Constitution and Molecular Species of Triacylglycerol in Meat and Giblet Lipids from Spent Laying Hen

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Abstract Constitution and molecular species of triacylglycerol (TG) from meats (breast and thigh) and giblets (liver, heart and gizzard) of White Leghorn hens were determined to evaluate the lipid quality of spent hen as foodstuff. Main fatty acids comprised in spent hen tissue TG were palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic (18:2) acids. TG from breast, thigh, heart and gizzard resembled in their fatty acid compositions, but TG from liver had higher contents of 18:0 and 18:1 acids, and lower content of 18:2 acid than TG of other tissues tested. Excepting liver, every tissue tested contained similar amounts of S₂D, SMD, SD₂ and MD₂, respectively, and it was supposed that these tissue lipids had comparatively resembled constitution of TG, while, liver alone was significantly differed from the other tissues, on the amount of SM₂, S₂D, M₂D, SD₂ and MD₂. In spent hen tissues, saturated fatty acids were esterified more on position 1 than positions 2 and 3 of TG, and unsaturated fatty acids predominated in position 2. Most abundant TG type was triunsaturated TG (sn-UUU), then followed by sn-SUU, sn-UUS, sn-SUS, sn-USU, sn-SSU and sn-USS in decreasing order, and sn-SSS was the least constituent in every tissue lipid. Also, assuming a 1-random, 2-random, 3-random arrangement, the major TG species in spent hen tissue lipids were considered as sn-OOO, sn-OOP, sn-POO, sn-OLO, sn-POP, sn-PLO and sn-OLP etc.

(Abbreviation used in this are as follows: S = saturated, U = unsaturated, M = monoenoic, D = dienoic, P = palmitic, O = oleic, L = linoleic acids, and sn- = stereospecifically numbered.)

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Key words: spent layer, giblet lipid, triacylglycerol, chromatography, stereospecific study

The egg industry is faced with large numbers of spent laying hens which are often difficult to market at a reasonable return. In U.S.A., whole spent layer carcasses are now utilized as an important source of usable poultry meat in the manufacture of the comminuted items and restructured products by mechanical deboning and flake processing^{10, 24)}. As poultry lipids are rich in highly unsaturated fats, poultry meat tends to become rancid faster than beef or

lamb¹³⁾. Studies by a number of reseachers have revealed that the neutral lipid fraction of various kinds of meat including poultry oxidized slowly, compared with the phospholipid^{7, 15)}. But, neutral lipids or TG constitute a relatively large portion of total lipids, and play an important role in the physical and rheological properties of lipids.

There have been many reports on fatty acid composition of broiler lipids^{1, 11, 22, 23)}, but a few

published data are available on the lipids from spent layers^{9,12}). Although TG constitutes a large portion of neutral lipid, TG of spent layers have not been studied by this time. The purpose of this study is to determine the constitution and molecular species of TG in various tissue lipids, and to elucidate the lipid quality of spent laying hen from the standpoint of the effective use of poultry by-products as foodstuff.

Materials and Methods

Birds and tissue samples

Spent laying hens used in this study were commercial strain layers of White Leghorn. Birds were kept in individual cage, and fed commercial formula feed. Five birds, 528 days of age, were sacrificed after fasting overnight. Breast, thigh, liver, heart and gizzard were removed from each carcass. Samples of each of five tissues were trimmed of all external fat and connective tissues, and kept at -15°C until analysis was carried out.

Extraction of lipid and preparation of triacylglycerol

Tissue samples were minced by a home meat chopper and used for lipid analysis. The total lipid was extracted from each sample according to the method of FOLCH *et al.*⁸), and was separated into neutral and phospholipid fractions by the procedure of MOERCK and BALL¹⁴). Triacylglycerol (TG) was isolated from the neutral lipid fraction of each tissue by silicic acid column chromatography¹⁴).

Argentation thin-layer chromatography

TG was fractionated by argentation thin-layer chromatography (AgNO_3 -TLC). The plate coated with Silica Gel G containing AgNO_3 was prepared by the method of WOOD and SNYDER²⁵), and separation of TG was performed by the method of CHRISTIE and MOORE⁴). The amount of TG fraction was calculated by reference to the methyl heptadecanoate added as internal standard. The TG types present in the fraction were estimated

from the results of fatty acid composition determined on each fraction, as described previously¹⁹).

Stereospecific analysis

Stereospecific analysis of TG was carried out essentially following the scheme proposed by BROCKERHOFF²⁾ and modified by CHRISTIE and MOORE³⁾, as described previously¹⁷).

Gas-liquid chromatography

The fatty acid composition of lipid was determined by the procedure described previously¹⁷), using Shimadzu GC-6 A PF Gas Chromatograph and Shimadzu Chromatopac C-R 2 A.

Results and Discussion

Fatty acid composition of TG of spent laying hen lipid

Fatty acid compositions of TG from meats (breast and thigh) and giblets (liver, heart and gizzard) of spent laying hens are shown in Table 1.

The major fatty acids present in tissue TG were palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic (18:2) acids. In addition to these acids, small amounts of myristic (14:0), palmitoleic (16:1) and eicosenoic (20:1) acids were found in hen tissues. Although the fatty acid compositions were mutually differed among tissues in details, fairly similar fatty acid patterns were observed in every tissue excepting liver TG which contained more 18:0 and 18:1, and less 18:2 than the other tissues tested.

The quality and composition of poultry lipids are influenced by several factors including age, sex, strain of bird, anatomical location and diet, and these factors have more effects on neutral lipid than polar lipid fraction^{13,15}). In spite of the difference in experimental conditions, breast, thigh and liver TG in the present study had similar fatty acid compositions to those reported by PIKUL *et al.*²⁰) on corresponding tissue lipids of 4 months aged chicken. However, our results on heart TG showed less 16:0 and more 18:2 than the results by

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Table 1. Fatty acid composition of triacylglycerol from spent laying hens

Fatty acid*	(mole %)				
	Breast	Thigh	Liver	Heart	Gizzard
14:0	1.2	1.2	0.6	1.4	1.3
16:0	25.2	23.0	25.4	22.3	24.4
16:1	4.0	5.2	4.0	4.8	4.4
17:0	0.4	0.6	0.4	0.4	0.4
17:1	0.2	0.2	0.2	0.2	0.2
18:0	6.9	5.9	8.4	5.4	5.5
18:1	40.3	41.3	49.4	40.9	42.2
18:2	19.7	21.0	10.8	22.1	20.1
18:3	0.7	0.7	0.3	0.9	0.7
20:1	0.8	0.7	0.4	1.1	0.7
20:2	0.1	tr**		0.1	tr
20:3	0.2	0.1	tr	0.1	tr
20:4	0.3	0.1	0.1	0.3	0.1
Sat. acid	33.7	30.7	34.8	29.5	31.6
Monoene	45.3	47.4	54.0	47.0	47.5
Diene	19.8	21.0	10.8	22.2	20.1
Polyene	1.2	0.9	0.4	1.3	0.8

* Number of carbon atoms : number of double bonds.

** tr=trace (below 0.1%). Sat. acid; saturated fatty acids.

Fig. 1. Argentation thin-layer chromatograms of triacylglycerols of tissue lipids from spent laying hens. Plates: Silica Gel G impregnated with 10%AgNO₃. Developing solvent system: hexane-ethyl ether-benzene-methanol (70/10/20/1 by volume). S: Saturated, M: Monoenoic and D: Dioenoic fatty acid.

PIKUL *et al.*²¹⁾

Fractionation of TG class by AgNO₃-TLC

Intact TG of every tissue lipid was fractionated by means of AgNO₃-TLC into different

classes depending on unsaturation and polarity of TG. The chromatograms of each TG are shown in Fig. 1.

Nine or more bands of TG classes were detected on the plates. The results of quantitative analysis of each tissue TG are shown in Table 2. Remainders contained many polyunsaturated or highly polarized TG components that were retained on near the origin. Those TG classes were not determined qualitatively.

Trisaturated TG (S₃) was a small TG component in every tissue lipid. Breast, thigh, heart and gizzard lipids contained similar amounts of TG classes of S₂D, SMD, SD₂ and MD₂, respectively. Accordingly, it was considered that those four tissue lipids had comparatively resembled TG constitution. However, liver TG had the most amount of SM₂ and the least of S₂D, M₂D, SD₂ and MD₂ among the tissues tested. Therefore, liver alone differed from the other tissues on the TG composition.

HUSBANDS¹²⁾ studied the lipids of liver and adipose tissue from layer of White Leghorn strain, and reported that SM₂ was contained about 40% in liver TG and that the amounts of

SM₂ and SMD were both estimated as about 20 % in adipose tissue TG of hens. Consequently, he recognized the different feature between liver and adipose tissue in reference to the TG compositions.

The contents of SM₂ in liver, also of SM₂ and SMD in adipose tissue from layers reported by HUSBANDS¹²⁾ agreed closely with our results obtained on the spent hen lipids.

Fatty acid distribution in spent hen tissue lipids

Positional distribution of fatty acids in TG molecule of spent layer tissue lipids was investigated by stereospecific analysis. The results obtained are shown in Table 3.

Each fatty acid which is comprised in TG possesses specific characteristics in relation to positional and proportional distribution on stereospecifically numbered positions of TG. In the lipids from spent layers, 14 : 0 and 16 : 1 were esterified more on position 3 than on positions 1 and 2 of TG. Although saturated

Table 2. Component triacylglycerol species of tissue lipids from spent laying hens (mole %)

TG type*	Breast	Thigh	Liver	Heart	Gizzard
S ₃	3.0	2.8	2.6	2.6	2.5
S ₂ M	14.7	12.5	15.5	12.0	14.6
SM ₂	20.6	19.0	38.3	17.4	21.3
S ₂ D	8.4	8.3	4.4	7.9	8.1
M ₃	9.1	11.0	10.9	11.4	10.2
SMD	20.7	21.5	17.1	21.0	20.8
M ₂ D	9.7	12.0	6.4	13.0	11.7
SD ₂	4.5	5.0	1.7	5.1	4.3
S ₂ T	0.7	0.8	0.5	0.8	0.7
MD ₂	2.9	2.7	0.3	3.2	2.4
Remainders	5.7	4.5	2.2	5.7	3.5

* S : Saturated, M : Monoenoic, D : Dienoic and T : Trienoic fatty acid.

Table 3. Positional distributions of fatty acids in triacylglycerols of tissue lipids from spent laying hens (mole %)

Tissue	Position in TG	Fatty acid								Unsat. acid
		14 : 0	16 : 0	16 : 1	18 : 0	18 : 1	18 : 2	Others*	Sat. acid	
Breast	1**	0.6	30.1	3.0	13.1	41.5	9.2	2.5	44.2	55.8
	2**	1.0	17.1	2.5	2.9	42.3	32.2	2.0	21.5	78.5
	3**	2.0	28.4	6.5	4.7	37.1	17.7	3.6	35.4	64.6
Thigh	1	1.1	27.2	5.5	8.2	39.7	15.6	2.7	37.1	62.9
	2	1.0	14.9	3.0	3.5	42.0	33.0	2.6	20.6	79.4
	3	1.5	26.9	7.1	6.0	42.2	14.4	1.9	34.4	65.6
Liver	1	0.4	38.3	3.5	10.9	42.4	3.4	1.1	50.0	50.0
	2	0.5	6.4	2.7	4.5	60.3	24.3	1.3	11.6	88.4
	3	0.9	31.5	5.8	9.8	45.5	4.7	1.8	42.8	57.2
Heart	1	0.7	25.7	5.2	12.3	45.9	8.2	2.0	39.1	60.9
	2	1.1	13.2	2.9	2.5	43.7	34.3	2.3	17.2	82.8
	3	2.4	28.0	6.3	1.4	33.1	23.8	5.0	32.2	67.8
Gizzard	1	1.2	29.9	5.3	7.7	40.2	13.6	2.1	39.3	60.7
	2	0.7	13.7	2.4	2.3	44.7	34.6	1.6	17.0	83.0
	3	2.0	29.6	5.5	6.5	41.7	12.1	2.6	38.5	61.5

* Contained 17 : 0, 17 : 1, 18 : 3, 20 : 1, 20 : 2, 20 : 3 and 20 : 4.

** Stereospecific number in triacylglycerol.

fatty acids, such as 16 : 0 and 18 : 0 were found less in position 2 than positions 1 and 3, 16 : 0 was rather evenly distributed in both positions 1 and 3. Position 1 of hen tissue TG was preferentially occupied by 18 : 0, except the liver in which 18 : 0 was distributed evenly in positions 1 and 3.

TG from layer tissues other than liver had comparatively even distribution of 18 : 1 in each position of TG. In every tissue, 18 : 2 was predominantly distributed in position 2, and proportional distribution of 18 : 2 in position 2 of liver TG was estimated as up to 75%.

In all, saturated fatty acids were found more in positions 1 and 3 than in position 2, while, unsaturated fatty acids present abundantly in position 2 of TG. Especially, position 2 of TG from liver was largely (about 88%) occupied by unsaturated fatty acids. In addition, proportional distribution of unsaturated acids in position 2 of TG was higher in giblets than in the breast and thigh.

This characteristic distribution patterns of fatty acids in poultry tissue TG were similarly recognized by CHRISTIE and MOORE^{5,6)} on the lipids of chicken liver, heart and adipose tissue, and also by OHTAKE¹⁸⁾ on the lipid of chicken bone marrow.

Molecular species of TG of spent laying hen

The amounts of TG types and individual molecular species of TG were determined on spent layer tissues. The results obtained are shown in Table 4.

The most abundant TG type in every tissue was sn-UUU estimating as 1/4 ~ 1/3 part of total TG, then sn-SUU, sn-UUS, sn-SUS, sn-USU, sn-SSU and sn-USS were followed in decreasing order on their contents in tissue TG. Trisaturated TG (sn-SSS) was the least constituent in every tissue TG, and the determined values of sn-SSS in each tissue are nearly equal to the results of S₃ contents obtained by AgNO₃-TLC procedure on corresponding tissue lipids, respectively.

The amounts of U₃ in this study on spent

layer was more and S₂U was less than the results obtained by OHTAKE and WATANABE¹⁶⁾ from the research on broiler meats. This suggests that spent layer lipids are more unsaturated than broiler lipids.

Triolein (sn-OOO) was the most abundant TG species in every tissue lipid, and other main TG species such as sn-POP, sn-POO, sn-PLO, sn-OOP, sn-OLP, sn-OOL and sn-OLO were found in spent laying hen tissue lipids. Not so large differences were found among the spent hen tissues tested, except liver, as to the amounts of each TG species. Lipid of liver had significantly higher amounts of certain TG species, *e.g.* sn-POP, sn-POO, sn-OOP and sn-OOO than the lipid of other tissues.

DAWSON and GARTNER⁷⁾ described in their review article that the nature, proportion and degree of unsaturation of fatty acids present in a lipid system or food would indicate the approximate susceptibility of that product to oxidative deterioration. A general survey of our study on the TG constitution of hen tissue lipids proved that spent layer lipids were rich in many TG species which comprised both 18 : 1 and 18 : 2. Present study showed that the total amount of SU₂ and U₃ formed about 3/4 part of total TG in hen tissues. Consequently, lipids from spent layer tissues have lower melting point and are more susceptible to oxidative rancidity as compared with the lipids from meats of domestic animals. Therefore, reasonable consideration to oxidation should be paid during processing and storage of spent layer tissues when they are used as ingredients to food.

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Table 4. Triacylglycerol composition and main component triacylglycerols of tissue lipids from spent laying hens (mole %)

TG type*	Breast	Thigh	Liver	Heart	Gizzard
sn-SSS	3.4	2.6	2.5	2.2	2.6
sn-PPP	1.5	1.1	0.8	0.9	1.2
sn-SSU	6.1	5.0	3.3	4.6	4.1
sn-PPO	1.9	1.7	1.1	1.1	1.7
sn-SUS	12.3	10.1	18.9	10.4	12.6
sn-POP	3.6	3.1	7.3	3.1	4.0
sn-POSt	0.6	0.7	2.3	0.2	0.9
sn-PLP	2.8	2.4	2.9	2.5	3.1
sn-StOP	1.6	0.9	2.1	1.5	1.0
sn-USS	4.2	4.5	2.5	3.4	4.0
sn-OPP	2.0	1.6	0.9	1.7	1.6
sn-SUU	22.4	19.3	25.3	22.0	20.1
sn-POO	4.7	4.8	10.5	3.7	5.6
sn-StOO	2.1	1.5	3.0	1.8	1.4
sn-POL	2.3	1.6	1.1	2.7	1.6
sn-PLO	3.6	3.8	4.2	2.9	4.3
sn-StLO	1.6	1.1	1.2	1.4	1.1
sn-PLL	1.7	1.3	0.4	2.1	1.3
sn-USU	7.8	8.5	3.3	7.1	6.3
sn-OPO	2.6	2.5	1.2	2.0	2.3
sn-UUS	15.5	17.2	18.9	16.2	19.4
sn-OOP	5.0	4.5	8.1	5.6	5.3
sn-OOST	0.8	1.0	2.5	0.3	1.2
sn-OLP	3.8	3.5	3.2	4.4	4.1
sn-UUU	28.3	32.8	25.3	34.2	31.0
sn-OOPt	1.1	1.2	1.5	1.3	1.0
sn-OOO	6.5	7.0	11.6	6.6	7.5
sn-OOL	3.1	2.4	1.2	4.8	2.2
sn-OLO	5.0	5.5	4.7	5.2	5.8
sn-LOO	1.4	2.8	0.9	1.2	2.5
sn-OLL	2.4	1.9	0.5	3.7	1.7
sn-LLO	1.1	2.2	0.4	0.9	2.0

* Calculated triacylglycerol type by means of 1-random, 2-random, 3-random distribution.

sn-: stereospecifically numbered.

S: Saturated, U: Unsaturated, P: Palmitic, Pt: Palmitoleic, St: Stearic, O: Oleic and L: Linoleic acid.

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廃鶏の肉および内臓脂質のトリアシルグリセロールの 構造ならびに分子種

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廃鶏の肉（むね、もも）および内臓（肝臓・心臓・砂のう）のトリアシルグリセロール（TG）の構造ならびに分子種を研究した。廃鶏 TG の主要な脂肪酸はパルミチン酸、ステアリン酸、オレイン酸およびリノール酸であった。むね、もも、心臓および砂のうの TG の脂肪酸組成はよく似ていたが、肝臓は他の組織に比べてステアリン酸とオレイン酸が多く、リノール酸が少なかった。肝臓を除く各組織は S₂D, SMD, SD₂ および MD₂ の含量がそれぞれ近似していて、比較的似た TG 構造であった。しかし、肝臓の SM₂, S₂D, M₂D, SD₂ および MD₂ の含量は他の組織と明らかに異なっていた。廃鶏において飽和脂肪酸は TG の 2 および 3 の位置より 1 の位置に多く、不飽和脂肪酸は 2 の位置に多く存在していた。廃鶏の TG タイプでは sn-UUU が最も多く、次いで sn-SUU, sn-UUS, sn-SUS, sn-USU, sn-SSU および sn-USS の順であり、sn-SSS はいずれの組織においても最も少ない成分であった。1-ランダム、2-ランダム、3-ランダム分布理論に基づいて計算した廃鶏 TG の主要な分子種は sn-OOO, sn-OOP, sn-POO, sn-OLO, sn-POP, sn-PLO および sn-OLP であった。（S=飽和脂肪酸, U=不飽和脂肪酸, M=モノエン酸, D=ジェン酸, P=パルミチン酸, O=オレイン酸, L=リノール酸, sn-=立体特異的番号をあらわす。）

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