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Chemical and Volatile Characterization of Structured Lipid from Soybean Oil Containing Conjugated Linoleic Acid

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Abstract

Structured lipid (SL) produced from soybean oil was enriched with conjugated linoleic acid (CLA). The SL had 21.9 mol% CLA isomers incorporated into SL-soybean after the 24-h reaction. Removal of tocopherols (73~84% loss from original soybean oil) was observed in the SL. Electronic nose can discriminate the aroma of SL-soybean from that of soybean oil. Many oxidative volatiles including pentenal, octenal, 2,4-decandienal, and nonenal were found in SL-soybean. Electronic nose, which is valuable for composite aroma analysis, can provide flavor information together with GC-MS that is useful for qualitative or quantitative analysis of each odor compound in SL.

Key words: structured lipid, conjugated linoleic acid, electronic nose, solid-phase microextraction (SPME)

INTRODUCTION

Structured lipids (SL) are lipids or fats that have been restructured to change their fatty acid composition by chemical or lipase-catalyzed esterification reactions (1, 2). Since SL can be synthesized from various fatty acids such as short-, medium-, long-chain, saturated, or unsaturated acyl moieties, it is possible to modify the physical or chemical characteristics of the original lipids or fats through esterification. Furthermore, nutritive or therapeutic benefits could be provided through incorporating functional fatty acids that impart physiological benefits on overall health. Generally, functional fatty acids include EPA (5,8,11,14,17-eicosapentaenoic acid), DHA (4, 7,10,13,16,19-docosahexanoic acid), α-linolenic acid (9, 12,15-octadecatrienoic acid), and conjugated linoleic acid (CLA) (3,4). As a functional fatty acid, CLA is known to have benefits that include immune modulation, anti-carcinogenesis, antiatherosclerosis and reduction of body fat (5-8).

CLA is a mixture of positional and geometric isomers of linoleic acid (LA) and contains conjugated double bonds at Δ9, 11 or Δ10, 12 while LA has at Δ9, 12. Generally, CLA is found in ruminant animal and dairy products because it is formed by microbial bio-hydrogenation of dietary linoleic acid in the rumen (9). Most plant oil does not contain CLA, and bioconversion of dietary LA to CLA does not occur in humans (10). Therefore, consumption of modified plant oils containing CLA as SL molecules may be beneficial.

In this study, CLA was incorporated into soybean oil through a lipase-catalyzed reaction that synthesizes SL. After isolation of SL, their fatty acid composition, saponification number, iodine value, color, and tocopherol content were characterized. Moreover, volatiles and flavor characteristics analyzed by solid-phase miro-extraction (SPME) along with GC-MS and electronic nose were studied.

MATERIALS AND METHODS

Materials

Immobilized enzyme (IM 60) was purchased from Novo Nordisk Biochem North America Inc. (Franklinton, NC, USA). Refined soybean oil was purchased from a local market (Daejon, Korea), and CLA fatty acid mixture (70 CLA^{TM}) produced from safflower oil was a gift from Livemax Co. (Sungnam, Korea). The main isomers of 70 CLA^{TM} were *cis*-9, *trans*-11 CLA (30.1%) and *trans*-10, *cis*-12 CLA (30.6%).

Synthesis of structured lipids

IM 60 (3 g, 5% by weight of total substrates) was added to the pre-mixture of soybean oil (30 g) and CLA (28.9 g) with 1 : 3 molar ratios in 250 mL Erlenmeyer flasks with screw caps (11). *n*-Hexane (9 mL) was added. To monitor the incorporated CLA content in SL, the reaction mixture was periodically withdrawn from the flask and placed in a shaking water bath (150 rpm) at 55°C. After

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incubation, the reaction product was passed through filter paper in vacuum to remove lipase and poured through an anhydrous sodium sulfate column, and the hexane was then evaporated by a rotary evaporator. To remove free fatty acids, the reaction mixture was mixed with 0.5 N KOH solution (20% ethanol, 120 mL) and *n*-hexane (225 mL) in a separatory funnel with a stopcock. The upper phase (hexane phase) was collected into a round bottom flask, 3~4 drops of phenolphtalein solution was added, and the solution was titrated with 0.5 N KOH solution (20% ethanol). After titration, saturated NaCl (60 mL) solution was vigorously mixed. The hexane phase was then isolated. After passing through an anhydrous sulfate column, the hexane phase containing SL was collected and the solvent was fully evaporated by rotary evaporator under vacuum.

Fatty acid analysis

Fatty acid analysis was performed on a 6890 Series Gas Chromatograph (GC) equipped with an auto injector, flame-ionization detector (Agilent Technology, Anondale, PA, USA), and a fused-silica capillary column (SP-Wax, 60 m×0.25 mm i.d.; Supelco, Bellefonte, PA, USA). The analysis conditions were carried out as previously described (11). Briefly, the column was held at 100°C for 5 min and increased to 220°C for 30 min at the rate of 4°C/min. The carrier gas was nitrogen, and the total gas flow rate in inlet was 52 mL/min (constant flow mode) with split mode (50:1). The temperatures of injector and detector were 250 and 260°C, respectively. Heptadecanoic acid (C17:0) was used as an internal standard.

Analysis of tocopherol, color, and chemical characteristics

An external standard curve, was used for quantitative analysis of α-, γ-, and δ-tocopherol in soybean oil and the SL-soybean (12). The tocopherols were separated and identified using an isocratic HPLC system consisting of a Yonglin SP930D dual pump (Anyang, Korea) with two detectors connected in series. Yonglin UV830 detector (Anyang, Korea) set at 295 nm was connected with a Sedex 75 evaporative light scattering detector (ELSD, Sedere, Alfortvill, France). The operation temperature of the ELSD was 40°C and nitrogen was used as a nebulizing gas at a pressure of 2.2 bar. The column was a Chromsep Catridge, LiChrosorb DIOL (5 μm, 3×100 mm, Chrompack, Raritan, NJ, USA). The mobile phase was a mixture of hexane fortified with 0.1% acetic acid. Flow rate was 0.5 mL/min. The area of each peak was integrated by Autochro-2000 software (Anyang, Korea). Color of 10 g soybean oil and SL-soybean was measured with by JC801 colorimeter (Color techno system Corp, Tokyo, Japan). Hunter L* (±, lightness/darkness), a (±, redness/greenness), and b* (±, yellowness/blueness) values were measured. Free fatty acid (FFA) value, iodine value (IV), and saponification value (SV) of SL-soybean and soybean oil were determined by AOCS Official Methods (13). FFA, IV, and SV of soybean oil used in this study were <0.6, 125, and 197, respectively.

Electronic nose and GC/MS

The Fox 3000 Electronic Nose (Alpha M.O.S., Sa, France) was equipped with a metal oxide sensor array (12 sensors; SY/AA, SY/G, SY/gcT, SY/gctL, SY/Gh, SY/LG, P10/1, P10/2, P40/1, PA2, T30/1, T70/2) and an auto-sampler. These sensors measured headspace volatile compounds, and the FOX 3000 software automatically collected and processed the data. A synthetic air (99.995% pure) was used as a carrier gas and relative humidity (20%) was controlled by an air conditioning unit (ACU). Five gram of soybean oil and SL-soybean with 8 replications was weighed into a 20 mL vial and sealed with a silicon/PTFE septum and aluminum cap with a hole (14). Each sample was incubated in a heating chamber at 50°C with agitation (600 rpm) for 30 min. Syringe temperature was set at 55°C. Then, headspace samples (2500 μL) were injected into the sensors. Acquisition and delay time for each sample was 120 sec and 30 min, respectively, and the airflow was adjusted to 150 mL/min. After acquisition, the FOX 3000 software processed the raw data and performed the principal component analysis (PCA).

Each 4 g of soybean oil and SL-soybean was weighed into a 22 mL vial and sealed with a silicon/PTFE septum and a cap with a hole. A solid-phase microextraction (SPME) fiber (Supelco, Co., Bellefonte, PA, USA) coated with divinylbenzen/carboxen on polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μm thickness) was inserted into a vial to adsorb the headspace volatiles (15). The vial was incubated with agitation for 30 min at 50°C. A Varian Star 3400CX Gas Chromatograph with a Varian Saturn 2000 MS and an HP-5 column (crosslinked 5% diphenyl and 95% dimethylpolysiloxane, 30 m×0.32 mm×0.25 μm film thickness) was used to quantify and identify the volatiles. The flow rate of carrier gas (helium) was adjusted to 1 mL/min. The temperature of the injection port was 270°C. The temperatures of the ion source, manifold, and transfer line in mass spectrometry were 180°C, 50°C, and 180°C, respectively. The column oven was held at 35°C for 5 min, and raised to 60°C at a rate of 4°C/min, to 140°C at 2°C/min, to 220°C at 4°C/min, and to 260°C at 15°C/min and held for 4 min. Comparing mass spectra with selected standards, NIST 98, or Saturn mass spectral library identified

the volatiles. All samples were run in triplicates.

Statistical analysis

SAS software (SAS Institute Cary, NC, USA) was used to perform statistical computations. Analysis of variance (ANOVA) and Student-Newman-Keul's (SNK) multiple range test were performed to determine a significance of difference at a level of $p < 0.05$. For analysis of aroma responses from electronic nose, principal component analysis (PCA) and multivariate analysis of variance (MANOVA) along with SNK were used (16).

RESULTS AND DISCUSSION

Fatty acid composition and chemical characteristics

CLA was gradually incorporated during the 24 h reaction. Most of the reaction occurred within 12 h, showing, 21.3 moL% CLA incorporation (Fig. 1). Incorporation of CLA was especially rapid between 5 and 12 h, and the esterification reaction reached equilibrium after 12 h (Fig. 1). Fig. 2 shows the fatty acid composition of soybean oil and SL-soybean that was produced after 24 h reaction. As expected, the major fatty acid of soybean oil was linoleic acid (C18:2, 51.3 mol%) and CLA isomers were not detected in the original soybean oil. After esterification, some parts of linoleic acids in soybean oil were replaced and 21.9 mol% CLA isomers were found in SL-soybean that was used for further study (Fig. 2). Free fatty acid (FFA) value, iodine value (IV), and saponification value (SV) of SL-soybean were <0.6, 125, and 195, respectively. The FFA value of SL indicated that alkali refining successfully removed free fatty acids, which are unreacted CLA and fatty acids hydrolyzed from triacyglycerols in soybean oil. IV and SV were not much different between SL-soybean and its counterpart due to the substitution of CLA for LA in triacylglycerol molecules. These fatty acids have 18 carbons with 2 double bonds in their molecules that have little affect on the change of IV or SV.

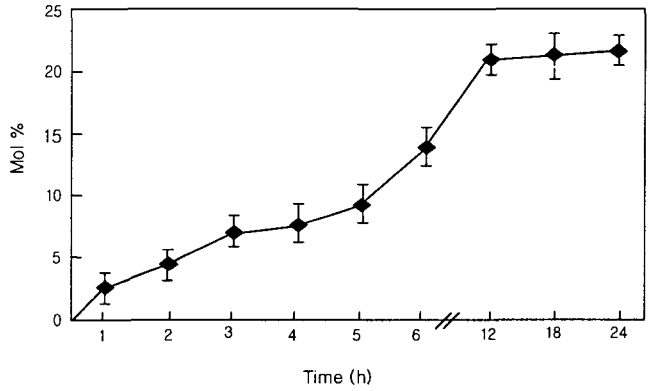

Fig. 1. Mol% of incorporated CLA in the structured lipid produced during the 24-h reaction.

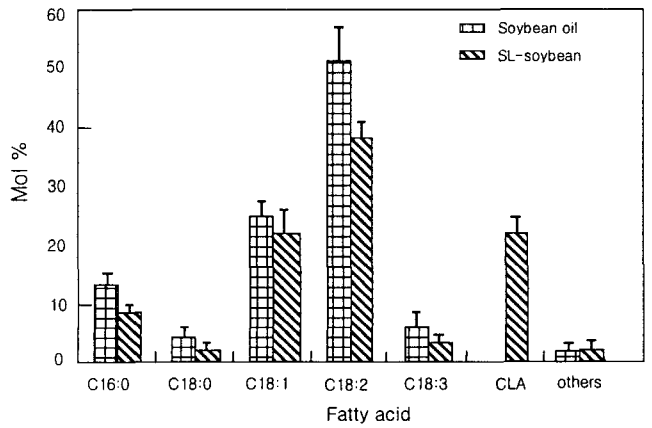

Fig. 2. Fatty acid composition (mol %) of soybean oil and SL-soybean.

Color and tocopherol analysis

After production of SL-soybean, loss of tocopherols and change of color were observed (Fig. 3 and 4). Among tocopherol isomers soybean oil (refined) contains in order of concentration, $\gamma > \delta > \alpha$-tocopherol. Soybean oil used in this study initially contained 0.833, 0.512, and 0.113 mg/g oil of γ, δ, and α-tocopherol,

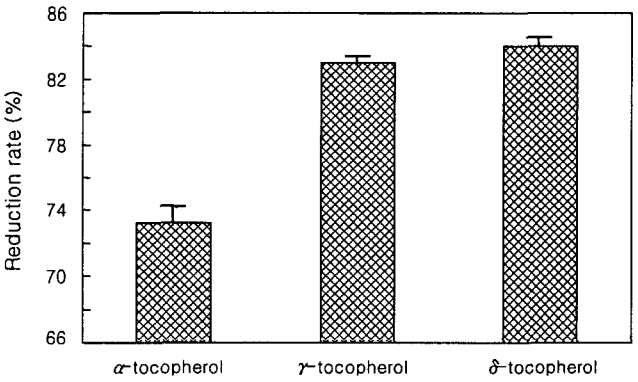

Fig. 3. Reduction rate of tocopherol isomers in SL-soybean.

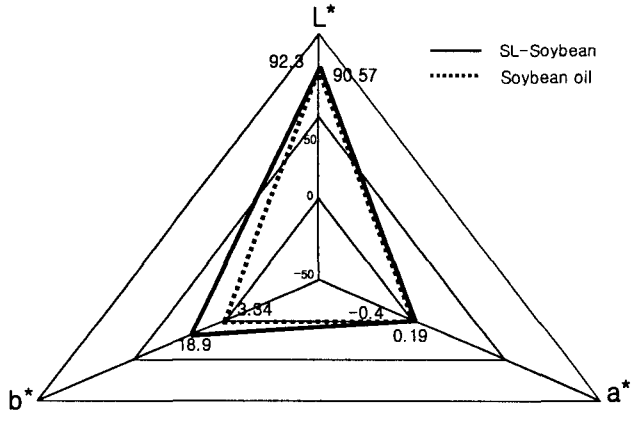

Fig. 4. Lightness (L*), red/greenness (a*), and yellow/blueness (b*) of soybean oil and SL-soybean.

respectively. However, in SL-soybean, 73% (α -tocopherol) to 84% (δ -tocopherols) reduction was observed after the processes (Fig. 3). Similar reductions in tocopherol after SL production was reported, previously (17). Because tocopherols play a very important role as an antioxidant, such reduction is not desirable. To protect SL from oxidation, therefore, additional antioxidant should be added. Color is also an important parameter that affects the quality of oil. Compared with soybean oil, yellowness (b*) of SL-soybean was considerably increased (Fig. 4).

Electronic nose and GC/MS

Electronic nose response ($(\Delta R)/R_0$) of soybean oil and SL-soybean was obtained by 12 metal oxide sensors (Table 1), and was analyzed using PCA and multivariate analysis of variance (MANOVA) statistical analysis. As shown in Fig. 5, PCA permits the classification of the composite aroma information in separate ellipses, discriminating the aroma of soybean oil from that of SL-soybean. The first principal component contains 77.92% of the information. The Wilk's Lambda value with MANOVA, used to compare groups of variables, also showed the overall aroma difference ($p<0.05$). Among the sensors, SY/LG, SY/gctL (sensors for polar compounds), SY/gcT (for polar compounds), SY/Gh, T30/1, and T70/2 (for aromatic compounds) significantly discriminated between the different aroma of soybean oil and SL-soybean ($p<0.05$).

The solid-phase micro-extraction (SPME) method was used for GC-MS analysis. SPME is a well-established technique for the extraction of headspace samples for gas chromatography. Since fibers are coated with various sorbents and these sorbents show different affinities to diverse species, the selection of a certain fiber is very

Table 1. Aroma intensities of soybean oil and SL-soybean[1)]

Sensors	Soybean oil	SL-soybean
SY/LG	0.0041[b2)]	0.0072[a]
SY/G	-0.0446[a]	-0.0566[a]
SY/AA	-0.0223[a]	-0.0251[a]
SY/Gh	-0.0265[a]	-0.0343[b]
SY/gctL	-0.0206[a]	-0.0279[b]
SY/gcT	-0.0160[a]	-0.0259[b]
T30/1	0.0567[b]	0.0667[a]
P10/1	0.0451[a]	0.0434[a]
P10/2	0.0291[a]	0.0296[a]
P40/1	0.0382[a]	0.0382[a]
T70/2	0.0436[b]	0.0576[a]
PA2	0.1270[a]	0.1198[a]

[1)]Values of each sensor were expressed as the means of 8 replicates.
[2)]Values with different superscript letters within the same row are significantly different ($p<0.05$).

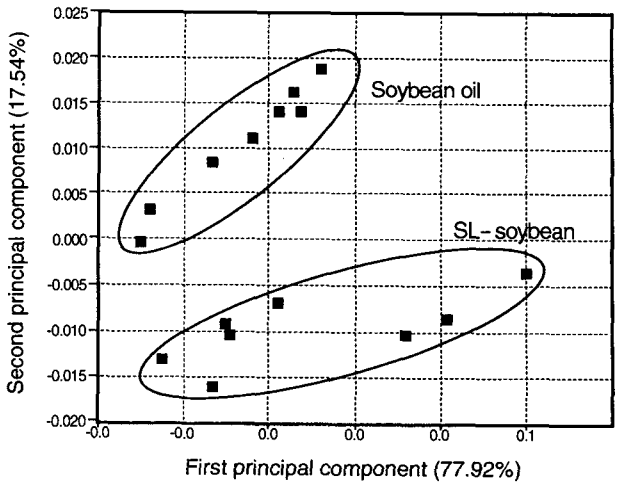

Fig. 5. Principal component analysis of soybean oil and SL-soybean aroma.

important for analyzing volatile compounds. In our study, fiber coated with divinylbenzen/ carboxen/ polydimethylsiloxane was chosen because this fiber provides the best detection of volatile compounds in vegetable oils compared to other fibers (18).

Acetic acid, heptanone, octanol, heptanol, and octane were identified as the major volatiles in soybean oil. SL-soybean has a more complex composition of volatiles than soybean oil (Table 2). Fig. 6 shows the chromatograms of volatile compounds of soybean oil and SL-soybean. In SL-soybean, oxidative compounds of polyunsaturated fatty acids including 2,4-pentadien-1-ol, 1, 10-decanediol, (E)-2-octenal, (E)-6-nonenal, (E)-2-tridecanal, and (E,E)-2,4-decadienal were found. These results suggest that these volatiles come from SL processing because they were not found in original soybean oil. Further, among volatile compounds cyclopentane and 2,4-decadienal could be also generated from the substrate CLA since these were identified in CLA (data not shown). SL-soybean contains high amount of polyunsaturated fatty acids including linoleic acid and CLA. Therefore, larger amounts of octenal, nonenal, and 2,4-decandienal, which are known as oxidative products of linoleic acid, were found in SL-soybean. The amount of 2,4-decadienal as well as total volatiles is well correlated with oxidative deterioration in oil (19,20). Pentenal derived from oxidation of linolenic acid was also observed in SL-soybean. Generally, various carbonyl compounds such as aldehydes, alcohols, ketones, and hydrocarbons are the secondary oxidative products after hydroperoxide breakdown, and have been known to generate off-flavors in oil. Thus, these different volatile profiles could cause different aroma patterns in electronic nose analysis. Electronic nose, which is valuable for composite aroma

Table 2. Volatile compounds of SL-soybean and soybean oil by SPME with GC-MS analysis[1)]

SL-soybean			Soybean oil		
Peak No.	Volatile compounds	Area	Peak No.	Volatile compounds	Area
1	(E)-2-Pentenal	419	1	Acetic acid, hexyl ester	648
2	4-Octenal, 2,2-dimethyl	3,226	2	4-Heptanone, 2,6-dimethyl	476
3	Cyclopentane, 1,2-dimethyl-3-(1-methylethyl)	3,203	3	1-Octanol	1,608
4	2,4-Pentadien-1-ol, 3-ethyl	2,869	4	1-Octanol, 2-methyl	2,513
5	1-Octen-3-ol	298	5	1-Pentanol, 4-methyl-2-propyl	192
6	2-Octanone	187	6	1-Octanol, 2-butyl	327
7	1,10-Decanediol	1,776	7	1-Heptanol, 2,6-dimethyl	278
8	(E)-2-Octenal	1,298	8	Octane, 1,1′-oxybis	621
9	1-Decene, 3,4-dimethyl	1,091			
10	2,4-Pentadien-1-ol, 3-propyl	2,282			
11	(E,E)-3,5-Octadien-2-one	561			
12	(E)-6-Nonenal	1,680			
13	1,12-Tridecadiene	236			
14	Decanoic acid, 3-methyl	1,486			
15	2,4-Dodecadienal	439			
16	2,4-Pentadien-1-ol, 3-pentyl	499			
17	E-2-Tridecenal	1,112			
18	Hexyl octyl ether	375			
19	7-Tetradecene	1,165			
20	1-Decanol, 2-ethyl	1,187			
21	(E,E)-2,4-Decadienal	1,203			
22	1-Octanol, 2-butyl	1,041			
23	Hexadecanoic acid, ethyl ester	811			
24	9,12-Octadecadienoic acid, ethyl ester	400			
25	Ethyl oleate	412			
	Total	29,256		Total	6,663

[1)]Peak areas (total ion counts $\times 10^3$) are the means of triplicates.

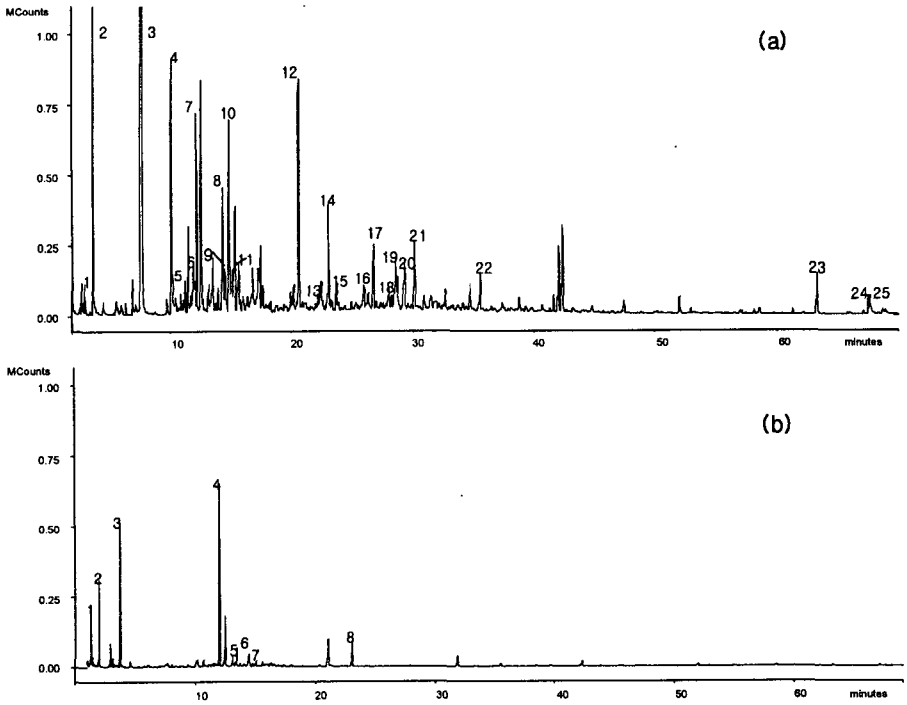

Fig. 6. Total ion chromatograms of volatile compounds from (a) SL-soybean and (b) soybean oil by SPME.

analysis, can provide powerful flavor information together with GC-MS that is useful for qualitative or quantitative analysis of each odorous compound.

CONCLUSION

Chemical and volatile characterization of structured

lipid from CLA with soybean oil was conducted in this study. The processes in SL production lead to changes in flavor features. Furthermore, significant amounts of tocopherols in soybean oil were removed during the process of SL production. Many volatile compounds found in SL were lipid oxidation products. Therefore, during SL production, careful concern for retarding lipid oxidation is needed to prevent deteriorative oxidation in SL. Electronic nose along with GC-MS analysis can successfully monitor flavor attributes of SL.

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