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Analytical Methods

Volatile compounds suitable for rapid detection as quality indicators of cold smoked salmon (*Salmo salar*)

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Abstract

Volatile compounds in cold smoked salmon products were identified by gas chromatography to study their suitability for rapid detection as indicators to predict sensory quality evaluated by quantitative descriptive analysis. Smoked salmon odour contributed by guaiacol, boiled potato- and mushroom-like odours characteristic for fish lipid degradation and sweet odours associated with the microbial metabolites 3-methyl-butanal and 3-hydroxybutanone were the most intense odours. Other key volatiles were present in high levels but contributed less to the odours. These included furan-like compounds originating from the smoking, spoilage compounds like ethanol, 3-methyl-1-butanol, 2-butanone, and acetic acid along with oxidatively derived compounds like 1-penten-3-ol, hexanal, nonanal and decanal. Partial least square regression models based on data from storage studies of cold smoked salmon from Iceland and Norway verified that selected key volatile compounds performed better as predictors to explain variation in sensory attributes (smoked, sweet/sour rancid and off odour and flavour) than traditional chemical and microbial variables.

Keywords: Quality indicators; Cold smoked salmon; Volatile compounds; Gas chromatography; Sensory analysis; Electronic nose

1. Introduction

Odour is the primary parameter determining the sensory quality of products and consequently it is of interest to study if key volatile compounds contributing to the characteristic odours can be measured as indicators of quality. Many factors influence the quality of smoked fish products including the properties of the fish flesh, maturity, age, seasonal variations and factors involved in the smoking procedure such as type of wood, composition of the smoke, temperature, humidity, velocity and density of the smoke. The composition of compounds produced in the smoking process depends on the amount of oxygen supplied for combustion, the temperature in the fire zone, the type of wood burnt, and the moisture content of the wood. Specific volatile compounds in particular phenolic compounds have been related to the different smoking techniques which directly influence the sensory characteristics of smoked salmon (*Salmo salar*) (Cardinal et al., 1997) and in herring (Cardinal et al., 2006).

The typical smoke flavours result from a number of chemicals found in the smoke, but is mostly attributed to the phenols. Phenolic compounds, which are mainly produced by pyrolysis of lignin, are important for preservation and flavour properties of smoked products. The content of phenolic compounds in these products depends on the nature of wood. Phenolic derivatives like guaiacol (2-methoxyphenol) and syringol (2,6-dimethoxyphenol) have been identified as the most characteristic smoke related compounds in smoked fish-like herring (*Clupea harengus*) (Sérot et al., 2004). Guillén et al. (2006) analyzed headspace components of cod and swordfish where groups of phenol,

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guaiacol, and syringol compounds derived from wood pyrolysis were most noticeable of the smoke flavour volatiles. In addition to phenolic compounds, furan-like compounds have been reported to be responsible for the smoked odour in smoked salmon while carbonyl compounds, such as heptanal and (E,Z)-2,6-nonadienal, where characteristic in unsmoked fish, giving the flesh its typical fishy odour (Varlet et al., 2006).

The handling and conditions during processing and storage influence the spoilage changes in smoked salmon products and the development of sweet, sour, bitter, faecal, ammonia and cabbage-like off-flavours caused by microbial growth and autolytic changes (Hansen, Rontved, & Huss, 1998). Sulphurous, acidic, rancid, rubbery, cheesy and acidic off odours in spoiled smoked salmon have been associated with various bacterial groups by inoculating pure cultures into sterile products (Stohr, Joffraud, Cardinal, & Leroi, 2001). Knowledge about the potential of the different microflora to produce the volatile compounds contributing to the spoilage of the products is important for the establishment of spoilage indicators. The relationship between bacteria, the composition of the volatile fraction and the sensory quality of smoked salmon by multivariate analysis was studied by Joffraud, Leroi, Roy, and Berdagué (2001). Volatile compounds, identified in vacuum packed cold smoked salmon (*Salmo salar*) during cold storage at 5 °C, were mainly alcohols (i.e. 2-methyl-1-butanol, 3-methyl-1-butanol, 1-penten-3-ol, and 1-propanol) produced by microbial activity and contributed to the spoilage off-flavour of cold smoked salmon as confirmed by gas chromatography–olfactometry (Jørgensen, Huss, & Dalgaard, 2001). A multiple compound quality index based on 1-propanol, 2-butanone, and 2-furancarboxaldehyde was suggested for cold smoked salmon (Jørgensen et al., 2001).

Currently there is a need for rapid, automated, in-situ and objective tools for process monitoring and quality assurance of perishable food products. The possibility to use electronic nose for rapid quality control of smoked salmon products is therefore of interest. The study presented herein was a part of a European project (QLK1-CT-2002-71304) where an electronic nose (FishNose) with application specific sampling unit interfaced with the sensor module was developed and adapted for quality monitoring of smoked salmon (Haugen et al., 2006). Quality criteria for cold smoked fish was established to use in models based on the FishNose responses to classify cold smoked salmon of different quality. The quality was defined by sensory attributes (sweet/sour-, off- and rancid odour) and microbial criteria based on total viable counts (TVC) and lactic acid bacteria counts (LAB) (Olafsdottir et al., 2005a).

The aim of the study presented herein was to identify the key characteristic volatile compounds in cold smoked salmon by gas chromatography olfactometry (GC–O) and gas chromatography mass spectrometry (GC–MS) and study their suitability for rapid detection as quality indicators to predict sensory quality. The objective was to verify if the variation in the volatile compounds could explain the differences observed by sensory, chemical and microbial measurements that were earlier found to correlate well with the electronic nose (FishNose) responses (Haugen et al., 2006; Olafsdottir et al., 2005a). This provides basis for further development of rapid devices based on detection of volatile compounds as quality indicators. Storage studies of different cold smoked salmon products from three different producers were performed at 5 and 10 °C to obtain samples of different quality.

2. Materials and methods

Cold smoked salmon products were obtained from three smokehouses in Iceland and Norway (B, C, and D). The smoking time and temperature varied in the different locations according to their specifications. The time and temperature of the smoking process was 14–18 h at 16–22 °C, 5 h at 22 °C and 12 h at 28 °C in smokehouses B, C and D, respectively. Humidity during smoking was 50–60%. The smoking was performed 2–3 days after slaughtering by traditional smoking and dry salting. The cold smoked salmon products were sliced and vacuum packed, but one producer (B) vacuum packed the products as whole fillets. Storage studies were carried out at 5 °C (B) and at 10 °C (C and D) in laboratories in the respective countries. The temperature conditions were chosen to reflect conditions often encountered during distribution and 10 °C is an example of abusive conditions. The samples from the different producers were selected to represent products of various quality as can be expected for commercial products on the market. The storage studies were part of the Fishnose, EU project performed in 2003.

Chemical analyses of water, total fat, and salt content, were done to characterize the different products as described in Olafsdottir et al. (2005a). The microbial analyses included total viable counts (TVC), using modified Long & Hammer's medium (LH) and incubation at 15 °C (Van Spreekens, 1974), lactic acid bacteria (LAB) counts using nitrite-actidione-polymyxin (NAP) medium slightly modified (Davidson & Cronin, 1973) and Enterobacteriacae (EB) counts (ISO 7402-1985).

2.1. Sensory analysis

Sensory analysis based on quantitative descriptive analysis (QDA) (Stone & Sidel, 1985) was used to develop a detailed sensory scheme for smoked salmon as described earlier (Olafsdottir et al., 2005a). Nine trained panellists (age range, 30–55 y) from the Icelandic Fisheries Laboratories' sensory panel participated in the sensory assessments. They were selected and trained according to international standards (Standardization, 1993), including detection and recognition of taste and odour, training in the use of scales, and in the development and use of descriptors. The members of the panel were familiar with the QDA method and trained according to International Standards ([ISO] International Organization for Standardization,

1994) for the QDA assessment. One 1.5-h session was used for training of the panel using freshly smoked salmon samples and samples that had been stored for three weeks at 5 °C. The panellists evaluated the attributes and developed vocabulary to describe changes occurring in smoked salmon during storage. The descriptive words were accumulated and consensus was reached to limit the number of attributes. Thereafter the panel was trained in the use of an unstructured scale (15 cm) for the selected attributes. Odour evaluation refers to sniffing the samples while flavour evaluation refers to masticating in the mouth. The odour and flavour attributes were the following. Smoked salmon attribute was described as fresh, characteristic smoked salmon odour, i.e. honey-ham, smokehouse odour, smoked meat products, bacon, ashes, butter/caramel. Metallic – fresh salmon attribute was characterized by the descriptors metallic-like, sea, salty water, green/freshly cut grass. Rancid attribute was reminiscent of oxidized lipids, herring-like, paint-like, plastic/citrus fruit. Sweet/sour fruity spoilage attribute had fruit-like, sour/sweet fruity (melon, pears), sour table cloth, queasy odour, sour/fermented and acid/vinegar characteristics. Finally, off-odour and flavour was described as spoilage odour; amine, rubber, cheese/feet, blue cheese/musty, hydrogen sulphide/egg, cabbage/gas/garlic and faecal. The assessors evaluated the samples each time by using 19 descriptors of odour, flavour, appearance and texture. Herein the results of odour and flavour attributes, salt and bitter taste and fat secretion will be presented. Samples from each sampling day were kept frozen (−30 °C) until analyzed by sensory analysis all at once at the end of the storage time.

2.2. Purge-and-trap sampling

Prior to GC–MS and GC–O analysis, samples were collected by a purge-and-trap sampling (Olafsdottir, Steinke, & Lindsay, 1985). Frozen samples were thawed overnight at 4 °C and then homogenized with a Moulinex mixer. Samples were prepared by weighing 100 ± 2 g and 100 ± 5 g of saturated aqueous solution of NaCl into a 250 mL round bottom flask. Saturated NaCl solution (200 ± 5 g) was prepared as a blank sample. Heptanoic acid ethyl ester was added as an internal standard to all samples by adding 1 mL of 10-ppm aqueous solution of the standard to the sample solution. The sample was purged at room temperature with nitrogen at about 100 mL/min for 2.5 h (15 L). Volatiles were collected on 250 mg Tenax 60/80 (Alltech Associates Inc., Deerfield, IL, USA) in stainless steel tubes (Perkin–Elmer, Buchinghamshire, UK) for the combined ATD 400 and GC–MS measurements. For GC–O measurements traps were prepared with 150 mg Tenax in a Pasteur pipette. Each sample was prepared in duplicate.

2.3. GC–MS Measurements

Volatile compounds were thermally desorbed (ATD 400, Perkin Elmer) from the Tenax tubes onto a DB-5 ms column (30 m × 0.25 mm i.d. × 0.25 μm, J&W Scientific, Folsom, CA) and detected by GC–MS (HP G1800C GCD, Hewlett-Packard, Palo Alto, CA). Helium was used as a carrier gas and the following temperature program was used: 50 °C for 7 min, 50 °C to 120 °C at 5 °C/min and from 120 °C to 220 °C at 10 °C/min. The injection temperature was 250 °C and the detector temperature was 280 °C. The mass detector ion range was 35–300 m/z. Semi-quantitative evaluation of the concentration of volatiles was based on comparison of peak area to the peak area of the internal standard.

2.4. GC–O Measurements

Volatiles were extracted from the Tenax traps with 1 mL diethyl ether. The sample was then concentrated by passing nitrogen over the solution leaving a small amount of sample (20–30 μL) and 1 μL sample was then injected splitless onto the column. Measurements were performed on a GC (HP 5890, Hewlett-Packard, Palo Alto, CA) with the same type of column and the same conditions as for the GC–MS measurements. The end of the column was split 1:1 between flame ionization detector (FID) and an ODO-1 olfactory detector outlet (SGE, UK). Nitrogen, bubbled through water to add moisture, was used to drive the sample up to the sniffer. Two assessors describing the odour sniffed the effluent. Intensity (quality and duration/retention times) of each odour was determined using an intensity from 0 to 5, 0: not present; 5: very strong. The assessors were trained in recognizing characteristic spoilage odours and smoke odours by injecting into the GC–O, mixtures of standard compounds (100 ppm) dissolved in ether and sniffing the effluent. Two mixtures were prepared, i.e. rancid odours (hexanal, *cis*-4-heptenal, 2,4-heptadienal, 2,6-nonadienal, 2-nonenal and 2,4-decadienal) and smoke odours (2-methoxy-4-methyl phenol, 2-methoxy-4-[2-propenyl] phenol (eugenol), iso-eugenol, 2-methoxyphenol (guaiacol), phenol and, 4-methylphenol (*p*-cresol)). All standards were purchased from Sigma–Aldrich. GC–O measurements were only performed for samples from producer B.

Identification of the volatiles was done by matching retention indices (RI), calculated according to Van den Dool and Kratz (1963) based on ethyl esters (e.g. RI of ethyl pentanoate is 500) and verified by the database Flavornet (Acree & Arn, 2004), and mass spectra of samples with authentic standards (Sigma–Aldrich Chemical Co. St. Louis, MO, USA). Tentative identifications were based on standard MS library data (Hewlett Packard Co, 1997) and manually checked against literature sources and the database Flavornet (Acree & Arn, 2004). For the GC–O results the FID responses were not useful for quantification mainly because some of the odour active compounds were present in very low concentrations and were hardly noticed as peaks in the chromatogram. In addition, since diethyl ether was used for extracting the volatiles from the TENAX traps for the GC–O analysis, some of the early

eluting low molecular compounds were lost in the solvent peak in the chromatogram. Quantification of low molecular weight polar compounds on the TENAX is also difficult because of their large breakthrough volume on the TENAX.

2.5. Data handling

Multivariate analysis was performed by the Unscrambler 9.5 software package (CAMO AS, Trondheim, Norway). The main variance in the data set was studied using principal component analysis (PCA). All the data were mean centred and scaled to equal variance prior to PCA. Cross validation was used in the validation method. Partial least square regression (PLSR) models were calculated with variables based on selected key volatiles obtained by quantification by GC–MS data as X predictors and sensory data as Y response factors.

3. Results and discussion

3.1. Chemical, microbial and sensory characterization of the smoked salmon samples

The proliferation of the microflora is shown in Fig. 1 for all the sample groups stored at 5 and 10 °C. The initial microbial counts varied in the samples. The highest initial and maximum counts were found in samples from smokehouse D stored at 10 °C and more rapid growth was observed in the samples stored at 10 °C compared to the sample group B stored at 5 °C as expected. At sensory rejection the TVC is typically 10^7–10^8 cfu/g in cold smoked products and the microflora differs depending on the processes involved in the different smokehouses (Hansen, Gill, & Huss, 1995). Only the D sample was of unacceptable microbial quality reaching higher counts than 10^7 cfu/g on day 4 of storage. The initial EB counts were low which indicated good hygienic conditions in the factories but more active growth was observed in samples stored at 10 °C (Fig. 1). The results show that LAB became predominant in all the samples. At 10 °C, LAB counts were similar to TVC throughout storage (samples C and D). At the lower storage temperature (5 °C), LAB development occurred slowly but dominated in the end of the storage period (Fig. 1). This is in agreement with other studies showing that the LAB appear to be well adapted in vacuum packages and resistant to the high salt content found in smoked salmon products (Leroi, Joffraud, Chevalier, & Cardinal, 1998).

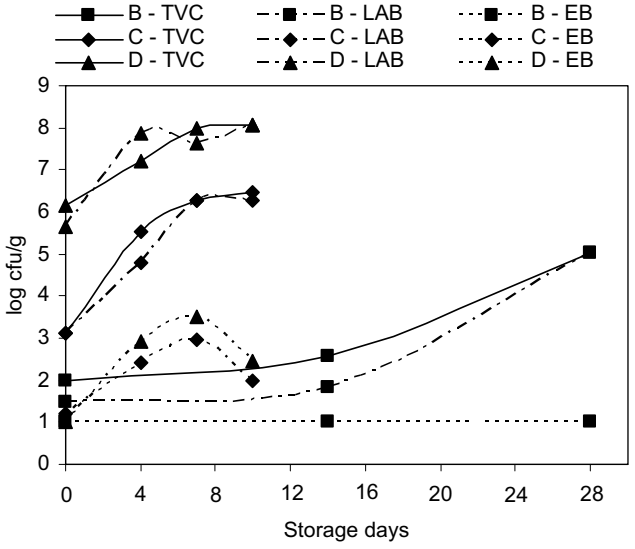

Fig. 1. Microbiological analysis (log cfu/g) of total viable counts (TVC)(—), lactic acid bacteria (LAB) (– – –), and Enterobacteriaciae (EB) (- - -), for samples from producer B (■) stored at 5 °C for 28 days and from producers C (♦) and D (▲) stored at 10 °C for 10 days.

Samples from producer B had the highest fat content of 15.6%, while fat content of samples C and D was 10.9% and 9.8%, respectively. The salt content was highest in samples from producer B (4.2%), 3.2% in samples from C and lowest in samples from D (2.8%). The high salt content in B (4.2%) may have contributed to slower spoilage rate and low microbial counts, however, the lower storage temperature (5 °C) most likely influenced slower growth of the microflora.

In parallel studies on smoked salmon products quality criteria for samples of marginal quality were established for microbial counts (TVC $< 10^5$ cfu/g, LAB $< 10^4$ cfu/g) and sensory odour scores (off odour <20, rancid odour <10, sweet/sour odour <20) (Olafsdottir et al., 2005a). According to these values the B samples were of acceptable quality throughout the storage study. Samples from producers C were not acceptable according to sensory sweet/sour odour criteria on day 0 (>20) and exceeded the TVC criteria on day 4. All samples from producer D were unacceptable according to the criteria for samples of marginal quality (Fig. 1 and Table 1).

Principal component analysis (Fig. 2) of all data obtained by the traditional quality measurements (sensory, microbial and chemical analysis) from different producers shows that 56% of the variance in the data set was explained by PC1 and 18% by PC2. PC1 and to some extent PC2, explained the difference between the samples that were clearly grouped according to producers. PC1 also explained the changes during storage as seen by the location of the B samples of acceptable quality on the left side of the plot and the marginally unacceptable D samples on the right with the highest scores for spoilage attributes and highest microbial counts.

The B samples were characterized by high smoke odour and flavour and low metallic and spoilage odours and flavours (Table 1 and Fig. 2). The bitter taste and fat secretion was also low in the beginning for the B samples and fat secretion increased significantly with time. Samples from producers C were characterized by high salt taste and metallic flavour (Fig. 2) while samples from producer D with the lowest salt 2.8% and fat content of 9.8% were characterized by high spoilage odour and flavour (Table 1 and

Table 1
Sensory scores, mean (standard deviation), of smoked salmon samples from producer B stored at 5 °C and producers C and D stored at 10 °C

Sensory attributes	Smokehouse B (5 °C) Days of storage						Smokehouse C (10 °C) Days of storage				Smokehouse C (10 °C) Days of storage			
	0	*n*	14	*n*	28	*n*	0	*n*	10	*n*	4	*n*	7	*n*
Smoked salmon odour	75.8	1	70.8 (6.4)	2	68.7 (0.7)	2	67.3 (5.8)	3	63.8 (3.2)	3	46.7 (7.5)	3	37.5 (9.4)	3
Metallic odour	29.5	1	32.0 (5.9)	2	39.2 (4.2)	2	37.9 (2.3)	3	36.0 (4.2)	3	37.8 (3.5)	3	37.0 (6.5)	3
Sweet/sour odour	3.7	1	4.5 (2.6)	2	6.3 (8.1)	2	26.5 (8.7)	3	30.3 (7.5)	3	25.5 (14.2)	3	21.1 (13.5)	3
Rancid odour	0.0	1	1.5 (2.1)	2	0.5 (0.7)	2	8.6 (10.0)	3	4.1 (7.1)	3	11.8 (6.3)	3	23.6 (20.0)	3
Off-odour	0.0	1	0.0	2	0.0	2	11.1 (11.6)	3	8.2 (12.6)	3	19.6 (16.1)	3	34.9 (27.7)	3
Smoked salmon flavour	74.2	1	66.4 (2.9)	2	68.9 (5.2)	2	67.9 (3.9)	3	68.8 (2.6)	2	48.5	1	–	0
Metal flavour	35.2	1	32.7 (7.5)	2	30.5 (7.8)	2	41.4 (3.8)	3	40.9 (1.2)	2	37.9	1	–	0
Sweet/sour flavour	5.5	1	7.2 (7.3)	2	9.0 (4.8)	2	28.1 (7.8)	3	34.5 (6.0)	2	39.9	1	–	0
Rancid flavour	0.0	1	1.7 (1.4)	2	1.0 (0.0)	2	9.2 (12.9)	3	1.7 (2.4)	2	9.3	1	–	0
Off-flavour	0.0	1	0.0	2	1.1 (1.6)	2	12.2 (6.8)	3	6.8 (4.8)	2	21.8	1	–	0
Salt taste	60.2	1	45.8 (6.5)	2	42.8 (11.3)	2	64.8 (14.3)	3	69.8 (13.7)	2	45.1	1	–	0
Bitter taste	8.3	1	13.8 (3.9)	2	11.7 (6.6)	2	42.0 (4.8)	3	43.4 (3.4)	2	40.4	1	–	0
Fat secretion	20.7	1	37.8 (4.1)	2	64.6 (20.4)	2	43.5 (6.1)	3	51.6 (0.1)	2	46.8	1	–	0

–: Not evaluated because of high bacteria count.

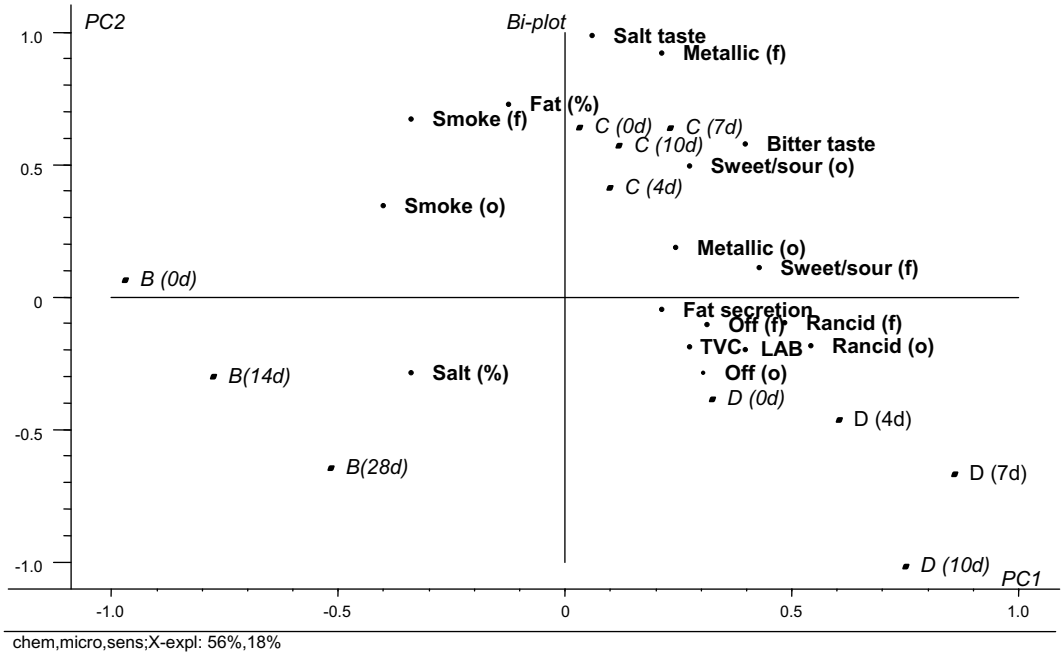

Fig. 2. PCA biplot of sensory odour (o) and flavour (f) attributes, salt and bitter taste, fat and salt content (%) and microbial results (log TVC and log LAB) for samples from producers C and D stored at 10 °C for 0, 4, 7 and 10d and B stored at 5 °C for 0, 14 and 28d.

Fig. 2). The characteristic smoked odour of the samples decreased in all samples in storage while the metallic odour only increased in sample B but remained similar in samples C and D (Table 1). The spoilage odours, sweet/sour-, rancid and off- odour scores increased with storage for the D sample stored at 10 °C and rancid and off-odour scores were highest in that sample in agreement with the highest bacterial counts in that sample.

The quality characterization of the samples, illustrated by the PCA (Fig. 2), demonstrates the impact and correlation of the different quality attributes and furthermore the need for the different methods to evaluate the overall quality. However, the long term aim is to provide a more simpler approach to characterize quality of products instead of lengthy and time consuming evaluation using laboratory based techniques and sensory analysis (Olafsdottir et al., 1997). Therefore, the aim of the GC analysis herein was to verify and give more background on the identity of volatiles that can be detected as quality indicators for smoked salmon products to underpin our earlier studies (Haugen et al., 2006; Olafsdottir et al., 2005a) and to stimulate further development of rapid detection means for quality. The selection of key volatiles that can be used to predict sensory quality is justified in the following sections based on analysis of samples of different defined quality from three producers. Criteria for selection of the key volatiles was based

on their role as odourants influencing the quality, their presence in samples in high enough levels to be quantified by GC–MS and also possible detection by rapid techniques. Finally their potential application to predict sensory attributes is demonstrated.

3.2. Characteristic odours and identification of key volatile compounds in smoked salmon

The main odours identified by GC–O analysis in freshly smoked and stored samples (14 and 28 days from producer B) are grouped in three classes according to their odour characteristics as illustrated in Fig. 3. The odour descriptions are listed in Table 2 according to retention times with corresponding compounds identified by GC–MS, but some of the components detected by GC–O were not identified. All the compounds that were identified in Table 2 have been reported earlier in smoked products (Cardinal et al., 1997, 2006; Guillén & Errecalde, 2002; Guillén et al., 2006; Joffraud et al., 2001; Jørgensen et al., 2001; Varlet et al., 2006). The most abundant compounds quantified by GC–MS in the samples were related to the smoking process and the onset of spoilage. However, they did not necessarily contribute to the most intense odours as can be explained by different odour thresholds.

3.2.1. Smoke related odour

The smoked salmon odour derived from the smoking process was pronounced and appeared to increase slightly during storage (odour scores 3–4) (Fig. 3). Guaiacol (2-methoxyphenol) was identified as the main compound contributing to the smokehouse odour detected by GC–O analysis of samples from producer B (Table 2). Guaiacol, 4-me-guaiacol and 2-me-phenol contributed to the "smokehouse, sweet", "wood smoke, sweet" and "smoke-like" odours, respectively (Fig. 3). The identity of other components (RI: 344–350, RI: 668 and RI: 831) contributing to the smoke like characteristic odours is unknown (Table 2).

The smoke related compounds phenol, methoxy phenol and furan derivatives were present in the highest level in the headspace of the samples from producer B that also had the highest smoke odour sensory characteristic (Table 1). The high intensity of smoke odour can possibly be

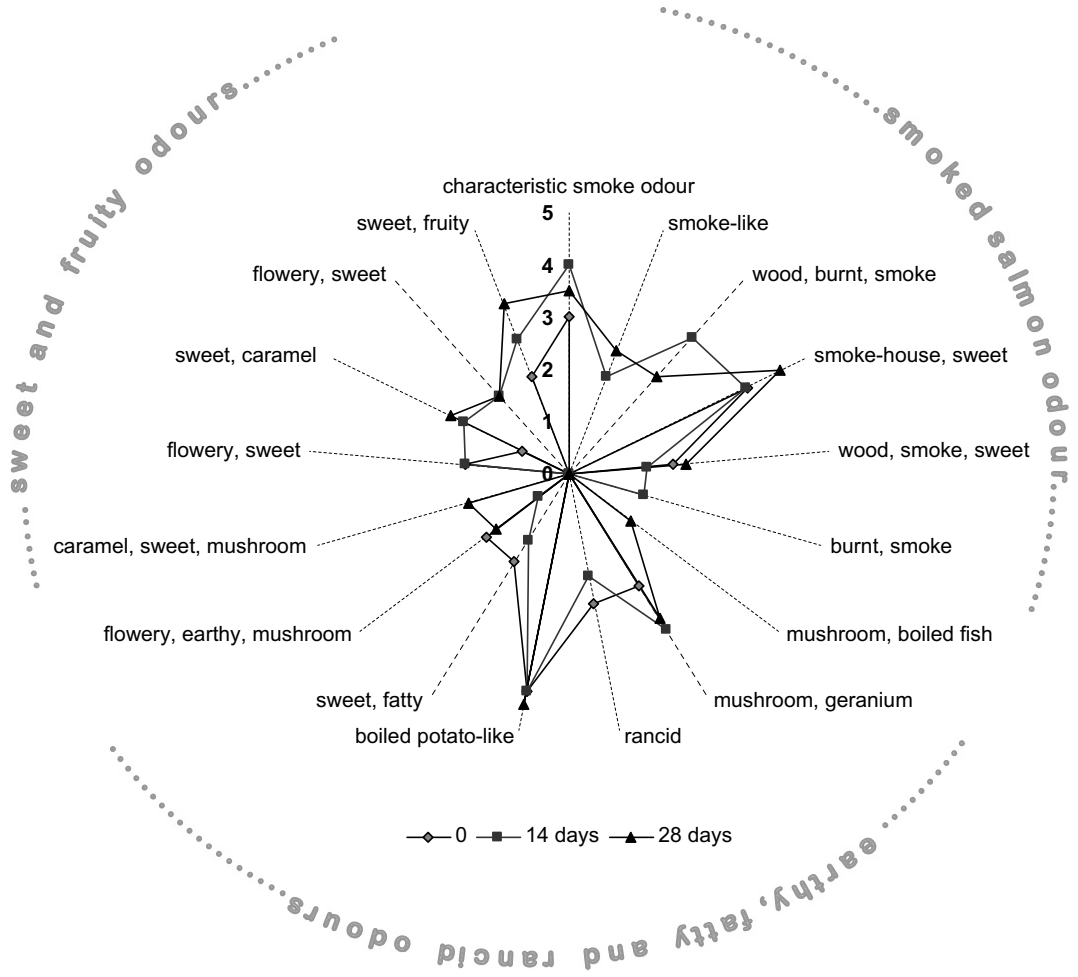

Fig. 3. GC–O odour evaluation of volatile compounds detected in cold smoked salmon samples from smokehouse B after 0, 14 and 28 days of storage at 5 °C. Odours also listed in Table 2 according to retention time.

Table 2
Volatile compounds in smoked salmon from producer B stored at 5 °C and producers C and D stored at 10 °C, odour evaluation by GC–O and quantification by GC–MS expressed as mean peak area ratio (stdv)

Compound	RI DB-5 ms[a]	Id means[b]	Odour description (GC–O)	Smokehouse B (5 °C) Days of storage			Smokehouse C (10 °C) Days of storage		Smokehouse D (10 °C) Days of storage	
				0	14	28	0	10	4	7
Ethanol	<165	MS	n.d.[c]	60.7 (17.4)	36.8 (25.3)	41.2 (15.7)	15.4	12.6 (9.3)	74.7 (6.7)	74.5 (87.8)
Acetic acid	188	MS	n.d.	14.5	5.9	64.8 (34.9)		333.0 (56.5)	17.3 (16.9)	41.7
2-Butanone	200	MS, 1	n.d.	106.6 (38.0)			160.7 (138.8)	72.5 (39.4)	77.1 (3.6)	93.9 (82.9)
Ethyl acetate	212	MS	n.d.				54.1 (44.8)		10.8 (0.3)	4.8
1-hydroxy-2-propanone	247	MS	n.d.	91.6 (8.6)		131.1 (60.7)	62.2	256.1		
2-Pentanone	259	MS	n.d.	35.2 (4.1)	22.3 (16.4)	24.0 (9.2)				
1-Penten-3-ol	271	MS, 1	n.d.				216.7 (138.1)	187.6	78.9 (45.5)	39.1 (26.4)
2,3-Pentanedione	282	MS	n.d.				93.6 (67.8)	74.9		
3-Hexanone	282	MS	n.d.					37.1		
3-Methyl-butanal	282	MS, 1, 2	Sweet, caramel, flowery		13.1 (5.4)	15.2				11.0
3-Hydroxy-2-butanone	294	MS, 1	n.d.	52.1 (5.4)	146.3	85.1	141.4 (88.6)	99.9 (82.3)	85.8 (19.6)	82.2
3-Methyl-1-butanol	316	MS, 1	n.d.	30.6 (2.2)					18.0 (0.2)	9.3 (5.5)
Unknown	344–350	2	Characteristic smoke odour							
Unknown	362–372	2	Bad, vomit							
Cyclopentanone	391	MS	n.d.	43.6 (12.5)	48.5 (31.7)	55.8 (19.2)	69.3 (38.8)	53.7 (30.4)	30.4 (0.1)	23.9
Hexanal	403	MS, 1	n.d.	32.7				69.0	29.2 (0.3)	41.3
3-Hydroxy-butanal	406	MS	n.d.			20.7				
1-Hydroxy-2-propanone	418	MS	n.d.		146.5					
Furfural	423	MS	n.d.	1158.7 (254.4)	1190.3 (230.8)	1309.6 (442.5)	164.2 (90.2)	31.8 (11.8)		15.0
2-Methyl-cyclopentanone	433	MS	n.d.						17.8 (0)	
3-Methyl-cyclopentanone	440	MS	n.d.			8.1 (4.2)				
Unknown	440–446	2	Flowery, sweet, alcohol							
2-Furanmethanol	442	MS	n.d.	212.1 (59.3)	355.5	416.4 (147.8)	114.9 (67.4)	113.2 (42.8)	24.6 (6.7)	19.1
Unknown	451–460	2	Flowery, earthy, mushroom							
2-Methyl-2-cyclopenten-1-one	493	MS	n.d.	210.0	170.3 (17.5)	168.9 (69.1)	108.3 (59.4)	87.8 (52.5)	22.2 (2.1)	25.4

1-(2-Furanyl)-ethanone	501	MS	n.d.	110.0 (23.0)	175.6 (17.4)	181.0 (66.3)	75.6 (41.1)	62.5 (31.7)	16.0 (0.8)	16.1
cis-4-Heptenal	497–500	1.2	Rancid							
Heptanal	505–509	1.2	Boiled potato, characteristic salmon							
5-Methyl-2-furancarboxaldehyde	506	MS	n.d.	28.0 (5.9)	38.9 (4.5)	38.9 (4.5)	10.4			
Unknown	558–565	2	Mushroom, boiled fish							
5-Methyl-2-furancarboxaldehyde	559	MS	n.d.	232.7 (49.7)	363.2 (20.0)	366.8 (129.8)				
1-Octen-3-ol	577–581	1,2	Mushroom, geranium							
Phenol	579	MS, 1	n.d.	28.2 (7.2)	27.8 (21.1)	36.6 (11.7)	38.4 (18.1)	41.7 (20.6)	8.4 (4.9)	5.6
(E,E)-2,4-heptadienal	612	MS, 1, 2	Sweet, fatty				12.3 (6.2)		4.2	
3,4-Dimethyl-2-cyclopenten-1-one	626	MS	n.d.			11.6 (5.7)			111.0 (14.0)	74.5
2,3-Dimethyl-2-cyclopenten-1-one	636	MS	n.d.		75.0 (0.7)	76.8 (33.8)				
Unknown	642–647	2	Caramel, sweet, mushroom							
Unknown	647–661		Flowery, sweet, heavy							
2-Methyl-phenol,	654	MS, 2	Smoke-like	15.9 (2.6)	18.6 (0.3)	20.0 (11.5)				
3-Ethyl-2-cyclopenten-1-one	662	MS	n.d.	13.3 (3.2)	18.6 (4.0)	18.8 (9.1)	14.5			
1-(2-Furanyl)-ethanone	665	MS	n.d.	32.3		42.3 (16.8)				
Unknown	668		Wood, burnt, smoke							
2-Methoxy-phenol	686	MS, 1, 2	Smoke-house, sweet, phenol	212.6 (46.7)	344.1 (14.0)	343.0 (131.6)	162.6 (90.3)	180.1 (88.2)	52.6 (16.2)	62.0
Nonanal	708	MS, 1	n.d.	15.7 (10.3)	38.1 (13.5)	34.8 (10.0)	48.7	29.4	18.4 (10.3)	18.8 (9.2)
Unknown	739–742	2	Sweet, fruity							
Naphthalene	787	MS	n.d.				35.0 (17.9)	41.2 (15.8)		
2-Methoxy-4-methyl-phenol	790	MS, 1, 2	Wood, smoke, sweet	70.6 (21.3)	94.9 (1.9)	97.4 (40.2)	61.3 (33.3)	71.9 (32.2)	14.3 (2.6)	
Decanal	813	MS, 1	n.d.	10.9	14.0	5.1 (0.1)			6.1 (3.1)	5.6 (2.2)
Unknown	831–835	2	Burnt, smoke							
Nonanoic acid	897	MS	n.d.	13.1	13.5					
4-Ethyl-2-methoxy-phenol	879	MS	n.d.	26.1 (1.7)	21.4 (0.5)		11.0 (6.1)	13.4 (4.6)		
2-Methoxy-4-vinylphenol	915	MS, 1	n.d.	6.7						
Undecanal	915	MS, 1	n.d.	14.9	23.2 (19.4)	13.0 (0.4)		5.7		

[a] Calculated ethyl ester retention index on DB-5 ms capillary column.
[b] Identification means: MS = mass spectra 1 = authentic standards; 2 = odour identification.
[c] n.d. = odour not detected by GC–O.

explained by the long smoking time and the type of wood used (Olafsdottir et al., 2005a). The samples from producer C contained higher level of smoking compounds than samples from producer D, despite similar smoking processing conditions. This could be explained by the fact that producer C used grinded beech wood, whereas producer D used grinded pine. Higher content of smoke compounds have been found in the water-insoluble fraction from pyrolysis oil (pyrolytic lignin) in beech compared to pine wood (Scholzea & Meier, 2001). The high levels of furfural in samples from producer B may be related to longer smoking time compared to producer C using the same type of wood. Furfural is a weak odourant and does therefore not contribute much to characteristic smoked aroma. Other furan-like compounds like 5-methyl-2-furancarboxyaldehyde and 1-(2-furanyl)-ethanone were identified and present in high levels in sample B and selected herein as potential key smoke related volatiles in addition to furfural, guaiacol, 4-me-guaiacol, 4-ethyl-guaiacol and phenol. Jørgensen et al. (2001) suggested earlier to use 2-furancarboxaldehyde along with other microbially derived compounds as quality indicators for cold smoked salmon.

3.2.2. Oxidatively derived earthy, fatty and rancid odours

Characteristic earthy, mushroom-like and fatty rancid odours derived from oxidative processes of the muscle constituents remained similar (odour scores 2–3) and slightly lower than the smoke like odours. The boiled potato like odour which contributed to the overall characteristic salmon odour was the most intense odour detected by GC–O (Fig. 3). Characteristic mushroom and geranium like odour was identified as 1-octen-3-ol originating from oxidation of polyunsaturated fatty acids in fish and known to contribute to the characteristic mild, fresh, plant-like aromas of fresh salmon (Josephson, Lindsay, & Stuiber, 1984). This compound has a an odour threshold of 10 ppb in water and was only identified by GC–O and confirmed by authentic standard. It is possible that other coeluting compounds with lower odour threshold may be responsible for the observed geranium like note.

Compounds contributing to the boiled potato like odour with a hint of characteristic smoked salmon as perceived by GC–O were identified as a combination of *cis*-4-heptenal and heptanal using authentic standards. Smoke related compounds were also identified in the same region by GC–MS (Table 2) contributing to the characteristic smoke odours. *cis*-4-Heptenal is derived from lipid oxidation of *n*-3 unsaturated fatty acids (McGill, Hardy, Burt, & Gunstone, 1974) and the odour has been described as boiled potato like (Josephson & Lindsay, 1987). Cardboard or paint-like odour and furthermore the'cold storage flavour' of cod has also been associated with *cis*-4-heptenal (McGill et al., 1974; Hardy, McGill, & Gunstone, 1979). The odour threshold in water for *cis*-4-heptenal is very low (0.04 ppb) (McGill et al., 1974) and can therefore have a high flavour impact.

cis-4-Heptenal and heptanal were not detected by GC–MS and 2,4-heptadienal was detected by GC–MS in very low concentration. These characteristic rancid type compounds could therefore not be used as predictors for sensory attributes in the PLSR model.

The oxidatively derived saturated aldehydes, hexanal, nonanal and decanal originating from n-9 unsaturated fatty acids were identified and quantified by GC–MS (Table 2). Their odour threshold is higher than the unsaturated lipid derived aldehydes but their sweet, green like and fatty characteristics may contribute to the overall sweet and fatty like odour of smoked salmon. Although their levels did not change much during storage, they were present in detectable levels, and were selected as potential indicators to predict quality. Lipid derived aldehydes, like hexanal and decanal have been reported to follow the same trend in cod fillets and contributed to the characteristic fish-like, sweet odours of fillets during chilled storage (Olafsdottir, Jonsdottir, Lauzon, Luten, & Kristbergsson, 2005b).

3.2.3. Microbially derived sweet and fruity spoilage odours

The compounds contributing to spoilage characteristics were present in lower concentration in smoked salmon samples during storage than the smoke related compounds (Table 2). This is in agreement with sensory analysis showing high intensity of smoked salmon odour/flavour and lower intensity of the spoilage attributes (Table 1). Among the spoilage related compounds selected as key spoilage indicators were short chain alcohols, aldehydes and ketones (e.g. ethanol, 2-butanone, 2-pentanone, 3-methylbutanal, 3-hydroxy-2-butanone and 3-methyl-1-butanol) (Table 2). Earlier studies have shown that microbially produced ketones, aldehydes and alcohols were abundant in the headspace of cold smoked salmon products during storage (Joffraud et al., 2001; Jørgensen et al., 2001). The spoilage related alcohols ethanol and 3-methyl butanol were highest in the D sample in agreement with the highest microbial counts. Alcohols have typically lower odour threshold than aldehydes and ketones and do not contribute as much to the spoilage odours. 2-Butanone was in the highest concentration in the D sample, in accordance with its highest spoilage level and correlated to sweet/sour and off odours and flavour attributes (Fig. 3). These compounds have been suggested earlier as microbial spoilage indicators in smoked salmon (Joffraud et al., 2001; Jørgensen et al., 2001).

Acetoin (3-hydroxy-2-butanone) was detected in high levels in all the samples. The role of acetoin in smoked salmon products appears to be related to its contribution to the characteristic pleasant "butter" like odour of the product. LAB that often predominate in smoked salmon products may contribute to the formation of this compound but lactic acid bacteria do not appear to be involved in the development of offensive spoilage odours (Leroi et al., 1998). The high levels of acetoin in samples B with low spoilage characteristic may be related to LAB being predominant throughout the storage. The increasing concentration and high levels suggest that acetoin may be useful as quality indicator for smoked salmon products.

The characteristics of smoked salmon related to the onset of spoilage were also explained by the presence of acids and esters. Lactobacillus ssp in combination with other bacteria strains are known to be able to produce acids, esters and sulphides (Joffraud et al., 2001). These compounds contribute to the sour, sickenly sweet and putrid odours that are characteristic for the later stages of spoilage when the obvious spoilage signs have developed with high sensory off odour scores. None of the samples in this study reached the advanced stage of spoilage where these off odours are typically dominating but acetic acid was detected in all the samples and ethyl acetate was present in samples C and D in agreement with their higher microbial load compared to sample B.

Ideally, quality indicators should demonstrate clear increasing or decreasing levels with storage time. However, this was not a clear trend for all the key volatiles selected (Table 2). It also needs to be acknowledged that there is generally a high variation in repeatability of measurements of volatile compounds by GC. Earlier studies on levels of microbial metabolites in fish during chilled storage have shown that their levels are indicative for the dominating spoilage bacteria and do not necessarily exhibit a consistent increase but rather may reach a plateau and then decrease (Olafsdottir et al., 2005b). Therefore, multivariate data analysis is important to explore the overall trend of the main quality indicators taking into account the complexity of the spoilage processes caused by the diversity of the microflora and their different spoilage potential.

3.3. Prediction of sensory quality by key volatiles in smoked salmon

To study the correlation of the key volatiles with sensory data a PLSR model was calculated to predict the sensory scores of flavour and odour attributes based on GC–MS data (Fig. 4). The key volatiles selected for the PLSR model were compounds present in high levels in the headspace and some of them contributed to the characteristic smoke odour, sweet like spoilage odours and characteristic fatty and sweet like odours as discussed before. The model based on samples from all the producers ($N = 7$) had the correlation, $r^2 = 0.59$ with a RMSEP of 8.8. The first two PLSR components explained 71% of the x-variables (key volatiles) and 86% of the y-variables (sensory attributes). For comparison it was of interest to study if data on fat and salt content and microbial counts (TVC and LAB) could predict sensory quality of the smoked salmon products. PLSR model based on the same data as used for the PCA (Fig. 2) had a correlation of $r^2 = 0.82$ and RMSEP of 7.2 where 96% of the X variables (chemical and microbial) were explained by the first two components and 58% of the Y variables (sensory attributes). This shows that a model based on the volatiles as predictors explained much better the variation in the sensory attributes than the traditional chemical and microbial measurements (86% and 58%, respectively).

It needs to be mentioned that earlier studies on identification of quality indicators to calibrate the FishNose system showed significant correlation of sensory and microbial data with gas sensor responses while the correlation with chemical parameters fat and salt content was low.

Smoked salmon odour and flavour correlated with the presence of high levels of furan-like compounds e.g. furfural, 5-methyl-2-furancarboxyaldehyde and 1-(2-furanyl)-ethanone, methoxy phenols like guaiacol, 4-methylguaiacol and 4-ethylguaiacol and phenol (Fig. 4). Samples with high scores of sensory related spoilage attributes (sweet/sour, rancid and off odour and flavour) were characterized by ethanol, 3-methyl-1-butanol, 3-methyl-butanal, 3-hydroxybutanone, 2-butanone, and acetic acid. The samples from the different producers were distributed similar as seen for the PCA of chemical, microbial and sensory attributes (Fig. 2). The odour characteristics specifically related to the smoking process show a clear discrimination of the samples from producer B on the left side explained by higher levels of the smoke compounds and low spoilage attributes. Oxidatively derived compounds contributing to rancidity like 1-penten-3-ol, hexanal, nonanal and decanal were detected in the samples and contributed to the overall fish-like sweet odour but did not explain much of the variation in the microbial and sensory quality of the samples. This is in agreement with earlier studies on cold smoked salmon products from the same smokehouses showing that the rancid attribute was not important in discriminating the spoilage level of samples (Olafsdottir et al., 2005a). Instead, sweet and sour spoilage odour appeared to explain the spoilage level and different quality of the products in agreement with high microbial counts and correlated well with the responses of the gas sensors in the Fishnose prototype. The sensors in the Fishnose were not selective towards individual compounds but appeared to have general selectivity towards very volatile compounds derived from microbial metabolism. Principal component analysis of gas sensor responses for samples of different quality from different producers clearly grouped samples according to spoilage level but discrimination between different smokehouses was not as obvious as seen for the volatiles herein. The sensors were not sensitive enough to detect increasing concentrations of the less volatile smoke related compounds like furfural and guaiacol (Olafsdottir et al., 2005a). Therefore, the Fishnose was suitable only to detect spoilage changes, but not differences in the quality related to the smoking process and content of phenolic compounds (Haugen et al., 2006; Olafsdottir et al., 2005a).

4. Conclusions

Key characteristic volatile compounds in smoked salmon responsible for the different quality of the products have been studied and compared with traditional methods to demonstrate that variation in the level of smoke related, spoilage and oxidatively derived compounds explains the different quality of the products. PLSR model with vola-

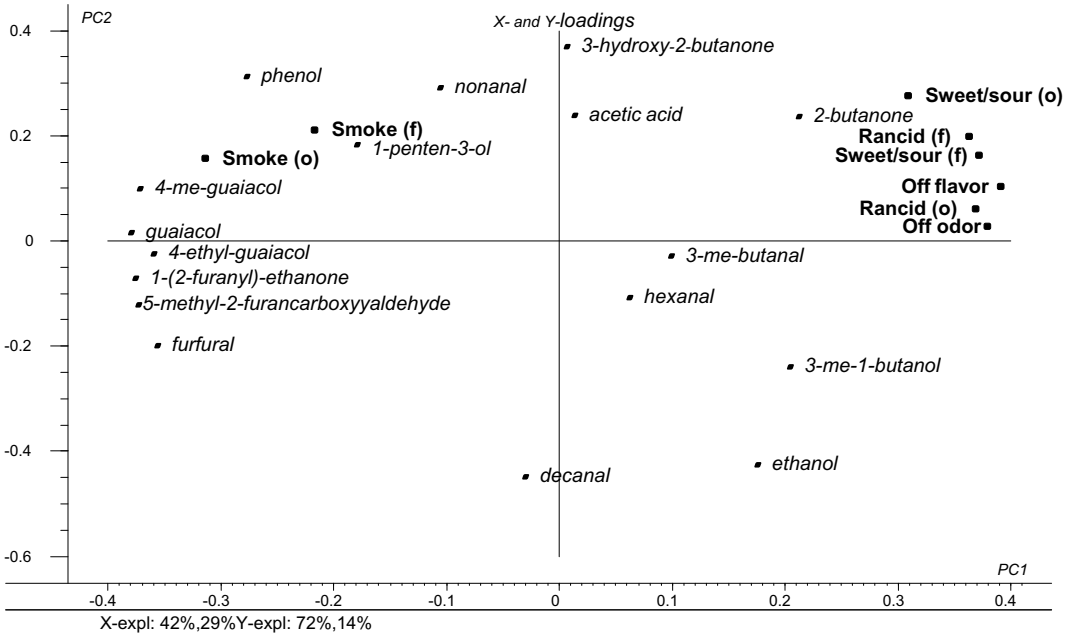

Fig. 4. Correlation loading plot from a partial least squares regression (PLSR) model based on volatile compounds as predictors and sensory odour (o) and flavour (f) attributes as response variables.

tiles as predictors for sensory attributes explained the contribution of the volatiles to the smoked, sweet/sour rancid and off odour and flavour. The studies have confirmed that smoke related compounds such as furfural, phenol, guaiacol and 4-methyl guaiacol are useful as indicators to discriminate products from different producers using various handling and smoking process. The spoilage related compounds like ethanol, 3-methyl-1-butanol, 3-methyl-butanal, 3-hydroxy-butanone, 2-butanone, and acetic acid are useful as quality indicators for monitoring spoilage level of smoked salmon products. However, oxidatively derived lipid degradation compounds are not important to discriminate products of different quality but their presence contributes to the characteristic fish odour of smoked salmon products. This information is useful when developing rapid instrumental devices like electronic nose to monitor the quality of cold smoked salmon. Some volatile compounds may be non odourants and may have neither an impact on the odour nor the quality. However, if present in high concentrations they may influence the response of the electronic nose. Therefore, it is important to identify key odourous or non odourous quality indicating volatiles and apply selective sensors for their detection. Innovative electronic noses equipped with sensors with known selectivity and sensitivity to key compounds could improve the performance of electronic noses for quality evaluation of smoked fish products.

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