**ARTICLE**

# Characteristics of Purified Horse Oil by Supercritical Fluid Extraction with Different Deodorants Agents

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**Abstract**   This study investigated the impact of activated carbon, palm activated carbon, and zeolite on horse oil (HO) extracted from horse neck fat using supercritical fluid extraction with deodorant-untreated HO (CON) as a comparison. The yield and lipid oxidation of deodorant untreated HO (CON) were not significantly affected by the three deodorants. However, deodorant-treated HOs exhibited significantly elevated levels of $\alpha$-linolenic acid (C18:3n3) and eicosenoic acid (C20:1n9) compared to CON ($p<0.05$), while other fatty acids remained consistent. Zeolite-purified HO demonstrated significantly lower levels of volatile organic compounds (VOCs) than other treatments ($p<0.05$). Remarkably, zeolite decreased the concentration of pentane, 2,3-dimethyl (gasoline odor), by over 90%, from 177.17 A.U. $\times10^6$ in CON to 15.91 A.U. $\times10^6$. Zeolite also effectively eliminates sec-butylamine (ammonia and fishy odor) as compared to other deodorant-treated HOs ($p<0.05$). Additionally, zeolite reduced VOCs associated with the fruity citrus flavor, such as nonanal, octanal, and D-limonene in HO ($p<0.05$). This study suggests that integrating zeolite in supercritical fluid extraction enhances HO purification by effectively eliminating undesirable VOCs, presenting a valuable approach for producing high-quality HO production in the cosmetic and functional food industries.

**Keywords**   horse oil, supercritical fluid extraction, purification, deodorants, zeolite

## Introduction

Horse oil (HO) is extracted from the adipose tissue of horses and has long been used in various Asian countries owing to its anti-inflammatory, anti-bactericidal, and anti-pruritic properties. Off late, HO has gained increasing attention as an ingredient in cosmetic formulations because of its potential to restore the skin barrier and provide skin-moisturizing benefits (Park and Kim, 2021). HO can originate from different anatomical regions of the horse, including meat, bones, and abdominal fat. Notably, it

contains elevated levels of unsaturated fatty acids (UFA) that closely resemble those found in the human skin, rendering it a desirable component for dermatological application (Maeng et al., 2018). Linoleic acid (C18:2n6, LA) and α-linolenic acid (C18:3n3, ALA) are the highest fatty acid among the polyunsaturated fatty acids (PUFA) in HO, known for their anti-inflammatory properties (Lee et al., 2018). LA and ALA (a precursor to omega-3 eicosapentaenoic acid and docosahexaenoic acid) may reduce the production and influence gene expressions of pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6; Ren and Chung, 2007; Saiki et al., 2017). Also, they are important for maintaining skin homeostasis as they cannot be endogenously synthesized in the human body and must be acquired through external consumption (Simard et al., 2022). The anti-inflammatory activity of HO has already been reported by Kim et al. (2020) using UVB-induced photoaging and inflammation in hairless mice. Incorporating HO topically significantly inhibited IL-6 and TNF-α production induced by UV-B radiation. This feature underscores the potential therapeutic and cosmetic applications of HO in skin care.

The extraction process plays a crucial role in animal oil production and significantly influences its yield, quality, and chemical composition of the extracted oil. Traditional extraction methods that rely on heat and flammable solvents present environmental and human health hazards and impact the quality and bioactive constituents of the oil itself (Gaber et al., 2018). In contrast, supercritical fluid extraction has emerged as an environmentally sustainable technique that has been employed in diverse industries, including food, pharmaceuticals, and cosmetics, since the 1970s. This method uses solvents such as water, carbon dioxide, and ethanol, which are widely recognized as safe and operate at lower temperatures than commercial extraction processes. This low-temperature approach preserves the appearance and nutritional content of the extracted oils. Supercritical fluid extraction has been widely used to extract bioactive compounds from various plant-based matrices, including green tea (Kim et al., 2007), orange peel (Lee et al., 2001), ginseng (Woo et al., 2011), and leaves of *Eurya japonica* (Li et al., 2020). However, the use of supercritical fluid extraction to extract oil from animal-based sources remains has not been explored in detail.

Additional purification processes are required to improve the quality and eliminate undesired odors from the extracted oil. Deodorization represents the final phase of the oil refining process and is designed to eliminate any undesired off-odors and off-colors that may persist from the earlier stages (Hamm, 2003). The conventional approach for deodorizing animal oil involves conducting the process under vacuum conditions at a temperature of 180°C. This specific temperature regime is primarily employed to mitigate the risks of polymerization and geometrical isomerization during the purification process (Carvajal and Mozuraityte, 2016). An innovative approach to deodorization, diverging from its reliance on high temperatures, is the utilization of various deodorizing agents such as activated carbon (AC) and zeolite (Gibon et al., 2007). AC is renowned for its highly porous structure and substantial adsorptive surface area and has been used in oil purification processes. It can adsorb molecules from liquid and gas phases through physical and chemical adsorption mechanisms, including van der Waals forces (Castillejos et al., 2012). AC can be obtained from various sources, including cassava peels, rice husks, bamboo, and palm kernels. Palm kernels are a by-product of palm oil production and can be employed to produce palm activated carbon (PAC). PAC exhibits microporous properties like those of AC, endowing it with a significant surface area for the adsorption of undesired off-odors and off-colors in oil. Zeolite, characterized by their nanoporous structures and substantial surface areas, excel at adsorbing polar oxidation (Fuss et al., 2021). These materials, including AC, PAC, and zeolite, are widely used for catalysis, separation, and selective adsorption decontamination. Nevertheless, their effectiveness in purifying HO using the supercritical fluid extraction method, followed by deodorization, remains ambiguous and requires further exploration.

Therefore, the objective of this study was to elucidate the potential use of supercritical fluid extraction for HO extraction and investigate the efficacy of AC, PAC, and zeolite as deodorizers in HO purification.

## Materials and Methods

### Horse oil extraction

Subcutaneous fat was obtained from the necks of 27-month-old Jeju horses from a local slaughterhouse on Jeju Island, Korea. The collected horse fat was cut into dimensions of 0.5×0.5 cm and subjected to supercritical fluid extraction following the method described by Li et al. (2020), with slight modifications. Subsequently, a four-step purification process was performed. The extracted horse fat was purified using a supercritical $CO_2$ extraction system (ISA-SEFE0-500-0700-080 system, Ilshin Autoclave, Daejeon, Korea) at the Nano Bio Research Center in Naju, Korea. The primary extraction gas used was pure $CO_2$ with $C_2H_3OH$ as the co-solvent. The sample was exposed to a pressure of 400 bar and the extracted HO was collected at 400 bar and 40℃ for 8 h. The extracted HO was then stored at –20℃ for subsequent use.

### Horse oil purification

The purification of HO from supercritical fluid extraction involves four stages: degumming, acid removal, washing and fractionation, bleaching and deodorization. In the degumming stage, 1% phosphoric acid (85%, Samchun Pure Chemical, Seoul, Korea) was added to the supercritical fluid extracted HO to remove the fat-soluble gums. Subsequently, 1% of 3N NaOH was added to remove free fatty acids, trace metals, and pigments during the acid removal process. The sample underwent two cycles of washing using distilled water, followed by stirring in a 50℃ water bath with an automatic stirrer for 20 min to eliminate moisture. Separation was accomplished using a separation funnel during the washing and fractionation phases. In the final stage of bleaching and deodorization, various deodorants, including AC, PAC, and zeolite, were added at a concentration of 2% to remove the off-odor and undesirable color from the HO. The treatment groups were control group with no deodorant treatment (CON), AC-purified HO (ACHO), PAC-purified HO (PACHO), and zeolite-purified HO (ZHO). The deodorization process transpired in a shaking incubator for 30 min at a temperature of 50℃, and the ultimate purified HO from supercritical fluid extraction was obtained through vacuum filtration. Stringent safety protocols were adopted throughout each phase of the study.

### Quality characteristics analysis

#### Yield

The yields of HO purified from supercritical fluid extraction using various deodorant purification processes were analyzed and quantified using the equation established by Gudmundsson and Hafsteinsson (1997).

$$\text{Yield (\%)} = [\text{Weight of the yielded sample (purified HO) (g) / Initial weight of horse fat (g)}] \times 100 \tag{1}$$

#### Thiobarbituric acid reactive substance

The malondialdehyde content in the samples was determined in accordance with the method outlined by Buege and Aust (1978). A total of 15 mL of distilled water was added to 5 g of sample and 0.05 μL of 7.2% butylated hydroxytoluene was

then added. After thorough homogenization, 2 mL of 20 mM 2-Thiobarbituric acid (in 15% trichloroacetic acid) was added to 1 mL of the homogenate mixture. The mixture was then kept in a 90℃ water bath for 15 min. After cooling for 10 min, the sample was centrifuged at 2,000×g for 10 min. Thiobarbituric acid reactive substance (TBARS) was determined using a UV/VIS spectrophotometer (M2e, Molecular Devices, Sunnyvale, CA, USA) by measuring the absorbance at 531 nm with distilled water as the blank sample. The TBARS value was calculated by multiplying the absorbance value by a factor of 5.88.

### Color

Instrumental color analysis was conducted using a Chroma meter CR-400 colorimeter (Minolta, Osaka, Japan) to measure the CIE L*, CIE a*, and CIE b* of the samples. Each sample was subjected to ten measurements to ensure accuracy and reliability.

### Fatty acids composition

Fatty acid analysis was conducted by examining fatty acid methyl esters (FAME) in the samples using a gas chromatography (GC)/flame-ionization detection instrument (detailed operating conditions are listed in Table 1; Folch et al., 1957). Fat was extracted from the samples using a Folch solution (chloroform:methanol=2:1) and then transferred to a test tube. NaOH methanol solution (1.5 mL of 0.5 N) was subsequently added, followed by heating at 100℃ for 5 min and cooling in a cold-water bath. The mixture was then homogenized with 2 mL of boron trifluoride-methanol solution (approximately 10%, Supelco, Bellefonte, PA, USA) to convert the fatty acids into methyl esters. After 2 min of heating at 100℃ and another cooling phase, FAME was extracted by adding 2 mL iso-octane to the mixture. To complete the process, 1 mL of saturated NaOH was added and mixed thoroughly. Centrifugation was performed at 783 ×g for 3 min to facilitate the separation of the supernatant layer containing FAME, which was then transferred to a GC vial for subsequent analysis.

### Volatile compound analysis

The profiles of volatile organic compounds (VOCs) in the samples were determined via headspace solid-phase microextraction (HS-SPME) analysis according to the method of Del Pulgar et al. (2013). The analysis comprises three key

**Table 1.** Gas chromatography operating conditions for determination of horse oil fatty acid composition

| Item | Condition |
| --- | --- |
| Instrument | Agilent 6890N (Agilent Technologies, Palo Alto, CA, USA) |
| Column | Omegawax250 (30 m×0.25 mm id, 0.25 μm film thickness; Supelco, Bellefonte, PA, USA) |
| Detector | Flame ionization detector |
| Carrier gas | Helium (99.99%, research purity) |
| Column flow rate | 1.2 mL/min |
| Split ratio | 100:1, 1 μL (injection volume) |
| Injection port temperature | 250℃ |
| Detection port temperature | 260℃ |
| Oven temperature | 150℃, hold for 2 min<br>4℃/min up to 220℃, hold for 30 min |

stages: 1) VOC extraction, 2) Gas chromatography-mass spectrometry (GC-MS) analysis, and 3) VOC flavor analysis. For VOC extraction, 5 g of the sample was introduced into a 20 mL glass vial sealed with septa and a crimp cap. Equilibration of VOCs in the headspace was initiated within a sealed vial and placed in a constant-temperature water bath (40℃) for 20 min. An SPME fiber (50/30 μm DVB/Carboxen/PDMS, Supelco) was employed to absorb the VOCs by exposing the needle on the headspace, allowing for a 30 min absorption period.

The VOCs absorbed onto the SPME fibers were analyzed by GC-MS (Agilent 8890 GC/5977B MSD, Agilent Technologies, Palo Alto, CA, USA). Helium served as the mobile phase, and a DB-5MS analytical column (30 m×0.25 mm id, 0.25 μm) was utilized at a flow rate of 1.3 mL/min. The inlet temperature was set at 250℃, the detector temperature was 280℃, and the oven was initially held at 40℃ for 10 min in gradient mode, increased to 250℃ at a rate of 5℃/min, and then held for 5 min. The fragrance component absorbed onto the SPME fiber was desorbed by exposure to an injector for 15 min.

The VOC flavor within the samples, as derived from the GC-MS results, was identified using the linear retention index (LRI) and mass spectral library data (NIST21 mass spectral library, National Institute of Standards and Technology, Gaithersburg, MD, USA), along with alkane standard materials (C10–C26). The VOC components were expressed in units of $1×10^6$ of the area (A.U. $×10^6$). Additionally, the flavor characteristics of each VOC were analyzed in accordance with Magagna et al. (2016) using FlavorDB (https://cosylab.iiitd.edu.in/flavordb/), FooDB (www.foodb.ca/), and Flavornet (http://www.flavornet.org).

### Statistical analysis

Statistical analysis of the data (n=3) was performed using a one-way analysis of variance, followed by Tukey's range test as the post-hoc test. Statistical significance was set at p<0.05. SAS 9.4 (SAS Institute, Cary, NC, USA) was used for the analysis, and superscripts indicated significant differences among treatments. The identified VOC compounds were subjected to additional partial least squares-discriminant analysis (PLS-DA) and heatmap analysis using MetaboAnalyst 5.0 online analysis software.

## Results and Discussion

### Yield, thiobarbituric acid reactive substance, and color

The TBARS assay is a rapid and convenient method to determine the levels of oxidative products derived from fatty acids in animal products (Oancea et al., 2022). In agreement with the yield percentages, the TBARS values of CON remained consistent throughout the purification process involving various deodorants (Table 2). Interestingly, the TBARS value of CON in this study was found to be below the established standard TBARS value of oil designated for human consumption, which is typically within the range of 7–8 mg MDA/kg of oil (Monte et al., 2015). Deodorants are frequently used to remove impurities from oils, often producing transparent final products. Additionally, deodorants such as AC, PAC, and zeolite possess catalytic properties that expedite the decomposition of oxidizing compounds during oil purification (Hamm, 2003). These versatile substances have applications in various industries including deodorization, purification, decolorization, and catalysis. Functioning as deodorizers, they efficiently eliminate unwanted volatile off-odors through van der Waals forces while adsorbing particles responsible for undesirable coloration on their microporous surfaces during purification (Castillejos et al., 2012).

**Table 2. Yield, TBARS, and color of purified horse oil by supercritical fluid extraction and three deodorants**

| Items | | CON | ACHO | PACHO | ZHO | SEM |
|---|---|---|---|---|---|---|
| Yield (%) | | 68.51 | 67.51 | 67.58 | 67.45 | 1.367 |
| TBARS (mg MDA/kg) | | 2.10 | 2.19 | 1.91 | 2.05 | 0.084 |
| Color | CIE L* | 56.02[a] | 54.99[b] | 54.52[c] | 55.84[a] | 0.054 |
| | CIE a* | 0.18[b] | 0.24[a] | 0.23[a] | 0.14[c] | 0.008 |
| | CIE b* | –0.72[b] | –0.83[c] | –0.86[d] | –0.59[a] | 0.007 |

[a–d] Mean values within the same rows with the different superscripts are significantly different among treatments ($p<0.05$).
TBARS, thiobarbituric acid reactive substance; CON, supercritical fluid extracted HO without deodorant; ACHO, supercritical fluid extracted HO purified with activated carbon; PACHO, supercritical fluid extracted HO purified with palm activated carbon; ZHO, supercritical fluid extracted HO purified with zeolite; HO, horse oil.

The use of supercritical fluid extraction with various deodorizing agents during the purification process yielded substantial alterations in the color attributes of CON. Color is a pivotal quality parameter of vegetable and animal oils. The CON exhibited lower CIE L* and CIE a* values (54.52–56.02 and 0.14–0.24, respectively) compared to foal perirenal fat, which recorded a CIE L* and CIE a* value of 68.2 and 2.4, respectively (Álvarez et al., 2015). Similarly, in a comparative analysis of fat color among Jeju horses, Halla horses, lard, and beef-tallow, Park et al. (2019) reported that the liquid state of fat from Jeju and Halla horse meat, when subjected to temperatures below 60℃, yielded higher CIE L* values and lower CIE a* and CIE b* values, in contrast to CON and HO with deodorants in this study. The color profile of animal fat is an important quality indicator for both producers and consumers. In this regard, the CIE L*, CIE a*, and CIE b* values have been widely used to evaluate animal fat color. Notably, the CIE b* serves as a prominent factor in evaluating the color of animal fats. It has been found that animal fat with lower CIE b* values is often preferred by the majority of consumers due to its favorable sensory attributes (Ardeshiri and Rose, 2018). It is crucial to acknowledge that various factors, including feed intake and processing methods, can influence CIE b* values (Moloney et al., 2013).

### Fatty acids composition

The fatty acid compositions of the CON and HO with deodorants are shown in Table 3. CON and HO with deodorants exhibited higher concentrations of UFA than those of saturated fatty acids (SFA). CON and HO with deodorants had UFA concentrations between 60%–65% of all fatty acids present, as also observed previously (Maeng et al., 2018; Piao et al., 2019). The predominant UFAs found in CON and HO with deodorants were oleic acid (C18:1n9), LA (C18:2n6), and palmitoleic acid (C16:1n7). Oleic acid was found in CON and HO with deodorants with an average amount of 33.75%–33.81%, aligning with previous studies (Kim et al., 2020; Piao et al., 2019). Oleic acid has been reported to modulate inflammation and contribute to beneficial processes in skin tissue repair (Pereira et al., 2008). Although LA plays a vital role in maintaining the skin membrane fluidity, the levels observed in CON and HO with deodorants were slightly lower compared to those reported by Kim et al. (2020). Additionally, studies have found that palmitoleic acid in HO is associated with skin injury healing, and its proportion in HO exceeds that observed in lard and tallow (Park et al., 2019).

The SFA composition of CON and HO with deodorants ranged from 39.27% to 39.45%. Palmitic acid (C16:0) was the predominant SFA, followed by myristic acid (C14:0) and stearic acid (C16:0). This pattern is similar to findings that identified palmitic acid as the predominant SFA in HO, regardless of the extraction method employed (Piao et al., 2019). Notably, palmitic acid has been associated with reducing facial wrinkles and fine lines (Husein el Hadmed and Castillo,

**Table 3.** Comparison of the fatty acid composition of purified horse oil by supercritical fluid extraction and three deodorants

| Fatty acid composition (%) | CON | ACHO | PACHO | ZHO | SEM |
|---|---|---|---|---|---|
| Myristic acid (C14:0) | 5.42 | 5.37 | 5.39 | 5.34 | 0.012 |
| Palmitic acid (C16:0) | 30.50 | 30.36 | 30.38 | 30.38 | 0.057 |
| Palmitoleic acid (C16:1n7) | 9.11 | 9.00 | 8.83 | 8.85 | 0.081 |
| Stearic acid (C18:0) | 3.53 | 3.53 | 3.55 | 3.55 | 0.005 |
| Oleic acid (C18:1n9) | 33.70 | 33.72 | 33.81 | 33.77 | 0.046 |
| Vaccenic acid (C18:1n7) | 1.87 | 1.87 | 1.87 | 1.87 | 0.002 |
| Linoleic acid (C18:2n6) | 10.74 | 10.67 | 10.67 | 10.69 | 0.024 |
| γ-linolenic acid (C18:3n6) | 0.02 | 0.02 | 0.02 | 0.02 | 0.003 |
| α-linolenic acid (C18:3n3) | 4.57[b] | 4.90[ab] | 4.95[a] | 4.99[a] | 0.076 |
| Eicosenoic acid (C20:1n9) | 0.46[b] | 0.47[a] | 0.46[b] | 0.47[ab] | 0.002 |
| Arachidonic acid (C20:4n6) | 0.05 | 0.05 | 0.05 | 0.04 | 0.003 |
| Eicosapentaenoic acid, EPA (C20:5n3) | 0.00 | 0.00 | 0.00 | 0.00 | 0.000 |
| Adrenic acid (C22:4n6) | 0.03 | 0.03 | 0.03 | 0.03 | 0.001 |
| Docosahexaenoic acid, DHA (C22:6n3) | 0.00 | 0.00 | 0.00 | 0.00 | 0.000 |
| SFA | 39.45 | 39.27 | 39.31 | 39.27 | 0.055 |
| UFA | 60.55 | 60.73 | 60.69 | 60.73 | 0.055 |
| MUFA | 45.14 | 45.07 | 44.97 | 44.96 | 0.072 |
| PUFA | 15.41 | 15.67 | 15.72 | 15.77 | 0.088 |
| MUFA:SFA | 1.14 | 1.15 | 1.14 | 1.14 | 0.003 |
| PUFA:SFA | 0.39 | 0.40 | 0.40 | 0.40 | 0.003 |

[a,b] Mean values within the same rows with the different superscripts are significantly different among treatments (p<0.05).
CON, supercritical fluid extracted HO without deodorant; ACHO, supercritical fluid extracted HO purified with activated carbon; PACHO, supercritical fluid extracted HO purified with palm activated carbon; ZHO, supercritical fluid extracted HO purified with zeolite; HO, horse oil; SFA, saturated fatty acid, UFA, unsaturated fatty acid, MUFA, monounsaturated fatty acid, PUFA, polyunsaturated fatty acid.

2016). Overall, the results suggest that the supercritical fluid extraction method combined with various deodorants (AC, PAC, and zeolite) effectively purified HO without causing significant changes in the proportions of palmitoleic acid and LA.

Notably, this study found significant differences in the levels of ALA and eicosenoic acid (C20:1n9) in HO treated with deodorants (p<0.05). CON exhibited the lowest ALA content compared to HO with deodorants. The ALA contents in the CON, ACHO, PACHO, and ZHO treatments were 4.57%, 4.90%, 4.95%, and 4.99%, respectively. ALA, a PUFA, serves as a precursor to produce eicosapentaenoic acid (EPA) and docosahexaenoic acid. Its beneficial effects against various diseases have been also extensively studied, as evidenced by numerous scientific investigations (Yue et al., 2021). Additionally, ALA plays a crucial role in the formation of the skin barrier, and its anti-inflammatory properties contribute to skin health (Fan et al., 2020). Recent research conducted by Simard et al. (2022) underscored the significance of ALA in maintaining skin health, including its role in enhancing skin hydration and mitigating the risk of skin diseases. Additionally, the ratio of monounsaturated fatty acids (MUFA) to SFA showed no significant differences among the treatments, and the ratio of PUFA to SFA did not exhibit significant differences across the treatments. These findings indicate that the supercritical fluid extraction method combined with various deodorants (AC, PAC, and zeolite) did not significantly affect the overall ratio of MUFA and PUFA to SFA in purified HO.

### Volatile organic compounds

Odor represents a critical quality parameter for HO. Oils derived from animal sources often have an unpleasant and distinctive odor. Given the subjectivity of sensory evaluation, which can vary among individuals (Moloney et al., 2013), it is imperative to analyze the odor of HO through chemical analyses, such as the identification of VOCs. The VOCs in the CON and HO with deodorants are listed in Table 4. The VOCs were classified into seven groups: alcohols, aldehydes, esters, furans, hydrocarbons, ketones, and other unclassified compounds, with 127 identified compounds. Among these, the hydrocarbons group emerged as the most prominent category among the other VOC groups across all treatments. In this study, the presence of undesirable odors in HO may be attributed to compounds such as 1-octen-3-ol and nonanal. While we cannot state that these compounds are solely responsible for the undesirable odor in HO, their detection in our study aligns with findings from horse meat in previous studies (Beldarrain et al., 2022; Maggiolino et al., 2019; Sujiwo et al., 2024; Tateo et al., 2020). These VOCs were also present among VOCs detected in other meat sources (Bleicher et al., 2022) and salami (Moretti et al., 2004). According to Maggiolino et al. (2019), nonanal is characterized by grassy, tea, vegetable, lemony, sour, beefy odor profile. VOC such as nonanal, heptanal, octanal, decanal, (E)-2-decenal and (E)-2-undecenal are relevant contributors of cooked horse meat aroma have been reported as major oxidation products derived from oleic acid (Beldarrain et al., 2022). Furthermore, 1-octen-3-ol has been recognized as a significant contributor to the overall flavor profile of boiled pork due to its higher odor activity values (Vilar et al., 2022).

Notably, the utilization of zeolite led to a significant reduction in hydrocarbons such as dodecane (alkane aroma profile), methane, isocyanato- (cabbage and horseradish-like odor), and pentane, 2,3-dimethyl- (gasoline scent) in ZHO ($p<0.05$). The microporous nature of the pores in zeolite may demonstrate a high capacity for adsorbing VOCs, making it an effective deodorizing agent. Furthermore, zeolite exhibited an exceptional capability to reduce the levels of sec-butylamine, a contributor to the ammonia and fishy odor observed in CON, to an insignificant amount from its initial level of 16.05 A.U. $\times 10^6$. Similarly, the level of cyclotetrasiloxane, octamethyl- (without aroma description) was reduced from 108.93 A.U. $\times 10^6$ to 37.86 A.U. $\times 10^6$ ($p<0.05$). Furthermore, the presence of pentane, 2,3-dimethyl (known for its gasoline-like odor) was dramatically reduced from 177.17 A.U. $\times 10^6$ to 15.91 A.U. $\times 10^6$ in the ZHO compared to the CON. Interestingly, this study also revealed that zeolite was highly effective at significantly reducing the levels of fruit-like aromatic VOCs in HO, such as nonanal (citrus, lime, or orange-peel scent), octanal (lemon, citrus, or orange-peel scent), and d-limonene (mint, lemon citrus scent; $p<0.05$). This result is consistent with a recent study by Fuss et al. (2021), demonstrating the role of zeolite as a molecular sieve material, proficient in capturing and eliminating odors in the oil refining process.

Supercritical fluid extraction is a widely adopted method for extracting volatile compounds from various beverage samples and is valuable for volatile compound analysis. This method is particularly suitable for extracting non-polar analytes (Vafaei et al., 2022). Additionally, the inclusion of a small quantity of a polar cosolvent during the process expands its capabilities to include the extraction of more polar analytes from oil (Herrero et al., 2010). Supercritical fluid extraction was found to enhance both the diffusion coefficient and pore penetration properties of extracted oil, which is advantageous for the purification process. The increased pore penetration of HO extracted by supercritical fluid extraction, facilitates the adsorption of impurities by microporous deodorants through van der Waals forces (Castillejos et al., 2012). The deodorization process for oil purification is designed to eliminate VOCs and free fatty acids, ultimately yielding odorless and tasteless oils. AC is commonly utilized to remove impurities such as odors, colors, and tastes from liquids via adsorption (Castillejos et al., 2012; Gibon et al., 2007).

**Table 4.** Volatile organic compounds of purified horse oil by supercritical fluid extraction and three deodorants (A.U. ×10[6])

| Volatile organic compounds | Flavor profile[1] | m/z | LRI | CON | ACHO | PACHO | ZHO | SEM |
|---|---|---|---|---|---|---|---|---|
| **Alcohols** | | | | | | | | |
| (S)-(+)-3-Methyl-1-pentanol | Cocoa, cognac, fruity, fusel, green | 56.1 | 783 | 0.80 | 0.81 | 0.80 | 0.60 | 0.188 |
| 1-Heptanol | Leafy, coconut, herbal, peony, strawberry, chemical, musty, sweet, woody, violet, green | 70 | 957 | 0.53 | 0.68 | 0.71 | 0.51 | 0.199 |
| 1-Hexanol, 2-ethyl- | Citrus, fresh, floral, oily, sweet, fatty, fruity | 57 | 1,029 | 13.09[a] | 13.03[a] | 11.60[a] | 1.67[b] | 0.434 |
| 1-Octen-3-ol | Raw, fishy, oily, earthy, fungal, chicken, mushroom, green | 57 | 968 | 7.15 | 9.02 | 8.54 | 5.13 | 0.998 |
| 1-Pentanol, 4-methyl-2-propyl- | NA | 71 | 1,100 | 0.00[b] | 2.16[a] | 0.00[b] | 1.57[ab] | 0.445 |
| 3-Octen-2-ol, (E)- | Creamy, melon rind, mushroom, waxy | 81 | 988 | 19.95[a] | 19.48[a] | 21.18[a] | 4.68[b] | 1.338 |
| Benzenemethanol, alpha,alpha-dimethyl- | NA | 121.1 | 1,089 | 0.00 | 0.13 | 0.00 | 0.10 | 0.030 |
| Ethanol, 2-(2-butoxyethoxy)- | Faint butyl, mild ether | 57.1 | 1,190 | 0.00[c] | 0.41[a] | 0.23[ab] | 0.16[bc] | 0.043 |
| Ethanol,2-(2-butoxyethoxy)-, acetate | Mild, not unpleasant | 87.1 | 1,370 | 0.00[b] | 0.28[a] | 0.11[b] | 0.13[ab] | 0.036 |
| p-Menth-4(8)-en-9-ol | NA | 136 | 1,283 | 0.00[b] | 0.13[a] | 0.00[b] | 0.00[b] | 0.004 |
| Sub total | | | | 41.53[a] | 46.13[a] | 43.17[a] | 14.56[b] | 1.696 |
| **Aldehydes** | | | | | | | | |
| 2,4-Decadienal, (E,E)- | Fried, wax, fat | 81.1 | 1,320 | 0.43 | 0.23 | 0.16 | 0.31 | 0.146 |
| 2,4-Decadienal, (E,Z)- | Fat, fatty, fried, geranium, green, wax | 81.1 | 1,297 | 0.34 | 0.20 | 0.17 | 0.26 | 0.107 |
| 2,4-Heptadienal, (E,E)- | Cinnamon, hazelnut | 81 | 1,005 | 14.18[a] | 15.02[a] | 14.18[a] | 3.38[b] | 1.372 |
| 2,4-Nonadienal, (E,E)- | NA | 81 | 1,214 | 1.12[a] | 1.17[a] | 1.22[a] | 0.19[b] | 0.103 |
| 2-Decenal, (E)- | Orange, coriander, rose, tallow, waxy, oily, fatty, earthy, floral, aldehydic, mushroom, green | 70.1 | 1,265 | 0.21 | 0.15 | 0.16 | 0.14 | 0.049 |
| 2-Nonenal, (E)- | Aldehydic, citrus, cucumber, fat, fatty, green, paper | 70.1 | 1,163 | 0.40 | 0.48 | 0.37 | 0.45 | 0.116 |
| 2-Octenal, (E)- | Green, nut, fat | 83 | 1,049 | 2.42[a] | 2.32[a] | 2.53[a] | 0.40[b] | 0.105 |
| 4-Nonenal, (E)- | Fruity | 69.1 | 1,103 | 0.00 | 0.26 | 0.41 | 0.30 | 0.103 |
| 4-Oxohex-2-enal | NA | 83 | 944 | 0.00[b] | 0.81[a] | 0.67[a] | 0.00[b] | 0.032 |

**Table 4. Volatile organic compounds of purified horse oil by supercritical fluid extraction and three deodorants (A.U. ×10$^6$) (continued)**

| Volatile organic compounds | Flavor profile[1] | m/z | LRI | CON | ACHO | PACHO | ZHO | SEM |
|---|---|---|---|---|---|---|---|---|
| 5-Ethylcyclopent-1-enecarboxaldehyde | NA | 67 | 1,028 | 1.57 | 1.56 | 1.48 | 1.59 | 0.311 |
| Benzaldehyde, 2,4-dimethyl- | Almond, cherry, naphthyl, spice, vanilla | 57 | 1,178 | 0.00$^b$ | 0.09$^a$ | 0.08$^a$ | 0.00$^b$ | 0.009 |
| Benzaldehyde, 3,4-dimethyl- | NA | 133 | 1,228 | 0.05 | 0.04 | 0.00 | 0.01 | 0.013 |
| Benzaldehyde, 4-ethoxy- | Sweet, spicy, floral, anisic | 121 | 1,324 | 0.09 | 0.14 | 0.14 | 0.18 | 0.054 |
| Benzeneacetaldehyde | Hawthorn, honey, sweet | 91.1 | 1,043 | 0.27 | 0.27 | 0.19 | 0.19 | 0.069 |
| Decanal | Soap, orange peel, tallow | 57 | 1,206 | 0.24$^a$ | 0.16$^b$ | 0.2$^{ab}$ | 0.02$^c$ | 0.013 |
| Hexanal, 5-methyl- | NA | 70 | 845 | 9.18$^a$ | 8.54$^a$ | 9.17$^a$ | 1.07$^b$ | 0.440 |
| Nonanal | Citrus, lime, orange peel, rose, fat, green, fishy, waxy, fresh, fatty, peely, aldehydic, orris, grapefruit | 57.1 | 1,110 | 4.15$^a$ | 4.75$^a$ | 4.23$^a$ | 0.51$^b$ | 0.238 |
| Octanal | Lemon, citrus, soap, orange peel, fat, waxy, fatty, aldehydic, green | 57 | 997 | 2.78$^a$ | 3.25$^a$ | 3.08$^a$ | 0.73$^b$ | 0.131 |
| Propanal, 2,2-dimethyl- | NA | 57.1 | 554 | 14.10 | 0.00 | 19.15 | 14.10 | 5.078 |
| Sub total | | | | 51.53$^a$ | 39.45$^{ab}$ | 57.58$^a$ | 23.83$^b$ | 5.788 |
| Esters | | | | | | | | |
| 1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester | Slight ester | 149 | 1,872 | 0.98$^a$ | 0.77$^a$ | 0.75$^a$ | 0.00$^b$ | 0.103 |
| 2,2,4-Trimethyl-1,3 pentanediol diisobutyrate | NA | 71.1 | 1,600 | 5.19$^a$ | 1.91$^{ab}$ | 0.84$^b$ | 0.30$^b$ | 0.767 |
| 2-Ethylhexyl salicylate | Slight floral | 120 | 1,808 | 0.00$^b$ | 0.09$^a$ | 0.00$^b$ | 0.00$^b$ | 0.011 |
| 2-Ethylhexyl trans-4 methoxycinnamate | NA | 178 | 2,321 | 0.22 | 0.23 | 0.08 | 0.40 | 0.202 |
| Arsenous acid, tris (trimethylsilyl) ester | NA | 207 | 698 | 65.62 | 43.74 | 66.5 | 41.49 | 9.653 |
| Benzoyl isothiocyanate | NA | 105 | 604 | 0.51$^a$ | 0.37$^a$ | 0.41$^a$ | 0.00$^b$ | 0.058 |
| Borinic acid, diethyl-, methyl ester | NA | 43 | 549 | 3.12 | 7.75 | 2.37 | 0.00 | 3.201 |
| Butyl isocyanatoacetate | NA | 57 | 554 | 0.00 | 9.41 | 13.34 | 1.74 | 4.096 |
| Dibutyl phthalate | Faint | 149 | 1,965 | 2.42$^a$ | 1.85$^{ab}$ | 1.33$^b$ | 0.29$^c$ | 0.176 |

**Table 4.** Volatile organic compounds of purified horse oil by supercritical fluid extraction and three deodorants (A.U. ×10⁶) (continued)

| Volatile organic compounds | Flavor profile[1] | m/z | LRI | CON | ACHO | PACHO | ZHO | SEM |
|---|---|---|---|---|---|---|---|---|
| Diphosphoric acid, diisooctyl ester | NA | 83 | 933 | 9.54ᵃ | 11.37ᵃ | 10.92ᵃ | 3.61ᵇ | 1.058 |
| Hydrogen isocyanate | NA | 43 | 537 | 1.50ᵃ | 1.31ᵃᵇ | 1.11ᵃᵇ | 0.50ᵇ | 0.204 |
| Methyl propionate | Sweet, fruity, rum-like | 57.1 | 630 | 8.00ᵃᵇ | 12.84ᵃ | 6.49ᵃᵇ | 0.00ᵇ | 2.708 |
| Methyl salicylate | Mint, wintergreen, peppermint | 120 | 1,194 | 0.73 | 0.53 | 0.57 | 0.43 | 0.109 |
| Oxalic acid, isohexyl neopentyl    ester | NA | 57 | 1,503 | 0.00ᵇ | 0.00ᵇ | 0.09ᵃ | 0.00ᵇ | 0.004 |
| Propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester | NA | 71 | 1,355 | 5.95ᵃ | 6.35ᵃ | 1.85ᵇ | 0.08ᵇ | 0.610 |
| Sub total | | | | 103.78ᵃᵇ | 98.50ᵃᵇ | 106.65ᵃ | 48.84ᵇ | 12.335 |
| **Furans** | | | | | | | | |
| Furan, 2,5-dihydro- | NA | 70.1 | 567 | 9.44ᵃ | 9.50ᵃ | 8.96ᵃ | 3.92ᵇ | 0.048 |
| Furan, 2-methoxy- | NA | 83.1 | 727 | 0.00ᵇ | 0.18ᵃ | 0.21ᵃ | 0.00ᵇ | 1.087 |
| Furan, 2-pentyl- | Green bean, butter | 81.1 | 982 | 4.98 | 5.23 | 5.21 | 3.37 | 0.012 |
| cis-2-(2-Pentenyl) furan | NA | 136.1 | 995 | 0.00 | 0.21 | 0.19 | 0.15 | 0.049 |
| Sub total | | | | 14.41ᵃ | 15.11ᵃ | 14.57ᵃ | 7.44ᵇ | 1.163 |
| **Hydrocarbons** | | | | | | | | |
| 1,6-Heptadien-3-yne, 5-methyl- | NA | 91.1 | 756 | 1.31ᵃᵇ | 0.00ᵇ | 1.82ᵃ | 0.00ᵇ | 0.332 |
| 1-Isopropylcyclohex-1-ene | NA | 81.1 | 918 | 0.2ᵃᵇ | 0.30ᵃ | 0.29ᵃ | 0.00ᵇ | 0.055 |
| 1-Pentene, 3-methyl- | NA | 56.1 | 561 | 3.21 | 2.74 | 4.32 | 1.23 | 1.067 |
| 2,6,10-Trimethyltridecane | NA | 57 | 1,465 | 0.00ᵇ | 0.08ᵃ | 0.00ᵇ | 0.00ᵇ | 0.001 |
| 2-Methyl-1-hepten-3-yne | NA | 79.1 | 799 | 0.04 | 0.07 | 0.05 | 0.00 | 0.016 |
| 3,4-Heptadiene | NA | 81 | 858 | 5.66ᵃ | 4.37ᵃᵇ | 5.97ᵃ | 1.40ᵇ | 0.735 |
| 3-Ethyl-2-methyl-1-heptene | NA | 69 | 1,103 | 0.00ᵇ | 0.38ᵃ | 0.00ᵇ | 0.03ᵇ | 0.011 |
| 3-Hexene, 2,2-dimethyl- | NA | 83 | 735 | 0.00ᵇ | 0.15ᵃ | 0.00ᵇ | 0.00ᵇ | 0.007 |
| 7-Oxabicyclo [4.1.0]heptane | NA | 69.1 | 745 | 1.46 | 1.14 | 1.49 | 1.01 | 0.272 |
| Benzene,1,2,4-trimethyl- | Cauliflower, sweet cherry, black walnut, corn | 105 | 980 | 0.41 | 0.61 | 0.58 | 0.28 | 0.125 |

**Table 4.** Volatile organic compounds of purified horse oil by supercritical fluid extraction and three deodorants (A.U. ×10$^6$) (continued)

| Volatile organic compounds | Flavor profile[1] | m/z | LRI | CON | ACHO | PACHO | ZHO | SEM |
|---|---|---|---|---|---|---|---|---|
| Benzene,1,3-bis(1,1-dimethylethyl)- | NA | 175.2 | 1,258 | 0.91$^a$ | 0.37$^b$ | 0.48$^{ab}$ | 0.40$^b$ | 0.094 |
| Benzene,1-methyl-3-(1-methylethyl)- | Blackcurrant, sweet basil | 119 | 1,021 | 0.00 | 0.00 | 0.00 | 0.43 | 0.150 |
| Benzene, 1-methyl-4-propyl- | NA | 105.1 | 1,052 | 0.16 | 0.20 | 0.00 | 0.16 | 0.062 |
| Bicyclo[3.1.0]hex-2-ene,4- methyl-1-(1-methylethyl)- | NA | 93 | 954 | 0.23 | 0.32 | 0.20 | 0.21 | 0.098 |
| Borane, diethyl(decyloxy)- | NA | 85.1 | 1,267 | 0.18$^a$ | 0.11$^{ab}$ | 0.07$^b$ | 0.10$^{ab}$ | 0.021 |
| Butane, 2-azido-2,3,3-trimethyl- | NA | 57 | 627 | 1.58 | 0.00 | 1.97 | 1.47 | 0.548 |
| Cyclohexane, azido- | NA | 55 | 612 | 9.35 | 8.59 | 8.57 | 6.36 | 1.589 |
| Cyclohexene, 3-(1-methylethyl)- | NA | 81 | 919 | 0.00$^b$ | 0.34$^a$ | 0.00$^b$ | 0.00$^b$ | 0.002 |
| Cyclopentene, 1-ethenyl-3-methylene- | NA | 91.1 | 820 | 0.00 | 0.74 | 0.59 | 0.52 | 0.205 |
| Cyclotetrasiloxane, octamethyl- | NA | 281.1 | 1,002 | 108.93$^{ab}$ | 110.84$^{ab}$ | 113.48$^a$ | 37.86$^b$ | 16.314 |
| Decane | Alkane | 57 | 994 | 8.11 | 5.98 | 6.13 | 4.18 | 0.925 |
| Decane, 2,3,5-trimethyl- | NA | 57 | 1,399 | 0.00$^c$ | 0.00$^c$ | 0.10$^a$ | 0.03$^b$ | 0.004 |
| Decane, 2,4-dimethyl- | NA | 57.1 | 1,106 | 0.32$^a$ | 0.18$^{ab}$ | 0.21$^{ab}$ | 0.12$^b$ | 0.036 |
| Decane, 3-methyl- | NA | 57.1 | 1,057 | 0.21$^a$ | 0.00$^b$ | 0.00$^b$ | 0.00$^b$ | 0.005 |
| D-Limonene | Mint, lemon, citrus, orange, fresh, sweet | 68 | 1,024 | 0.77$^a$ | 0.82$^a$ | 0.78$^a$ | 0.21$^b$ | 0.051 |
| Dodecane | Alkane | 57 | 1,199 | 1.57$^a$ | 1.45$^a$ | 1.50$^a$ | 0.19$^b$ | 0.072 |
| Dodecane, 4,6-dimethyl- | NA | 71.1 | 1,330 | 0.09$^a$ | 0.00$^b$ | 0.00$^b$ | 0.00$^b$ | 0.003 |
| Ethane, isocyanato- | NA | 71 | 585 | 0.85$^b$ | 0.00$^b$ | 3.87$^a$ | 0.00$^b$ | 0.255 |
| Guanidine | NA | 43.1 | 868 | 0.00$^b$ | 0.22$^a$ | 0.00$^b$ | 0.00$^b$ | 0.001 |
| Heptane, 2,2,4,6,6-pentamethyl- | NA | 57 | 977 | 9.05$^a$ | 8.61$^{ab}$ | 8.05$^{ab}$ | 4.65$^b$ | 0.960 |
| Hexane, 1,1-diethoxy- | Cognac, pear, floral, hyacinth, apple, fruity | 103 | 1,102 | 0.09 | 0.06 | 0.06 | 0.00 | 0.020 |
| Hexane, 3-ethyl- | NA | 43.1 | 766 | 5.55$^a$ | 2.42$^{bc}$ | 3.41$^b$ | 1.15$^c$ | 0.331 |
| Hexane, 3-ethyl-2-methyl- | NA | 57.1 | 842 | 0.23 | 0.15 | 0.31 | 0.00 | 0.071 |

**Table 4.** Volatile organic compounds of purified horse oil by supercritical fluid extraction and three deodorants (A.U. ×10$^6$) (continued)

| Volatile organic compounds | Flavor profile[1] | m/z | LRI | CON | ACHO | PACHO | ZHO | SEM |
|---|---|---|---|---|---|---|---|---|
| Methane, dichloronitro- | NA | 83.1 | 559 | 4.77 | 3.55 | 4.60 | 2.57 | 0.927 |
| Methane, isocyanato- | Cabbage, horseradish | 57.1 | 580 | 6.48$^a$ | 0.36$^{ab}$ | 0.15$^b$ | 1.62$^{ab}$ | 1.393 |
| Methane-d, trichloro- | NA | 84 | 545 | 0.06 | 0.05 | 0.08 | 0.08 | 0.022 |
| Naphthalene | Dry, pungent, tarry, tar | 128 | 1,181 | 0.07$^{ab}$ | 0.14$^a$ | 0.11$^a$ | 0.00$^b$ | 0.019 |
| Nonane, 2,5-dimethyl- | NA | 57 | 1,011 | 1.33$^a$ | 0.60$^b$ | 0.69$^{ab}$ | 0.68$^{ab}$ | 0.160 |
| Nonane, 2,6-dimethyl- | NA | 71.1 | 1,021 | 2.28 | 1.15 | 1.23 | 1.17 | 0.262 |
| Octane, 2,4,6-trimethyl- | NA | 57 | 963 | 1.01 | 0.50 | 0.61 | 0.57 | 0.121 |
| o-Cymene | NA | 119 | 1,020 | 0.66$^a$ | 0.67$^a$ | 0.58$^a$ | 0.00$^b$ | 0.024 |
| Pentadecane | Alkane | 57.1 | 1,503 | 0.00$^c$ | 0.13$^{ab}$ | 0.15$^a$ | 0.05$^{bc}$ | 0.019 |
| Pentane, 2,3-dimethyl- | Gasoline | 56.1 | 663 | 177.17$^a$ | 175.05$^a$ | 193.31$^a$ | 15.91$^b$ | 5.594 |
| Tetradecane | Alkane, mild, waxy | 57.1 | 1,400 | 0.14$^a$ | 0.16$^a$ | 0.12$^a$ | 0.00$^b$ | 0.011 |
| Tridecane | Alkane | 71 | 1,284 | 0.21 | 0.20 | 0.19 | 0.17 | 0.045 |
| Undecane | Alkane | 57 | 1,106 | 0.32$^a$ | 0.21$^b$ | 0.22$^b$ | 0.00$^c$ | 0.020 |
| Undecane, 2,6-dimethyl- | NA | 57 | 1,215 | 0.28$^a$ | 0.00$^b$ | 0.00$^b$ | 0.00$^b$ | 0.002 |
| Undecane, 2,8-dimethyl- | Alkane | 71 | 1,223 | 0.08$^a$ | 0.05$^b$ | 0.06$^{ab}$ | 0.00$^c$ | 0.006 |
| Undecane, 3,6-dimethyl- | Alkane | 57 | 1,215 | 0.00$^b$ | 0.15$^a$ | 0.11$^{ab}$ | 0.00$^b$ | 0.028 |
| Undecane, 3-methyl- | NA | 57 | 1,173 | 0.12$^a$ | 0.00$^b$ | 0.11$^a$ | 0.00$^b$ | 0.010 |
| Undecane, 5-methyl- | NA | 57.1 | 1,160 | 0.12$^a$ | 0.00$^b$ | 0.00$^b$ | 0.00$^b$ | 0.013 |
| Sub total | | | | 355.56$^a$ | 334.20$^a$ | 366.62$^a$ | 84.8$^b$ | 16.558 |
| Ketones | | | | | | | | |
| 2,3-Butanedione | Butter, caramel, oily, creamy, sweet, pungent, strong | 43.1 | 581 | 0.00$^b$ | 1.29$^a$ | 0.00$^b$ | 0.00$^b$ | 0.271 |
| 2-Octanone | Soap, gasoline | 58.1 | 983 | 0.28$^a$ | 0.26$^{ab}$ | 0.2$^{ab}$ | 0.08$^b$ | 0.480 |
| 3,5-Octadien-2-one | Fat, fruity, mushroom | 95.1 | 1,098 | 5.27$^a$ | 6.10$^a$ | 5.68$^a$ | 0.97$^b$ | 0.386 |
| 3,5-Octadien-2-one, (E,E)- | Fruity, grassy, green | 95.1 | 1,075 | 9.02$^a$ | 9.62$^a$ | 9.27$^a$ | 1.72$^b$ | 0.379 |
| 3-Heptanone, 2-methyl- | Fruity, green, leafy | 43 | 890 | 0.00$^b$ | 0.00$^b$ | 0.57$^a$ | 0.21$^b$ | 0.056 |
| 3-Hexanone, 2-methyl- | Fruity | 71.1 | 788 | 0.38$^a$ | 0.33$^{ab}$ | 0.35$^{ab}$ | 0.14$^b$ | 0.042 |
| 3-Octen-2-one | Crushed bug, nut, herbal, earthy, hay, sweet, blueberry, mushroom, spicy | 55 | 1,040 | 0.98$^a$ | 1.09$^a$ | 1.14$^a$ | 0.20$^b$ | 0.060 |

**Table 4. Volatile organic compounds of purified horse oil by supercritical fluid extraction and three deodorants (A.U. ×10$^6$) (continued)**

| Volatile organic compounds | Flavor profile[1] | m/z | LRI | CON | ACHO | PACHO | ZHO | SEM |
|---|---|---|---|---|---|---|---|---|
| N,N'-Bis(2,6-dimethyl-6- nitrosohept-2-en-4-one) | NA | 83.1 | 612 | 1.48 | 2.54 | 1.28 | 0.61 | 0.423 |
| Sub total | | | | 17.41$^a$ | 21.24$^a$ | 18.49$^a$ | 3.93$^b$ | 1.937 |
| Others | | | | | | | | |
| 1H-1,2,3,4-Tetrazol-5-amine, 1-methyl- | NA | 57 | 628 | 0.00$^b$ | 1.36$^a$ | 0.00$^b$ | 0.00$^b$ | 0.028 |
| 1H-Imidazol-2-amine | NA | 83 | 1,036 | 1.60$^{ab}$ | 0.00$^b$ | 2.27$^a$ | 0.00$^b$ | 0.455 |
| 1H-Tetrazol-5-amine | NA | 43 | 572 | 0.73 | 0.84 | 0.81 | 0.30 | 0.481 |
| 4-Pyridinecarboxamide | NA | 122 | 1,388 | 0.00 | 0.00 | 0.06 | 0.00 | 0.014 |
| 5-Amino-2-methyl-2H-tetrazole | NA | 43.1 | 836 | 1.18 | 1.18 | 1.22 | 0.81 | 0.256 |
| 5H-Tetrazol-5-amine | NA | 57 | 1,064 | 0.00$^b$ | 1.11$^{ab}$ | 1.75$^a$ | 0.05$^b$ | 0.373 |
| Aluminum, triethyl- | NA | 57 | 818 | 1.15$^{ab}$ | 1.68$^a$ | 1.89$^a$ | 0.14$^b$ | 0.300 |
| Benzothiazole | Rubber, vegetable, gasoline, sulfury, meat, rubbery, coffee, nutty, cooked | 135 | 1,222 | 0.14 | 0.11 | 0.16 | 0.12 | 0.049 |
| Camphor | Bitter, herbal, medicainal, minty, phenolic, woody | 95.1 | 1,147 | 0.12 | 0.11 | 0.11 | 0.08 | 0.025 |
| Caprolactone oxime, (NB)-O-[(diethylboryloxy)(ethyl)boryl]- | NA | 76.7 | 707 | 0.00$^b$ | 3.65$^a$ | 0.00$^b$ | 0.00$^b$ | 0.805 |
| Diethylcyanamide | NA | 55.1 | 911 | 0.00$^b$ | 0.12$^a$ | 0.00$^b$ | 0.00$^b$ | 0.021 |
| Dimethylphosphinic fluoride | NA | 81.1 | 586 | 8.25 | 6.49 | 7.43 | 6.19 | 0.986 |
| Formamide, N,N-dibutyl- | Odorless | 72.1 | 1,307 | 0.23 | 0.27 | 0.16 | 0.21 | 0.053 |
| Formamide, N,N-diethyl- | NA | 101.1 | 907 | 0.63 | 0.85 | 0.76 | 0.43 | 0.143 |
| n-Butyl ether | Ethereal | 57 | 854 | 0.00$^b$ | 1.56$^a$ | 1.43$^a$ | 0.98$^{ab}$ | 0.269 |
| n-Hexadecanoic acid | Fatty, slightly waxy | 73 | 1,959 | 0.31 | 0.07 | 0.15 | 0.07 | 0.090 |
| Phosphoramidous difluoride | NA | 85.1 | 682 | 0.33 | 0.22 | 0.41 | 0.00 | 0.107 |
| sec-Butylamine | Ammonia, fishy | 44.1 | 584 | 16.05$^a$ | 14.78$^a$ | 15.97$^a$ | 0.00$^b$ | 0.358 |
| Triisobutyl phosphate | NA | 99.1 | 1,523 | 0.35$^a$ | 0.2$^{ab}$ | 0.00$^b$ | 0.05$^b$ | 0.053 |
| Trimethylene oxide | NA | 58 | 542 | 52.82 | 42.31 | 41.64 | 32.93 | 6.607 |
| Sub total | | | | 31.07$^a$ | 34.60$^a$ | 34.58$^a$ | 9.43$^b$ | 1.937 |

**Table 4.** Volatile organic compounds of purified horse oil by supercritical fluid extraction and three deodorants (A.U. ×10$^6$) (continued)

| Volatile organic compounds | Flavor profile[1] | m/z | LRI | CON | ACHO | PACHO | ZHO | SEM |
|---|---|---|---|---|---|---|---|---|
| Total | | | | 615.29$^a$ | 589.23$^a$ | 641.66$^a$ | 192.83$^b$ | 25.562 |

[1] Flavor profile of horse oil volatile organic compounds analyzed using FlavorDB (https://cosylab.iiitd.edu.in/flavordb/), FooDB (www.foodb.ca/), and Flavornet (http://www.flavornet.org).
a–c Mean values within the same rows with the different superscripts are significantly different among treatments (p<0.05).
NA, not available; No aroma/flavor description found for the volatile organic compounds.
LRI, linear retention index; CON, supercritical fluid extracted HO without deodorant; ACHO, supercritical fluid extracted HO purified with activated carbon; PACHO, supercritical fluid extracted HO purified with palm activated carbon; ZHO, supercritical fluid extracted HO purified with zeolite; HO, horse oil.

Zeolite is renowned for its exceptional adsorption capacity, particularly for polar molecules such as ammonia and aromatic hydrocarbons, surpassing its performance for non-polar molecules, as supported by recent research (Fuss et al., 2021). Its distinctive molecular porosity, ranging from 0.1 to 1 nm, significantly influences its effectiveness as a deodorant in removing VOCs from oils. The adsorption process is especially efficient for smaller molecules, which are quickly absorbed by zeolite compared with larger molecules. The introduction of zeolite as a deodorant in oil purification allows the effective absorption of undesirable components on the oil surface by employing a combination of physical adsorption and chemisorption mechanisms. This is because of the molecular porosity of zeolite, which provides a larger surface area, leading to an increased adsorption capacity (Fuss et al., 2021). In addition to its deodorizing properties, zeolite possesses catalytic properties that make it valuable in oil purification processes. The tetrahedral units in zeolite create three distinct pore systems, resulting in enhanced catalytic activity compared to that of conventional catalysts. The zeolite surface exhibited a unique combination of properties from both ordinary crystal surfaces and crystal lattices, allowing it to interact strongly with the detected VOCs in the HO. The capability of zeolite to remove carbon from adsorbed molecules continuously increases the hydrogen content in the oil, ultimately leading to improved catalytic efficiency (Morris and Allan, 2017).

PLS-DA is a powerful multivariate statistical tool for analyzing and classifying data with multiple inter-correlated dependent variables (Ruiz-Perez et al., 2020). In this study, the PLS-DA model effectively separated volatile compounds using two principal components (PC1: 32.4%, PC2: 15.1%; Fig. 1). Although the VOCs of CON, ACHO, and PACHO were similar, the VOCs of ZHO were significantly different from those of the other treatments. To further evaluate the contribution of individual VOCs to the PLS-DA classification based on deodorant treatment, variable importance in projection (VIP) scores were calculated (Fig. 2). The VIP score is a relevant index for determining the importance of experimental variables in defining the classification model. In this study, variables with VIP scores above average (>1.2) were considered better indicators for differentiating the effects of the deodorant compounds (Alcantara et al., 2021).

In this study, 30 VOCs were identified as important for discriminating the effects of different deodorants on HO purification (VIP scores>1.2). Dibutyl phthalate, which contributes to a faint odor, had the highest VIP score (>1.8) among the other VOCs and was the most significant contributor to flavor/odor profile discrimination in CON and HO with deodorants. The concentration of dibutyl phthalate was the highest in the HO purified with deodorants. Undecane had the second-highest VIP score and played a role in differentiating the alkane odor of HO, along with other compounds, such as 2,8-dimethyl-, tetradecane, and dodecane (VIP score>1.2). Compounds such as 1-Hexanol, 2-ethyl, nonanal, and D-limonene, with VIP scores higher than 1.2, may contribute to the discrimination of VOCs related to citrus odors in CON and HO with deodorants. The relative contents of VOCs between CON and HO with deodorants were also analyzed and clustered separately in a clustered heatmap (Fig. 3), where the VOCs in ZHO were clustered differently from the other HO with
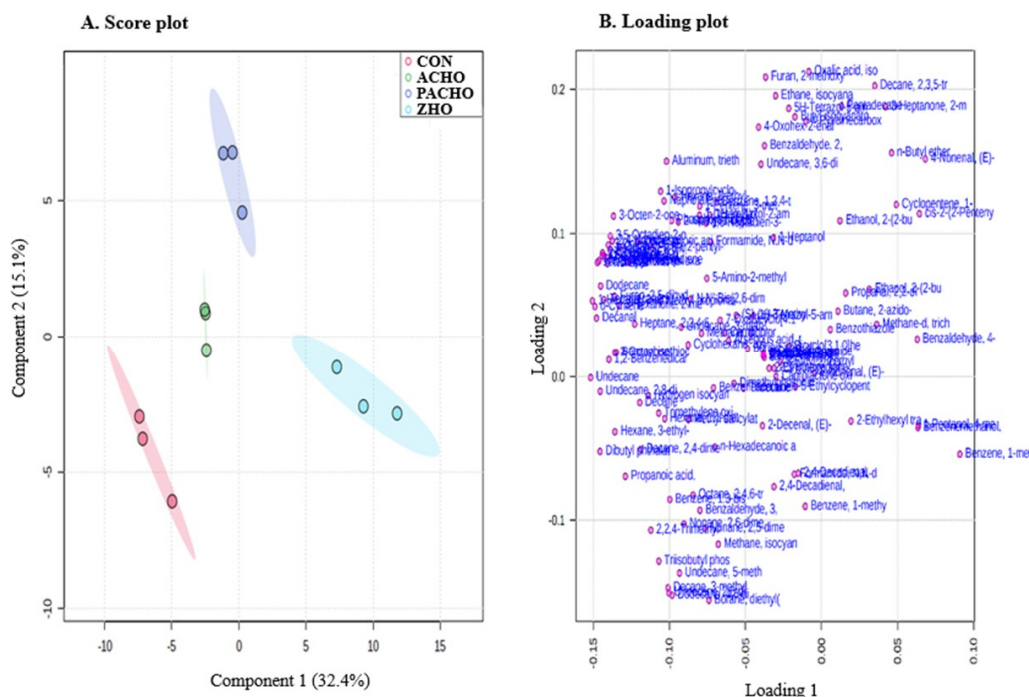
**Fig. 1. Score plot (A) and loading plot (B) of partial least-squares discriminant analysis (PLS-DA) of volatile organic compounds from purified horse oil by supercritical fluid extraction and three deodorants.** CON, supercritical fluid extracted HO without deodorant; ACHO, supercritical fluid extracted HO purified with activated carbon; PACHO, supercritical fluid extracted HO purified with palm activated carbon; ZHO, supercritical fluid extracted HO purified with zeolite; HO, horse oil.
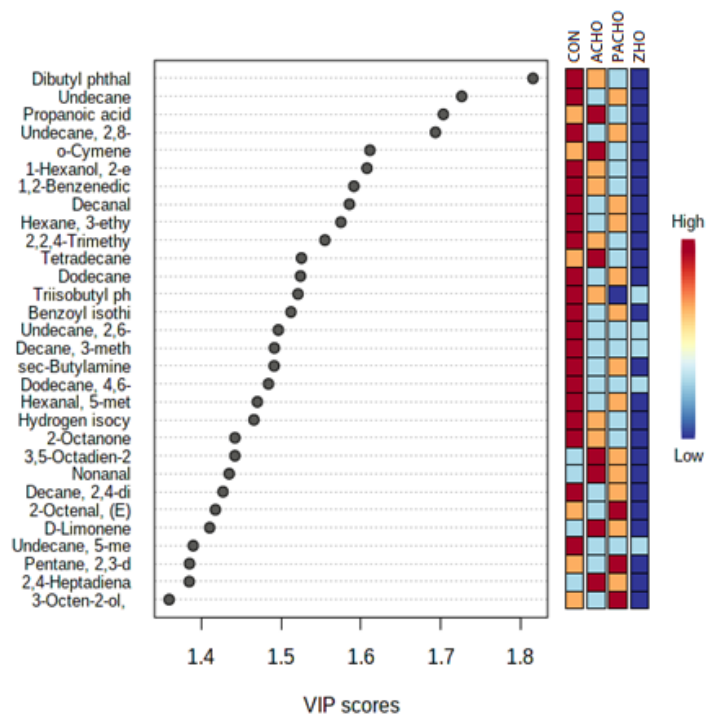


**Fig. 2. Multivariate analysis and list of high variable importance in projection scores (VIP scores>1.2) of purified horse oil by supercritical fluid extraction and three deodorants.** CON, supercritical fluid extracted HO without deodorant; ACHO, supercritical fluid extracted HO purified with activated carbon; PACHO, supercritical fluid extracted HO purified with palm activated carbon; ZHO, supercritical fluid extracted HO purified with zeolite; HO, horse oil.
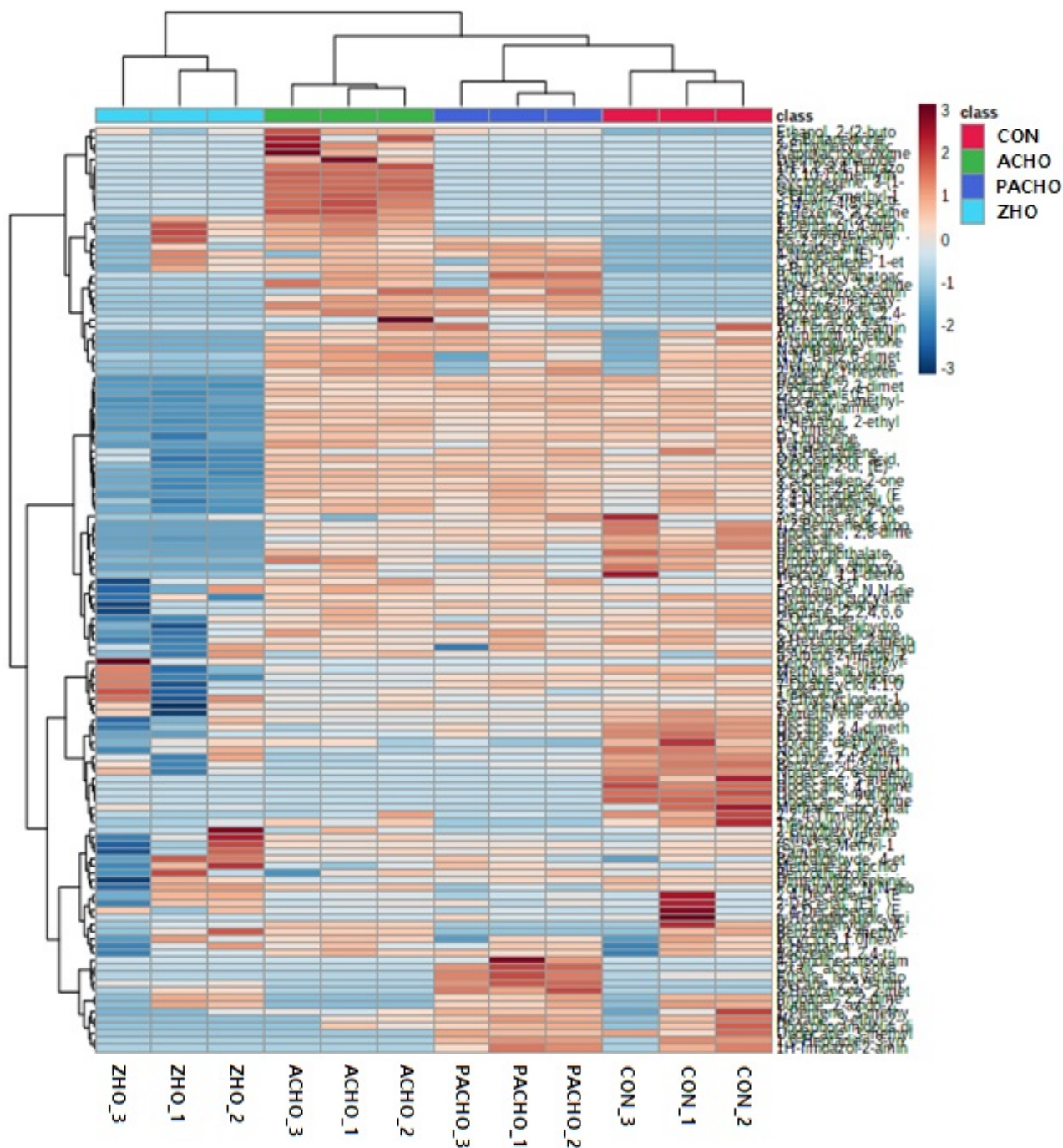
**Fig. 3. Overview of heatmap analysis based on the volatile organic compounds of purified horse oil by supercritical fluid extraction and three deodorants.** CON, supercritical fluid extracted HO without deodorant; ACHO, supercritical fluid extracted HO purified with activated carbon; PACHO, supercritical fluid extracted HO purified with palm activated carbon; ZHO, supercritical fluid extracted HO purified with zeolite; HO, horse oil.

deodorant treatments (ACHO and ZHO). In contrast, the VOCs from CON and PAC-HO were more closely related to each other than those from ACHO and ZHO.

## Conclusion

The use of supercritical fluid extraction and various deodorants, including AC, PAC, and zeolite, in the purification of HO did not negatively affect the oil yield, TBARS values, or fatty acid composition. Thirty relevant VOCs, including dibutyl phthalate, undecane, 2,8-dimethyl, tetradecane, dodecane, 1-hexanol, 2-ethyl, nonanal, and D-limonene, showed scores greater than 1.2, indicating their importance in discriminating deodorant effects on HO. Additionally, the use of zeolite as a deodorant resulted in a significant reduction in sec-butylamine and cyclotetrasiloxane, octamethyl-, as well as the total counts of VOCs in purified HO ($p < 0.05$), which may be beneficial for further use. This study provides preliminary data on the characteristics of HO purified using these deodorants; however, further research is needed to understand the mechanisms underlying their effects on VOCs and the changes in off-odor-related VOCs produced before, during, and after the purification process.

## Conflicts of Interest

The authors declare no potential conflicts of interest.

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## Author Contributions

Conceptualization: Anneke, Kim HJ, Kim D, Jang A. Data curation: Anneke, Kim HJ, Jang A. Formal analysis: Kim HJ, Kim D, Shin DJ, Jang A. Methodology: Kim HJ, Kim D, Shin DJ, Do KT, Yang CB, Jeon SW, Jang A. Software: Kim HJ, Kim D, Shin DJ. Validation: Jang A. Investigation: Kim HJ, Kim D, Shin DJ, Do KT, Yang CB, Jeon SW, Jung JH, Jang A. Writing - original draft: Anneke, Kim HJ, Kim D, Do KT, Yang CB, Jeon SW, Jung JH, Jang A. Writing - review & editing: Anneke, Kim HJ, Kim D, Shin DJ, Do KT, Yang CB, Jeon SW, Jung JH, Jang A.

## Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

## References

Alcantara GMRN, Dresch D, Melchert WR. 2021. Use of non-volatile compounds for the classification of specialty and traditional brazilian coffees using principal component analysis. Food Chem 360:130088.

Álvarez R, Meléndez-Martínez AJ, Vicario IM, Alcalde MJ. 2015. Carotenoids and fat-soluble vitamins in horse tissues: A comparison with cattle. Animal 9:1230-1238.

Ardeshiri A, Rose JM. 2018. How australian consumers value intrinsic and extrinsic attributes of beef products. Food Qual Prefer 65:146-163.

Beldarrain LR, Morán L, Sentandreu MÁ, Barron LJR, Aldai N. 2022. Effect of ageing time on the volatile compounds from cooked horse meat. Meat Sci 184:108692.

Bleicher J, Ebner EE, Bak KH. 2022. Formation and analysis of volatile and odor compounds in meat: A review. Molecules 27:6703.

Buege JA, Aust SD. 1978. Microsomal lipid peroxidation. In Methods in enzymology. Fleischer S, Packer L (ed). Academic Press, Cambridge, MA, USA.

Carvajal A, Mozuraityte R. 2016. Fish oils: Production and properties. Elsevier, Amsterdam, The Netherlands.

Castillejos E, Rodríguez-Ramos I, Guerrero-Ruiz A. 2012. Catalytic removal of water-solved aromatic compounds by carbon-based materials. In Novel carbon adsorbents. Tascón JMD (ed). Elsevier, Amsterdam, The Netherlands.

Del Pulgar JS, Roldan M, Ruiz-Carrascal J. 2013. Volatile compounds profile of sous-vide cooked pork cheeks as affected by cooking conditions (vacuum packaging, temperature and time). Molecules 18:12538-12547.

Fan R, Kim J, You M, Giraud D, Toney AM, Shin SH, Kim SY, Borkowski K, Newman JW, Chung S. 2020. α-linolenic acid-enriched butter attenuated high fat diet-induced insulin resistance and inflammation by promoting bioconversion of n-3 PUFA and subsequent oxylipin formation. J Nutr Biochem 76:108285.

Folch J, Lees M, Sloane Stanley GH. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497-509.

Fuss VLB, Bruj G, Dordai L, Roman M, Cadar O, Becze A. 2021. Evaluation of the impact of different natural zeolite treatments on the capacity of eliminating/reducing odors and toxic compounds. Materials 14:3724.

Gaber MAFM, Tujillo FJ, Mansour MP, Juliano P. 2018. Improving oil extraction from canola seeds by conventional and advanced methods. Food Eng Rev 10:198-210.

Gibon V, De Greyt W, Kellens M. 2007. Palm oil refining. Eur J Lipid Sci Technol 109:315-335.

Gudmundsson M, Hafsteinsson H. 1997. Gelatin from cod skins as affected by chemical treatments. J Food Sci 62:37-39.

Hamm W. 2003. Vegetable oils: Oil production and processing. In Encyclopedia of food sciences and nutrition. Caballero B (ed). Academic Press, Cambridge, MA, USA.

Herrero M, Mendiola JA, Cifuentes A, Ibáñez E. 2010. Supercritical fluid extraction: Recent advances and applications. J Chromatog A 1217:2495-2511.

Husein El Hadmed H, Castillo RF. 2016. Cosmeceuticals: Peptides, proteins, and growth factors. J Cosmet Dermatol 15:514-519.

Kim HJ, Kim D, Kim NY, Kim JS, Jang A. 2020. Anti-wrinkle and anti-inflammatory effects of a combination of topically applied horse oil and dietary enzyme hydrolysates from horse bone. Process Biochem 90:257-267.

Kim WJ, Kim JD, Oh SG. 2007. Supercritical carbon dioxide extraction of caffeine from Korean green tea. Sep Sci Technol 42:3229-3242.

Lee C, Eom YA, Yang H, Jang M, Jung SU, Park YO, Lee SE, Jung H. 2018. Skin barrier restoration and moisturization using horse oil-loaded dissolving microneedle patches. Skin Pharmacol Physiol 31:163-171.

Lee CH, Row KH, Lee YW, Kim JD, Lee YY. 2001. Supercritical fluid extraction of perillyl alcohol in Korean orange peel. J Liq Chromatogr Relat Technol 24:1987-1996.

Li L, Jin R, Li Y, Nho JH, Choi W, Ji YS, Yoon HJ, Yoon KC. 2020. Effects of *Eurya japonica* extracts on human corneal epithelial cells and experimental dry eye. Exp Ther Med 20:1607-1615.

Maeng Y, Kim KT, Zhou X, Jin L, Kim KS, Kim YH, Lee S, Park JH, Chen X, Kong M, Cai L, Li X. 2018. A novel

microbial technique for producing high-quality sophorolipids from horse oil suitable for cosmetic applications. Microb Biotechnol 11:917-929.

Magagna F, Valverde-Som L, Ruíz-Samblás C, Cuadros-Rodríguez L, Reichenbach SE, Bicchi C, Cordero C. 2016. Combined untargeted and targeted fingerprinting with comprehensive two-dimensional chromatography for volatiles and ripening indicators in olive oil. Anal Chim Acta 936:245-258.

Maggiolino A, Lorenzo JM, Marino R, Della Malva A, Centoducati P, De Palo P. 2019. Foal meat volatile compounds: Effect of vacuum ageing on semimembranosus muscle. J Sci Food Agric 99:1660-1667.

Moloney AP, Mooney MT, Kerry JP, Stanton C, O'kiely P. 2013. Colour of fat, and colour, fatty acid composition and sensory characteristics of muscle from heifers offered alternative forages to grass silage in a finishing ration. Meat Sci 93:608-615.

Monte ML, Monte ML, Pohndorf RS, Crexi VT, Pinto LAA. 2015. Bleaching with blends of bleaching earth and activated carbon reduces color and oxidation products of carp oil. Eur J Lipid Sci Technol 117:829-836.

Moretti VM, Madonia G, Diaferia C, Mentasti T, Paleari MA, Panseri S, Pirone G, Gandini G. 2004. Chemical and microbiological parameters and sensory attributes of a typical sicilian salami ripened in different conditions. Meat Sci 66:845-854.

Morris RE, Allan PK. 2017. Structure determination. In Zeolites in catalysis: Properties and applications. Čejka J, Morris RE, Nachtigall P (ed). Royal Society of Chemistry, London, UK.

Oancea AG, Untea AE, Dragomir C, Radu GL. 2022. Determination of optimum TBARS conditions for evaluation of cow and sheep milk oxidative stability. Appl Sci 12:6508.

Park YH, Cho MJ, Kim HJ. 2019. Comparison of physicochemical characteristics of horse fat, lard, and beef-tallow. Korean J Food Sci Technol 51:1-6.

Park YH, Kim HJ. 2021. Formulation and stability of horse oil-in-water emulsion by HLB system. Food Sci Biotechnol 30:931-938.

Pereira LM, Hatanaka E, Martins EF, Oliveira F, Liberti EA, Farsky SH, Curi R, Pithon-Curi TC. 2008. Effect of oleic and linoleic acids on the inflammatory phase of wound healing in rats. Cell Biochem Funct 26:197-204.

Piao MJ, Kang KA, Zhen AX, Kang HK, Koh YS, Kim BS, Hyun JW. 2019. Horse oil mitigates oxidative damage to human HaCaT keratinocytes caused by ultraviolet B irradiation. Int J Mol Sci 20:1490.

Ren J, Chung SH. 2007. Anti-inflammatory effect of α-linolenic acid and its mode of action through the inhibition of nitric oxide production and inducible nitric oxide synthase gene expression via NF-κB and mitogen-activated protein kinase pathways. J Agric Food Chem 55:5073-5080.

Ruiz-Perez D, Guan H, Madhivanan P, Mathee K, Narasimhan G. 2020. So you think you can PLS-DA? BMC Bioinformatics 21:2.

Saiki P, Kawano Y, Van Griensven LJLD, Miyazaki K. 2017. The anti-inflammatory effect of *Agaricus brasiliensis* is partly due to its linoleic acid content. Food Funct 8:4150-4158.

Simard M, Tremblay A, Morin S, Martin C, Julien P, Fradette J, Flamand N, Pouliot R. 2022. α-linolenic acid and linoleic acid modulate the lipidome and the skin barrier of a tissue-engineered skin model. Acta Biomater 140:261-274.

Sujiwo J, Lee S, Kim D, Lee HJ, Oh S, Jung Y, Jang A. 2024. Physicochemical features and volatile organic compounds of horse loin subjected to sous-vide cooking. Foods 13:280.

Tateo A, Maggiolino A, Domínguez R, Lorenzo JM, Dinardo FR, Ceci E, Marino R, Malva AD, Bragaglio A, De Palo P.

2020. Volatile organic compounds, oxidative and sensory patterns of vacuum aged foal meat. Animals 10:1495.

Vafaei N, Rempel CB, Scanlon MG, Jones PJH, Eskin MNA. 2022. Application of supercritical fluid extraction (SFE) of tocopherols and carotenoids (hydrophobic antioxidants) compared to non-SFE methods. AppliedChem 2:68-92.

Vilar EG, O'sullivan MG, Kerry JP, Kilcawley KN. 2022. Volatile organic compounds in beef and pork by gas chromatography-mass spectrometry: A review. Sep Sci Plus 5:482-512.

Woo HC, Shin BK, Cho I, Koo H, Kim M, Han J. 2011. Anti-obesity effect of carbon dioxide supercritical fluid extracts of *Panax ginseng* C. A. Meyer. J Korean Soc Appl Biol Chem 54:738-743.

Yue H, Qiu B, Jia M, Liu W, Guo XF, Li N, Xu ZX, Du FL, Xu T, Li D. 2021. Effects of α-linolenic acid intake on blood lipid profiles: A systematic review and meta-analysis of randomized controlled trials. Crit Rev Food Sci Nutr 61:2894-2910.