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Modeling the Sensory Impact of Defined Combinations of Volatile Lipid Oxidation Products on Fishy and Metallic Off-Flavors

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The volatiles (*E*,*Z*)-2,6-nonadienal, 1-penten-3-one, (*Z*)-4-heptenal, and (*E*,*E*)-2,4-heptadienal were added to milk containing 1.5% fat according to a central composite design, to evaluate the individual and combinatory effects of these volatiles on sensory properties. The milk samples with added volatiles were subjected to sensory descriptive analysis for fishy and metallic off-flavors. The data were analyzed using partial least-squares regression and multiple linear regression to develop mathematical models. The models revealed significant main effects of (*E*,*Z*)-2,6-nonadienal and 1-penten-3-one and highlighted the importance of two-factor interactions for contribution toward off-flavors. The results suggest that (*E*,*Z*)-2,6-nonadienal and 1-penten-3-one could be useful markers for fishy and metallic off-flavors in fish oil and fish oil enriched foods. Within the addition levels of the volatiles there was a curvature effect of (*E*,*Z*)-2,6-nonadienal, a compensatory effect of (*Z*)-4-heptenal and (*E*,*E*)-2,4-heptadienal, and a synergistic effect of (*E*,*Z*)-2,6-nonadienal and (*Z*)-4-heptenal in the development of fishy off-flavors.

KEYWORDS: (*E*,*Z*)-2,6-Nonadienal; 1-penten-3-one; (*Z*)-4-heptenal; (*E*,*E*)-2,4-heptadienal; fishy off-flavor; metallic off-flavor; milk emulsion; PLSR; MLR; response surface plots

INTRODUCTION

Lipid oxidation is the most critical parameter affecting the quality and shelf life of fish oil and the food emulsions in which fish oils have been incorporated. The oxidative deterioration of fish oil involves the formation of hydroperoxides from polyunsaturated fatty acids in triglycerides, and the further progress of autoxidation give rise to a complex mixture of secondary oxidation products. Although lipid hydroperoxides are tasteless and odorless, the secondary oxidation products are responsible for the changes in the aroma and flavor properties of foods caused by the oxidation (*1*).

Widely used methods for assessing lipid oxidation include peroxide value (PV), anisidine value, 2-thiobarbituric acid value, and conjugated dienes. Although the data obtained by the use of these methods indicate the state of lipid oxidation, it has been shown that none of these methods correlate well to the sensory data of fish oil (*2*). Furthermore, Jacobsen (*3*) showed that there is no correlation between PV and the taste panel response on fish oil enriched spreads. However, the data on volatile compounds obtained by headspace methods have been demonstrated to correlate well with sensory data (*4*). On the basis of the gas chromatographic analysis of volatile compounds coupled with sniffing experiments and sensory analysis, odors and flavors associated with a large number of volatile oxidation compounds in oils of vegetable and animal origin have been described (*5*, *6*). However, the sensory impact of individual or combinations of volatile oxidation compounds in real food emulsions has not been studied.

Several volatile components have been characterized in fish oil (*7*−*9*), in fish itself (*10*−*12*), and in fish oil enriched foods such as mayonnaise (*13*) and milk (*14*). Sixty different volatiles comprising alkenals, alkadienals, alkatrienals, and vinyl ketones have been identified in fish oil enriched milk (*14*). Furthermore, the most potent odorants identified in this system by gas chromatography−olfactometry (GC-O) were 1-penten-3-one, (*Z*)-4-heptenal, 1-octen-3-one, 1,5-octadien-3-one, (*E*,*E*)-2,4-heptadienal, and (*E*,*Z*)-2,6-nonadienal, but despite their potency, none of the separated individual volatiles produced a fishy or metallic odor. It was therefore hypothesized that the fishy and metallic off-flavors were due to a combination of some of the potent odorants identified in the study.

1-Penten-3-one has been suggested to contribute to unpleasant off-flavors described as sharp-fishy in fish oil (*5*) or rancid and

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Table 1. Combinations of Design Variables Used in the Central Composite Design along with Response Variables Obtained by Sensory Analysis

sample	(*E*,*Z*)-2,6-nonadienal (ng/100 g of milk)	1-penten-3-one (ng/100 g of milk)	(*Z*)-4-heptenal (ng/100 g of milk)	(*E*,*E*)-2,4-heptadienal (ng/100 g of milk)	fishy odor (mean ± SD)	metallic odor (mean ± SD)	fishy flavor (mean ± SD)	metallic flavor (mean ± SD)
1	0	3000	250	500	1.26 (±1.45)	1.02 (±0.82)	1.26 (±1.28)	1.12 (±0.98)
2	1000	3000	250	500	1.84 (±1.55)	1.24 (±1.09)	2.72 (±1.65)	2.15 (±1.46)
3	500	0	250	500	1.41 (1±.38)	0.78 (±0.92)	2.01 (±1.47)	1.54 (±1.48)
4	500	6000	250	500	2.77 (±1.91)	2.19 (±1.45)	2.89 (±1.51)	2.36 (±1.08)
5	500	3000	0	500	1.63 (±1.38)	0.92 (±1.13)	2.09 (±1.37)	1.71 (±1.37)
6	500	3000	500	500	1.02 (±0.88)	0.86 (±0.85)	2.41 (±1.39)	1.75 (±1.58)
7	500	3000	250	0	1.85 (±1.43)	1.15 (±1.14)	2.50 (±1.97)	2.01 (±1.48)
8	500	3000	250	1000	1.57 (±1.39)	1.28 (±1.07)	2.11 (±1.42)	1.91 (±1.39)
9	250	1500	125	250	1.20 (±1.36)	0.77 (±0.85)	1.88 (±1.06)	1.24 (±0.87)
10	750	1500	125	250	0.88 (±1.16)	0.70 (±0.78)	1.60 (±1.52)	1.63 (±1.18)
11	250	4500	125	250	1.09 (±1.44)	0.78 (±0.77)	2.43 (±1.40)	1.89 (±1.24)
12	750	4500	125	250	2.57 (±1.50)	1.86 (±1.29)	2.83 (±1.64)	2.04 (±1.35)
13	250	1500	375	250	1.09 (±1.08)	0.57 (±0.70)	1.83 (±1.06)	1.54 (±1.40)
14	750	1500	375	250	1.56 (±1.41)	0.91 (±0.85)	2.28 (±1.41)	1.63 (±1.27)
15	250	4500	375	250	2.33 (±1.88)	1.29 (±1.41)	2.65 (±1.46)	2.12 (±1.53)
16	750	4500	375	250	2.61 (±1.46)	1.52 (±1.00)	3.46 (±1.85)	2.26 (±1.59)
17	250	1500	125	750	1.59 (±1.39)	0.99 (±0.84)	2.78 (±1.34)	2.03 (±1.44)
18	750	1500	125	750	1.67 (±1.26)	1.00 (±1.09)	1.86 (±1.75)	1.51 (±1.44)
19	250	4500	125	750	1.91 (±1.47)	1.31 (±1.31)	2.64 (±1.42)	2.05 (±1.18)
20	750	4500	125	750	2.10 (±1.69)	1.15 (±0.86)	3.28 (±1.57)	1.98 (±1.58)
21	250	1500	375	750	0.73 (±1.13)	0.41 (±0.63)	1.20 (±1.27)	1.31 (±1.39)
22	750	1500	375	750	1.38 (±1.62)	0.88(±1.09)	2.33 (±1.49)	1.58 (±0.97)
23	250	4500	375	750	2.13 (±1.49)	1.46 (±1.10)	2.57 (±1.45)	1.95 (±1.25)
24	750	4500	375	750	2.62 (±1.76)	1.81 (±1.18)	3.31 (±1.35)	2.23 (±1.00)
25	500	3000	250	500	2.34 (±2.12)	1.31 (±1.23)	2.76 (±1.28)	2.11 (±1.26)
26	500	3000	250	500	1.84 (±1.13)	1.05 (±0.89)	2.38 (±1.24)	2.18 (±1.47)
27	500	3000	250	500	1.11 (±1.37)	0.76 (±0.92)	2.54 (±1.48)	1.81 (±1.46)
28	500	3000	250	500	1.97 (±1.71)	0.90 (±0.90)	2.60 (±1.47)	1.64 (±0.99)
29	500	3000	250	500	2.30 (±1.44)	1.74 (±1.14)	2.92 (±1.04)	2.06 (±0.92)

plastic in fish oil enriched mayonnaise (*13*) and fish oil enriched milk (*14*). The increase in fishy, metallic, and rancid off-flavors has been correlated to high concentrations of (*E*,*E*)-2,4-heptadienal in fish oil enriched mayonnaise (*3*, *15*). The perception of off-flavors has been correlated to the development of 1-penten-3-one, (*E*,*E*)-2,4-heptadienal, and (*E*,*Z*)-2,6-nonadienal in fish oil enriched milk (*16*). The volatiles (*Z*)-4-heptenal and (*E*,*Z*)-2,6-nonadienal have been associated with fishy off-flavors in oxidized fish oil (*7*) and in related fish oil products (*13*). On the basis of these reports along with the results of volatile secondary oxidation products identified in fish oil enriched milk by GC-O (*14*), the volatiles (*E*,*Z*)-2,6-nonadienal, 1-penten-3-one, (*Z*)-4-heptenal, and (*E*,*E*)-2,4-heptadienal were considered in the present study to draw the relationships between the combinatory effects of these volatiles and the development of off-flavors. We did not include 1,5-octadien-3-one in the present experiment, even though it had been identified as a potent odorant in fish oil enriched milk by GC-O (*14*). However, 1,5-octadien-3-one was not detected in fish oil enriched milk having a strong fishy off-flavor after only 1 day of storage in our previous study (*16*), which is why we opted not to include 1,5-octadien-3-one in this combinatory study.

Multivariate analysis can be of help in modeling the effects of volatile secondary lipid oxidation products on the development of off-flavors. The only model available in the literature describing the quantitative relationship of volatiles and fishy taste in fish oil was developed by Macfarlane et al. (*2*) and was based on three volatiles, 2,6-nonadienal, 4-heptenal, and 3,6-nonadienal. To keep the model simple, Macfarlane et al. studied only the main effects of three volatiles and did not include other sensory descriptors such as metallic, rancid, and paintlike, which are commonly perceived in oxidized fish oil.

The objective of the present study was to develop models using partial least-squares regression (PLSR) and multiple linear regression (MLR) describing the relationships between the concentrations of 1-penten-3-one, (*E*,*Z*)-2,6-nonadienal, (*Z*)-4-heptenal, and (*E*,*E*)-2,4-heptadienal and the intensity of off-flavors in a milk emulsion. To achieve response surface models, different concentrations of volatiles were added to the milk following a central composite design (CCD). Furthermore, due to the importance of combinatory effects of volatiles on sensory properties, the interactions and square effects were studied in addition to the main effects.

MATERIALS AND METHODS

Materials. Pasteurized milk with fat content of 1.5% was purchased locally. The chemicals (*E*,*Z*)-2,6-nonadienal (95%), 1-penten-3-one (97%), and (*E*,*E*)-2,4-heptadienal (90%) were purchased from Aldrich-Chemie, and (*Z*)-4-heptenal (> 85%) was purchased from TCI.

Experimental Design. A CCD was followed to reduce the number of possible combinations of the selected volatiles to a manageable size. Five levels were chosen for each design variable in the form −2, −1, 0, +1, and +2, where −2 and +2 were the lowest and highest levels, respectively, with 0 level as the center point. Five replications of the center points were used in the design. The 29 combinations obtained are shown in **Table 1**. The milk samples were prepared by adding 1-penten-3-one, (*E*,*Z*)-2,6-nonadienal, (*Z*)-4-heptenal, and (*E*,*E*)-2,4-heptadienal to pasteurized milk according to the runs of the CCD. The samples were shaken for 5−10 min to ensure that the added components were mixed properly in the milk. Then, the samples were kept at 5 °C for 1 h and served for sensory evaluation.

Descriptive Sensory Analysis and Evaluation of Panelist Performance. Descriptive sensory analysis was performed using 16 panelists (eight females and eight males) who had previous experience in performing descriptive analysis on milk samples and fishy off-flavors. ISO Standards 8586, 6658, and 6564 were generally followed for training and sensory analysis methods, respectively. The panelists were trained in six sessions to define the descriptors by a consensus method using either fish oil enriched milk samples having distinct fishy and metallic off-flavors or pure milk to which the four selected compounds had been added in different concentrations and combinations. Concentrations used for the four compounds were as follows: (*E*,*Z*)-2,6-nonadienal, 0−1000 ng/100 g; 1-penten-3-one, 0−3000 ng/100 g; (*Z*)-4-heptenal, 0−1000 ng/100 g; (*E*,*E*)-2,4-heptadienal, 0−2000 ng/100 g. During these sessions the panelists were trained to use a 9 cm line scale to quantify the intensity of selected flavor descriptors fishy odor, fishy flavor, metallic odor, and metallic flavor. The samples (5 °C) were presented one at a time, and the samples were examined in individual sensory evaluation booths. During the experiment each sample was evaluated only once by each panelist. The samples were assessed quantitatively with respect to four descriptive responses: fishy odor, fishy flavor, metallic odor, and metallic flavor. Six samples were assessed at each sensory session including one center sample, which was evaluated in each session. Apart from this restriction, the order of the evaluation of the samples was randomized.

The performance of the panel was evaluated by calculating the overall signal-to-noise ratio of each assessor. Moreover, the response from the individual assessors was compared with the mean value for each descriptor. On the basis of this evaluation, the responses from two of the assessors were ignored and not included in the calculations of the models described below.

Multivariate Analysis. The tasks of PLSR and MLR were performed using the Unscrambler 7.6 SR-1 (Oslo, Norway). The modeling with main effects, interaction effects, and square effects in *X* was attempted in both PLSR and MLR on the basis of the polynomial equation

$$Y = b_0 + b_1N + b_2P + b_3H + b_4D + b_5N \times P + b_6N \times \mathrm{H} + b_7N \times D + b_8P \times H + b_9P \times D + b_{10}H \times D + b_{11}N^2 + b_{12}P^2 + b_{13}H^2 + b_{14}D^2 + E$$

where *Y* is the predicted response variable and *N*, *P*, *H*, and *D* are design variables (*E*,*Z*)-2,6-nonadienal, 1-penten-3-one, (*Z*)-4-heptenal, and (*E*,*E*)-2,4-heptadienal, respectively. The regression coefficients b_1-b_4 are the main effects, b_5-b_{10} are interaction effects, $b_{11}-b_{14}$ are square effects, and b_0 is the intercept. *E* is the residual error term.

Each variable was weighed with the inverse of its standard deviation (1/SD) before modeling. Full cross-validation (leaving out one sample at a time) and jack-knifing were applied in the PLSR model to assess the statistical reliability of the individual estimated responses (*17*). The validity of the MLR model was assessed by ANOVA. In the final models, only the coefficients characterized by $P < 0.1$ were used to evaluate the effects of linear, quadratic, and interactive terms. The significance level $P < 0.1$ was considered in view of the relatively large standard deviations observed in the quantitative descriptive analysis of the sensory attributes in the present study.

Response surface plots displaying the levels of a response as a function of two selected variables with other variables being held constant were used for interpretation of the final models. The parameters studied to compare the individual models were the root-mean-square error of prediction (RMSEP), the regression coefficients, and the offset of the regression curve (intercept).

RESULTS AND DISCUSSION

Screening Experiment. The selected volatiles (*E*,*Z*)-2,6-nonadienal, 1-penten-3-one, (*Z*)-4-heptenal, and (*E*,*E*)-2,4-heptadienal were screened to assess their possible role in the development of off-flavors. The concentrations chosen for each compound corresponded to the concentrations observed in fish oil enriched milk emulsions exhibiting distinct fishy and metallic flavors (data not shown). When the volatile compounds were added individually to the milk, none of these compounds imparted fishy or metallic off-flavors to the milk. Addition of two or three volatiles in specific combinations and addition of all four volatiles resulted in development of fishy and metallic off-flavors. The highest intensities of fishy and metallic off-flavors were perceived when the volatiles were added in the following concentrations per 100 g of milk: (*E*,*Z*)-2,6-nonadienal, 500 ng; 1-penten-3-one, 3000 ng; (*Z*)-4-heptenal, 250 ng; and (*E*,*E*)-2,4-heptadienal, 500 ng. By taking these concentrations as a center point, five levels were followed in the central composite design (**Table 1**). A concentration of zero was chosen as the lowest level for all of the variables.

Descriptive Sensory Analysis. The data obtained from the sensory analysis as per the runs of CCD are detailed in **Table 1**. The values of standard deviation (SD) for each sensory response are given in parentheses, and it was observed that the relative values of SD appear to be high in the case of fishy and metallic odor descriptors compared to those of flavor descriptors. This can partly be explained by the fact that the threshold values are generally lower for flavor than for odor (*18*).

The design followed in the study allowed us to ascertain the contribution of selected volatiles to the development of off-flavors. The sensory responses observed in samples 1 (without nonadienal), 3 (without penten-3-one), 5 (without heptenal), and 7 (without heptadienal) tended to be lower compared to that of central samples 25−29 (**Table 1**). This observation demonstrated the combined effect of the four selected volatiles on flavor attributes. Particularly, the effect of nonadienal (samples 1 and 2) and penten-3-one (samples 3 and 4) was pronounced with respect to fishy flavor. Furthermore, the samples with concentrations of three volatiles (16 and 20) and four volatiles (24) at +1 level were found to exhibit high sensory scores of off-flavors (**Table 1**).

Multivariate Analysis. The mean values of sensory responses were used for statistical analysis. The data were modeled according to polynomial equations by multivariate analysis. A so-called ANOVA PLSR (APLSR) model was calculated, in which the design variables (concentrations of volatiles) were used as *X* variables and the response variables (sensory data) were used as *Y* variables (*19*). The purpose of the APLSR model was to determine the relationship between the volatiles plus their interactions and the sensory responses and at the same time to provide a graphical overview of these relationships. In parallel, a traditional MLR model was calculated on the same data. The MLR model was compared with the APLSR model with respect to the values of the intercepts and regression coefficients and statistical significance.

ANOVA PLSR. In view of the absence of fishy and metallic off-flavors associated with the individual volatiles in the screening experiment, all possible two-, three-, and four-factor interactions and square terms were considered for modeling. In the first attempt, none of the variables showed significance. After some variables with small contributions and large uncertainty limits were ignored, the model showed significant coefficient values for 1-penten-3-one and $N \times P \times D$ (for interpretation of volatile names, refer to **Table 2**). However, in an attempt to improve the model by ignoring some other nonsignificant interactions, the model revealed that the main effects of 1-penten-3-one and the $N \times H$ interaction were significant, with $N \times P \times D$ becoming nonsignificant. Owing to this inconsistency and instability of the models, three- and four-factor interactions were not considered for the final models. Then, APLSR analysis was repeated with all linear, two-factor interactions and square terms. Again, the model for each descriptor was improved by ignoring some variables with small contributions and large uncertainty limits.

The final APLSR model for fishy flavor was based on four main effects and three interaction terms ($N \times P$, $N \times H$, and $H \times D$). The model consisted of two principal components (PC), in which 64% of the *Y* variance and 28% of the *X* variance were explained. The model with the same main effects and two-

Table 2. Regression Coefficients, RMSEP, and Intercept of the Final APLSR Models of the Sensory Responses (Significance Level $P < 0.1$ in the Final Model)

factor	fishy odor	metallic odor	fishy flavor	metallic flavor
(*E*,*Z*)-2,6-nonadienal (*N*)	0.0008	0.0006	0.0008	0.0005
1-penten-3-one (*P*)	0.0003	0.0002	0.0003	0.0002
(*Z*)-4-heptenal (*H*)	*a*	*a*	*a*	*a*
(*E*,*E*)-2,4-heptadienal (*D*)	*a*	*a*	*a*	*a*
$N \times P$	*a*		*a*	*a*
$N \times H$	*a*		0.0859	*a*
$P \times H$		0.0804		
$H \times D$	*a*		−0.1021	*a*
P^2		0.0409		
H^2		−0.0609		
intercept	0.4509	0.1529	1.0544	1.0946
RMSEP	0.4468	0.3100	0.3995	0.2386

[a] The factor was included in the model but found to be not significant.

factor interaction terms was found to be optimum for both fishy odor and metallic flavor, but the model for fishy flavor had more significant interactions compared with the models for fishy odor and metallic flavor (**Table 2**). The optimized model for metallic odor was based on four main effects, two-factor interaction $P \times H$ and the square terms of penten-3-one and heptenal, and it contained two PCs with similar explained variance ($Y = 64\%$ and $X = 29\%$). The relatively low percentage of explained variance in X indicated that only a part of the variation in the design was used to describe the obtained sensory data. It may be mentioned that the low percentage of explained variance has been reported in other sensory experiments with fish oil enriched products (*15*, *16*). The polynomial equation for each sensory descriptor can be drawn from **Table 2**. For example, the equation for fishy flavor is

$$\text{fishy flavor} = 1.0544 + 0.0008\,[(E,Z)\text{-2,6-nonadienal}] + 0.0003\,(\text{1-penten-3-one}) + 0.0859\,(N \times H) - 0.1021\,(H \times D) + E \quad (1)$$

The main relationships in the data, revealed by the APLSR, were depicted in correlation loadings plot (**Figure 1**). All of the response variables describing the fishy/metallic odor and flavor were located far to the right in the correlation loadings diagram. Thus, the horizontal dimension (PC1) was clearly related to the development of undesirable off-flavors, indicating that both fishy and metallic descriptors were perceived together in the study. This is in agreement with the observation that fishy and metallic attributes have been reported together in oxidized fish oil and fish oil enriched foods (*3*, *16*). Among the seven variables selected for the modeling, 1-penten-3-one, (*E*,*Z*)-2,6-nonadienal, and the interaction of $N \times H$ showed strong correlation to the off-flavors. The vertical dimension (PC2) discriminated between the odor and flavor attributes as they differed in their intensity (**Figure 1**).

Discriminant PLSR. To ensure that the data structure observed in the APLSR model was sound and valid, the opposite model, a so-called discriminant PLSR (DPLSR) model, was calculated (*19*). The sensory variables were used as X variables and the design variables considered in the final APLSR model as Y variables. Cross-validation showed that two components were optimum in describing the data. The two principal components explained 93% of the X variance and 22% of the Y variance. The main relationships between X and Y variables in the DPLSR model were found to be almost similar to the pattern observed in the APLSR model. The similarities between the DPLSR and APLSR models verify that the data structure observed in the APLSR model is real, and therefore this model is sound and valid. Furthermore, the DPLSR model revealed the fishy flavor, fishy odor, and metallic odor as significant. A DPLSR model without two-factor interactions showed only fishy flavor as significant, indicating that the interaction terms were important for the final model.

MLR in Comparison with APLSR. The MLR analysis was performed in the same way as APLSR by ignoring the variables with small contribution and large uncertainty for achieving a model with optimum interactions. Separate models were derived for four different flavor attributes. The MLR model obtained for fishy flavor contained two main effects and four two-factor interaction terms in the following form:

$$\text{fishy flavor} = 2.511 + 0.001\,[(E,Z)\text{-2,6-nonadienal}] + 0.0003\,(\text{1-penten-3-one}) + 0.118\,(N \times P) + 0.176\,(N \times H) - 0.141\,(H \times D) - 0.096\,(N^2) + E \quad (2)$$

Similarly, equations for other sensory descriptors can be drawn from **Table 3**. All of the models generated by MLR explained >70% variations of the responses with R^2 values > 0.7 (**Table 3**). The highest correlation coefficient was observed with respect to fishy flavor ($R^2 = 0.784$). The explained variability can be considered adequate in view of the observed probability level in all of the models of $P < 0.0002$.

The significance estimates of the regression coefficients in the jack-knifed PLSR model and MLR model are listed in **Tables 2** and **3**, respectively. The volatiles (*E*,*Z*)-2,6-nonadienal and 1-penten-3-one were found to be significant for all of the response variables in both MLR and APLSR models. On the basis of these models it can be concluded that the increase in the concentrations of (*E*,*Z*)-2,6-nonadienal and 1-penten-3-one contribute significantly to the development of off-flavors. It should be emphasized that these volatiles have been reported to result from the oxidation of $n-3$ fatty acids (*3*, *20*). The results from the present study suggest that (*E*,*Z*)-2,6-nonadienal and 1-penten-3-one could be useful markers for evaluating fishy and metallic off-flavors in fish oil and fish oil enriched foods. Extensive application of fish oil in different foods has been limited by the development of fishy and metallic off-flavors due to the formation of volatiles resulting from the oxidation of fish oil. Propanal, a major breakdown product of $n-3$ polyunsaturated fatty acids (PUFA), has been used as a marker for monitoring the oxidation of $n-3$ PUFA (*21*). Usually, three sampling methods (static headspace, dynamic headspace, and direct injection) have been employed to measure the volatiles. It has been observed that the static headspace method showed large proportions of low molecular weight propanal compared to other GC methods, which produced large proportions of high molecular weight 2,4-alkadienals (*4*). The findings of the present study are thus significant in view of the difficulty in quantitative measurement of propanal by dynamic headspace technique and direct injection of GC methods.

In addition to demonstrating the significant main effects of (*E*,*Z*)-2,6-nonadienal and 1-penten-3-one, the present study highlighted the importance of two-factor interactions and square effects in the development of off-flavors. The two-factor interactions of $N \times P$, $N \times H$, and $H \times D$ and the square effect of nonadienal were found to be significant in the MLR model with respect to fishy flavor. Among these interactions, the effects of $N \times P$ and $N \times H$ were observed as positive and the interaction of $H \times D$ and the square effect of (*E*,*Z*)-2,6-nonadienal were observed as negative (eq 2). The strongest

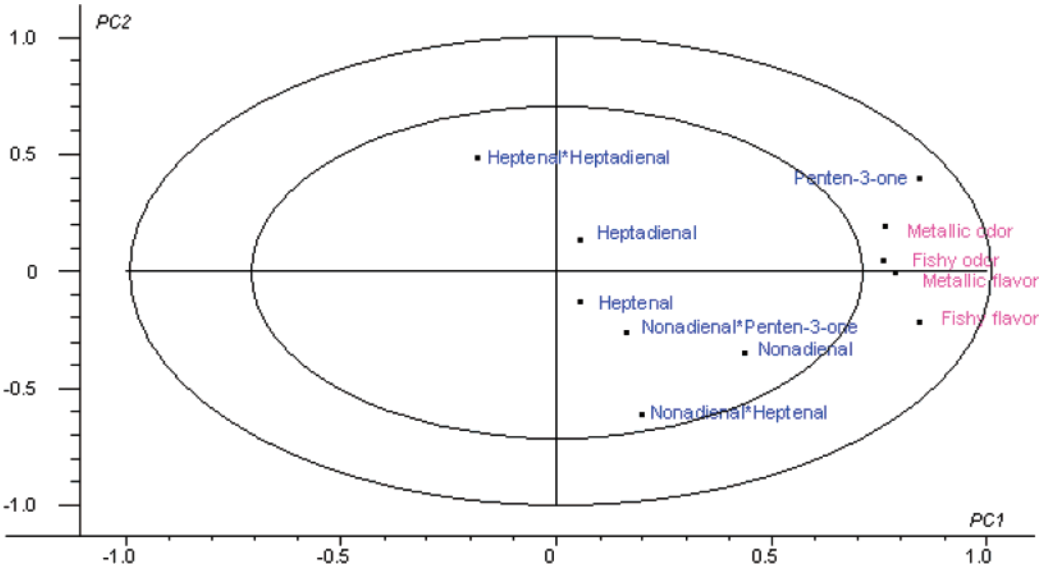

Figure 1. Correlation loading plot of PC1 versus PC2 obtained by APLSR model for fishy odor, fishy flavor, metallic odor, and metallic flavor. The ellipses represent 50 and 100% explained variance.

Table 3. Regression Coefficients and Intercepts of the Final MLR Models of the Sensory Responses (Significance Level $P < 0.1$ in the Final Model)

factor	fishy odor	metallic odor	fishy flavor	metallic flavor
(*E*,*Z*)-2,6-nonadienal (*N*)	0.0008	0.0005	0.0010	0.0005
1-penten-3-one (*P*)	0.0003	0.0002	0.0003	0.0002
(*Z*)-4-heptenal (*H*)	*a*	*a*	*a*	*a*
(*E*,*E*)-2,4-heptadienal (*D*)	*a*	*a*	*a*	*a*
$N \times P$	*a*		0.118	
$N \times H$			0.176	
$P \times H$	0.139			
$H \times D$	*a*		–0.141	*a*
N^2	*a*		–0.096	–0.069
P^2		*a*	*a*	
H^2	–0.12	–0.082	*a*	*a*
intercept	1.923	1.107	2.511	1.955
$R^{2\,b}$	0.721	0.741	0.784	0.705
$P^c <$	0.0009	0.00001	0.00001	0.0002

[a] The factor was included in the model but found to be not significant. [b] Coefficient of regression. [c] Significance level of the model.

positive effect of two-factor interactions $N \times H$ and $N \times P$ to fishy flavor was revealed by their high regression coefficients compared to that of individual main effects (eq 2). The negative coefficient of nonadienal for its square effect was in agreement with the observed cucumber taste in samples in which nonadienal was at maximum levels. The negative contribution of nonadienal at high concentrations toward fishy flavor was presumed to be due to the masking effect of the cucumber taste. In the APLSR model, a positive two-factor interaction of $N \times H$ and a negative two-factor interaction of $H \times D$ were also noticed for the fishy flavor (eq 1). A positive two-factor interaction indicates a synergistic effect between the involved design factors. The assumptions and hypotheses made in earlier studies (*7*, *13*, *22*, *23*), stating that the fishy off-flavors are due to complex mixtures of carbonyl compounds, were thus substantiated by these results.

The MLR model revealed a significant effect of $P \times H$ and a square effect of heptenal for fishy odor and a square effect of nonadienal for metallic flavor (**Table 3**). The square terms of penten-3-one and heptenal and the interaction of $P \times H$ were found to be significant in the optimized APLSR model for metallic odor, whereas only the square term of heptenal was found to be significant in addition to the main effects in the MLR model.

Relatively large values of intercept for all of the response variables were noticed in both of the models examined (**Tables 2** and **3**). The observed high values of intercept can be explained on the basis of the fact that all 29 samples studied in the experiment resulted in sensory scores >0.4. This is because all of the samples in the design, except four samples in which one variable was omitted in each, contained the four selected variables at various concentrations. When the sensory data of real milk samples (without added volatiles) was added to the data set, the intercept value was reduced considerably and approached zero in the model (data shown). The intercept values obtained by the APLSR models were much smaller compared to those of the MLR models.

The estimated values of root-mean-square error prediction (RMSEP) were obtained by the APLSR analysis for all of the sensory descriptors. The RMSEP values are expressed in the same units as the original responses. The RMSEP values obtained in the study (**Table 2**) are low compared to the standard deviations observed in the sensory data.

Response Surface Plots. The three-dimensional surface plots, reconstructed from the final APLSR and MLR models with optimum interactions, illustrated information on the main and interactive effects of the volatiles. In the final MLR model for fishy flavor (eq 2), the two factors (*E*,*Z*)-2,6-nonadienal and 1-penten-3-one showed clear main effects. The strong main effect of 1-penten-3-one was evident from the slope of the surface. The (*E*,*Z*)-2,6-nonadienal showed a curvature effect, and the probable reason for the curvature might be due to strong sensory masking effects by its cucumber taste as discussed earlier (**Figure 2a**). Interestingly, the variables (*Z*)-4-heptenal and (*E*,*E*)-2,4-heptadienal had a compensatory effect on fishy flavor (**Figure 2b**). The maximum value of fishy flavor was observed when the (*Z*)-4-heptenal was at its maximum and (*E*,*E*)-2,4-heptadienal was at its minimum and vice versa. In other words, the combination of these two variables at their lowest or highest levels was associated with the minimum fishy flavor.

The effect of interaction between (*Z*)-4-heptenal and (*E*,*Z*)-2,6-nonadienal was found to be statistically significant for fishy

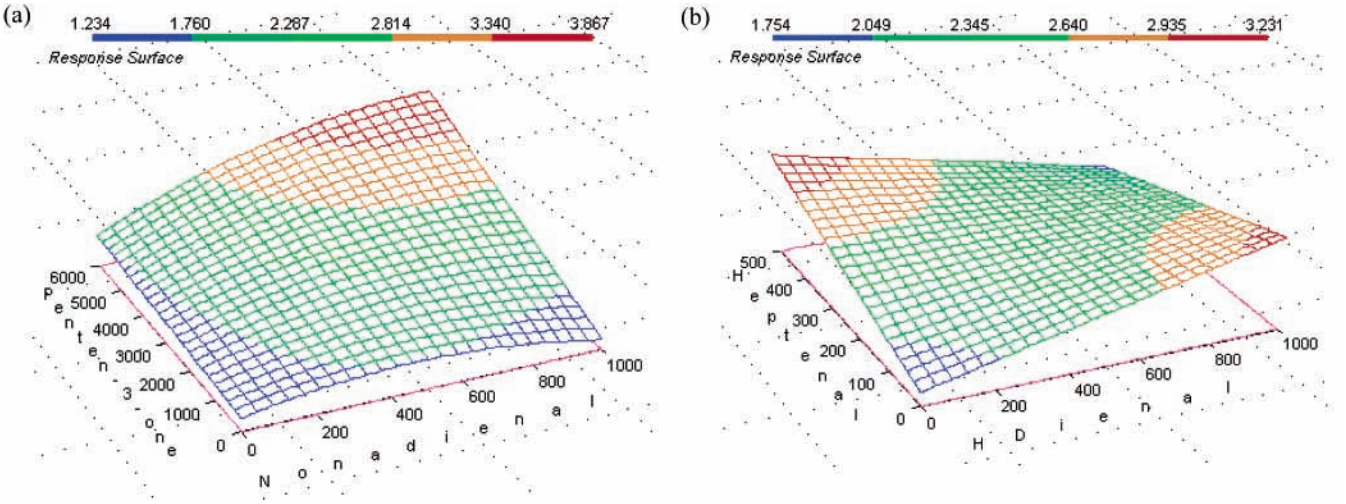

Figure 2. Response surface plots reconstructed from the MLR model: the estimated fishy flavor as a function of (*E*,*Z*)-2,6-nonadienal and 1-penten-3-one at heptenal 250 and heptadienal 500 (**a**); (*Z*)-4-heptenal and (*E*,*E*)-2,4-heptadienal at nonadienal 500 and penten-3-one 3000 (**b**). The units of volatiles are the same as those in **Table 1**.

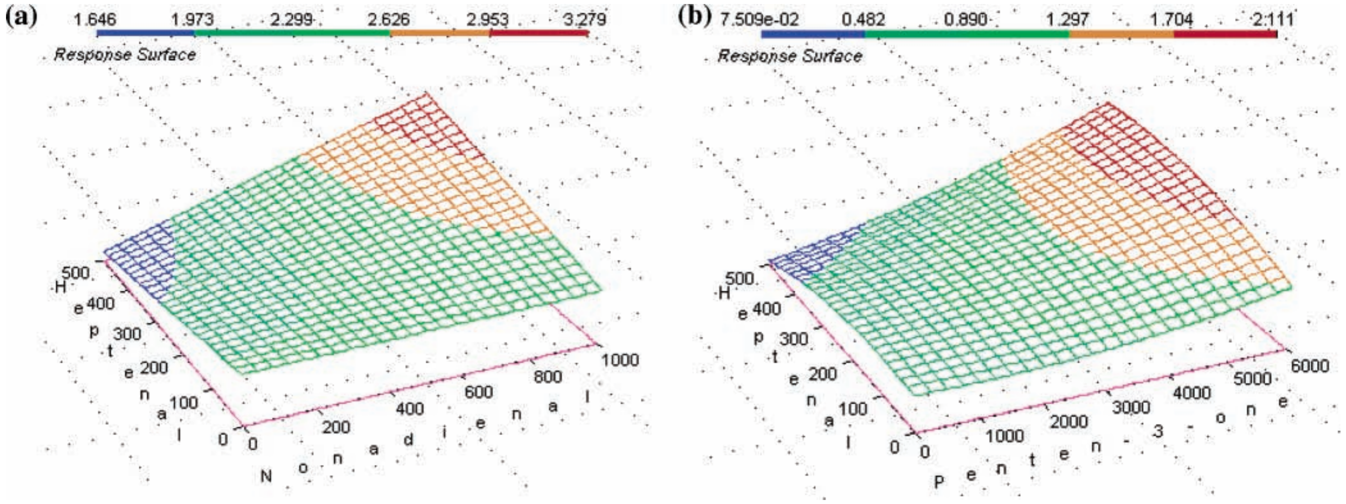

Figure 3. Response surface plots reconstructed from the APLSR model: the estimated fishy flavor as a function of (*Z*)-4-heptenal and (*E*,*Z*)-2,6-nonadienal at penten-3-one 3000 and heptadienal 500 (**a**); estimated metallic odor as a function of (*Z*)-4-heptenal and 1-penten-3-one at nonadienal 500 and heptadienal 500 (**b**). The units of volatiles are the same as those in **Table 1**.

flavor in both MLR and APLSR models. Accordingly, the response surface plot drawn from the APLSR model (eq 1) for fishy flavor shows that the contribution of (*Z*)-4-heptenal to the fishy flavor increases with the increase in the concentration of (*E*,*Z*)-2,6-nonadienal (**Figure 3a**). In the absence of nonadienal, an increase in the amount of heptenal minimizes the intensity of fishy flavor. Moreover, in the absence of (*Z*)-4-heptenal, increasing the concentration of (*E*,*Z*)-2,6-nonadienal will not increase fishy off-flavor. The interaction of these two variables is expected to play a significant role in real foods because (*Z*)-4-heptenal was known to result from the degradation of (*E*,*Z*)-2,6-nonadienal (*20*). A similar response surface was observed for the interaction of heptenal and penten-3-one with respect to metallic odor in which the heptenal enhances the effect of penten-3-one toward the development of metallic odor (**Figure 3b**). These findings support the observation of Lindsay (*24*) on the role of heptenal. According to him, (*Z*)-4-heptenal has the ability to give potency to the character of other flavor compounds rather than making a readily recognizable flavor contribution of its own. He also noted that at higher concentrations (*Z*)-4-heptenal could provide, or exacerbate, an off-flavor.

The results obtained in the present study by the two different methods, PLSR and MLR, were found to be rather similar. This is in agreement with many other studies, where PLSR and conventional MLR gave similar significance estimates (*19*). To our knowledge the work presented here happened to be the first study to investigate the effect of volatile secondary lipid oxidation product interactions on the development of off-flavors, by incorporating the volatiles in a real food emulsion and subsequently evaluating the sensory scores.

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LITERATURE CITED

(1) Frankel, E. N. Review. Recent advances in lipid oxidation. *J. Sci. Food Agric.* **1991**, *54*, 495−511.

(2) Macfarlane, N.; Salt, J.; Birkin, R. The FAST index−A fishy scale. *Int. News. Fats, Oils Relat. Mater.* **2001**, *12*, 244−249.

(3) Jacobsen, C. Sensory impact of lipid oxidation in complex food systems. *Fett/Lipid* **1999**, *101*, 484−492.

(4) Frankel, E. N. *Lipid Oxidation*; The Oily Press: Dundee, Scotland, 1998; pp 99−114.

(5) Grosch, W. Low-MW products of hydroperoxide reactions. In *Autoxidation of Unsaturated Lipids*; Chan, H. W. S., Ed.; Academic Press: London, U.K., 1987; pp 95−139.

(6) Rørbæk, K. Oxidation and flavours in fish oil. Ph.D. Thesis, Technical University of Denmark, 1994.

(7) Karahadian, C.; Lindsay, R. C. Evaluation of compounds contributing characterizing fishy flavors in fish oils. *J. Am. Oil Chem. Soc.* **1989**, *66*, 953−960.

(8) Hsieh, T. C. Y.; Williams, S. S.; Vejaphan, W.; Meyers, S. P. Characterization of volatile components of menhaden fish (*Brevoortia tyrannus*) oil. *J. Am. Oil Chem. Soc.* **1989**, *66*, 114−117.

(9) Aidos, I.; Jacobsen, C.; Jensen, B.; Luten, J. B.; van der Padt, A.; Boom, R. M. Volatile oxidation products formed in crude herring oil under accelerated oxidative conditions. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 808−818.

(10) Milo, C.; Grosch, W. Detection of odor defects in boiled cod and trout by gas chromatography-olfactometry of headspace samples. *J. Agric. Food Chem.* **1995**, *43*, 459−462.

(11) Milo, C.; Grosch, W. Changes in the odorants of boiled salmon and cod as affected by the storage of the raw material. *J. Agric. Food Chem.* **1996**, *44*, 2366−2371.

(12) Yoshiwa, T.; Morimoto, K.; Sakamoto, K.; Ishikawa, Y.; Tokita, M.; Morita, M. Volatile compounds of fishy odor in Sardine by simultaneous distillation and extraction under reduced pressure. *Nippon Suisan Gakkaishi* **1997**, *63*, 222−230.

(13) Hartvigsen, K.; Lund, P.; Hansen, L. F.; Hølmer, G. Dynamic headspace gas chromatography/mass spectrometry characterization of volatiles produced in fish oil enriched mayonnaise during storage. *J. Agric. Food Chem.* **2000**, *48*, 4858−4867.

(14) Venkateshwarlu, G.; Let, M. B.; Meyer, A. S.; Jacobsen, C. Chemical and olfactometric characterization of volatile flavor compounds in a fish oil enriched milk emulsion. *J. Agric. Food Chem.* **2004**, *52*, 311−317.

(15) Jacobsen, C.; Hartvigsen, K.; Lund, P.; Thomsen, M. K.; Skibsted, L. H.; Adler-Nissen, J.; Hølmer, G.; Meyer, A. S. Oxidation in fish oil enriched mayonnaise: 3. Assessment of the influence of the emulsion structure on oxidation by discriminant partial least squares regression analysis. *Eur. Food Res. Technol.* **2000**, *211*, 86−98.

(16) Let, M. B.; Jacobsen, C.; Frankel, E. N.; Meyer, A. S. Oxidative flavour deterioration of fish oil enriched milk. *Eur. J. Lipid Sci. Technol.* **2003**, *105*, 518−528.

(17) Martens, H.; Høy, M.; Westad, F.; Folkenberg, D.; Martens, M. Analysis of designed experiments by stabilized PLS regression and jack-knifing. *Chemom. Intell. Lab. Syst.* **2001**, *58*, 151−170.

(18) Meijboom, P. W. Relationship between Molecular Structure and Flavor perceptibility of Aliphatic Aldehydes. *J. Am. Oil Chem. Soc.* **1964**, *41*, 326−328.

(19) Martens, H.; Martens, M. *Multivariate Analysis of Quality. An Introduction*; Wiley: Chichester, U.K., 2001.

(20) Josephson, D. B.; Lindsay, R. C. Retro-aldol degradations of unsaturated aldehydes: Role in the formation of *cis*-4-heptenal from *trans*-2,*cis*-6-nonadienal in fish, oyster and other flavours. *J. Am. Oil Chem. Soc.* **1987**, *64*, 132−138.

(21) Frankel, E. N. In search of better methods to evaluate natural antioxidants and oxidative stability in food lipids. *Trends Food Sci. Technol.* **1993**, *4*, 220−225.

(22) Badings, H. T. Fishy off-flavours in autoxidized oils. *J. Am. Oil Chem. Soc.* **1973**, *50*, 334.

(23) Ke, P. J.; Ackman, R. G.; Linke, B. A. Autooxidation of polyunsaturated fatty compounds in mackerel oil: formation of 2,4,7-decatrienals. *J. Am. Oil Chem. Soc.* **1975**, *52*, 349−353.

(24) Lindsay, R. C. Fish flavours. *Food Rev. Int.* **1990**, *6*, 437−455.

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