# Plasmalogen fingerprint alteration and content reduction in beef during boiling, roasting, and frying

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**Abstract**

Plasmalogens are dietary phospholipids with beneficial health effects. In this work, plasmalogen characteristics and changes in beef during boiling, frying, and roasting were comprehensively investigated by liquid-chromatography-mass spectrometry. The alteration of plasmalogen fingerprint during cooking processes was found by untargeted omics approach, in which time of boiling, temperature of roasting, and meat core/surface of frying were responsible for the observed variations. Moreover, the targeted determination of representative plasmalogen species showed significant loss with a temperature- and time-dependent manner in roasting and frying. And frying even showed an extra loss in meat surface compared with core. Furthermore, an artificial neural network-based predictive model elucidated the dynamics of plasmalogen species during cooking. Finally, batter-coating pretreatment was performed to show its protection against plasmalogens loss during frying. These results might provide a potential strategy to better control and improve the quality of functional foodstuffs during cooking processes.

**Keywords**

Plasmalogen; cooking process; food fingerprints; LC-MS quantitation; artificial neural network modeling; batter-coating.

## 1. Introduction

Plasmalogen is a class of glycerophospholipids containing a fatty alcohol with a vinyl-ether bond at the *sn*-1 position, a commonly unsaturated fatty acyl at the *sn*-2 position, and a phosphate headgroup, typically choline (PlsCho) or ethanolamine (PlsEtn) (Braverman & Moser, 2012). Serving as structural components of cell membrane, plasmalogens are widely distributed in mammalian cells and exert beneficial bioactivities, such as prevention of oxidative damage (Luoma et al., 2015; Wu et al., 2019), maintenance of mitochondrial function (Bozelli, Lu, Atilla-Gokcumen, & Epand, 2020), inhibition of inflammation (Sejimo, Hossain, & Akashi, 2018), reduction of atherosclerotic lesions and serum LDL-c levels (Ding et al., 2020), and suppression of neuronal apoptosis in nerve cells (Garcia et al., 2012; Yamashita et al., 2016). Therefore, plasmalogens are regarded as potential functional ingredients with health benefits.

Although the vinyl-ether bond in plasmalogen is considered sensitive to acid, there has been a report that the synthesized plasmalogen (PlsEtn p16:0/22:6) showed considerable in vitro stability in acid and oral bioavailability in mice (Fallatah et al., 2020). Animal studies with rats also demonstrated that bovine-derived plasmalogens could be absorbed in the intestine and delivered to various tissues, showing the sufficient stability in gastric acid (Nishimukai, Wakisaka, & Hara, 2003). Moreover, in clinical trials on Alzheimer's disease patients, oral administration of scallop- and chicken-derived plasmalogens improved cognitive function (Fujino et al., 2017) and attenuated memory loss (Hossain, Tajima, Kotoura, & Katafuchi, 2018), respectively. Thus, there is growing interest in research on dietary plasmalogen

62 supplementation.

63 Plasmalogens are abundant in fresh meat (such as beef, chicken, pork, and sheep), fish (such
64 as salmon, anchovy, hake, and trout), and shellfish (such as oyster, scallop, clam, and mussel)
65 (Boselli, Pacetti, Lucci, & Frega, 2012; Getz, Bartley, Lurie, & Notton, 1968; Hanuš, Levitsky,
66 Shkrob, & Dembitsky, 2009; Yamashita et al., 2016). Most of the aforementioned meats are
67 thoroughly cooked to ensure microbiological safety and enhance flavor. Popular home cooking
68 techniques include, taking beef as an example, roasting, frying, grilling, simmering, and others.
69 It is known that cooking alters the composition and content of the nutrients in the food, which
70 may reduce protein, vitamins, minerals, as well as oxidize lipids (Espe, Nortvedt, Lie, &
71 Hafsteinsson, 2002; Zeng, 2013). However, even though plasmalogens are known susceptible
72 to oxidation and degradation due to the vinyl-ether bond (Engelmann, 2004), there has yet not
73 been investigation in the aspect of nutrition loss. The questions, such as how plasmalogens
74 change during cooking, whether different cooking methods with various conditions affect these
75 influences, and how to possibly minimize the loss of plasmalogens, remain to be answered.

76 Most of earlier studies focused on the total plasmalogens (Garcia et al., 2012; Yamashita et
77 al., 2016), rather than the individual molecular species. Previously, we have confirmed that
78 plasmalogen species differ in terms of their antioxidative and cytoprotective effects (Wu et al.,
79 2019), indicating that the molecular composition including headgroups and fatty chains of
80 dietary plasmalogens might affect their beneficial effects. Therefore, not only the vinyl-ether
81 linkage but also the headgroups and the fatty chains are worthy investigating. Besides, the
82 concept of “food fingerprints” was recently proposed to represent the characteristics or specific

conditions of food for improved quality control (Medina, Pereira, Silva, Perestrelo, & Câmara, 2019). Thus, a "plasmalogen-omics" approach could identify alterations in plasmalogen fingerprint during cooking, especially, to figure out the possible associations between the changes and distinctive plasmalogen species, which will help us to better understand and improve the daily plasmalogens intake.

Hence, the present work aimed to investigate the changes on quantity and quality of dietary plasmalogens during daily cooking processes. Lean beef was chosen as the representative meat type, while boiling, roasting, and frying were selected as typical cooking methods. Originally, the global plasmalogen species were identified and profiled to explore the plasmalogen fingerprint by high-performance liquid chromatography coupled to high-resolution tandem mass spectrometry (HPLC-HRMS/MS) combined with chemometric tools. Moreover, the LC-MS-based quantitative method was established to monitor the representative species, and the artificial neural network (ANN)-based estimation was performed to reveal the detailed losses during cooking. In addition, the variations among plasmalogen species under different cooking conditions were compared. Finally, the batter-coating treatment as an improved cooking method for plasmalogen preservation during frying was evaluated and discussed.

## 2. Materials and Methods

### 2.1. Chemicals

Spectral grade solvents and reagents for lipid extraction and LC-MS measurements were purchased from Sigma-Aldrich (St. Louis, MO, USA). The plasmalogen standards for quantitation, namely PlsCho p16:0/18:1 (PlsCho-oleic), PlsCho p16:0/18:2 (PlsCho-linoleic), PlsCho p16:0/20:5 (PlsCho-EPA), PlsEtn p16:0/18:1 (PlsEtn-oleic), PlsEtn p16:0/18:2 (PlsEtn-linoleic), PlsEtn p16:0/20:5 (PlsEtn-EPA), as well as the internal standards (IS) PlsCho p16:0/17:0 (PlsCho-17:0) and PlsEtn p16:0/17:0 (PlsEtn-17:0), were synthesized in our laboratory (Hui, Chiba, & Kurosawa, 2011). Other chemicals were of analytical grade and purchased from Kanto Chemical Co., Inc. (Tokyo, Japan) unless otherwise specified.

### 2.2. Sampling and cooking

Fresh lean beef was purchased from a local supermarket. The meat samples were cut into uniform pieces (approximately 3 cm × 3 cm × 1 cm, each weighing approximately 10 g) and processed by boiling, roasting, or frying. For boiling, the beef samples were cooked in a pot with boiling water at 97 ºC for 0.5, 1, 2, 5, 10, or 30 min. Roasting was conducted in an oven preheated to 180 ºC, 200 ºC, or 220 ºC for 3, 5, 10, 20, or 30 min. While frying was performed by using a deep fryer at 160 ºC, 180 ºC, or 200 ºC for 0.5, 1, 2, 3, 4, or 5 min. The commercial fresh rapeseed oil (Nisshin OilliO Group, Ltd., Tokyo, Japan) was used for frying, which contained 51.8% of oleic acid, 30.8% of linoleic acid, 8.3% of linolenic acid, 5.9% of palmitic acid, and 1.4% of stearic acid as the major components. For the batter-coating, the commercial wheat flour (100 g) was mixed with water (150 mL) to make the batter, and the meat pieces

were dipped into the batter to coat it on all sides immediately before frying.

**2.3. Lipid extraction**

Each piece of cooked beef meat sample was separated into core and surface parts on the basis of the clear texture differences (shown in **Figure S1.1**). Total lipid was extracted according to Folch's method (Folch, Lees, & Sloane Stanley, 1957) with some modifications. Briefly, approximately 50 mg of ground freeze-dried sample was weighed in a 1.5 mL Eppendorf tube and added to 600 uL of ice-cold chloroform/methanol 2:1 (v/v, with 0.002% butylated hydroxytoluene) together with the IS (PlsCho-17:0 and PlsEtn-17:0, 0.2 nmol for each), followed by the Folch partition and organic layer recovery. After extraction twice, the total lipid extracts were dried under vacuum. Then, the lipid sample was dissolved in methanol, filtered to remove any residue, and stored at −80 °C until analyses. All the samples were prepared within 1 hour to avoid lipid auto-oxidation and degradation.

**2.4. Plasmalogen fingerprinting by untargeted lipidomic approach**

A Prominence HPLC (Shimadzu Corp., Kyoto, Japan) coupled to an LTQ Orbitrap mass spectrometer (Thermo-Fisher Scientific Inc., Waltham, MA, USA) was utilized to profile the plasmalogen in the beef samples. A Luna C18(2) column (2 × 150 mm, 3 μm, Phenomenex, Ltd, Torrance, CA, USA) was equipped for chromatographic separation, with the oven temperature 40 °C and flow rate 200 μL/min. The mobile phase consisted of 10 mM aqueous ammonium acetate (A), isopropanol (B), and methanol (C), and the following gradient elution was applied: initial 0.5 min, 15% A, 10% B, 75% C; 0.5–3.0 min, 10% A, 40% B, 50% C; 3.0–15.0 min, 5% A, 80% B, 15% C; 15.0–20.0 min, 2% A, 85% B, 13% C; 20.0–20.5 min

returned to initial gradient and kept to 23 min for re-equilibration. High-resolution MS in ESI-negative mode was utilized for detection under these conditions: Spray voltage, 3 kV; capillary temperature, 330 °C; sheath gas (nitrogen) pressure, 50 psi, auxiliary gas (nitrogen) pressure, 5 psi; resolving power, 60,000; scan speed, 2 Hz; scan range, *m/z* 650–900. The MS/MS fragmentation was acquired by collision induced dissociation (CID) and run in data-dependent mode, with the collision energy set at 35 V. To minimize the running time-induced variation, the data acquisition sequence was randomized. Meanwhile, to monitor the performance and stability of the method, the quality control (QC) samples, which consisted of a representative average of samples pooled from different final sample extracts, were spiked at the beginning, end, and randomly throughout the sequence (Sangster, Major, Plumb, Wilson, & Wilson, 2006; Theodoridis, Gika, & Wilson, 2008).

### 2.5. Quantitation of plasmalogen species

The absolute amounts of plasmalogens were also investigated for all the samples. Six representative plasmalogens were simultaneous determined by LC-MS/MS method using a TSQ Quantum Access MAX Triple Quadrupole mass spectrometer (Thermo-Fisher Scientific Inc.), of which the major instrumental parameters were according to our previously reported protocol (Wu et al., 2019) as described in **Supplementary Material 3**. The optimized multiple reaction monitoring (MRM) conditions for all analytes are shown in **Table S3.1**. Standard solutions with a series of diluted concentrations were prepared to draw calibration curves, in which the area ratio of each plasmalogen to its corresponded IS (x axis) and the plasmalogen amount (y axis) was calculated. The limit of detection (LOD) and the limit of quantitation

(LOQ) were determined by diluting the standards until reaching the signal-to-noise ratio (S/N) equaled to 3 and 10, respectively. Precision was investigated by repeated inter-day and intra-day analysis and expressed as the coefficient of variation (CV) of eight replicated measurements. Recovery, which reflected the accuracy of this method, was evaluated by adding equivalent standards prior to determination (n = 8) and comparing the measured quantity against the known amount of standard as follows:

$$\text{Recovery} = \frac{\text{Amount found} - \text{Amount original}}{\text{Amount spiked}} \times 100\%$$

**2.6. Data processing and statistical analyses**

The raw data generated by MS was processed by the workstation Xcalibur 2.1 (Thermo-Fisher Scientific Inc.). All experiments were conducted in quadruplicate (four pieces), and the results were expressed as the means ± standard deviations (SD). Two-tailed Student's *t* test and two-way ANOVA (using the Tukey *post hoc* test) were calculated using GraphPad Prism 8 (La Jolla, CA, USA), of which the differences were considered significant at $P < 0.05$. Principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were performed to reveal and distinguish the plasmalogen characteristics among various cooking methods, conditions, and meat sample parts using R software 3.6.1 (R Development Core Team, 2008). The ANN model was established to predict plasmalogen loss using time and temperature as the input layer nodes and three sigmoid transfer functions (TanH) to construct the hidden and the output layers, which was run by JMP® 14 pro (SAS Institute Inc., Cary, NC,

184 USA) according to previously reported procedure (Behkami, Zain, Gholami, & Khir, 2019).

185

186 **3. Results and Discussion**

187 **3.1. Plasmalogen fingerprint variation under different cooking conditions**

188 **3.1.1. LC/MS analysis of plasmalogen profile in beef samples**

189 The boiled, roasted, and fried beef meat samples (typical appearances are shown in **Figure S1.2**)
190 were firstly analyzed by non-targeted LC-MS. The typical total ion chromatograms of beef
191 meats are shown in **Figure S2.1**, and the fatty chain composition of these molecular species
192 was elucidated by MS/MS fragmentation characteristics of different phosphate headgroups.
193 For PlsCho, taking 34:1 as an example, the major fragments of *m/z* 728, 464, and 281 were
194 assigned as the loss of acetate adduct together with methyl group from precursor ion, the further
195 loss of *sn*-2 acyl chain, and the *sn*-2 $RCOO^-$ ion (Khaselev & Murphy, 2000), indicating
196 p16:0/18:1 (**Figure S2.2**). While for PlsEtn, taking 34:2 as an example, the MS/MS showed
197 the major fragments of *m/z* 436, 418, and 279, which were assigned as the loss of sn-2 acyl
198 chain as ketene or acid, and the *sn*-2 $RCOO^-$ ion (Hsu & Turk, 2007), indicating p16:0/18:2
199 (**Figure S2.3**). Finally, a total of 33 plasmalogen species (12 PlsCho and 21 PlsEtn) were
200 annotated, in which 8 PlsCho and 13 PlsEtn were elucidated for their fatty chain composition
201 (**Table S2.1**).

202 **3.1.2 Alterations of plasmalogen characteristics in three cooking methods**

203 The intensities of all the identified plasmalogens as peak areas were exported and normalized
204 for multivariate statistical analysis. The unsupervised PCA using the plasmalogen species

variables was performed for all the samples of the three cooking methods (sample number: 12 for raw, 48 for boiling, 120 for roasting, and 144 for frying, 324 in total), and a biplot graph (score plot stacked with loading plot) was generated (**Figure 1A**). The first two principal components (PC) accounted for 82.5% of the total variation (PC1, 61.3%; PC2, 21.2%), adequately explaining most of variance (Lever, Krzywinski, & Altman, 2017). The QC samples, which evaluated the accuracy and robustness of the whole analytical procedure, were clustered tightly in the center of the graph, thereby confirming the reliability of the data acquisition (Lee et al., 2013). Distinctive clustering of raw, boiled, roasted, and fried samples was revealed, indicating that the plasmalogen profiles substantially differed among the three cooking methods. Specifically, the fried samples distributed on right side, the roasted samples gathered in the lower left corner, while the boiled samples grouped in the upper left corner and were near the raw samples. The order of plasmalogen fingerprint similarity in the cooked meat samples to the raw meat samples was boiling > roasting > frying. Moreover, the plasmalogen variables which orientated to positive x-axis consisted of PlsCho (such as p16:0/20:5, 38:6, 40:6) as well as PlsEtn with n-3 fatty acyls (such as p18:1/20:5, 38:7, p18:1/22:5, p18:0/22:6), with loading matrix in PC1 more than 0.95 (**Table S2.2**). Whereas the variables belonging to PlsEtn containing n-6 or n-9 fatty acyls (such as p18:0/18:1, p18:0/18:2, p16:0/20:4) were orientated towards the negative x-axis, suggesting that frying lost more of these plasmalogen species compared with other two methods. On the contrary, PlsCho and n-3-contained PlsEtn lost more in boiling and roasting than frying. Therefore, the primary variations among different cooking methods were exhibited in headgroups and fatty acyls of plasmalogens.

In order to explore the dominant factors that influence the plasmalogen characteristics during cooking, PCA was performed for boiling, roasting, and frying, individually. In boiling, as the processing time increased, the spots in score plot migrated along the negative x-axis (**Figure 1B**). Since the variables with significant positive loading in PC1 were almost PlsCho, whereas those with negative loading were PlsEtn (**Table S2.3**), our data suggested that boiling primarily affected the plasmalogen phosphate headgroup. Particularly, PlsEtn was more likely to loss during long-time boiling compared with PlsCho.

Roasting showed another pattern, in which the 180 °C-treated samples were obviously away from those treated at 200 °C and 220 °C in x axis of score plot (**Figure 1C**). Considering the positive loading matrix in PC1 mainly referred to polyunsaturated fatty acyl-contained plasmalogen species such as PlsCho p16:0/20:5 and PlsEtn p18:0/22:6) (**Table S2.3**), these results indicated that roasting influenced the plasmalogen *sn*-2 fatty acyls. Furthermore, we calculated the relative changes of each fatty acyl in plasmalogens, and found that the *sn*-2 fatty acyls with more double bonds (e.g. 20:5, 22:5, 22:6) lost more than those with less double bonds (e.g. 18:1, 18:2), indicating that the polyunsaturated fatty acyls were comparatively more sensitive to the high temperature during roasting (**Table S2.4**).

In terms of frying, neither temperature nor time markedly altered the plasmalogen profile (**Figure 1D**). However, OPLS-DA disclosed a distinct separation between the cores and the surfaces of the meat samples (**Figure 1G**), in which the most contributive variables were assembled in the corners of the S-plot (**Figure S2.4**). In surface samples, the potential chemical markers were with shorter fatty acyls, especially for PlsCho, while in core samples, all of them

247 were PlsEtn with longer and more unsaturated fatty acyls. This discrepancy could be explained
248 by the direct/indirect contact of the meat with the hot oil and the different temperature in frying.
249 Interestingly, boiling and roasting failed to show similar observation (**Figure 1E** and **F**),
250 indicating that it was oil rather than water or air that differentiated the plasmalogen
251 characteristics between core and surface of the meat samples during cooking processes.

252 **3.2. Plasmalogen loss during cooking processes**

253 **3.2.1. Validation of the quantitation method**

254 Under the optimized LC-MS/MS condition, all the targeted plasmalogen species showed
255 identical peaks within 10 min, and retention times were similar in solution of standard mixtures
256 and beef extracts (**Figure S3.1)**, indicating acceptable selectivity and specificity. The obtained
257 calibration curves exhibited excellent linearity ($R^2 > 0.999$ for all; **Figure S3.2**). The LOD of
258 these analytes ranged from 0.010 pmol (PlsEtn-EPA) to 0.023 pmol (PlsCho-oleic), while the
259 LOQ ranged from 0.038 pmol (PlsEtn-EPA) to 0.092 pmol (PlsCho-oleic), suggesting
260 sufficient sensitivity. For precision, the CV of each analyte obtained was below 10% in both
261 intra- and inter-day experiments. The average recoveries achieved for all these plasmalogens
262 ranged from 85.1% ± 5.5% (PlsEtn-EPA) to 103.0% ± 7.4% (PlsEtn-linoleic), with all the CV
263 less than 10% (**Table 1**). Thus, the developed method was considered reliable to investigate the
264 loss of plasmalogen in the following studies.

265 **3.2.2. Changes of plasmalogen content in three cooking methods**

266 By using the established quantitation method, six plasmalogen species were determined in the
267 beef samples. In the raw meat, PlsCho-linoleic was the predominant species

(1719.5 ± 133.3 nmol/g), followed by PlsCho-oleic (801.5 ± 66.2 nmol/g), whereas the PlsCho-EPA (192.1 ± 15.4 nmol/g) showed the lowest content, followed by PlsEtn-EPA (217.9 ± 15.4 nmol/g) (**Table S3.2**). As to cooked beef samples (all the contents compared with raw were listed in **Table S3.3**), during the 30 min of boiling, all these plasmalogens, as well as their total amount, showed negligible losses relative to the raw samples in both core and surface parts (**Figure S3.3**).

On the other hand, roasting caused significant change in plasmalogen content compared with the raw samples (**Figure 2A**, **B**). Time- and temperature-dependent plasmalogen reduction occurred within 30 min (**Figure 2C**−**H**). In particular, the content curves fell more drastically in the first five minutes, followed by steady decrease until the end of the roasting process. After 60 min, the final contents for all the plasmalogen species even reached undetectable (beef samples became charcoal, and no peak on MRM chromatogram). Moreover, the influences of roasting temperature depended on species, especially for the EPA-contained plasmalogen species PlsCho-EPA and PlsEtn-EPA which were more susceptible, with the remained contents in the core part decreased by 36.6% and 25.0% from 180 °C to 200 °C, respectively (**Figure 2E** and **H**), while other four species were reduced by only 16.2%−21.1% (**Figure 2C**, **D**, **F**, **G**). These results suggested the heat sensitivity difference among these plasmalogens, especially for EPA-contained species, and were consistent with the trends revealed by PCA (**Figure 1C** and **Table S2.3**). In addition, there was no such difference between the core and the surface of the meat.

In terms of frying, a similar change pattern of plasmalogens was observed, of which both

289 the total plasmalogens and all the individual species showed a time- and temperature-dependent
290 manner of loss (**Figure 3A**−**H**). However, the interesting point appeared that after sharply
291 falling within the first minute, the content curves tended to be flat until the fifth minute. Besides,
292 the temperature accelerated plasmalogen loss more than roasting, especially for PlsEtn
293 (**Figure 3F**, **G**, **H**). Furthermore, it was worth noticing that there was an obvious distinction of
294 plasmalogen loss between the core and surface parts of the meat samples, reaching to
295 26.6%−35.3% vs. 36.6%−52.9% in 160 °C, 37.5%−52.5% vs. 47.6%−60.6% in 180 °C, and
296 51.0%−62.1% vs. 61.9%−77.4% in 200 °C for 5 min as the loss ranges of the six species.
297 Especially, during the 200 °C of frying, the losses of PlsEtn species in the surface part were
298 extremely serious (71.2% ± 1.7% for PlsEtn-oleic, 77.4% ± 3.6% for PlsEtn-linoleic, and
299 75.8% ± 2.7% for PlsEtn-EPA) in comparison with those in the core part (51.0% ± 3.4%,
300 60.9% ± 1.3%, and 62.1% ± 3.6%, respectively) ($P < 0.001$ for all). Moreover, the differences
301 between the core and the surface parts were even more severe for PlsEtn (20.2% for PlsEtn-
302 oleic, 16.5% for PlsEtn-linoleic, and 13.7% for PlsEtn-EPA) than PlsCho (5.9% for PlsCho-
303 oleic, 5.8% for PlsCho-linoleic, and 6.9% for PlsCho-EPA) (**Figure 3I**). For the possible
304 explanation to these results, as Safari et al. previously reported, when the heat transfers from
305 surface to core of the food during frying, the intensive rate of water loss at first results in a fast
306 dehydration in surface and “crust” formation, forming large bubbles around the surface to slow
307 down the heat transfer rate (Safari, Salamat, & Baik, 2018), which played the role of barrier on
308 protecting the plasmalogen in the core part from heating damage. Besides, Rabeler et al. proved
309 that the temperature difference between core and surface caused different physical properties

310 (e.g. hardness) (Rabeler & Feyissa, 2018). While our study elucidated the differences in the
311 aspect of chemical component, i.e. the composition and contents of plasmalogens, which might
312 provide new insights into the changes of the substantial basis in daily cooked foods.

313 **3.3. Prediction of plasmalogen losses during roasting and frying**

314 To thoroughly investigate the plasmalogen losing behavior during cooking, ANN modeling
315 was performed to estimate the remained content of each plasmalogen species, which is
316 considered suitable for solving the non-linear, multi-modal, and poorly understood problems
317 (Agatonovic-Kustrin & Beresford, 2000; Xia, Ni, & Kokot, 2013). The models for roasting and
318 frying were established individually, in which approximately 80% of the measured data was
319 used for network training, while approximately 20% was used for validation. The fitted values
320 from the model were compared against the measured values by linear regression, of which the
321 satisfied consistency was observed in both the training data (**Figure S4.1**) and the validation
322 data (**Figure S4.2**), with slope close to 1 and R square values higher than 0.94 for all, indicating
323 the satisfied robustness of established model (Parker et al., 2012).

324 The three-dimensional surface charts of the remained plasmalogen species versus cooking
325 time and temperature are illustrated in **Figure 4**. The contour lines showed the suggested
326 cooking conditions which would remain the desired plasmalogen content, taking PlsEtn-
327 linoleic as an example, remaining more than 80% of plasmalogen required less than 1 min of
328 roasting or 0.5 min of frying, which seemed quite risky for food safety. In order to remain more
329 than 60%, a 180 °C roasting for no more than 20 min could be possible, but with temperature
330 increasing to 220 °C, the permitted time shortened to 5 min. While in frying, the low

temperature from 160 °C to 180 °C could support as long as 5 min. However, if the frying temperature continued increasing, the tolerable time sharply shortened to 2 min for 190 °C or 1 min for 200 °C, indicating that 180 °C might be a key frying temperature. To be worse, a combination of 190−200 °C for 5−1 min even resulted in less than 50% of PlsEtn-linoleic remained. Similar trends were also found in other conditions and even for other species, suggesting the regular pattern that a possibly lower-temperature and shorter-time cooking could contribute to a more beneficial meat cooking in the aspect of plasmalogen content.

It should be noted that, there are more factors affecting the plasmalogen loss. As shown in our results, the intact profile of plasmalogen species, including their fatty acyl composition, affected the plasmalogen loss during roasting. Besides, there have been reports on the lipid composition variation on fat content of meat (Davenel, Riaublanc, Marchal, & Gandemer, 1999). Therefore, for different meats, the polyunsaturated fatty acyl-enriched species (e.g. shrimp) are supposed to loss more plasmalogens than the saturated fatty acyl-enriched ones (e.g. pork); while within the same origin, the lean meat might loss more plasmalogens than the fatty meat. Other properties such as protein and moisture contents, might also influence the plasmalogen loss. In addition, though we conducted the three representative approaches in the present work, there are numerous other methods to considered in the future work. Nevertheless, by fixing the possible variables, our study provided a potential strategy for meat dish makers and related food product suppliers to better estimate, predict, and even control functional food ingredients during processing.

### 3.4. Beneficial effect of batter-coating on attenuate plasmalogen losing during frying

352 The fact that significant difference existed between the core and the surface of the meat in
353 frying but not in roasting attracted our interests. Since the surface part served as the protective
354 barrier for the core part against heat transfer and oil contact during frying, it is worth verifying
355 whether an extra barrier of the meat during frying could protect the original surface part. The
356 coated frying for foodstuffs is known and appreciated worldwide, which preserves and
357 enhances food quality during frying (Carvalho & Ruiz-Carrascal, 2018). Therefore, we
358 conducted the batter-coating treatment and compared the differences between directly frying
359 and batter-coated frying on plasmalogen content. The remained content (compared with raw)
360 of each plasmalogen species is listed in **Table S5.1** and shown in **Figure 5**. **Figure 5A** shows
361 the appearance of meat samples with and without coating (at the typical condition, 180 °C,
362 2 min), in which there was obviously less scorching on the surface in the batter-coated meat
363 than the directly fried one. Subsequently, the total amount of the six plasmalogen species was
364 compared in the core and the surface. In the core part, there was no plasmalogen amount change
365 between batter-coated and directly fried meat ($P > 0.05$ for all the conditions except for 200 °C,
366 5min) (**Figure 5B**). Whereas in the surface, the remained plasmalogen amount were
367 significantly higher in batter-coated frying ($P < 0.05$ for all the conditions) (**Figure 5C**).
368 Furthermore, the increase of the remained amount for the measured plasmalogens varied
369 among different frying conditions. Under 160−200 °C from 0.5−2 min, the plasmalogens
370 increased by 12.0%−13.9%, while under 200 °C for 5 min only 6.2% was protected. These
371 results indicated that the plasmalogens were indeed protected by the extra batter-coating, but
372 this protective effect could be attenuated under the extreme frying condition, i.e. excessively

high temperature and long time. Therefore, in the aspect of plasmalogen intake, the coating process would be applied as a beneficial way in frying, but whether direct or coated frying was chosen, it is recommended to lower oil temperature and shorten frying time.

For a healthy cooking in daily dishes, there have been various means to improve food quality in cooking processes, e.g. polyphenol from bamboo leave could prevent the formation of aldehydes during frying clam (Liu et al., 2020), and beer marinades could reduce polycyclic aromatic hydrocarbons produced in grilled pork (Viegas, Yebra-Pimentel, Martínez-Carballo, Simal-Gandara, & Ferreira, 2014). Our current experiment, focusing on dietary plasmalogen intake, provided a potential strategy for healthy diet.

## 4. Conclusions

This work revealed the changes of plasmalogen composition and content during boiling, roasting, and frying. Firstly, the untargeted omics approach disclosed that all these cooking methods influenced plasmalogen fingerprint, in which boiling time and roasting temperature played critical roles on the plasmalogen profile variation, while in frying the core and surface of the meat showed characteristic differences. Then, based on the developed quantitation method, the content losses of the plasmalogens during cooking process were determined. A temperature- and time-dependent manner of plasmalogen reduction was observed in roasting and frying, but not in boiling. Frying even showed an extra loss of plasmalogens in surface of the meat. Moreover, the prediction model was established to elucidate the dynamic remaining of each plasmalogen species. Furthermore, a batter-coating pretreatment of the meat was

proposed and proved to effectively protect plasmalogen from losing during frying. These results not only contributed a further understanding toward nutritional component loss in daily cooking, which focused on the new viewpoint of bioactive phospholipid intakes, but also provided a potential strategy to better control and even improve the quality of functional foodstuffs during cooking processes.

**Acknowledgments**

This work was supported by (1) Japan Society for the Promotion of Science (JSPS) KAKENHI Grant No. 19K16531 to Zhen Chen, and (2) Hokkaido University "Research Fund Program for Early Career Scientists" to Zhen Chen.

**Abbreviations**

ANN, artificial neural network; IS, internal standards; LOD, limitation of detection; LOQ, limit of quantitation; MRM, multiple reaction monitoring; OPLS-DA; orthogonal partial least squares discriminant analysis; PC, principal component; PCA, principal component analysis; PlsCho, plasmalogen choline; PlsEtn, plasmalogen ethanolamine; QC, quality control

**Conflict of Interest**

The authors declare no competing financial interests.

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547 **Figure Captions**

548

549 **Figure 1**. Multivariate statistics of the beef meat samples: Biplot of the samples under different
550 cooking processes (♦, Raw; ●, Boiling; ■, Roasting; ▲, Frying) obtained by the PCA of
551 plasmalogen variables, together with QCs (×) (**A**); PCA score plots of plasmalogen profiles
552 grouped by boiling time (**B**), roasting temperature (**C**), and frying temperature (**D**); OPLS-DA
553 score plots for discriminating the core and the surface parts of meat samples under boiling (**E**),
554 roasting (**F**), and frying (**G**).

555

556 **Figure 2**. Changes of the plasmalogens remained in meat samples during 30 min of roasting.
557 (**A**) The total plasmalogen amount in core, (**B**) The total plasmalogen amount in surface;
558 columns with different superscripted letters represent significant differences at the 0.05
559 probability level. (**C**) PlsCho-oleic, (**D**) PlsCho-linoleic, (**E**) PlsCho-EPA, (**F**) PlsEtn-oleic, (**G**)
560 PlsEtn-linoleic, (**H**) PlsEtn-EPA; the colors of blue, green, and orange indicate the roasting
561 temperatures of 180 °C, 200 °C, and 220 °C, respectively; the solid and the dotted lines
562 represent the core and the surface parts of the meat within the same roasting condition,
563 respectively.

564

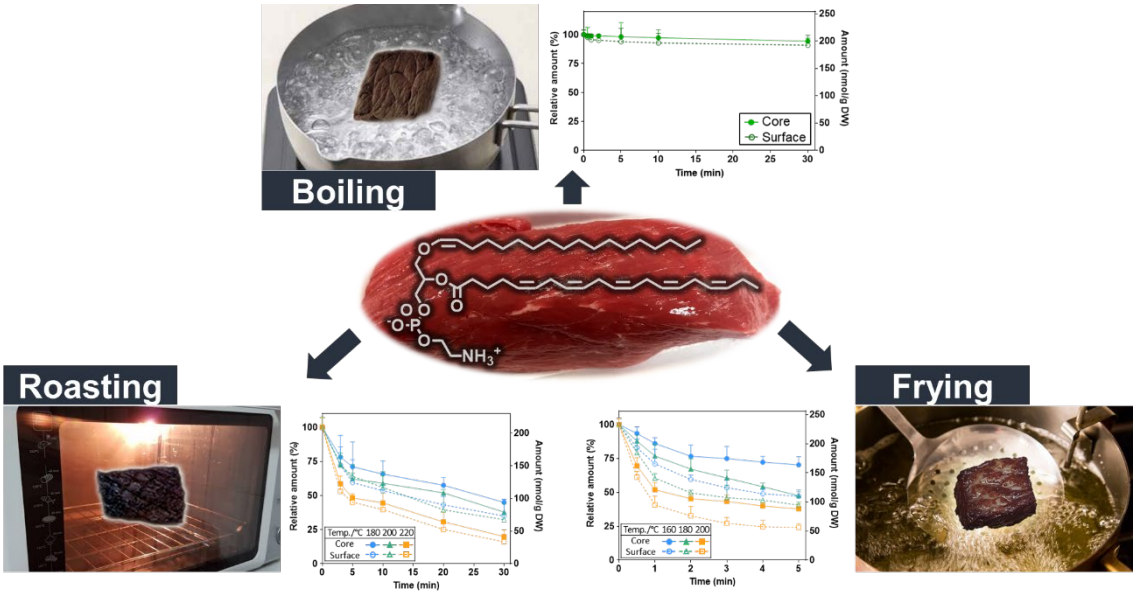
565 **Figure 3**. (**A**–**B**) Changes of total plasmalogens remained in core and surface of the meat
566 samples, respectively, during 5 min of frying; columns with different superscripted letters
567 represent significant differences at the 0.05 probability level. (**C**–**H**) Changes of the

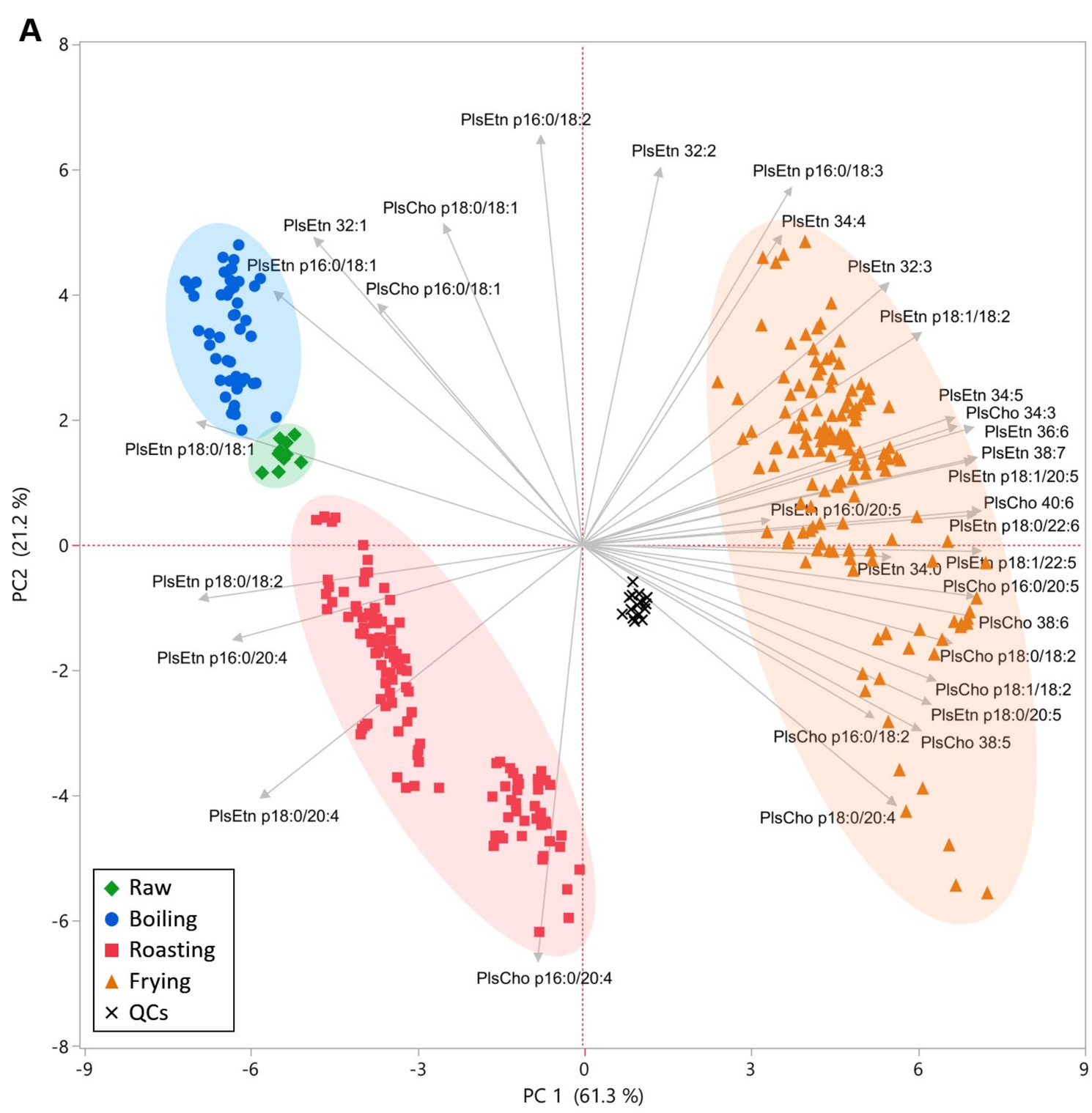
plasmalogen species contents; the colors of blue, green, and orange indicate the frying temperatures of 160 °C, 180 °C, and 200 °C, respectively; the solid and the dotted lines represent the core and the surface parts of the meat within the same frying condition, respectively. (**I**) Comparison of plasmalogen loss rate as relative percentages between the core and the surface parts after 5 min of frying under 200 °C; *** $P < 0.001$, ns, not significant.

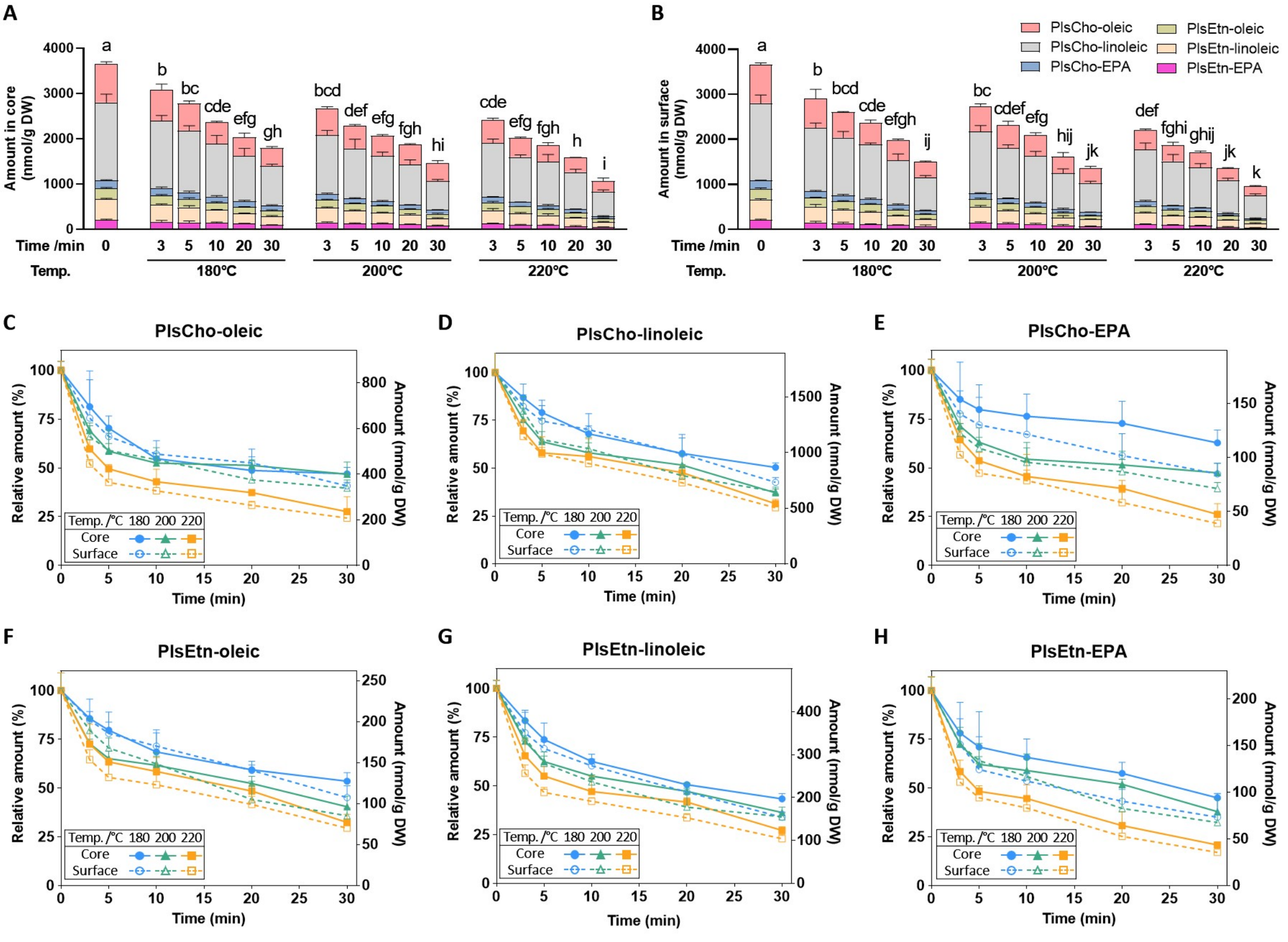
**Figure 4**. The predictive three-dimensional surface charts of remained plasmalogens during roasting (**A**) and frying (**B**) by using the established neural networks.

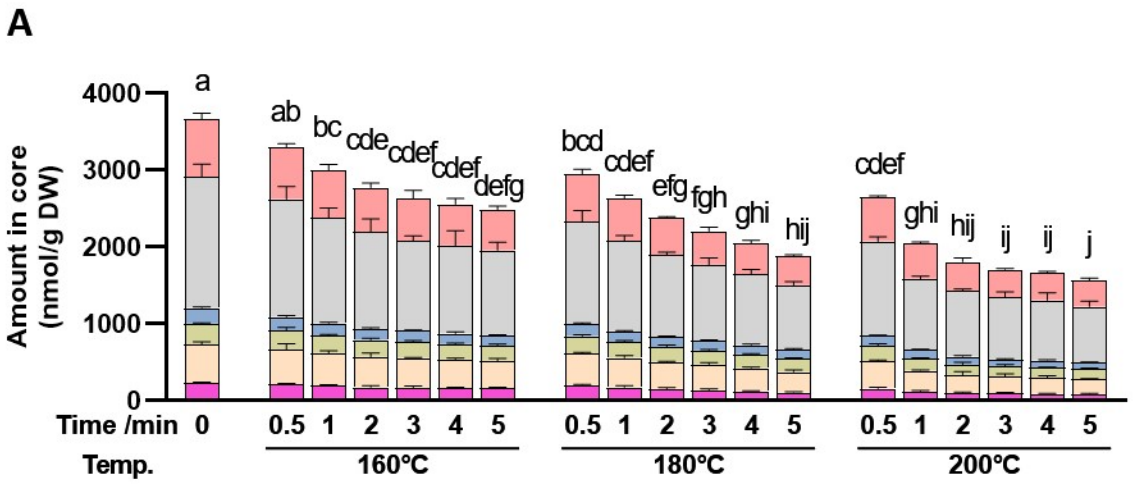
**Figure 5**. The effects of batter-coating on preventing plasmalogen loss during frying. (**A**) The representative photos of meat samples: (left) the directly fried meat; (middle) the coated fried meat, with batter-coating; (right) the coated fried meat, with coating removed. And the comparison of plasmalogens remained between the frying with/without batter-coating in the core (**B**) and in the surface (**C**) of meat. The value above each column indicates the percentage of the total remained mount of the six measured plasmalogen species.

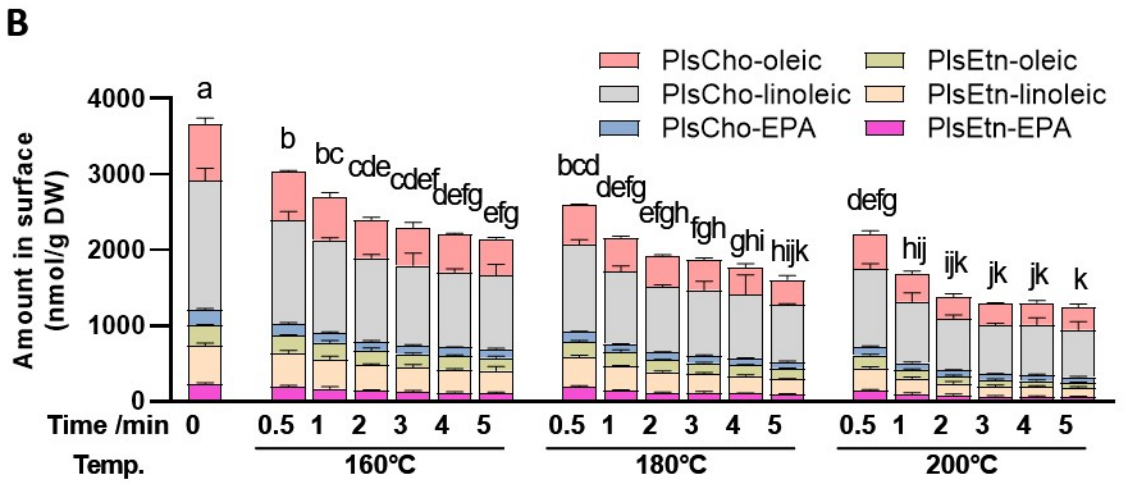
**Table of contents graphic**

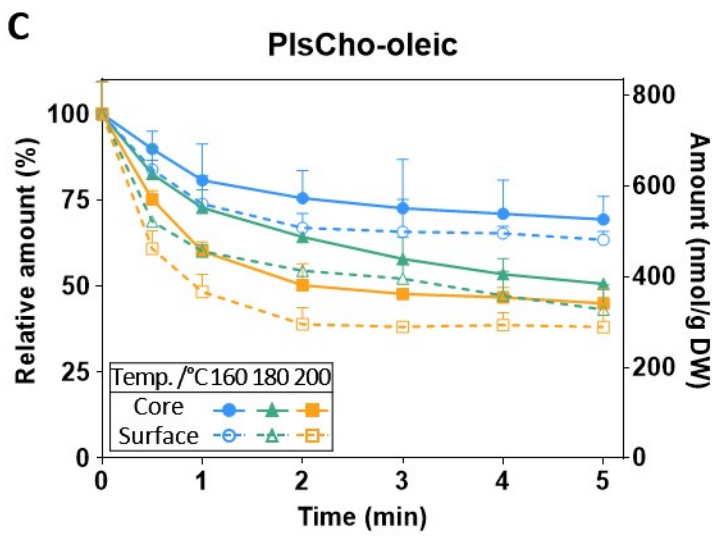


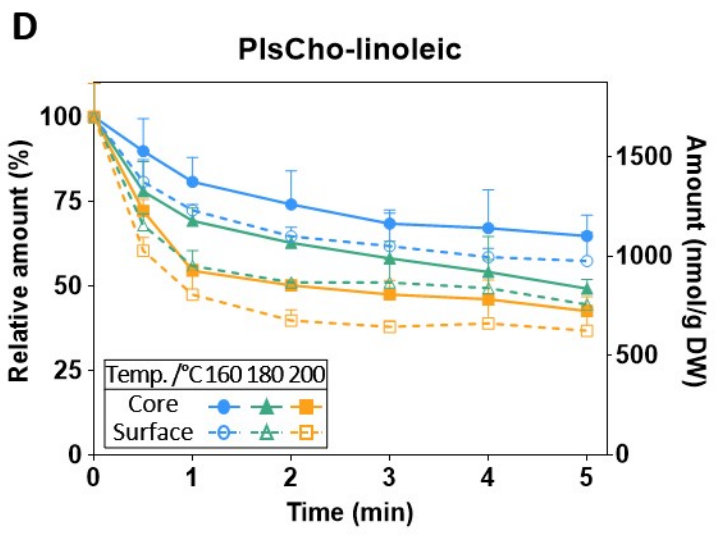

A
PlsEtn p16:0/18:2
PlsEtn 32:2
PlsEtn p16:0/18:3
PlsCho p18:0/18:1
PlsEtn 32:1
PlsEtn 34:4
PlsEtn p16:0/18:1
PlsEtn 32:3
PlsCho p16:0/18:1
PlsEtn p18:1/18:2
PlsEtn 34:5
PlsCho 34:3
PlsEtn 36:6
PlsEtn p18:0/18:1
PlsEtn 38:7
PlsEtn p18:1/20:5
PlsEtn p16:0/20:5
PlsCho 40:6
PlsEtn p18:0/22:6
PlsEtn 34:0
PlsEtn p18:1/22:5
PlsEtn p18:0/18:2
PlsCho p16:0/20:5
PlsCho 38:6
PlsEtn p16:0/20:4
PlsCho p18:0/18:2
PlsCho p18:1/18:2
PlsEtn p18:0/20:5
PlsCho p16:0/18:2
PlsCho 38:5
PlsEtn p18:0/20:4
PlsCho p18:0/20:4
PlsCho p16:0/20:4
Raw
Boiling
Roasting
Frying
QCs
PC2 (21.2 %)
PC 1 (61.3 %)
B
Raw
0.5 min
1 min
2 min
5 min
10 min
30 min
Boiling
PC2 (22.4 %)
PC1 (30.7 %)
E
Core
Surface
Boiling
Orthogonal T score [1]
C
Raw
180ºC
200ºC
220ºC
Roasting
PC2 (15.1 %)
PC1 (57.6 %)
F
Core
Surface
Roasting
Orthogonal T score [1]
D
Raw
160ºC
180ºC
200ºC
Frying
PC2 (27.3 %)
PC1 (41.6 %)
G
Core
Surface
Frying
Orthogonal T score [1]
T score [1]

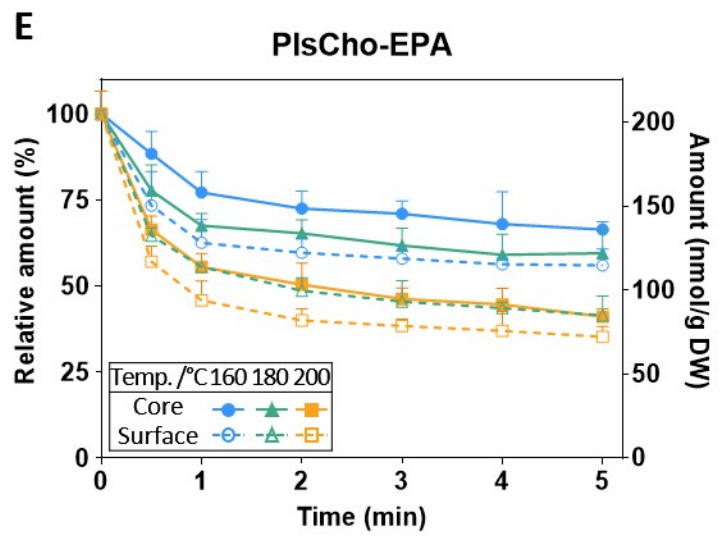

A
B
PlsCho-oleic
PlsCho-linoleic
PlsCho-EPA
PlsEtn-oleic
PlsEtn-linoleic
PlsEtn-EPA
Amount in core (nmol/g DW)
Amount in surface (nmol/g DW)
Time /min
Temp.
180°C
200°C
220°C
C
PlsCho-oleic
D
PlsCho-linoleic
E
PlsCho-EPA
F
PlsEtn-oleic
G
PlsEtn-linoleic
H
PlsEtn-EPA
Relative amount (%)
Amount (nmol/g DW)
Time (min)
Temp./°C 180 200 220
Core
Surface

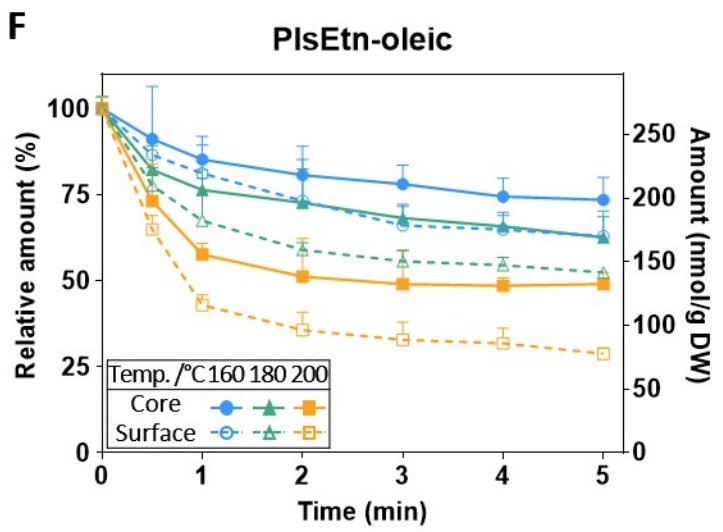

A
Amount in core (nmol/g DW)
Time /min
Temp.
160°C
180°C
200°C

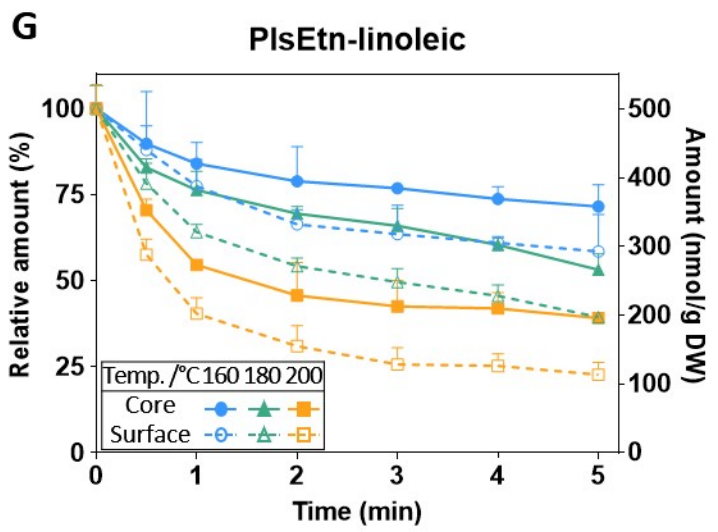

B
PlsCho-oleic
PlsCho-linoleic
PlsCho-EPA
PlsEtn-oleic
PlsEtn-linoleic
PlsEtn-EPA
Amount in surface (nmol/g DW)
Time /min
Temp.
160°C
180°C
200°C

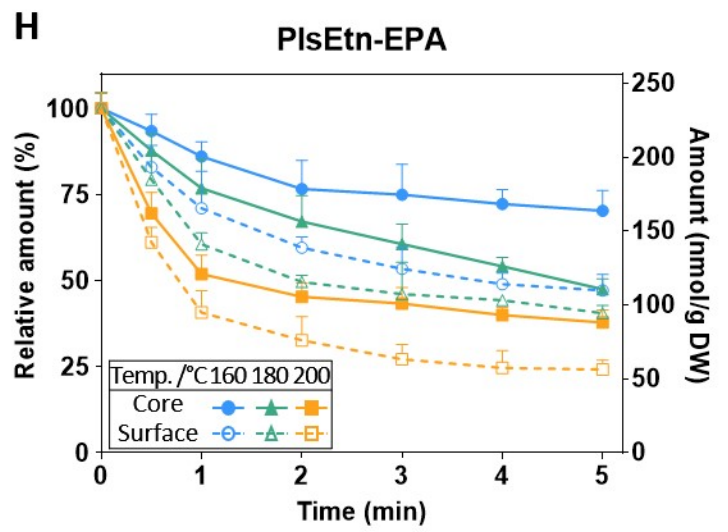

C
PlsCho-oleic
Relative amount (%)
Amount (nmol/g DW)
Temp. /°C 160 180 200
Core
Surface
Time (min)

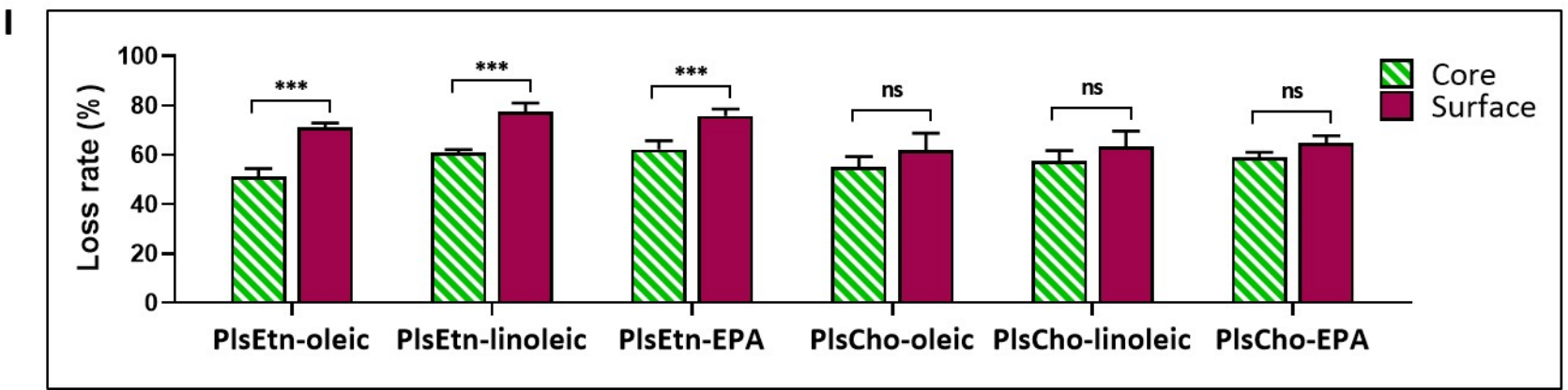

D
PlsCho-linoleic
Relative amount (%)
Amount (nmol/g DW)
Temp. /°C 160 180 200
Core
Surface
Time (min)


E
PlsCho-EPA
Relative amount (%)
Amount (nmol/g DW)
Temp. /°C 160 180 200
Core
Surface
Time (min)


F
PlsEtn-oleic
Relative amount (%)
Amount (nmol/g DW)
Temp. /°C 160 180 200
Core
Surface
Time (min)

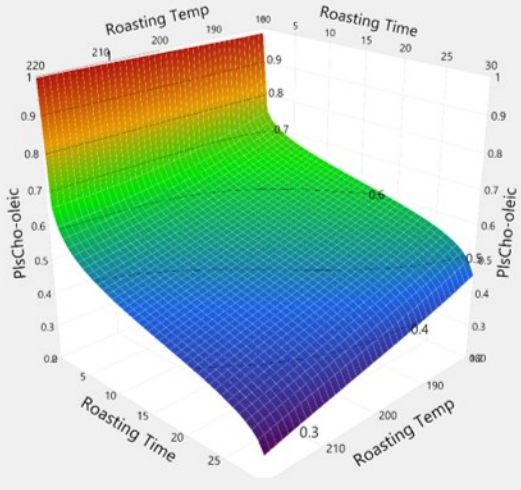

G
PlsEtn-linoleic
Relative amount (%)
Amount (nmol/g DW)
Temp. /°C 160 180 200
Core
Surface
Time (min)


H
PlsEtn-EPA
Relative amount (%)
Amount (nmol/g DW)
Temp. /°C 160 180 200
Core
Surface
Time (min)

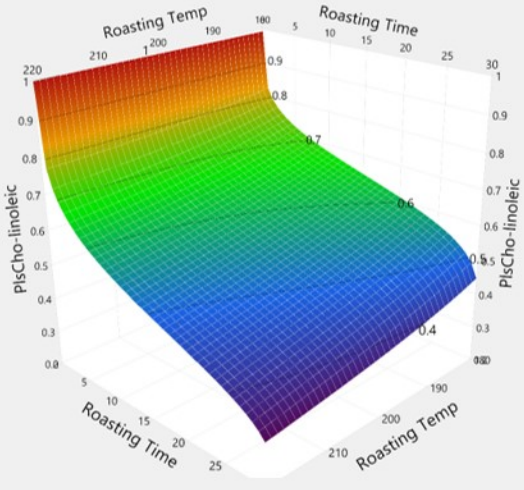

I
Loss rate (%)
***
***
***
ns
ns
ns
Core
Surface
PlsEtn-oleic
PlsEtn-linoleic
PlsEtn-EPA
PlsCho-oleic
PlsCho-linoleic
PlsCho-EPA

# A Roasting

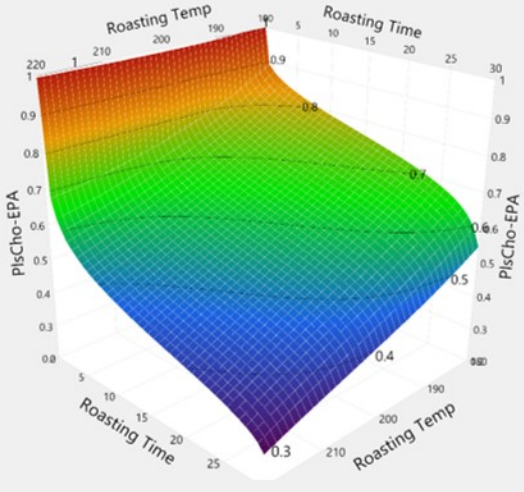
## PlsCho-oleic



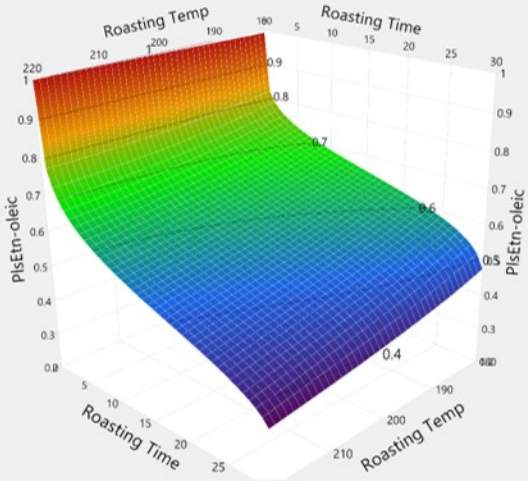

## PlsCho-linoleic



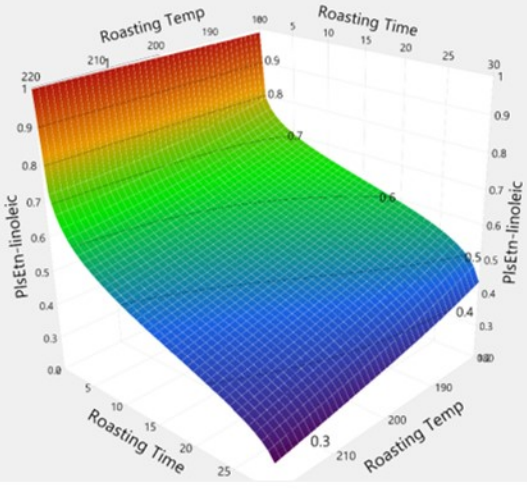

## PlsCho-EPA



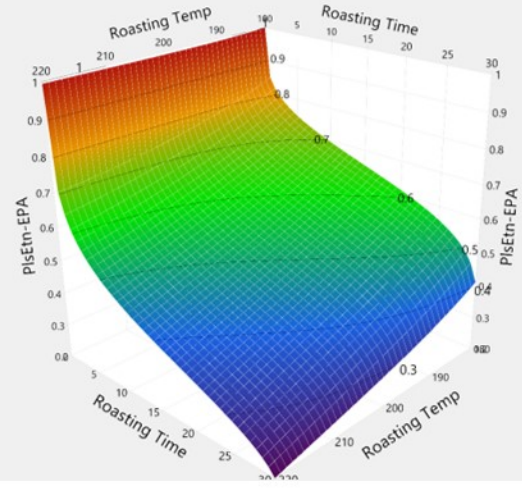

## PlsEtn-oleic




## PlsEtn-linoleic

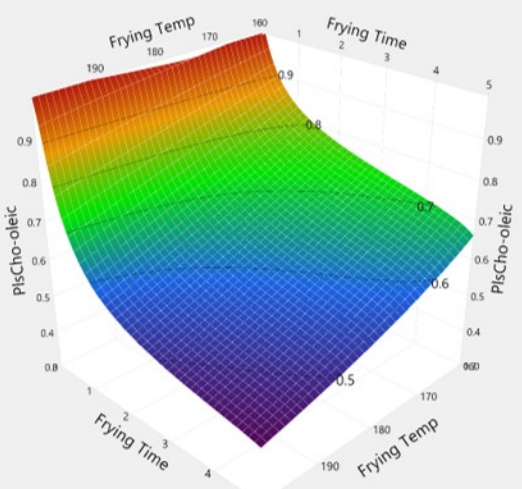



## PlsEtn-EPA




# B Frying

## PlsCho-oleic

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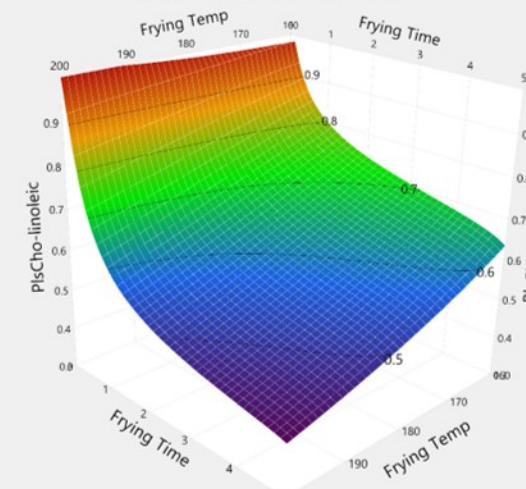
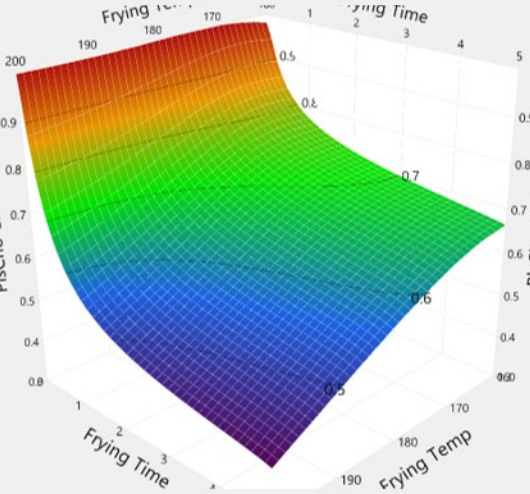



## PlsCho-EPA

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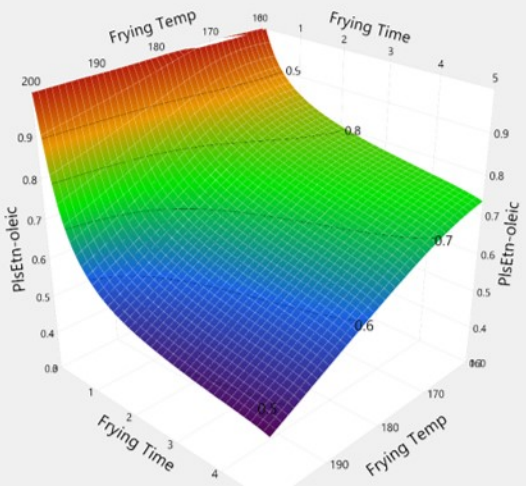


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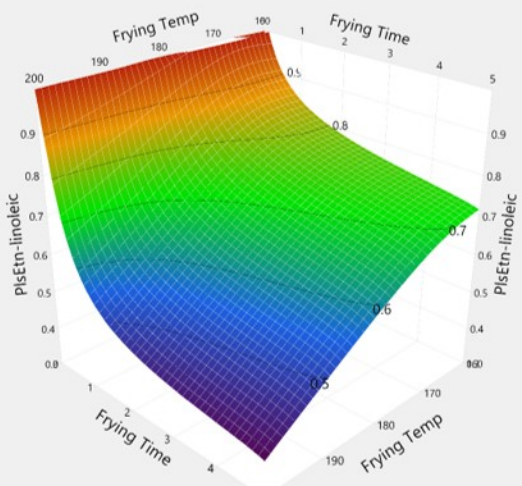


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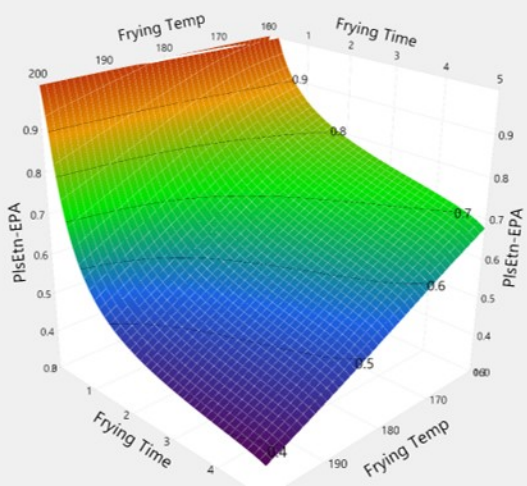

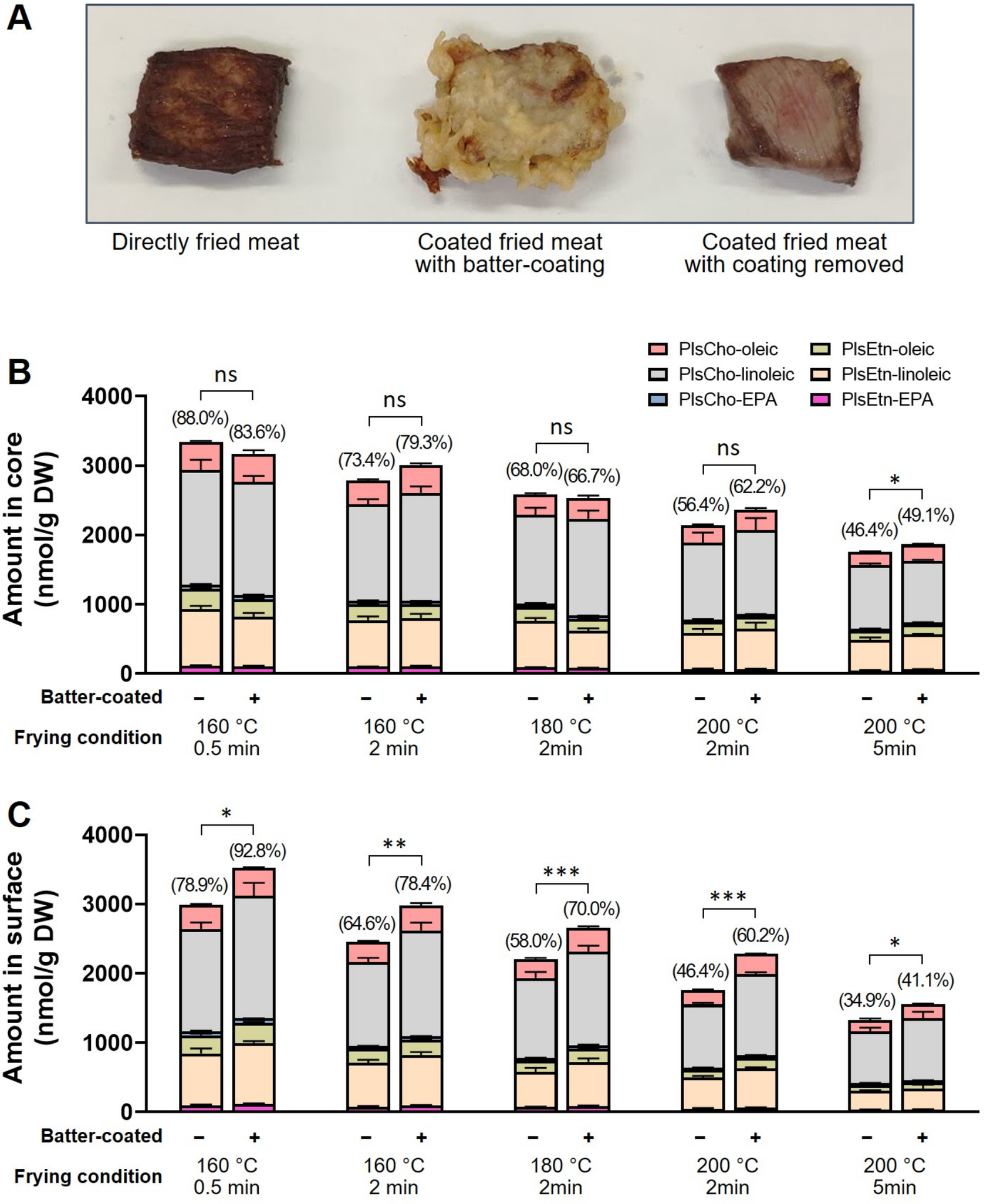
A
Directly fried meat
Coated fried meat with batter-coating
Coated fried meat with coating removed
B
PlsCho-oleic
PlsCho-linoleic
PlsCho-EPA
PlsEtn-oleic
PlsEtn-linoleic
PlsEtn-EPA
Amount in core (nmol/g DW)
4000
3000
2000
1000
0
ns
(88.0%)
(83.6%)
ns
(73.4%)
(79.3%)
ns
(68.0%)
(66.7%)
ns
(56.4%)
(62.2%)
*
(46.4%)
(49.1%)
Batter-coated
– +
Frying condition
160 °C 0.5 min
160 °C 2 min
180 °C 2min
200 °C 2min
200 °C 5min
C
Amount in surface (nmol/g DW)
4000
3000
2000
1000
0
*
(78.9%)
(92.8%)
**
(64.6%)
(78.4%)
***
(58.0%)
(70.0%)
***
(46.4%)
(60.2%)
*
(34.9%)
(41.1%)
Batter-coated
– +
Frying condition
160 °C 0.5 min
160 °C 2 min
180 °C 2min
200 °C 2min
200 °C 5min