Polycyclic aromatic hydrocarbons in seasoned-roasted laver and their reduction according to the mixing ratio of seasoning oil and heat treatment in a model system

Se-Jin Kang¹ · Sung-Yong Yang^{1,2} · Jin-Won Lee³ · Kwang-Won Lee¹

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Abstract In food processing, polycyclic aromatic hydrocarbons (PAHs) can be generated during heat treatment, and the PAHs in seasoned-roasted (SR) laver can be reduced by checking points during manufacturing. Benzo (*a*) anthracene, chrysene, benzo (*b*) fluoranthene, and benzo (*a*) pyrene have been identified in SR laver via GC/MS. We confirmed that in practice, the PAHs in SR laver form from the mixed oil (57%) and roasting process (43%). To mitigate the formation of PAHs, we used a model system to change the mixing ratio of oil, roasting temperature, and time. A significant reduction (35%) was observed in the PAH level as the perilla oil was removed from the mixed oil composition and roasting continued at 350 °C for 10 s. These results show that the composition of

the mixing oil and the parameters of the heat treatment are crucial factors that contribute to the formation of PAHs in roasted laver.

Keywords Processed food · Seasoned-roasted laver · Reduction · Polycyclic aromatic hydrocarbons · GC–MS

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are chemical compounds with two or more fused aromatic rings that result from the incomplete combustion of any organic matter used to generate heat over 200 °C (Phillips, 1999). Foods can be contaminated with PAHs during processing or cooking, e.g., preparing charbroiled food or smoking meat (Larionow and Soboleva, 1938). The formation of PAHs has been reported in grilled foods (Larsson et al., 1983), roasted foods (De Kruijf et al., 1987), smoked foods (Wang et al., 1999), dried foods (Jo et al., 2009), and edible oils (Dost and İdeli, 2012). The importance of monitoring PAHs in processed foods using vegetable oils was also reported (Alomirah, et al., 2010). PAHs also enter the food chain through contaminated soil and water (IARC, 1989). Mutagenicity, carcinogenicity and epidemiological studies by the WHO International Agency for Research on Cancer (IARC) have classified benzo (*a*) pyrene (B(*a*)P), one of PAHs as a group 1 carcinogen (IARC, 1989). B(*a*)P is a potent pro-carcinogen formed through the condensation of smaller organic compounds by either pyrolysis or pyro-synthesis at a high temperature by forming stable polynuclear aromatic compounds (Rengarajan, et al., 2015). Previous studies have reported that B(*a*)P can be generated in several processed foods during heating treatment (Kazerouni et al., 2001; Li et al., 2011), and the health hazard

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✉ Kwang-Won Lee
kwangwon@korea.ac.kr

Se-Jin Kang
sjk0104@korea.kr

Sung-Yong Yang
yangssi42@naver.com

Jin-Won Lee
ljw7542@hanmail.net

¹ 212 CJ Food Safety Hall, Department of Biotechnology, College of Life Science and Biotechnology, Korea University, Anam-Dong, Sungbuk-gu, Seoul 02841, South Korea

² Present Address: Quality Evaluation Team, Samsung BIOEPIS, Incheon 21987, Republic of Korea

³ Faculty of Health and Food/Medicinal Plant, Suwon Women University, Suwon, Gyeonggi-do 16632, Republic of Korea

associated with dietary exposure to B(a)P has also been investigated (Hu et al., 1999; Uno et al., 2004).

Since the European Scientific Committee on Food (SCF) on Food had issued a document on the risks to human health of PAHs in food (Scientific Committee on Food, 2002), it concluded through an *in vivo* study that 16 PAHs showed clear evidence of genotoxicity (Domingo and Nadal, 2015). Also, European Food Safety Authority (EFSA) concluded that PAH4, including benzo (a) anthracene (B(a)A), chrysene (CHR), benzo (b) fluoranthene (B(b)F), and benzo (a) pyrene (B(a)P) have been suggested among 15 priority PAHs to be the most suitable indicators of PAHs in foods (Commission, 2006; Lee et al., 2016).

Seasoned-roasted (SR) laver is a processed food that uses various oils and undergoes a high heating process above 350 °C, and it has attracted popularity as a healthy snack (Park et al., 2016). Although the roasting process generates PAHs (Speer et al., 1990), it is difficult to find a study on the formation of PAHs in roasted laver. Kim et al. reported that PAHs were not found in commercial dried laver, which has little chance of exposure to PAHs from the environment during its short period of growth (Kim et al., 2008). Another study reported that the PAH concentration is higher with sesame oil than with perilla oil or olive oil (Shi et al., 2016). Although the specific PAHs were not identified in their study, the toxic equivalent (TEQ) level of PAHs in roasted laver is higher (1.234 ng TEQ/g) than that in fried chicken (0.788 ng TEQ/g) (Yoon et al., 2007). These authors reported that the processing method for SR laver caused the formation of the PAHs, however, the critical points contributing to the generation of PAH during manufacturing have not been explored. The occurrence of the PAHs in the roasted laver with oil was not detected in roasted laver in the absence of oil, suggesting that the primary factors inducing the formation of PAH in roasted laver is the oil itself and the heat treatment with oil.

Several studies have shown that raw materials contaminated with PAHs are the primary source of formation of PAHs in processed foods due to the transfer of PAH to the final product (Moret and Conte, 2000; Moret et al., 2000). Reducing the portion of sesame oil and perilla oil, which usually contain PAHs, in the mixed oil can significantly decrease the PAHs levels in laver roasted with oil. Also, the smoke points of sesame oil and perilla oil can contribute to the formation of PAHs in laver roasted with oil because the smoke point of oil is related to its thermal stability (Morgan, 1942). Heating oil beyond its smoke point can degrade the products into fatty acids (Chung and Choe, 2001), so oil with a higher smoke point is more suitable for cooking at high temperatures (Sarwar et al., 2016).

In the present study, SR laver products from domestic markets in Korea and samples collected from industrial

plant processing SR laver were analyzed to assess the PAH levels. In addition, the roasted laver samples were also analyzed in a model system to obtain a strategy for to reduce the PAHs by investigating the effects of the roasting temperature/time and mixing ratio of oils.

Materials and methods

Chemicals and materials

The four standards of B(a)A, CHR, B(b)FA, B(a)P, chrysene-d12 (CHR-d12), and benzo(a)pyrene-d12 (B(a)P-d12) were obtained from Supelco (Bellefonte, PA). The standard mixture consisted of four PAHs and the two deuterated internal standards (IS). All stock standard solutions were prepared at 1000 µg/mL in dichloromethane (DCM), placed in silanized amber vials, and stored at 4 °C for further dilution. All solvents were of HPLC grade. Used ethanol, n-hexane, and DCM were from Burdick & Jackson (Muskegon, MI), and also N,N-dimethylformamide (DMF) were from Sigma-Aldrich (St. Louis, MO). Potassium hydroxide (KOH) was purchased from Showa Denko (Tokyo, Japan), and anhydrous sodium sulfate was obtained from Yakuri Pure Chemicals (Kyoto, Japan). Sep-Pak Vac Bond Elut silica-based cartridges (1 g/6 mL) were purchased from Agilent technologies (New Castle, DE).

Sample acquisition for screening

SR laver (n = 10) and dried laver (n = 3) were obtained from several markets in the Republic of Korea. Dried laver was used as a control without any processing.

Sample collection in an industrial manufacturing process

The manufacturing process of SR laver in a manufacturing plant includes four steps: 1) dried laver is exposed to primary roasting (240 °C, 2–3 s), 2) mixed oils and NaCl are consecutively spread on the primary roasted laver, 3) the laver is transferred to secondary roasting (340–380 °C, 4–6 s), and 4) cut laver is finally packed into sterile plastic bags. To determine the critical control points where the PAH levels increased, the samples including dried laver, primary roasted laver, SR laver, salt and four oils combined to prepare the mixed oil were used. The mixing composition of brown rice oil, perilla oil, sesame oil, and chili oil was 83.5, 13.5, 2.4, and 0.6%.

Roasting with a frying pan in a model system

The effect of oil in the oil mixture

To apply the model system, dried laver and four oils were collected from a manufacturing plant. The laver was roasted on a frying pan (bottom width: 25 × 25 cm and height: 6 cm), which was heated, and the surface temperature of the pan was measured using an infrared thermometer (Fluke, Everett, WA) three times continuously. The oils were mixed according to the mixing ratio described above, and 3.5 g of mixed oil were sprayed onto each laver sheet (size 19 × 21 cm) using an oil sprayer. This oil-applied laver sheet was roasted for 10 s and was turned over three times. Laver without oil application was also roasted under the same conditions. The roasted laver sheet was measured for its moisture content immediately by using a moisture analyzer (MX50, A&D Co., Japan). This preparation was replicated at least five times (Supplementary Scheme 1).

Modulation of the mixing oils and roasting condition

Two experiments were performed to investigate the effect of the mixing oils and roasting condition on the PAH levels. In experiment 1, three different oils were prepared by changing the level of sesame oil (1.2%, 0.6%, and 0%) in the mixing formulation: mixed oils 1, 2 and 3. In this case, brown rice oil was added at 84.7%, 85.3% and 85.9%, respectively, in the formulation. In experiment 2, three different oils were prepared by changing the level of perilla oil (6.8%, 3.4% and 0%) in the mixing formulation to obtain mixed oils 4, 5 and 6. In this case, brown rice oil was added at 90.2%, 93.6% and 97%, respectively. These six mixed oils were used to roast the laver at 350 °C or 380 °C for 10 s and 15 s in the model system.

Sample preparation for GC/MS

The sample was prepared for the GC/MS according to the procedure described in the “Guideline for benzopyrene analysis” (MFDS, 2011). The extraction of PAHs in the oil matrix is based on liquid–liquid extraction by using DMF with alkaline saponification of lipids. 10 g of oil sample was weighed into a separator funnel and was shaken with 90% DMF (50 mL) and *n*-hexane (100 mL) with IS mixture (200 ng/mL). After separation, DMF (25 mL) was added to the *n*-hexane layer twice, and 1% sodium sulfate solution (100 mL) and *n*-hexane (50 mL) were added. Then, the *n*-hexane layer was moved to another separator funnel, and *n*-hexane (35 mL) was added into the remaining water layer, followed by shaking and collecting the *n*-hexane layer, with this step repeated twice. After

pooling the *n*-hexane layers, distilled water (50 mL) was mixed with the pooled *n*-hexane fraction, and the water layer was discarded as a cleaning step that was repeated. After dehydrating the cleaned *n*-hexane fraction with anhydrous sodium sulfate, the *n*-hexane fraction was thoroughly concentrated using a rotary evaporator at 40 °C. The concentrated residue was dissolved into *n*-hexane (5 mL) and this solution was loaded on a silica cartridge that was previously activated with DCM (10 mL) and *n*-hexane (20 mL). After eluting with *n*-hexane (5 mL) and *n*-hexane:DCM (3:1, v/v) (15 mL), eluent (20 mL) was collected in the test tube and was completely evaporated under a steady stream of nitrogen gas. This dried sample was dissolved with 200 µL of DCM and was filtered with 0.45 µm of PTFE membrane filter for the GC/MS analysis.

For the laver, 1 g of the sample was weighed in a round-bottomed flask, and 100 mL of 1 M KOH-ethanol spiked with IS mixture (200 ng/mL) were added. The laver sample was extracted, and oil on the surface of the laver was saponified in a water bath at 80 °C for 3 h. With rapid cooling, the extract was transferred to a separator funnel, and 50 mL each of *n*-hexane, ethanol:*n*-hexane (1:1, v/v), and distilled water were added into the separate layers. Then, 50 mL of *n*-Hexane were added to the water layers, and the *n*-hexane layer was cleaned with 50 mL of distilled water twice. Thereafter, the following steps for the GC/MS analysis were the same as for the sample preparation for oil.

Determination of PAHs using GC/MS

The PAHs were analyzed using a 7890A gas chromatography device (Agilent Technologies, Santa Clara, CA) and 5975C MS detector (Agilent technologies, Santa Clara, CA). An HP-Analytical column was 5MS Ultra Inert column (30 m × 0.25 mm × 0.25 µm) (Agilent technologies J&W Scientific, USA) and carrier gas was helium (1.2 mL/min). The initial temperature of the oven was held at 80 °C for 1 min, increased to 180 °C at a rate of 10 °C/min, and increased consecutively to 245 °C at a rate of 4 °C/min, increased to 270 °C at a rate of 5 °C/min, and maintained at this final temperature during 10 min. The injection volume was 1 µL equipped with splitless injector, and the temperature was set to 310 °C. The mass detector was operated in the selective ion monitoring (SIM) mode, and the mass spectrometry of compounds was acquired by electron ionization at 70 eV. PAH4 and IS were analyzed based on their quantitative ions and retention times (Supplementary Table 1).

Validation of the analytical method

The PAH measurements for the oil and laver matrices were validated according to the linearity, limit of detection (LOD), limit of quantification (LOQ), recovery, and precision, all of which were carried out using the single-laboratory validation methods of AOAC (Taverniers et al., 2004). The calibration curves for PAH4 were assessed at six concentrations in the range from 1 to 100 ng/g using blank sample (Supplementary Figures 1&2). The LOD and LOQ were examined based on the standard deviation of the response and slope at the lowest detectable concentration of analyte. Five different concentrations of the PAH4-mixture were also added to the unpolluted samples with PAHs, and this procedure was repeated six times. The LOD and LOQ were set at three times and ten times by obtaining the SD/slope of the calibration curve, respectively. The analytical recovery was then tested to analyze the accuracy. 1 ng/g and 10 ng/g PAH4-mixture with deuterated 200 ng/g IS were individually spiked into blank samples in three replicates. The recovery of PAH4 were calculated the difference between spiked and actual standard level of the samples, and the repeatability for the single-laboratory methods was determined by assessing the relative standard deviation (RSD) via recovery tests within a day under the same conditions.

Statistical analysis

All results are expressed using the mean and standard deviation ($n = 3$) in nano grams for PAH4 per gram of sample. The significant differences in the PAH4 levels was assessed using Duncan's multiple range tests ($p < 0.05$). All statistical analyses were conducted using SAS (version 9.4) software package (SAS institute, Cary, NC, USA).

Results and discussion

Analytical method validation

The analytical measurements were evaluated for linearity, LOD, LOQ, recovery, and precision (Table 1). Correlation coefficients (r^2) above 0.99 were obtained for the linearity of the spiked blank samples over the concentration range. The LODs of PAH4 were 0.07–0.10 ng/g for the oil matrix and 0.07–0.13 ng/g for the laver matrix. The LOQs of PAH4 were 0.21–0.31 ng/g for the oil matrix and 0.21–0.39 ng/g for the laver. The mean values of the analytic recoveries for the repeatability of the PAH4 from the oil sample were range from 67.02 to 80.99% at spiked 1 ng/g and from 77.60 to 106.65% at spiked 10 ng/g. Their relative standard deviations (RSDs) were 2.65–9.25% for

1 ng/g and 0.69–4.95% for 10 ng/g. In the case of laver, the mean values of the recoveries were range from 87.35 to 110.90% and from 86.35 to 115.14% at spiking levels of 1 ng/g and 10 ng/g, respectively. The RSDs ranged from 1.69 to 6.93% for 1 ng/g and from 1.53 to 5.20% for 10 ng/g. The validation parameters were satisfied with the recommendation of the AOAC (Taverniers et al., 2004).

PAH concentrations of seasoned-roasted laver and dried laver

SR laver ($n = 10$) and dried laver ($n = 3$) were purchased from commercial markets in Korea (Table 2a). The sum of the concentrations of PAH4 in SR laver was 1.88–4.85 ng/g, except for one sample which none was detected. The level of CHR was from 1.73 to 4.37 ng/g in 7 of 10 samples, and all dried laver samples did not contain PAH4. The mean value of the PAHs in the roasted laver with oil was 2.58 ng/g, and PAHs were not detected in roasted laver in the absence of oil, suggesting that the primary factors inducing the formation of PAH in roasted laver is the oil itself and the heat treatment with oil. On the other hand, as shown in Table 2b, the levels of PAH4 in the SR laver were different according to the type of oil that was applied (Table 2b). Laver with sesame oil of brand A contained a higher PAH4 content (4.85 ng/g) than that with perilla oil (2.21 ng/g), and PAH4 were not detected in the one with olive oil. Brand B samples also showed a similar tendency, with which the mean level for laver with sesame oil was higher (4.72 ng/g) than that for perilla oil (3.14 ng/g).

Factor analysis of PAH formation of SR laver in the processing lines

To investigate the points at which the PAH formation increases in the processing lines, all materials were collected from the line (Table 3). The factory used mixed oil composed of brown oil, perilla oil, sesame oil, and chili oil with the following formulation: 83.5%, 13.5%, 2.4%, and 0.6%. First, PAH4 were not found in brown rice oil, but were found in perilla oil (0–7.19 ng/g), sesame oil (0–13.89 ng/g), and chili oil (0–2.98 ng/g). The mean concentration of PAH4 in the mixed oil, which is sprayed on the surface of the primary roasted laver, was 1.90 ng/g. Based on the formulation of the mixed oil, the level of PAH4 calculated in mixed oil was 1.59 ng/g, accounting for 83.7% of the actual level in mixed oil. No PAH4 were detected in the salt added on the laver for seasoning purpose. In addition, PAH4 were not detected in dried laver and primary roasted laver, and the mean concentration of PAH4 in roasted laver processed with secondary roasting after spraying mixed oil and salts was 1.76 ng/g. Considering that the amount of mixed oil that was sprayed was

Table 1 Validation data for oil and laver matrices

Matrix	PAH4	Linear equation ($y = ax + b$)	Correlation coefficient (R^2)	LOD (ng/g)	LOQ (ng/g)	Low spike (1 ng/g)		High spike (10 ng/g)	
						Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Oil	B(a)A	$y = 0.006x + 0.0126$	0.9956	0.07	0.21	73.04 ± 1.94	2.65	104.80 ± 5.19	4.95
	CHR	$y = 0.0066x + 0.0275$	0.9990	0.09	0.28	67.02 ± 6.20	9.25	104.05 ± 1.29	1.24
	B(b)FA	$y = 0.0048x + 0.0231$	0.9993	0.10	0.31	78.44 ± 2.87	3.66	106.65 ± 1.65	1.55
	B(a)P	$y = 0.0049x - 0.01$	0.9965	0.10	0.31	80.99 ± 3.19	3.93	77.60 ± 0.54	0.69
Laver	B(a)A	$y = 0.0063x + 0.0069$	0.9972	0.08	0.24	105.70 ± 4.70	4.44	86.35 ± 1.32	1.53
	CHR	$y = 0.006x + 0.0151$	0.9981	0.07	0.21	110.90 ± 2.17	1.96	115.14 ± 2.59	2.25
	B(b)FA	$y = 0.005x + 0.0176$	0.9973	0.10	0.30	100.84 ± 1.70	1.69	105.56 ± 3.16	3.00
	B(a)P	$y = 0.005x - 0.0038$	0.9994	0.13	0.39	87.35 ± 6.05	6.93	113.83 ± 5.91	5.20

PAH4 polycyclic aromatic hydrocarbons (PAH4), including benzo (a) anthracene (B(a)A), chrysene (CHR), benzo (b) fluoranthene (B(b)F), and benzo (a) pyrene (B(a)P); LOD limit of detection; LOQ limit of quantification; RSD relative standard deviation for intra-day precisions of the PAH4

Table 2 (A) Primary screening of the concentration of PAH4 in seasoned-roasted (SR) laver (n = 10) and dried laver (n = 3) and (B) concentrations of PAH4 in SR laver according to different types of seasoning oils (unit: ng/g)

	#	B(a)A	CHR	B(b)FA	B(a)P	Total
(A)						
SR lavers	1	Tr	2.99 ± 1.22	0.40 ± 0.25	Tr	3.38 ± 0.97
	2	0.48 ± 0.16	4.37 ± 0.00	Tr	Tr	4.85 ± 0.16
	3	Tr	2.21 ± 0.05	N.D.	N.D.	2.21 ± 0.05
	4	N.D.	N.D.	N.D.	Tr	N.D.
	5	N.D.	N.D.	N.D.	3.11 ± 2.19	3.11 ± 2.19
	6	N.D.	N.D.	N.D.	1.88 ± 1.33	1.88 ± 1.33
	7	N.D.	3.14 ± 0.33	N.D.	N.D.	3.14 ± 0.33
	8	0.69 ± 0.09	1.98 ± 0.14	Tr	N.D.	2.62 ± 0.23
	9	0.52 ± 0.34	2.09 ± 0.14	Tr	N.D.	2.62 ± 0.18
	10	0.54 ± 0.24	1.73 ± 0.11	N.D.	N.D.	2.39 ± 0.05
Dried lavers	1	N.D.	N.D.	N.D.	N.D.	N.D.
	2	N.D.	N.D.	N.D.	N.D.	N.D.
	3	N.D.	N.D.	N.D.	N.D.	N.D.
Raw materials		Brand A			Brand B	
		Sesame oil	Perilla oil	Olive oil	Sesame oil	Perilla oil
(B)						
Dried laver (%)		44.5	44.5	44.5	54.1	54.1
Oil (%)		5.2	10.5	5.2	9.0	6.6
PAH4 (ng/g)		4.85 ± 0.16^a	2.21 ± 0.05^b	N.D.	4.72 ± 1.08^a	3.14 ± 0.33^{ab}

The values are expressed as mean $\pm$ SD (n = 3). Different letters indicate significant differences at $p < 0.05$ using Duncan's multiple range test

Tr trace (LOD $< x < LOQ$); N.D. not detected ($x < LOD$)

0.53 g per 1 g of laver, 1.76 ng of PAHs/g roasted laver with oil can be calculated to have been derived from mixed oil, accounting for 1.01 ng of the PAHs (57%), and from the roasting process, accounting for 0.75 ng of the PAHs (43%).

Effect of the mixed oil and roasting process on PAH4 levels in the roasted laver

We then configured a model system to investigate the effect of oils on the production of PAH4 in roasted laver

Table 3 Detection of PAHs during different stages of the SR laver manufacturing process: raw materials, primary roasting and secondary roasting (final products) (unit: ng/g)

	Dried laver	Primary roasting	Mixed oil	Brown rice oil (83.5%)	Perilla oil (13.5%)	Sesame oil (2.4%)	Chili oil (0.6%)	Salt	Secondary Roasting with oil
B(a)A	N.D.	N.D.	N.D.	N.D.	N.D.	0.50 ± 0.04	1.14 ± 0.24	N.D.	N.D.
CHR	N.D.	N.D.	1.15 ± 0.03	N.D.	N.D.	3.69 ± 0.21	N.D.	N.D.	1.76 ± 0.31
B(b)FA	N.D.	N.D.	0.76 ± 0.01	N.D.	7.19 ± 1.68	1.90 ± 0.21	1.84 ± 0.39	N.D.	N.D.
B(a)P	N.D.	N.D.	N.D.	N.D.	N.D.	7.81 ± 0.34	N.D.	N.D.	N.D.
Total	N.D.	N.D.	1.90 ± 0.03	N.D.	7.19 ± 1.68	13.89 ± 0.48	2.98 ± 0.61	N.D.	1.76 ± 0.31

The values are expressed as mean ± SD (n = 3)

because it was not possible to alter the production lines in practice (Supplementary Scheme 1). The other batch of mixed oil and dried laver were provided from the lines, and the PAH4 level of the mixed oil that was used and of laver were 1.25 ng/g and not detected (N.D.), respectively (Table 4). PAH4 were not detected in the roasted laver at 350 °C for 10 s without mixed oil treatment, and the laver roasted with oil at 350 °C for 10 s showed the formation of PAH4, 2.58 ng/g (n = 5). In the model system, considering that the sprayed amount of mixed oil was 0.66 g per 1 g of laver, 2.58 ng of PAHs/g roasted laver with oil can be calculated to be derived from the mixed oil, accounting for 0.83 ng of the PAHs (32%), and from the roasting process, accounting for 1.75 ng of the PAHs (68%).

Effect of changing the amount of sesame oil and perilla oil in the mixed oil and roasting condition on the formation of PAHs in roasted laver

Lu et al. (2017) reported that the effect of oil and interaction between type of oil and in the cooking temperature on the formation of PAHs were significant in their model pork patties, which were formulated with 40% fat replacement by vegetable oil, including sunflower oil, olive oil or grape seed oil and cooked at 180 °C or 220 °C. In our model system, we changed the mixing portion of oil in order to decrease the formation of PAHs in roasted laver

with oil during processing. When the mixing portion of sesame oil and perilla oil in the mixed oil was reduced, the level of PAH4 significantly decreased (Fig. 1). In this experiment, 3 different mixed oils were prepared by changing the percentage of sesame oil in the mixed oil to 1.2%, 0.6%, and 0% of the mixed oil (Mixed oils 1–3). In this case, the portion of brown rice oil was changed to 84.7%, 85.3%, and 85.9% of the mixed oil, respectively (Fig. 1A). The total PAH levels in those mixed oils decreased to 2.16 ng/g, 1.83 ng/g, and 1.71 ng/g as the portion of sesame oil of the total decreased (Fig. 1B). Different letters indicate significant differences at $p < 0.05$ using Duncan's multiple range tests. Although sesame oil (13.89 ng/g) has a higher level of PAH4 than perilla oil (7.19 ng/g), portion within the mixing oil (2.4%) is less than that for perilla oil (13.5%), indicating that the contribution of perilla oil in reducing the PAH levels in mixed oil is greater than that of sesame oil. We also, prepared the other sets of mixed oils by changing the percentage of perilla oil in the mixed oil to 6.8%, 3.4% and 0% (Mixed oils 4–6). The portion of brown rice oil changed to 90.2%, 93.6% and 97%, respectively (Fig. 1A), and the total PAH level in those mixed oils decreased to 1.70 ng/g, 1.56 ng/g, and 1.15 ng/g as the portion of perilla oil decreased (Fig. 1B). Different letters indicate significant differences at $p < 0.05$ using Duncan's multiple range test.

Table 4 Comparison between the dried laver, roasted laver and SR lavers (unit: ng/g)

	Mixed oil	Dried laver	Roasted laver without oil	Roasted laver with oil
B(a)A	0.43 ± 0.00	N.D.	N.D.	1.57 ± 0.15
CHR	0.28 ± 0.04	N.D.	N.D.	0.36 ± 0.15
B(b)FA	0.54 ± 0.06	N.D.	N.D.	0.64 ± 0.06
B(a)P	ND	N.D.	N.D.	N.D.
Total	1.25 ± 0.10	N.D.	N.D.	2.58 ± 0.18

The dried laver was a raw material, roasted laver without oil was treated at 350 °C for 10 s, and SR laver with oil was treated at 350 °C for 10 s. The roasting experiments were replicated 3 times. The values are expressed as mean ± SD (n = 3)

Fig. 1 Mixing ratio (A) and concentrations (B) of PAH4 in mixed oils according to different ratio of sesame oil and perilla oil. The values represent the mean $\pm$ SD of three independent experiments performed in triplicate. Difference letters indicate significant differences at $p < 0.05$ using Duncan's multiple range test

The formation of PAHs is increased during industrial processing due to pyrolysis of organic matter in food (Dost and İdeli, 2012), and the highest level of PAHs has been found to be from the pyrolysis of oil (Bartle, 1991). The direct contact of oils with intense heat can generate PAHs that adhere to the food surfaces (Jägerstad and Skog, 2005). Therefore, when laver with oil is roasted by passing through a compartment generating heat over 350 °C, PAHs are possibly generated. We investigated the reduction of the PAH4 level in roasted laver using these 3 mixed oils, which have different percentage of sesame oil in the mixed oil to 1.2%, 0.6%, and 0% of the mixed oil (Mixed oils 1–3), by changing the roasting temperature and time in the model system (Fig. 2A). The level of PAH4 in laver at 350 °C for 10 s only significantly ($p < 0.05$) decreased from 2.71 ng/g with mixed oil 1–1.78 ng/g with mixed oil 3. On the other hand, laver roasted at 350 °C for 15 s or at

380 °C for 10 s or 15 s did not show a significant decrease in the PAH level. This result indicates that the laver roasted at 350 °C for 10 s with mixed oil 3, lacking sesame oil in mixed oil, had the lowest PAH level ($p < 0.05$), with a 31% reduction in the PAH level at the same roasting temperature and time. Since the mixed oil was sprayed at 0.66 g per 1 g of laver, 1.78 ng/g of PAH4 in laver roasted with mixed oil 3 at 350 °C for 10 s can be calculated to have been derived from the mixed oil (1.12 ng/g PAH4), accounting for 63% of the total level, and roasting process, (0.66 ng/g PAH4) accounting for 37%.

We also investigated the reduction in the PAH level in laver using these 3 mixed oils, which have different percentage of perilla oil in the mixed oil to 6.8%, 3.4%, and 0% of the mixed oil (Mixed oils 4–6), by changing the roasting temperature and time in the model system (Fig. 2B). Roasting the mixed oils at 350 °C for 10 s and

Fig. 2 Laver roasted with different mixing ratio of (A) sesame oil and (B) perilla oil at different roasting temperatures and times. Values represented mean $\pm$ SD of three independent experiments performed

15 s resulted in PAH4 levels of 2.47 and 2.73 ng/g, respectively, for mixed oil 4, 2.03 and 2.30 ng/g, respectively, for mixed oil 5, and 1.69 and 1.89 ng/g, respectively, for mixed oil 6. Roasting laver with mixed oil 6 at 350 °C for 10 s resulted in the lowest PAH level in which perilla oil is missing from the mixing oil. The PAH level decreased from 2.47 ng/g to 1.69 ng/g, which is a reduction of 32% at the same roasting temperature and time using the model system. The 1.69 ng PAH/g roasted laver with mixed oil 6 at 350 °C for 10 s can be calculated to have been derived from the mixed oil, accounting for 0.76 ng PAHs (45%), and from the roasting process, accounting for 0.93 ng PAHs (55%). Heating oil beyond its smoke point can degrade the products into fatty acids (Chung and Choe, 2001), so oil with a higher smoke point is more suitable for cooking at high temperatures (Sarwar et al., 2016). The smoke points of sesame oil, perilla oil, and brown rice oil are 165 °C (Kim and Choe, 2005), 161 °C (Detwiler and Markley, 1940), and 257 °C (Jennings and Akoh, 2009), respectively. Thus, a lower portion of perilla oil and sesame oil and greater portion of brown rice oil in the mixed oil resulted in a reduction in the amount of PAHs in laver roasted with oil.

In conclusion, the most important factor contributing the increase of PAHs in SR laver is the composition of mixed oil. It is highly recommended for oil containing the least amount of PAHs to be used during manufacturing of SR laver. The second factor is the heat treatment during the roasting process. The roasting temperature and time should not be greater than 350 °C for 10 s to maintain the minimal formation of PAHs during roasting.

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Compliance with ethical standards

Conflict of interest None of the authors of this study has any financial interest or conflict with industries or parties.

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