils. Although lipid oxidation is, in general, accelerated with the rise of temperature, it hastens to equilibrate the formation of peroxides in the lipids. From these facts it is easily understood that qualitative and quantitative differences of produced lactones take place with the roasting degree of fish.

In the present experiment, the yield of lactones was only 0.05% of the sample lipid, which may influence the improvement of the roasted fish flavor.

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