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Volatile compounds and sensory profile of suspensions of fresh and stored oat flour from batches of pure Finnish cultivars

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ABSTRACT

Oats are healthy and suitable for versatile food applications, yet seldomly studied as pure cultivars. Due to their high content of unsaturated lipids and required dehulling and kilning processes, lipid oxidation affects the sensory properties of oat products. In this study, twenty batches of oat flour, each from a pure cultivar grown in Finland during the same growing season, were stored for six months. Volatile compounds and sensory profiles were analyzed using an oat suspension model. The overall flavor was described as mild. The panelists could differentiate the batches based on oat, bitter, and green flavor, texture, and appearance, which correlated with volatile profiles, and contents of moisture, fiber, β-glucan, and avenanthramides. A gas chromatography-olfactometry test of four selected flour batches revealed odor-active volatiles. Panelists could discriminate between zero- and six-month samples based on increases in odor and flavor intensity, and bitter flavor at six months linked to oxidation processes.

1. Introduction

Oats (*Avena sativa*) are a nutritious, well-balanced, and essential staple in the diets of the Nordic countries and many other regions across Europe and beyond. Their ability to thrive in sub-Arctic conditions makes oats the most cultivated cereal in Finland, alongside barley (Natural Resources Institute Finland). In 2023, Finland ranked among the top oat producers and exporters worldwide, coming in fourth for production and third for export volume (FAOSTAT; Tridge Database). Oats are valued for their nutritional benefits, such as a high content of dietary fiber, protein, beta-glucan, lipids, and other nutrients (Holopainen-Mantila et al., 2023), as well as their adaptability to various food applications and industrial processes. Interest in oats within the context of health-conscious, plant-based, and gluten-free diets is growing globally (Grand View Research, 2024; Holopainen-Mantila et al., 2023).

Oat groats are mainly processed into flakes and flour in the food industry (Holopainen-Mantila et al., 2023; Jokinen et al., 2021). In addition to traditional baked goods like bread, cookies, muffins, pancakes, and waffles, breakfast foods such as porridge and granola, and pasta, oat flour can be used, for example, in spoonable and drinkable products (Decker et al., 2014; Holopainen-Mantila et al., 2023), and infant foods (Webster, 2011). Furthermore, oat flour can act as a binder in plant-based foods, such as meat substitutes (Holopainen-Mantila et al., 2023), and as a thickener for soups and sauces. Oat flour can be further processed into oat protein isolates (Holopainen-Mantila et al., 2024) or extruded products (Lampi et al., 2015; Nikinmaa et al., 2023).

The volatile compounds of oats are a complex mixture, with aldehydes, ketones, and alcohols dominating (McGorrin, 2019). However, non-volatile compounds such as phenolics, sugars, amino acids, small peptides, free fatty acids, and lipids also affect oat flavor (Salmenkallio-Marttila et al., 2011). The final volatile profile of oat raw materials differs based on variations in the initial composition, pretreatment, and industrial processing steps. Heat treatments such as kilning, toasting, or extrusion promote the formation of pyrazines, pyrroles, furans, and other volatiles (McGorrin, 2019). Overall, the chromatographically detected volatile composition can be quite extensive (McGorrin, 2019; Puganen et al., 2024). However, not all detected volatile compounds are aroma-active, and even more importantly, not all aroma-active compounds are responsible for the overall aroma (Zhou et al., 1999).

Previously, the aroma of oats has been described as grainy, grassy, nutty, earthy, and buttery, while the flavor of oats is considered sweet, nutty, earthy, and cereal-like (Molteberg et al., 1996; Heiniö et al., 2002;

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Salmenkallio-Marttila et al., 2011). Raw oats have a mild aroma profile and develop desirable "oat-like" and "nutty" odor properties during heat treatment and further processing. Volatiles such as hexanal ("green" and "grassy") and 1-octen-3-ol ("mushroom-like" and "earthy") have been associated with fresh oats, while pyrazines and furans derived from Maillard reaction products during thermal treatment are responsible for the "nutty" and "caramel-like" aroma and flavor notes (McGorrin, 2019; Salmenkallio-Marttila et al., 2011). Heat treatment (e.g., kilning) is a necessary step in the industrial processing of oats to deactivate lipolytic enzymes and ensure product stability during storage. However, even heat treatment and proper storage conditions cannot fully prevent lipid autoxidation. As the non-enzymatic process progresses, an increase of aldehydes (pentanal, hexanal, octanal, nonanal), ketones, alcohols, and other volatiles is seen (Heiniö et al., 2002; Lampi et al., 2015; Molteberg et al., 1996; Puganen et al., 2024), and a rancid, soap-like, fatty, grassy, "painty," and bitter off-flavors appear (Cognat et al., 2014; Heiniö et al., 2002; Molteberg et al., 1996).

While a fair amount of research has been conducted mainly on commercial oats during the past two decades, literature on the volatile and sensory profiles of industrially processed oat flour of pure cultivars, especially from modern Northern oat cultivars, is to date minimal. Moreover, most articles discuss volatile or aroma compounds of oat flakes (Klensporf & Jeleń, 2005; Klensporf & Jeleń, 2008; Li et al., 2022; Li et al., 2023; Li et al., 2024; Schuh & Schieberle, 2005), oat pastry (Cognat et al., 2014; Dach & Schieberle, 2021a; Dach & Schieberle, 2021b), or extrudates (Lampi et al., 2015; Parker et al., 2000). Furthermore, there is limited scientific research on sensory evaluations of oat flours, especially regarding storage. Earlier storage studies focused on lipids and volatiles in extruded oat flour (Lampi et al., 2015; Sjövall et al., 1997), with samples stored for up to 15 or 18 weeks. In contrast, a more recent study on oat flake volatiles (Li et al., 2023) used longer storage times but employed accelerated storage conditions. Two studies from over two decades ago included sensory analysis as part of storage trials of oat flours, such as Molteberg et al. (1996) and Heiniö et al. (2002), but only tested three and one cultivar, respectively. As the freshness of raw oat materials affects the sensory properties of the products, it is important to determine how the flour holds up during storage and whether storage causes noticeable changes in its sensory quality.

Previously, we investigated the volatile profiles of dry oat flour and their changes related to lipid oxidation during a nine-month storage period (Puganen et al., 2024). Here, we link the volatile compounds determined from wet oat flours (suspension matrix) and their sensory properties to each other and to previously measured volatiles from dry oat flours, with all comparisons greatly benefiting from uniform processing of batches of pure cultivars in the same commercial mill. Studied oat batches represent the scarcely researched modern pure Northern cultivars, all grown during the same season. We hypothesize that while the volatile profiles of the batches vary, trained sensory panelists can distinguish between oat batches, as well as between fresh samples and those stored for six months, when evaluated as oat flour-water suspensions. This study was part of a comprehensive research project (OatHow) examining the relationships between the chemical and physical properties of oats and their processability, as described previously (Jokinen et al., 2021, 2022). As OatHow results follow uniform sample codes, the results obtained here are linked with previously published results on total tocol content, avenanthramides, and saponins (Pöysä et al., 2024), further enhancing the scientific and industrial relevance of our research. Regarding methodology, the volatiles were analyzed as suspensions by headspace solid-phase microextraction gas chromatography–mass spectrometry (HS-SPME-GC–MS), and the sensory assessments were carried out using trained sensory panels utilizing generic descriptive analysis (GDA), discrimination tests, and gas chromatography-olfactometry (GC-O) to identify aroma-active compounds.

2. Materials and methods

2.1. Oat samples

The sample material for this study consisted of 20 batches of wholegrain and heat-treated oat flour from pure oat cultivars grown in Finland in 2019. The oat grains were obtained from Boreal Plant Breeding Ltd. (Jokioinen, Finland), Lantmännen Agro Ltd. (Vantaa, Finland), Peltosiemen Ltd. (Forssa, Finland), Plantanova Ltd. (Ruukki, Finland), Raisio plc (Raisio, Finland), and Vääksyn Mylly Ltd. (Vääksy, Finland). Native hulled grains were equally treated with an industrial-scale milling process at Vääksy Mylly Ltd. (Asikkala, Finland) by drying, dehulling, kilning, flaking, and milling. Enzyme deactivation was ensured and confirmed by lipase activity measurements. This study did not control for factors such as soil conditions, fertilization, and threshing time. Thus, the samples are referred to as batches of pure cultivars rather than cultivars. The composition and detailed processing parameters of the oat batches are described in Jokinen et al. (2021), and the same sample codes are used here.

The flour samples were stored in paper bags in a storage room cabinet at room temperature (22 °C) (Puganen et al., 2024). The fresh oat batches originate from the zero-time point ($t = 0$), and the stored oat batches were collected after a six-month storage period ($t = 6$). For the volatile profile and GC-O analysis, oat flours were frozen in borosilicate glass laboratory bottles and, for the sensory evaluations, in plastic bags. Freshly milled and stored samples were frozen immediately after collection and kept at −20 °C until use.

This study was conducted as part of the research project OatHow, as previously described by Jokinen et al. (2021, 2022). According to Jokinen et al. (2021), the protein content of the oat batches (F11–F30) ranged from 13.1% to 18.2% of dry matter (DM). The starch content varied between 57.8% and 63.8% (DM), and the lipid content ranged from 6.0% to 9.4% (DM). The total dietary fiber content was between 9.5% and 13.2%, and the β-glucan content varied from 2.9% to 4.6% (DM). Furthermore, the total tocol content of the oat batches was 29.5–43.3 μg/g DM, the avenanthramide content ranged from 13.7 to 129.2 μg/g DM, and the saponin content fell between 0.28 and 0.59 μg/g DM (Pöysä et al., 2024). The lipid profile of the extracted oat oil and the volatile profile of the dry flour samples were reported by Puganen et al. (2024). Additionally, the same batches of oat flour were used in parallel studies for bread-making (Pöysä et al., 2024; Sammalisto et al., 2021) and extruded products (Nikinmaa et al., 2023).

The suspension matrix, a mixture of oat flour and water, was selected to address the sensory evaluations. Suspension samples were prepared in a ratio of 1:2.5 (w/w) based on the pre-test. Additionally, a 10% NaH_2PO_4 salt was added to the suspension samples for GC–MS and GC-O analyses to enhance analyte extraction efficiency (Fiorini et al., 2015; Wardencki et al., 2004).

2.2. Chemicals

An aliphatic mix of C5-C12 (Sigma-Aldrich, USA) and saturated alkanes of C7-C30 (Supelco, Sigma-Aldrich, USA) were used as standards to calculate retention indices of compounds in HS-SPME-GC–MS and GC-O analysis. Sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$, 99.9%, VWR Chemicals, Belgium) was used for volatile analysis. External standard compounds used for volatile compound identification in GC-O are listed in **Supplementary Table 1**.

2.3. Analysis of the volatile profile of oat suspensions

The volatile compound composition was analyzed using an HS-SPME injector and a GC–MS instrument, the Thermo Scientific Trace 1300 GC, ISQ 7000 triple quadrupole MS, and TriPlus RSH autosampler (Waltham, MA, USA) with a DB-624 Ultra Inert (UI) capillary column (60 m × 0.25 mm i.d., 1.4 μm film thickness; Supelco, Bellefonte, PA, USA). For

sample preparation, oat flours were allowed to reach room temperature for 1.5 h, and 2.50 g of sample was weighed into a 20 mL SPME vial. The vials were placed in the SPME vial holder and then in a plastic box, where they were kept overnight at +4 °C in a cold room before analysis.

Oat suspensions for the analysis of the volatiles were prepared as follows: on the day of analysis, 6.25 g of a salt solution (10% NaH_2PO_4, 1:2.5, w/w) was added to the oat flour vials by pipetting 2 × 2.98 mL and mixing carefully with a thin metal spatula for 1 min. Each suspension sample was incubated in the instrument for 20 min at 50 °C. The extraction of volatile compounds was conducted at 50 °C for 30 min using a divinylbenzene/carboxy/polydimethylsiloxane (DVB/CAR/PDMS) fiber (50/30 μm film thickness; Supelco, Bellefonte, PA, USA), as described by Puganen et al. (2024). Analyses were performed in triplicate, and the samples were randomized. The volatile compounds were identified by comparing their MS library match numbers using the NIST MS Search library database (version 2.3, National Institute of Standards and Technology, Gaithersburg, Maryland, USA) and by calculating retention indices from alkane standards (C5-C12). In addition, the calculated retention indices and mass spectra of the compounds were compared with data from our previous study (Puganen et al., 2024) on dry oat flours, run on the same column, and retention indices calculated using Agilent GC column solvent retention table (Agilent Technologies, Inc, 2014) for DB-624. A tolerance for the RI of ±10 units was considered. Alkanes standards C7-C30 were not used for GC–MS compared to GC-O. Instead, retention indices of volatiles detected in GC-O, as well as those of external standards, were used as supporting evidence for the GC–MS results. GC–MS data were processed using Chromeleon 7.2.10 ES MUH software (Thermo Fisher Scientific, Waltham, MA, USA).

2.4. Sensory analysis

2.4.1. Ethics and design of sensory evaluations

The study was conducted in accordance with the principles outlined in the Declaration of Helsinki for research involving human subjects. Informed consent was obtained from all participants, and the European Union's General Data Protection Regulation (GDPR) was followed. Participants were informed of their right to withdraw from the study at any time without providing a reason. Our institution did not mandate pre-ethical approval for sensory studies at the time of the sensory evaluations.

Due to variations in the arrival of the sample batches, storage duration, and the large number of samples, the sensory evaluations were conducted in three parts. Two panels were used for the descriptive analysis, and a third panel was used for the discrimination test of storage-trial samples.

2.4.2. Generic descriptive analysis (GDA)

Two partially distinct panels were recruited from the staff and students at the University of Turku. The sensory studies were conducted in a sensory laboratory under controlled conditions in accordance with ISO 8589. Prior to the study, participants were briefed on the protocols and test settings, and written consents were secured. This was followed by two training sessions, during which the panelists were familiarized with the sensory attributes and instructed to evaluate the samples consistently. A total of 13 attributes (**Supplementary Table 2**) were predetermined through a pre-test, ensuring the selection of key attributes for this study (data not shown). The first panel (n = 11, 8 women) evaluated samples F11–F22 and the second panel (n = 10, 6 women) samples F23–F30. Samples were presented monadically to the panelists, and each sample was evaluated in triplicate.

Oat suspensions for the sensory analysis were prepared as follows: Approximately 10 g of each oat suspension (in a 1:2.5 ratio of oat flour to water, w/w) was prepared and served for each volunteer. The evaluation procedure and premises were as described in Nikinmaa et al. (2023), with the exception that the intensities of the attributes were rated on a line scale 0–10 (ranging from “mild” to “intense”), and a compulsory break between samples was set to 20 s. Control samples were used to compare the evaluations of different panels and sample sets. The data were collected using Compusense Cloud version 21.0 (Compusense Inc., Guelph, Ontario, Canada).

2.4.3. Triangle test (0 vs 6 months)

The third panel (n = 11; 8 women; consisting of staff and students from the University) underwent two training sessions (1 h/session) prior to the evaluations, in the same facilities used for GDA analysis. During the training sessions, assessors were acquainted with the triangle test procedure, which involves identifying the odd sample among three training samples based on odor, flavor, or texture. The samples included basic taste solutions, hexanal odor at two concentrations, and water suspensions of fresh and stored oat flours (in a 1:2.5 oat-to-water ratio).

The suspensions of each fresh and stored sample (F11–F30; 0 and 6-month time points) were evaluated by a panel in duplicate across three sessions. The duplicates were assigned to different sessions. The order of sample presentation within each set and the order of the sets were randomized. A 30-s break was implemented between each set as a compulsory rest period. After each triangle test, panelists were asked to rate their certainty about their choice (scale 1–3; 1 “I guessed,” 2 “I'm quite sure,” 3 “I'm sure”) and to select the reason for the observed difference (Check-All-That-Apply for four attributes: appearance, odor, flavor, texture; optional to describe the reason in an open-ended answer further). The data were gathered using Compusense Cloud.

2.4.4. GC-O panel recruitment and training

Panelists (n = 5) with previous experience of sensory evaluations were selected among the staff and students at the University of Turku. The first training session followed the sniffing bottles training method described by Aisala et al. (2019) with slight modifications. The session consisted of the panelists' training to describe odors (**Supplementary Table 3**, training 1 A) and rate the intensities of 1-octen-3-ol, 2,4-decadienal, and hexanal at three concentrations (**Supplementary Table 3**, training 1 A–C) on a scale from 0 (no odor) to 4 (recognizable, very strong odor). The odor solution of 5 μL was placed into a dark 30 mL glass vial containing a filter paper of 1 cm^2. The next training session was to familiarize participants with the GC-O equipment and evaluation method (Aisala et al., 2019). The second training session was conducted using an odor mixture (**Supplementary Table 3**, training 2), with a sniffing time of 18 min, while the third session used F25 with a sniffing time of 25 min.

2.4.5. GC-O sample preparation and analysis

Oat suspensions for the GC-O analysis were prepared as follows: A total of 12 g of oat flour was mixed with 30 g of 10% NaH_2PO_4 (1:2.5, w/w) and incubated for 10 min at 50 °C and 800 rpm using a magnetic stirrer (IKA® RCT basic, Staufen, Germany). Volatile compounds from the suspension headspace were collected using an SPME fiber (DVB/CAR/PDMS, 50/30 μm film thickness; Supelco, Bellefonte, PA, USA). The adsorption time was 45 min, followed by a desorption time of 5 min.

The analysis was performed using an HP 6890 series gas chromatograph (Agilent Technologies, Santa Clara, CA) equipped with a flame ionization detector (FID) and an olfactory detector port ODP-1 (Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany). The column effluent was equally split between the FID and the ODP. The sniffing port was mounted on the side wall of the GC and supplied with humidified air at a flow rate of 65–70 mL/min.

Two columns were used to separate the extracted compounds: DB-624 UI (60 m × 0.25 mm × 1.4 μm, Agilent Technologies, J&W Scientific, USA) and HP5MS (30 m × 0.25 mm × 0.25 μm, SGE). The injector temperature was set at 260 °C for HP5MS and 240 °C for DB-624 with splitless mode. Helium was the carrier gas, maintaining a constant linear flow velocity of 32 cm/s for HP5MS and 38 cm/s for DB-624. The FID temperature was held at 290 °C. For HP5MS, the initial oven temperature was set to 30 °C, held for 5 min, then increased at a rate of 5 °C/min

to 140 °C, followed by 20 °C/min to 240 °C, and held for 3 min. The total run time was 35 min, including an evaluation time of 25 min. For DB-624, the initial oven temperature was set to 40 °C, held for 6 min, then increased at a rate of 25 °C/min to 100 °C, followed by 7 °C/min to 220 °C, and held for 10 min. The total run time was 35.54 min, including an evaluation time of 25 min. The data were collected as outlined in Aisala et al. (2019), along with the intensities of each odor. The volatile compounds were identified using the NIST database, reference compounds (**Supplementary Table 1**), matching odor descriptions, retention indices, and comparisons with data from Section 2.3 and Puganen et al. (2024).

2.5. Data analysis

The volatile compound composition of oat suspensions was reported as average values of integrated peak areas. The Shapiro-Wilk test was used to assess the normality of the volatiles data. The statistical difference between fresh and stored oat flours, analyzed as suspensions, was measured using data from all oat batches for all possible volatiles at both the zero- and six-month time points. The significance of the difference between fresh and stored oat flours analyzed as suspensions was determined by the nonparametric Wilcoxon signed-rank test ($p < 0.05$), as the data were only partially normally distributed. To observe more distinctions between the various oat batches of the fresh and stored samples, principal component analysis (PCA) was applied. The chemometric analyses were conducted using Unscrambler® X version 11.0 (Camo Process AS, Oslo, Norway). The data were mean-centered and weighted (1/sdev). For GDA, one-way ANOVA and Tukey's post hoc test were utilized. These analyses were performed using IBM SPSS Statistics software (version 29.0.2.0, IBM, New York, USA). The linear regression and correlation test between chemical properties and sensory attributes (**Supplementary Table 4**) was performed using Origin 2016 Sr2 b9.3.2.303 (OriginLab Corporation, Northampton, MA, USA). The binomial distribution *p*-values for triangle test results were calculated according to Lawless and Heymann (2010).

3. Results and discussion

3.1. Volatile profile of oat suspensions

A total of 40 volatile compounds were identified from the analyzed fresh ($t = 0$ months) and stored ($t = 6$ months) samples (Table 1). Among

Table 1
Volatile compounds identified in fresh and stored oat samples analyzed as suspensions by HS-SPME-GC–MS using a semipolar column DB-624.

#	Compound	RT (min)	RI	NIST match	0 months*		6 months*		Wilcoxon test	Chemical group
					mean	SD	mean	SD	(*p*-value)	
1	Pentane	7.0	500	by standard	2.06	0.60	1.54	0.56	0.004	Alkane
2	2-Methylpropanal	10.7	593	907	4.35	0.26	1.78	0.49	<0.001	Aldehyde
3	1-Propanol	11.6	613	772	0.64 [a)]	0.42	n.d.	n.d.		Alcohol
4	Butanal	12.4	629	955	1.17	0.18	3.36	0.67	<0.001	Aldehyde
5	2,3-Butanedione	12.5	631	859	3.97	0.39	3.33	0.79	<0.001	Ketone
6	2-Butanone	12.9	639	948	7.62	0.99	4.26	1.05	0.073	Ketone
7	3-Methylbutanal	15.4	692	938	20.12	6.13	10.78	2.22	<0.001	Aldehyde
8	Acetic acid	15.6	696	921	15.06	3.60	9.11	2.46	<0.001	Acid
9	2-Methylbutanal	15.8	700	893	24.02	3.29	13.73	4.69	<0.001	Aldehyde
10	2-Pentanone	17.2	730	890	0.67	0.10	0.97	0.34	0.002	Ketone
11	Pentanal	17.5	735	953	6.59	0.96	39.22	6.88	<0.001	Aldehyde
12	4-Methyl-2-pentanone	19.6	782	865	0.84	0.16	0.48	0.08	0.167	Ketone
13	2-Propylfuran	20.8	808	910	n.d.	n.d.	1.57 [b)]	0.15		Furan
14	1-Pentanol	21.9	833	871	3.36	0.96	10.09	2.34	<0.001	Alcohol
15	Hexanal	22.1	839	977	62.14	6.26	537.86	60.33	<0.001	Aldehyde
16	(*E*)-2-Hexenal	25.0	907	952	n.d.	n.d.	0.94	0.11		Aldehyde
17	2-Butylfuran	25.1	910	900	n.d.	n.d.	1.26	0.14		Furan
18	2-Heptanone	26.0	934	873	2.70	0.93	17.04	2.30	<0.001	Ketone
19	Heptanal	26.4	942	880	1.63 [c)]	0.29	12.63	1.31	<0.001	Aldehyde
20	α-Pinene	26.8	954	940	92.58	10.90	7.92	1.22	0.911	Terpene
21	Camphene	27.6	974	959	6.78 [d)]	0.96	n.d.	n.d.		Terpene
22	2-Pentylfuran	29.0	1010	933	1.71	0.68	7.48	0.60	<0.001	Furan
23	Benzaldehyde	29.7	1027	929	2.87	0.98	5.19	0.59	0.017	Aldehyde
24	6-Methyl-5-hepten-2-one	29.8	1030	854	n.d.	n.d.	5.23	0.62		Ketone
25	3-Carene	29.9	1031	951	33.32	4.79	n.d.	n.d.		Terpene
26	1-Octen-3-ol	29.9	1033	844	n.d.	n.d.	3.37	0.44		Alcohol
27	Octanal	30.3	1042	959	n.d.	n.d.	4.74	0.45		Aldehyde
28	D-Limonene	30.6	1051	887	28.23 [e)]	6.19	n.d.	n.d.		Terpene
29	Hexanoic acid	30.6	1051	937	2.95 [f)]	0.56	10.51	1.73	0.001	Acid
30	*p*-Cymene	30.8	1054	956	4.37 [g)]	0.74	n.d.	n.d.		Terpene
31	β-Phellandrene	30.9	1058	888	3.09 [h)]	0.40	n.d.	n.d.		Terpene
32	(*E*)-3-Octen-2-one	32.3	1094	924	n.d.	n.d.	3.39	0.23		Ketone
33	(*E*)-2-Octenal	33.3	1117	949	n.d.	n.d.	2.88 [i)]	0.20		Aldehyde
34	(*E*,*Z*)-3,5-Octadien-2-one	33.2	1115	921	0.59	0.35	1.58	0.12	0.028	Ketone
35	Nonanal	34.5	1147	926	3.13	0.84	8.68	0.95	<0.001	Aldehyde
36	(*E*,*E*)-3,5-Octadien-2-one	34.9	1158	934	0.83	0.22	2.51	0.20	<0.001	Ketone
37	5-Ethyldihydro-2(3H)-furanone	35.2	1165	887	0.44	0.15	1.03	0.07	<0.001	Furanone
38	(*E*)-2-Nonenal	37.4	>1200	896	n.q.	n.q.	0.31	0.04		Aldehyde
39	Decanal	38.7	>1200	873	n.q.	n.q.	0.50	0.06		Aldehyde
40	(*E*,*E*)-2,4-Nonadienal	40.2	>1200	905	n.d.	n.d.	2.89 [j)]	0.41		Aldehyde

* Average peak area of volatile compound of all quantifiable samples in counts per s per 10^5 analyzed by headspace solid-phase microextraction with gas chromatography mass spectrometer detection (HS-SPME-GC–MS). In the table, RT presents retention time (min), RI calculated Kováts retention indices and NIST match number. The n.d. abbreviation stands for not detected and n.q. for detected but not quantifiable.

[a)] no data in F23, F27, F28, F29, and F30; [b)] only in F28; [c)] excluding data of F12, F14, F18 due to the other peak's interference; [d)] only in F11, F12, F14 and F18; [e)] only in F12; [f)] no data in F11, F12, F14, F18, and 23; [g)] only in F11, F12, F14, and F18; [h)] only in F11, F12, F14, and F18; [i)] excluding data of F12, F18, F19, F23, and F26 as peak co-eluted with another peak; [j)] only in F28.

these compounds were 15 aldehydes, 9 ketones, 6 terpenes, 3 furans, 3 alcohols, 2 acids, 1 alkane, and 1 furanone. Aldehydes and ketones were the most abundant compound groups, consistent with previous findings in oat samples (Heiniö et al., 2002; Klensporf & Jeleń, 2005; Klensporf & Jeleń, 2008; Li et al., 2022; Li et al., 2023; Puganen et al., 2024). The profiles of volatile compounds in suspensions were nearly identical to those in the corresponding flours (Puganen et al., 2024). Still, they were present at lower levels, highlighting the impact of water on volatile release. Klensporf and Jeleń (2005) observed that adding 40–60% water to oat flakes and milled powders decreased the extraction efficiency of volatile compounds. Our results showed a similar reduction in SPME efficiency and an increase in background noise in GC–MS with a flour-to-water suspension ratio of 1:2.5, resulting in 71.5% water in our samples.

3.1.1. Volatile compounds of fresh samples

The primary volatile compound found in suspensions of fresh samples was α-pinene, followed by hexanal, 3-carene, D-limonene, 2-methylbutanal, 3-methylbutanal, and acetic acid (Table 1). Other compounds appeared at lower intensities. Compared with our previous study (Puganen et al., 2024) on dry oat flours, suspension samples exhibited lower levels of terpenes, including α-pinene, 3-carene, and D-limonene. Interestingly, the water suspension matrix revealed clear peaks of 2,3-butanedione, hexanoic acid, and 6-methyl-5-hepten-2-one, previously undetected in the corresponding dry flour samples. In addition, the release of 3-methylbutanal increased (peak area doubled in the suspension model compared to dry oat samples), but the release of 2-methylbutanal remained approximately the same as in the dry oat samples. This behavior highlights matrix effects on the release of volatiles. The addition of water and salt changes the polarity of the matrix but can also cause changes in the conformation of compounds like starches or proteins, revealing new binding sites for volatiles (Damerau et al., 2014; Klensporf & Jeleń, 2005).

The PCA performed on volatiles from fresh samples (Fig. 1A and B) showed significant variability among oat batches in their volatile profiles. The most distinct samples were F12, located in the upper left corner of PC1, and F24 and F25, which were far from the other samples on the right side near the x-axis. The F12 sample differed from other batches due to its high levels of α-pinene and 3-carene, whereas samples F24 and F25 were associated with typical volatiles produced by autoxidation and the Maillard reaction.

3.1.2. Volatile compounds of stored samples vs. fresh samples

In the stored samples, the hexanal peak was the most abundant volatile detected in the suspensions (Table 1). Other volatiles with medium-sized peaks and lower intensities than hexanal included pentanal, 2-heptanone, 2-methylbutanal, heptanal, 3-methylbutanal, 1-pentanol, acetic acid, and hexanoic acid. Additional detected peaks appeared at minor levels. As expected, the levels of aldehydes (e.g., butanal, pentanal, hexanal, heptanal, octanal, nonanal) derived from lipid autoxidation of oleic and linoleic acid, as well as ketones (e.g., 2-

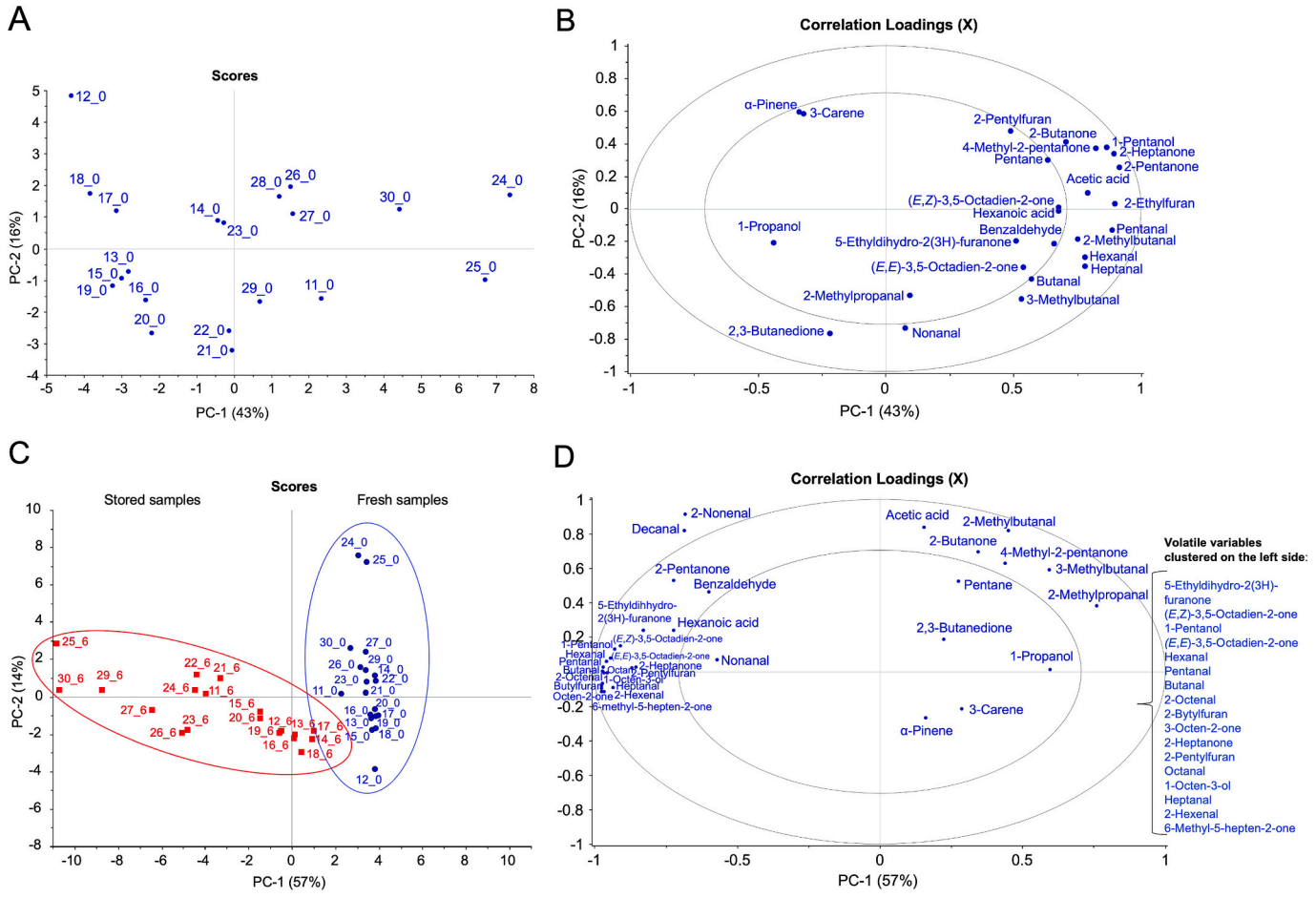

Fig. 1. Principal component analysis (PCA) of the volatile profile of oat suspension samples ($n = 20$). (A) PCA scores plot displaying fresh samples from different oat batches analyzed as suspensions. (B) PCA loadings plot showing volatile compound variables ($n = 26$) present only in samples at 0 months. (C) PCA scores plot displaying oat flours from different oat batches analyzed as suspensions during storage (blue ● = 0 months and red ■ = 6 months), excluding sample F28. (D) PCA loadings plot showing volatile compound variables ($n = 36$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Sensory attributes and their mean intensities of fresh flour samples analyzed as suspensions using the generic descriptive analysis method.

	Odors								Appearance and texture								Flavors											
	Intensity		Oat		Sweet		Roast		Color darkness		Dark particles		Thickness		Stickiness		Intensity		Oat		Sweetness		Bitterness		Green	
F11	3.87	a	4.30	a	1.16	a	1.31	a	4.15	abc	3.89	abcde	4.68	bc	3.46	bc	4.65	a	3.87	ab	1.02	a	2.09	abc	2.43	bcd
F12	3.57	a	3.94	a	0.96	a	1.00	a	4.15	abc	4.55	bcdef	4.85	bc	3.44	bc	5.29	a	3.89	ab	1.04	a	2.64	abcde	2.50	bcd
F13	3.68	a	3.85	a	1.09	a	1.36	a	4.52	abc	4.70	cdef	7.78	ghi	4.86	def	4.64	a	4.18	ab	1.15	a	1.63	a	1.58	ab
F14	3.75	a	4.00	a	1.14	a	1.27	a	4.29	abc	3.19	a	6.72	efg	4.68	def	4.82	a	4.18	ab	1.30	a	2.16	abc	2.45	bcd
F15	3.81	a	4.10	a	1.35	a	1.44	a	4.20	abc	3.78	abcd	8.81	ij	5.71	f	4.80	a	4.30	b	1.19	a	1.75	ab	1.88	abc
F16	4.19	a	4.29	a	1.04	a	1.63	a	4.89	c	4.84	def	9.28	j	5.69	f	4.78	a	3.97	ab	1.12	a	1.80	abc	2.38	abcd
F17	4.16	a	4.23	a	1.31	a	1.42	a	4.85	bc	4.03	abcde	6.72	efg	4.06	bcde	5.22	a	4.19	ab	0.94	a	2.98	cde	2.79	cd
F18	3.66	a	4.06	a	0.94	a	1.32	a	4.17	abc	4.13	abcde	7.53	fgh	3.93	bcd	4.40	a	4.02	ab	1.10	a	1.83	abc	1.70	abc
F19	3.65	a	3.93	a	1.17	a	1.38	a	4.44	abc	3.90	abcde	6.19	de	4.56	cdef	4.65	a	4.26	b	1.04	a	1.98	abc	1.89	abcd
F20	4.02	a	4.20	a	1.20	a	1.44	a	4.73	abc	5.37	f	8.28	hij	5.65	f	4.48	a	4.28	b	1.02	a	1.60	a	1.29	a
F21	4.00	a	4.00	a	1.20	a	1.30	a	4.88	c	5.05	ef	8.20	hij	4.68	def	4.41	a	4.21	b	1.11	a	1.51	a	1.73	abc
F22	3.99	a	4.20	a	1.08	a	1.49	a	4.29	abc	4.55	bcdef	6.41	ef	3.72	bcd	4.43	a	4.14	ab	1.18	a	1.86	abc	1.88	abc
F23	4.00	a	4.32	a	0.98	a	1.16	a	3.76	a	3.39	ab	4.83	bc	3.44	bc	4.71	a	3.86	ab	1.27	a	2.38	abcd	1.91	abcd
F24	4.33	a	4.63	a	1.31	a	1.48	a	3.82	ab	3.63	abc	4.13	b	3.33	b	5.38	a	3.37	a	0.89	a	3.84	e	2.99	d
F25	4.41	a	5.08	a	1.33	a	1.46	a	4.52	abc	5.73	f	4.33	b	3.06	b	4.83	a	4.13	ab	1.15	a	2.26	abc	1.94	abcd
F26	3.88	a	4.47	a	1.01	a	1.22	a	4.19	abc	4.64	cdef	5.12	bcd	3.28	b	4.50	a	3.96	ab	1.13	a	2.03	abc	1.57	ab
F27	3.98	a	4.47	a	1.14	a	1.19	a	4.42	abc	4.68	cdef	6.15	de	4.01	bcde	4.69	a	4.02	ab	1.20	a	2.23	abc	1.79	abc
F28	4.18	a	4.85	a	1.05	a	1.40	a	4.94	c	5.66	f	7.44	fgh	5.17	ef	5.32	a	3.57	ab	0.90	a	3.47	de	2.77	cd
F29	4.23	a	4.78	a	1.25	a	1.75	a	4.12	abc	4.85	def	5.59	cde	4.21	bcde	4.73	a	4.30	b	1.12	a	1.91	abc	1.46	ab
F30	3.86	a	4.36	a	1.12	a	1.41	a	4.48	abc	4.89	def	1.85	a	1.38	a	4.98	a	3.96	ab	1.31	a	2.91	bcde	2.16	abcd
mean	3.96		4.30		1.14		1.37		4.39		4.47		6.24		4.12		4.79		4.03		1.11		2.24		2.05	

Mean evaluations (scale 0–10) by two panels: n = 11*3 for samples F11–F22 and n = 10*3 for samples F23–F30 (each sample evaluated in triplicate). Statistically significant differences between samples in each attribute based on ANOVA and Tukey's post hoc test ($p < 0.05$) and shown with letters a-j.

heptanone, (*E*)-3-octen-2-one) and alcohols (e.g., 1-hexanol and 1-octen-3-ol), increased during the storage trial. Similar increases in lipid-derived volatiles during the storage period of oat products have been reported previously (Heiniö et al., 2002; Lampi et al., 2015; Li et al., 2023; Molteberg et al., 1996; Puganen et al., 2024). From the detected furans, 2-pentylfuran was present in fresh oat samples and increased in stored samples. The study by Lampi et al. (2015) indicated that a higher content of 2-pentylfuran, compared to hexanal, is related to lipoxygenase activity, and that a greater amount of hexanoic acid than hexanal was considered an indicator of intense oxidation during the storage of oat extrudates. In the present study, the levels of 2-pentylfuran and hexanoic acid were low compared to the intensity of hexanal, corroborating the nature of lipid autoxidation and indicating only low to moderate oxidation at six months, as seen in our previous study for dry flours (Puganen et al., 2024).

The difference between the fresh and stored samples was statistically significant ($p < 0.05$) or statistically highly significant ($p < 0.001$) for nearly all volatile compounds tested, except for 2-butanone, 4-methyl-2-pentanone, and α-pinene (Table 1). To compare fresh and stored samples, another PCA was conducted (**Fig. 1**C and D). Data for the most oxidized sample, F28, were removed from the PCA model, as it was identified as an outlier due to notably higher intensities of major lipid-derived volatiles. The difference in storage time was the main distinguishing factor separating fresh samples (0 months) from stored samples (6 months) (Fig. 1C). Most fresh samples were clustered closely and were linked to 2,3-butanedione, α-pinene, and 3-carene. Samples F24 and F25 were strongly associated with acetic acid, 2-methylpropanal, 2-butanone, 4-methyl-2-pentanone, 3-methylbutanal, and 2-methylbutanal (Fig. 1D). In oats, 2-methylbutanal and 3-methylbutanal are primarily formed via the Maillard reaction during heat treatment to inactivate lipases (Li et al., 2022; Li et al., 2024).

The stored samples were associated with volatiles indicative of lipid oxidation. Generally, the variance among the oat batches in stored samples was greater than in fresh samples. Suspension samples F13, F14, F16, F17, and F18, which had been stored for six months, were located very close to the fresh samples (Fig. 1C). These stored oat samples are likely more stable throughout the storage period and are grouped in the same cluster as the six-month samples F12, F15, F19, and F20. Our previous study observed a similar trend in dry oat flour samples F12, F14, F17, F18, F19, and F20, which were stored for six months (Puganen et al., 2024). Another cluster of stored samples, F11, F21, F22, F23, F24, F26, and F27 can be seen in the PCA. These samples exhibit greater variability in their volatile profiles due to oxidation changes. Based on the current PCA model (**Fig. 1**C and D), the most oxidized samples, following the excluded sample F28, were F25, F29, and F30. They were strongly associated with lipid oxidation-related aldehydes, including butanal, pentanal, hexanal, octanal, and 2-octenal. While F25 and F30 already showed higher levels of oxidation-related volatiles in fresh samples than in other samples, F28 and F29 did not distinguish from other batches as fresh. This highlights that the volatile content of the fresh sample can not be used to predict the storage stability of oat batches.

3.2. Sensory quality of oat suspensions

3.2.1. Sensory profile of fresh samples

The sensory characteristics of fresh flour samples were analyzed as oat-water suspensions and evaluated by two trained panels. Based on the control samples, both panels were evaluated similarly, and their results were combined. A total of 13 attributes were evaluated: four in the odor category (intensity, oat, sweet, roast), four in the appearance and texture category (darkness of color, dark particles, thickness, stickiness), and five in the flavor category (intensity, oat, sweetness, bitterness, green) (Table 2). Significant differences ($p < 0.05$) were detected in seven attributes: oat flavor, bitterness, green flavor, darkness of color, presence of dark particles, thickness, and stickiness.

Panelists rated the total odor intensity between 3.6 and 4.4 (average 4.0) out of 10, while the oat odor intensity ranged from 3.9 to 5.1 out of 10 (average 4.3) (Table 2). The samples had low levels of sweet and roasted odors, with average scores of 1.0 and 1.4, respectively. The darkness of the suspension samples and the presence of particles were scored slightly below the midpoint of the scale, with average scores of 4.4 and 4.5, respectively. Thickness and stickiness showed greater variation, from 1.9 to 9.3 (average 6.2) and from 1.4 to 5.7 (average 4.1), respectively. In addition to the odor, the flavor intensity and oat flavor of the fresh samples were perceived as mild. Panelists evaluated the overall intensity of the suspension samples as between 4.4 and 5.4 (average 4.8), and the oat flavor as 3.4 to 4.3 (average 4.0). Sweetness ranged from 0.9 to 1.3 (average 1.1), bitterness from 1.5 to 3.8 (average 2.2), and green flavor from 1.3 to 3.0 (average 2.1) (Table 2).

3.2.1.1. Sensory profile of odor attributes. Sensory analysis of fresh oat samples revealed a mild and neutral odor, and similar intensity levels for all odor attributes. While no differences were observed among the batches in odor attributes, their appearance and texture exhibited greater variability. A similar trend was found in the sensory study of cooked oatmeals by Lapveteläinen and Rannikko (2000), in which most samples showed very similar odor intensities and differed statistically from one another only in texture.

The PCA biplot (Fig. 2A and B) showed that the sensory panel's evaluation results indicate a notable range of variation among the samples. According to Table 2, the highest odor intensity was observed in samples F24 and F25. In addition, samples F25, F28, and F29 had stronger oat odors, while F15 and F25 had stronger sweet odors. Sample F29 exhibited a more pronounced roast odor. In contrast, the PCA separated samples F17, F25, and F28 (Fig. 2A and B) into the lower right quadrant, where they correlate with odor attributes. F28 correlates with total odor intensity and oat odor, and F25 is associated with roast odor. Lapveteläinen et al. (2001) found that the toasted odor of cooked oatmeal was positively correlated with maltose and negatively correlated with fat content in rolled oats, indicating that lower fat levels led to a stronger toasted odor. Similarly, sample F25 had the lowest total fat content (Puganen et al., 2024) and associated strongly with roast odor. In the PCA of volatile variables (Fig. 1A and B), sample F25 was connected with pentanal, heptanal, hexanal, and 2-methylbutanal, of which 2-methylbutanal is a Strecker aldehyde responsible for roasted aroma notes.

3.2.1.2. Sensory profile of appearance and texture attributes. The color of oat products has been reported to influence consumer acceptance. Specifically, dark-colored oat samples were associated with more processed products (Laaksonen et al., 2020). In the current study, sensory panelists observed color differences in oat flour samples and the presence of dark particles (Table 2). The samples F16, F21, and F28 were darker in color than the others, while sample F23 appeared lighter. The presence of dark particles was observed to be lower in sample F14. In contrast, samples F20, F25, and F28 contained a higher proportion of dark particles than the other samples. Based on the sensory PCA (Fig. 2A and B), particularly samples F16 and F29 were associated with darkness and dark particles. Some oat cultivars naturally have darker bran than

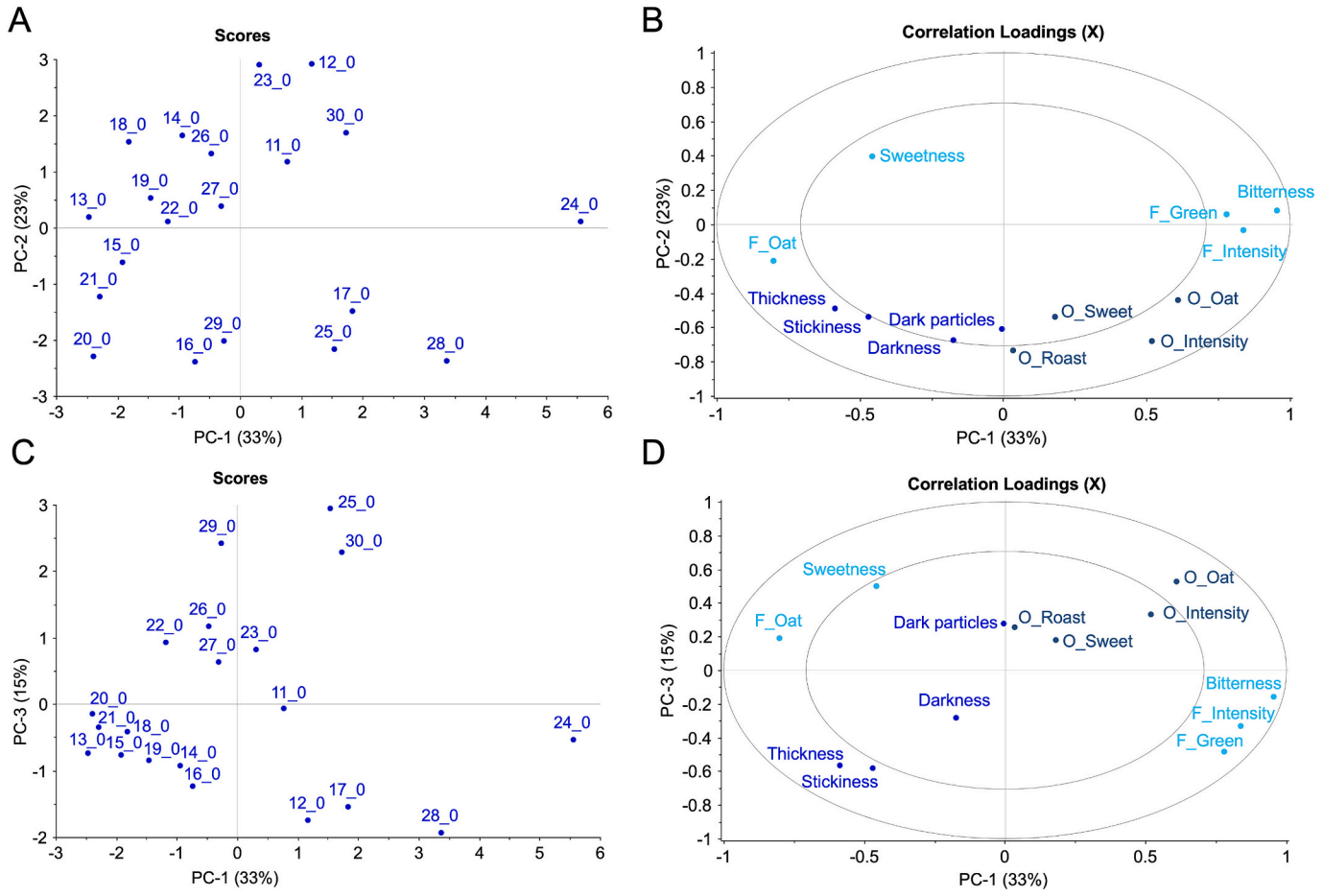

Fig. 2. Principal component analysis (PCA) of the sensory profile of oat suspension samples (n = 20). (**A**) PC1/PC2 scores plot showing oat flours from various oat batches evaluated as suspensions by a trained sensory panel using generic descriptive analysis (GDA). (**B**) PC1/PC2 loadings plot illustrating key sensory parameters (*n* = 13). (**C**) PC1/PC3 scores plot showing oat flours from various oat batches evaluated as suspensions. (**D**) PC1/PC3 loadings plot illustrating key sensory parameters (n = 13). In the correlation loadings plot, O = odor, and F = flavor.

others, which results in a darker and more intense overall color in whole-grain oat flour (Jokinen et al., 2021).

Typically, the dark color of oat groats changes during milling and heat treatment, and oat flours become lighter in color compared to the original groats (Jokinen et al., 2021). In the study by Lapveteläinen et al. (2001), the dark color of oatmeal correlated with the grain color value a*. Additionally, the dark color in cereals has been attributed to polyphenols (Heiniö et al., 2016). In our study, no correlation was found between darkness and flour color values (L*, a*, and b*) (Jokinen et al., 2021). Instead, the darkness showed a moderate correlation with type II avenanthramides ($r = 0.66$, $p < 0.05$), and dark particles correlated with 2c, 2p, and 2f types of avenanthramides ($r = 0.71$, $p < 0.05$), as well as with type II avenanthramides ($r = 0.67$, $p < 0.05$) and the total sum of avenanthramides ($r = 0.73$, $p < 0.05$) reported in Pöysä et al., 2024 (**Supplementary Table 4**).

The most noticeable variation in the evaluated sensory attributes was in the texture properties. Among the tested oat suspension samples, the thinnest texture was observed in sample F30, which received a very low score of 1.9 out of 10, while the thickest sample, F16, scored 9.3 out of 10 (Table 2). Regarding stickiness, sample F30 was less sticky (1.4), whereas sample F15 was the stickiest (5.7). In PC-1/PC-2 (**Fig. 2**A and B), only F15, F20, and F21 were linked to stickiness and thickness, while in PC-3 (**Fig. 2**C and D), several samples (F13, F14, F15, F16, F18, F19, F20, F21) grouped together based on similar stickiness and thickness. Interestingly, nearly the same samples (F13, F15, F16, F18, F19, F20, F21, F22) clustered closely in the PCA of the volatile profile of fresh samples (**Fig. 1**A and B), being associated with 1-propanal and 2,3-butanedione. According to evaluations by the sensory panelists, the samples that were thick were also sticky. Stickiness and thickness were strongly correlated with each other ($r = 0.94$, $p < 0.05$) (**Supplementary Table 4**). Furthermore, stickiness and thickness positively correlated with the content of dietary fiber ($r = 0.71$, $p < 0.05$; $r = 0.70$, $p < 0.05$) and β-glucan ($r = 0.75$, $p < 0.05$; $r = 0.85$, $p < 0.05$) of the same samples published previously (Jokinen et al., 2021). In contrast, no significant correlation with starch content was found. (**Supplementary Table 4**).

3.2.1.3. Sensory profile of flavor attributes. The study samples showed significant differences in oat, bitter, and green flavors (Table 2). Generally, oat samples identified as having a green flavor were also bitter. There was a positive correlation between bitterness and green flavor ($r = 0.80$, $p < 0.05$) (**Supplementary Table 3**). In this study, F15 and F29 were considered the most oaty samples, while F24 and F28 were identified as the most bitter and green, respectively (Table 2). Based on the sensory profile PCA (**Figs. 2AB and 2CD**), sample F24 was strongly linked to green, bitter, and overall flavor intensity. In the volatile profile PCA (Fig. 1A and B), F24 was more strongly associated with other volatiles like 1-pentanol, 2-pentanone, and 2-heptanone, in addition to hexanal, which may all contribute to a green flavor. Meanwhile, F28 was more connected to odor characteristics than to green or bitter flavor in the sensory profile PCA and did not associate with any specific volatile compounds in the volatile PCA.

Based on previous reports, non-volatiles, such as avenanthramides and saponins, can contribute to the bitter off-taste in oat flours (Günther-Jordanland et al., 2016; Günther-Jordanland et al., 2020). However, in our samples, no correlation was found between perceived bitterness and the levels of avenanthramides and saponins reported earlier by Pöysä et al. (2024). Instead, a moderate positive correlation ($r = 0.63$, $p < 0.05$) (**Supplementary Table 3**) was noticed between bitterness and the moisture content of fresh flours reported in Puganen et al. (2024). According to previous research, low moisture levels in oat samples are associated with freshness, oat odor, and oat flavor (Molteberg et al., 1996). In our samples, only oat flavor had a moderate negative correlation ($r = -0.62$, $p < 0.05$) with moisture, while oat odor correlated positively ($r = 0.69$, $p < 0.05$) (**Supplementary Table 3**).

3.2.2. Sensory differences in stored samples

In the discrimination test, apparent sensory differences were observed between the fresh and stored samples (Table 3). The panelists identified flavor properties as the most common descriptors for the differences between fresh and stored samples, followed by odor. The stored samples were perceived as having higher overall odor intensity, an oat-like and cereal-like odor, greater flavor intensity, a more pronounced bitter flavor, and a more noticeable pungent, green, and musty flavor than the fresh samples (**Supplementary Table 5**). However, the voluntary nature of the comment section resulted in too few responses to conduct a statistical analysis of the sensory characteristics for differentiating the individual samples.

The most oxidized sample among all batches, F28, which was removed from Fig. 1C as an outlier, was the most clearly recognized as different between fresh and stored samples and had the highest number of correct answers in the triangle test (Table 3). The differences were most often attributed to odor or flavor, which could arise from the higher level of hexanal in the stored F28 sample, as measured in suspension samples in this study and in previously published dry oat flours (Puganen et al., 2024). Of the 20 batches, only three flours (F19, F20, and F29) showed no distinct differences between fresh and stored samples (Table 3). In the volatile profile PCA (Fig. 1C), the stored samples of F19 and F20 were very close to the fresh samples, indicating minimal oxidation changes during the storage. This explains why panelists did not notice the difference between the stored and fresh samples of these flours. In contrast, the stored sample F29 was situated far from the cluster of fresh samples and was associated with hexanal, pentanal, 2-heptanone, 2-pentylfuran, and other lipid-derived oxidation volatiles (**Fig. 1**C and D).

Correct answers ($n = 284$) accounted for 65% of the total tests ($n = 440$) used to distinguish between fresh and stored samples. In the comment section of the triangle test, flavor was the most commented property in both fresh (74% of comments) and stored samples (84% of comments), while odor characteristics were described in less than half of the comments. As previously reported, long-term storage decreases the levels of oat odor, flavor, and sweetness, while lipid oxidation increases the rancidity, flavor intensity, and bitterness (Molteberg et al., 1996). Similarly, in our study, the stored flour samples were generally described as having a “stronger” odor (27% of comments), being more bitter (43%), pungent (18%), and having a “stronger” flavor (14%) compared to the fresh samples (**Supplementary Table 5**). However, oat odor (16%) and cereal odor (16%) were noted as being present in the stored samples, not in the fresh ones. Fresh flour batches did not associate with an oat flavor. Instead, they were more often described as having a mild odor (47%) and flavor (20%), with low levels of bitterness, oiliness, greenness, oatiness, and subtle pungency and mustiness (**Supplementary Table 5**).

3.3. GC-O profile of selected fresh samples

The oat batch samples F14, F21, F24, and F25 ($t = 0$ months) were selected for GC-O analysis based on the GDA results (Fig. 2), ensuring maximal differentiation among the samples. Using the DB-624 column, 32 odor-active compounds were detected, while 23 compounds were detected using the HP-5MS column (Table 4). In total, the panel recognized 45 aroma-active compounds, of which 20 were confirmed using reference compounds, odor descriptions, retention indices, and the NIST library. The most frequently detected compounds by the panelists were 2,3-butanedione, 3-methylbutanal, hexanal, 6-methyl-5-hepten-2-one, an unknown compound (13), and 1-octen-3-ol. Common descriptors for the odor-contributing compounds included sweet, fruity, green, musty, and notes of oatmeal and mushroom.

Altogether, the most commonly detected compounds in GC-O analysis were aldehydes and ketones. The average frequency and intensity of the identified compounds in four selected oat suspension samples are presented in Table 4, with more detailed information provided in

Table 3
The triangle test conducted on oat suspension samples comparing the storage periods of oat flours at room temperature for 0 and 6 months.

Sample	Correct answers[a]	*p*-value[b]	Certainty[c]	Reason for difference[d]			
				Appearance	Odor	Flavor	Texture
F11	19	<0.001	2.2	0	6	15	3
F12	15	0.001	2.3	0	10	13	3
F13	14	0.003	1.9	0	4	10	5
F14	16	<0.001	2.4	0	4	10	5
F15	14	0.003	2.3	1	8	13	4
F16	12	0.030	2.0	1	6	7	1
F17	15	0.001	2.3	0	7	15	2
F18	12	0.030	2.2	0	4	10	1
F19	11	0.074	2.0	0	9	8	1
F20	9	0.281	2.1	1	2	5	2
F21	13	0.011	1.8	0	6	9	0
F22	14	0.003	1.9	0	6	10	2
F23	14	0.003	2.1	0	4	10	2
F24	18	<0.001	2.3	0	6	17	2
F25	16	<0.001	1.9	0	4	10	2
F26	12	0.030	2.2	0	6	10	2
F27	14	0.003	2.3	0	5	10	5
F28	19	<0.001	2.7	3	16	18	1
F29	11	0.074	2.0	0	4	7	0
F30	16	<0.001	2.3	0	8	14	2

[a] Number of correct answers in the triangle tests by the panel (n = 11*2).
[b] Calculated binomial distribution *p* values (Lawless & Heymann, 2010); 12 correct answers are significant at the level 0.05, and 15 correct answers are significant at the level 0.001.
[c] Means for the self-assessed level of certainty of the correct answers on a scale 1–3 (1: "I guessed"; 2: "I'm quite sure"; 3: "I'm sure"). Data on incorrect answers is not included.
[d] Reason for the difference in the triangle test for correct answers (check-all-that-apply for the four choices).

Supplementary Table 6. Generally, both the average detection frequency and the average detection intensity were slightly higher in the DB624 column than in the HP5MS column. Additionally, more compounds were detected on the DB624 column than on the HP5MS column (Fig. 3). However, using two columns improved identification in cases when peaks overlapped. Hexanal (green, grassy), unknown sample 13 (sweet, fruity, candy), 6-methyl-5-hepten-2-one (musty, urine, spicy, pungent, metallic), and 3-methylbutanal (musty, chocolate, spicy, sweet), followed by 2,3-butanedione (sweet, butter, caramel) and 1-octen-3-ol (mushroom), were the most frequently detected aroma-active compounds in both columns, along with unknown compound 8 (sweet, fresh, metallic), assessed on the DB624 column. The highest aroma intensities on the scale of 0–4 were reported for 6-methyl-5-hepten-2-one (3.1, DB624; 3.1, HP5MS), 1-octen-3-ol (2.8, DB624; 2.5, HP5MS), and an unknown compound 13 (2.5, DB624; 2.0, HP5MS). Hexanal, 3-methylbutanal, and 2,3-butanedione were rated as having a mild odor, scoring 2 out of 4, while other compounds received lower scores, ranging from 1 to 2 (Table 4).

Based on PCA (Fig. 3), the selected sample batches differ in their GC-O profiles and are associated with different aroma-active compounds. For example, in the loadings plot of DB624 column (Fig. 3A), sample F24 strongly correlates with hexanal (green, grass), 3-methylbutanal (musty, spicy), and unknown compound 3 (sweet, fruity), supporting higher total flavor intensity, green and bitter flavor descriptions obtained in the sensory evaluations (Fig. 2). Additionally, the correlations of samples F24 and F25 with aroma-active compounds (**Fig. 3**A and B) are somewhat consistent with the volatile profile (**Fig. 1**A and B). The most intense odor of 6-methyl-5-hepten-2-one was associated with F14 in the PCA (Fig. 3A), but it did not align with the sensory evaluation results, where F14 was associated with a sweet flavor (Fig. 2). However, the sensory evaluation describes the overall flavor impression. This compound has been previously reported in extruded oat flours (Parker et al., 2000) and oat flakes (Li et al., 2024), showing that its formation is related to the steaming process of oats (Li et al., 2022).

Only a few studies have employed the chromatography-olfactometry method to analyze the aroma profiles of oat flakes (Klensporf & Jeleń, 2008; Schuh & Schieberle, 2005), oat pastry (Dach & Schieberle, 2021a), oat flour, and oat dough (Dach & Schieberle, 2021b), primarily using solvent-assisted flavor evaporation (SAFE) and aroma extract dilution analysis (AEDA). To our knowledge, the current study used the detection-frequency type of GC-O and the direct-intensity method for the first time in oats. Based on the work of Schuh and Schieberle (2005), the main oat-like odor-contributing compound in oat flakes was identified as (*E,E,Z*)-2,4,6-nonatrienal, which was later also found in oat flour, oat dough, and oat pastry (Dach & Schieberle, 2021a; Dach & Schieberle, 2021b). The volatile compound (*E,E,Z*)-2,4,6-nonatrienal was described as having an intense oatmeal-like odor at an extremely low concentration (0.0002 ng/L) and was suggested to form enzymatically in the oat kernel. In contrast to the results of Dach and Schieberle (2021b), no (*E,E,Z*)-2,4,6-nonatrienal was identified in the oat flour samples of the present study, due to a lack of commercial standards for this compound. Potentially, the unknown compound 22 detected (Table 4), with a musty, green, and oaty odor description and a retention index of 1281 on the HP5MS column, could be one of the isomers of 2,4,6-nonatrienal. However, the detection frequency for the unknown compound 22 was below 40% of evaluations, possibly due to panelists failing to detect and respond to its presence and odor, and/or low extraction efficiency of the compound by SPME with the used fiber.

The overlap of chemical, rose, honey, cauliflower, fatty, terpenic, and winey flavors has been suggested to explain the unique flavor of oats (Wang et al., 2023). In practice, even the most advanced GC–MS metabolomic analysis should be complemented by sensory science, as aroma-active compounds detected by the human nose are often not identifiable by GC–MS, and vice versa. Aroma-active volatile compounds exhibit different odor thresholds. Furthermore, the sample matrix is crucial. For instance, 1-octen-3-ol was not detected in fresh flour samples analyzed as oat suspensions by GC–MS in our study (Table 1). However, it was identified by the GC-O (Table 4). Conversely, 1-octen-3-ol was detected by GC–MS in dry oat flour samples (Puganen et al., 2024). Similarly, the musty odor of 6-methyl-5-hepten-2-one was among the strongest odor-active compounds in the GC-O analysis, but it was not detected in the GC–MS of fresh flours, appearing only in stored samples. The same trend is observed with the unknown aroma-active compound 22, which may be a potential 2,4,6-nonatrienal with an oat-like aroma.

Table 4
Olfactometric analysis (HS-SPME-GC-O) of volatile compounds in four selected oat suspension samples by using DB-624 and HP5MS columns.

#	Compound	Identification [a]		RI		Descriptors by the GC-O panel		Aver. frequency [b]		Aver. intensity [c]	
		DB624	HP5MS	DB624	HP5MS	DB624	HP5MS	DB624	HP5MS	DB624	HP5MS
1	Unknown 1					musty, rancid, sweet		2.3		2.1	
2	Unknown 2			539				2.0		1.0	
3	2-Methylpropanal	1, 3		597		fresh, green		1.3		1.4	
4	Uknown 3			624		sweet, fruity		2.0		1.0	
5	2,3-Butanedione	1, 2, 3	2, 3	633		sweet, butter, caramel	sweer, butter, caramel	4.3	3.0	2.1	1.7
6	Unknown 4			668		solvent, pungent		1.5		1.0	
7	2-Methylbutanal		2, 3		647		musty, cocoa, fruity		5.0		2.3
8	3-Methylbutanal	1, 2, 3	2, 3	695	655	musty, chocolate, spicy, sweet	musty, spicy	4.8	2.5	2.1	2.4
9	Unknown 5				706		liquorice		1.0		1.5
10	Unknown 6			723		musty, metallic		2.0		1.0	
11	Unknown 7			775		sweaty, fruity		1.3		1.4	
12	4-Methyl-2-pentanone	3, 4		782		sweet, fruity, fresh		2.0		1.4	
13	Unknown 8			824		sweet, fresh, metallic		4.0		1.3	
14	Unknown 9				786		vegetable, beef stock		1.0		1.5
15	Hexanal	1, 2, 3	1, 2, 3	841	798	green, grass	green, grass	5.0	4.5	2.3	2.2
16	Unknown 10				836		fruity, berry		1.0		2.0
17	Unknown 11				855		sweet, fruity, plastic		1.7		1.8
18	3-Methylbutyl ethanoate		1, 3		877		fresh, flowery		1.0		1.0
19	Heptanal		1		898		meaty, rotten		2.0		2.0
20	Unknown 12			857		leather		1.5		2.3	
21	Unknown 13			875	851	sweet, fruity, candy		5.0	4.8	2.5	2.0
22	3-Methyl pentanoic acid	1, 3		910		musty, urine, pungent, metallic, engine oil		1.0		1.8	
23	1-Hexyl formate	1, 3		925		fresh, fruity		1.0		1.0	
24	α-Pinene	1, 3	1, 3	961	931	pine, sweet, potato, butter	metallic, pine, pepper, vanilla	1.3	1.0	1.8	1.3
25	Camphene	4		979		potato, butter		2.3		1.8	
26	1-Octen-3-ol	1, 2, 3	1, 2, 3	1025	976	mushroom	mushroom	4.0	3.8	2.8	2.5
27	6-Methyl-5-hepten-2-one	1, 2		1034	962	musty, spicy, metallic, rotten	musty, urine, pungent, metallic, spicy	4.8	5.0	3.1	3.1
28	*p*-Cymene	1, 3		1062		fruity, fresh		1.0		2.3	
29	Nonanal	1, 2, 3		1145		sweet, fresh, solvent		2.0		1.2	
30	Unknown 14				1051				1.0		1.0
31	3,5-Octadien-2-one	1	1	1157	1058	roasted, musty, metallic	nitrogen	1.8	2.0	1.8	1.5
32	Unknown 15				1077		sweet, grainy		1.3		1.3
33	Camphor		1, 3, 4		1142		sweaty, rancid, citrus		1.0		1.3
34	Unknown 16				1160		grain, dry		1.0		1.7
35	Unknown 17			1183		sweet, green, fruity		1.0		1.5	
36	Unknown 18			1213		liquorice		1.0		3.0	
37	Unknown 19			1228		dry, grain		1.0		1.8	
38	Octanoic acid	1	1, 3	1244	1167	grain, oat, sweet	sweet, solvent, yeast	2.5	1.3	1.8	1.7
39	Decanal		1		1202		meaty		1.0		1.5
40	Unknown 20				1213		musty, spicy, rancid		2.3		1.5
41	Unknown 21			1296		fresh		1.0		1.0	
42	Unknown 22			1363	1281	musty, green, oat	green, musty, oat	1.8	1.5	2.8	1.8
43	Unknown 23			1379		oat, meat		1.0		2.0	
44	Unknown 24			1437		fresh, sweet, flower		2.0		1.6	
45	Unknown 25			1441		ground, oat		1.5		1.6	

[a] Identifications of compounds based on: 1 NIST database, 2 reference compound (Supplementary Table 1), 3 matching odor descriptors, 4 retention index (RI).
[b] Average detection freguency by the GC-O panel (n = 5) for four oat suspension samples. Data for four separate samples shown in Supplementary Table 4.
[c] Average intensity on a scale 0–4 by the GC-O panel (n = 5) for four oat suspension samples. Data for four separate samples shown in Supplementary Table 4.

Based on the retention indices (Dach & Schieberle, 2021b), 2,4,6-nonatrienal is expected to elute after decanal. Instead, no peaks corresponding to the mass spectra of 2,4,6-nonatrienal were observed in our samples after 40 min of running the GC–MS analysis program.

In the current study, the majority of aroma-active compounds detected by GC-O in oat flour exhibited mild odor intensities (Table 4). This supports the sensory evaluation results and aligns with the volatile profiles. Freshly produced and properly processed flours indeed have a neutral aroma. Further processing into pastries introduces roasty, popcorn-like compounds such as pyrrolines and nutty, earthy pyrazines (Dach & Schieberle, 2021a; McGorrin, 2019). Additionally, flakes and extrudates have been reported to contain pyrrolines and pyrazines in their profiles (Li et al., 2023; Li et al., 2024; Parker et al., 2000; Schuh & Schieberle, 2005), indicating a higher degree of heat treatment in their production. In contrast, flour samples from the current study, even when extruded, retained their mild odor and flavor (Nikinmaa et al., 2023). The roasted aroma produced by the volatiles from the Maillard reaction is often a desirable characteristic in food products rather than a neutral aroma. At the same time, a neutral aroma may indicate the freshness of the oat flour and help ensure the quality and storage stability of further-processed products.

4. Conclusions

Volatile profiles of twenty batches of oat flour, each from pure cultivars grown in Finland during the same growing season, analyzed as suspensions, revealed significant between-sample differences, particularly in the content of lipid oxidation and Maillard reaction compounds, as well as terpenes. Trained sensory panelists mostly discriminated fresh oat batches from one another, especially in terms of oat, bitter, and

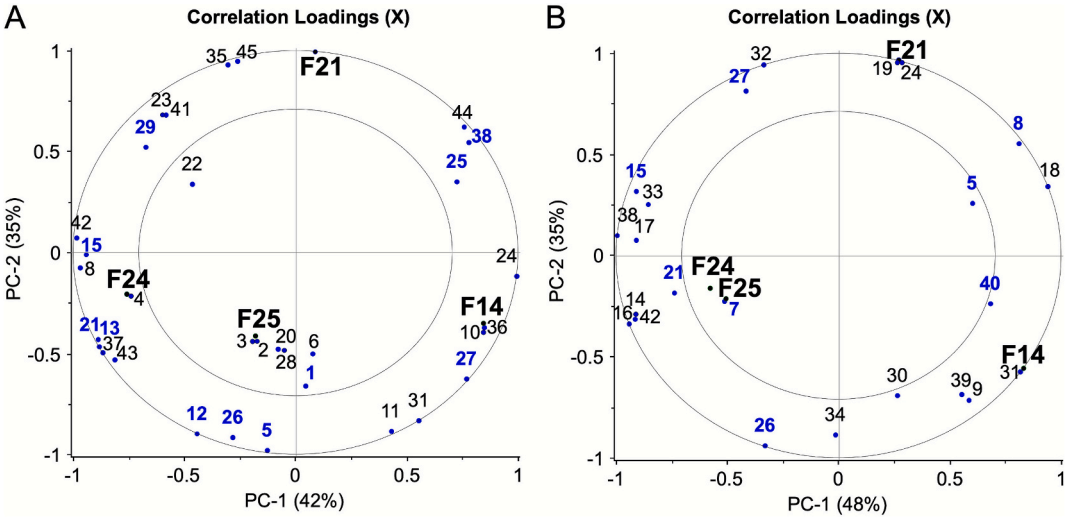

Fig. 3. Correlation loading plots of principal component analysis (PCA) models for averaged rated GC-O intensities (**Supplementary Table 6**). (**A**) DB624 column (n = 32 variables). (**B**) HP5MS column (n = 23 variables); Samples shown in the plots as downweighted variables. Variable numbers refer to Table 4. Blue and bolded variables detected by a minimum of three GC-O panelists in at least one sample. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

green flavors, texture, and color. The bitter flavor was associated with green flavor and moisture content, while the oat flavor had a mild negative association with moisture content. Texture differences correlated with fiber and β-glucan content, while color differences correlated with avenanthramide content. GC-O revealed that the most frequently and intensely rated volatiles were hexanal ("green"), 1-octen-3-ol ("mushroom"), and 2,3-butanedione ("fatty").

The volatile profiles of fresh and six-month-stored oat flours were significantly different. The main differences occurred through the accumulation and appearance of autoxidation compounds and the loss of terpenes during storage. In only three flours out of the twenty, the effect of storage was not distinguishable by the trained sensory panel. These samples also showed only minor changes in the volatile profiles. However, the effect of storage could not be predicted based on the fresh profile. No correlation was found between perceived bitterness and the levels of avenanthramides or saponins.

The fresh oat flours had a mild odor and flavor, reflecting the mild heat treatment, which was high enough to inactivate enzymes but low enough not to induce lipid oxidation and intense roast aroma. While also previous studies have shown that the flavor of fresh oat is mild, our results indicate that even within the mild profile, different oat batches vary, for example, in green and bitter flavor. Our study shows that, despite low flavor profiles, a trained sensory panel can reliably distinguish between fresh and stored samples. This is the first systematic sensory discrimination study across 20 pure-cultivar flours processed in equal industrial conditions. While the overall flavor of the oat flours was mild, different batches representing different cultivars varied significantly among one another in certain flavor attributes, making them suitable for different applications. The methodology used can be applied to screen batches or to develop suitable cultivars to preserve quality during storage, e.g., for products requiring a long shelf life.

CRediT authorship contribution statement

Anna Puganen: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Annelie Damerau:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Conceptualization. **Sari A. Hakanen:** Writing – original draft, Supervision, Methodology, Investigation, Formal analysis. **Baoru Yang:** Writing – review & editing, Supervision, Resources. **Oskar Laaksonen:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization. **Kaisa M. Linderborg:** Writing – review & editing, Writing – original draft, Resources, Project administration, Funding acquisition, Conceptualization.

Ethical statement

The authors confirm that the work described was conducted in accordance with the World Medical Association's Code of Ethics (Declaration of Helsinki) for sensory evaluation experiments involving humans. At the time of the evaluations, our institution did not require pre-ethical approval for sensory studies, and hence, no formal documentation process took place. The authors confirm that the appropriate protocols to protect the rights and privacy of all participants were followed during the research. The panelists were informed about the study protocols, their right to withdraw at any time, and that their informed consent to participate and have their personal data stored during the study was obtained.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2026.148136.

Data availability

Data will be made available on request.

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