LWT - Food Science and Technology 185 (2023) 115177

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Flavor-protein interactions for four plant protein isolates and whey protein isolate with aldehydes

Silvia J.E. Snel [a,b], Mirela Pascu [c], Igor Bodnár [c], Shane Avison [c], Atze Jan van der Goot [b], Michael Beyrer [a,*]

[a] Institute of Life Technologies, University of Applied Sciences and Arts Western Switzerland, CH-1950, Sion, Switzerland
[b] Food Process Engineering, Agrotechnology and Food Sciences Group, Wageningen University & Research, Bornse Weilanden 9, 6708 WG, Wageningen, the Netherlands
[c] Firmenich S.A., Rue de la Bergère 7, Meyrin 2, CH-1217, Geneva, Switzerland

ARTICLE INFO

Keywords:
Pea
Soy
Chickpea
Faba bean
Covalent interactions

ABSTRACT

Aldehydes are important flavor molecules to consider in plant-based products. Here, the flavor retention of a series of saturated aldehydes and mono-unsaturated aldehydes (2-alkenals) with different chain lengths (C4, C6, C8, and C10) in dispersions with protein isolates of pea, soy, fava bean, chickpea, and whey (as reference) was analyzed with APCI-TOF-MS. The headspace concentrations of alkenals were lower than aldehydes, meaning alkenals were retained more than saturated aldehydes. The retention was modeled by assuming hydrophobic interactions and covalent interactions. The ratio between the hydrophobic interaction parameter and the covalent parameter showed that covalent interactions are mainly important for butanal and butenal (C4). For the other aldehydes, hydrophobic interactions became increasingly important. Correlations were found between the chemical interaction parameters and the cysteine and methionine content of the different proteins. The obtained model parameters for each set of proteins and flavors allow the prediction of flavor retention when developing a flavored product with high protein content.

1. Introduction

Diet patterns must change to be able to feed the growing world population in a sustainable matter (Aiking & de Boer, 2018; Broekema et al., 2020). A route to reach this goal is to replace meat and dairy with plant-based products. Nevertheless, plant-based products are considered less appealing by consumers, because of their taste and off-notes (Michel et al., 2021). Currently, a key success factor for plant products that should replace meat is a high similarity in texture, flavor, and nutritional value. In case of meat, flavors are developed when heating the product (Ramalingam et al., 2019). The heating of meat generates different volatile flavor compounds such as alkenes, alcohols, aldehydes, ketones, ethers, esters, carboxylic acids, and sulfur-containing compounds (Kale et al., 2022). Plant-based products do not undergo a similar flavor development and often have distinct flavors of their own, which are frequently perceived as off-notes (Wang et al., 2022). In order to have a similar flavor profile, flavor compounds are added to plant-based products that both mask plant flavor and create meat-like flavors. However, flavors can strongly interact with the proteins present in plant-based products, making them less effective (Guichard, 2002). Protein-flavor retention is mostly hydrophobic, but depending on the type of flavor also irreversible covalent interactions, reversible hydrogen bonds, ionic bonds, and van der Waals's forces can lead to flavor retention (Wang & Arntfield, 2014). Aldehydes, for example, can interact covalently with the amine or thiol groups of the proteins, apart from hydrophobic interactions (Anantharamkrishnan & Reineccius, 2020b). Aldehydes can react in Schiff base formation, whereas alkenals are also capable of forming Michael adducts (Anantharamkrishnan et al., 2020a).

An efficient route to determine flavor retention is through comparing the equilibrium headspace concentration in a flavored protein dispersion to a control without protein (Gremli, 1974; Wang & Arntfield, 2015; Zhou & Cadwallader, 2006). However, this approach is time-consuming and therefore often only a few chemicals are studied, which makes it challenging to get a full overview of flavor retention in protein products. A more pragmatic method is to model experimental data to predict flavor partitioning. Harrison and Hills developed a mathematical model to predict flavor release for both hydrophilic and hydrophobic

* Corresponding author.
E-mail address: michael.beyrer@hevs.ch (M. Beyrer).

https://doi.org/10.1016/j.lwt.2023.115177
Received 21 April 2023; Received in revised form 5 August 2023; Accepted 8 August 2023
Available online 14 August 2023

compounds from a liquid containing macromolecules (Harrison & Hills, 1997). This model was applied to describe flavor retention in whey and sodium caseinate dispersions (Viry et al., 2018). For esters and alcohols, good predictions were obtained by only assuming hydrophobic interactions to explain flavor retention (Viry et al., 2018). For aldehydes, stronger retention was observed, which was attributed to the specific covalent interactions that proteins and aldehydes can undergo (Viry et al., 2018). Therefore, the model was extended with a covalent interaction parameter to describe aldehyde retention (Viry et al., 2018). Recently, this model was applied to predict flavor retention in four plant proteins and whey dispersions with esters, and ketones, assuming hydrophobic interactions only (Snel et al., 2023). Apart from esters and ketones, aldehydes are an important chemical class to consider for the flavoring of plant-based products such as meat analogues. Therefore, probing the interactions between plant proteins and aldehydes is essential and could give great insights into the applicability of the model when covalent interactions are involved. Furthermore, it would highlight the relative contribution of hydrophobic and covalent interactions for aldehyde retention.

This study describes the retention of aldehydes by plant proteins and applies a flavor partitioning model to analyze the results. The proteins studied are pea protein isolate (PPI), soy protein isolate (SPI), chickpea protein isolate (CPPI), and fava bean protein isolate (FBPI). Besides, whey protein isolate (WPI) will be included as a control. The investigated flavors include a series (C4, C6, C8, C10) of saturated aldehydes and 2-mono-unsaturated aldehydes (alkenals), from now on addressed as aldehydes and alkenals. Furthermore, the obtained partitioning parameters will be correlated with amino acid composition.

2. Theory: flavor partitioning models

Harrison and Hills (1997) developed a first-order mathematical model to predict flavor release from an aqueous solution containing polymers at equilibrium conditions. In the case of proteins, flavors can interact with the proteins through either hydrophobic interactions or specific covalent interactions. The flavor-partitioning model will be shortly summarized here. The partition coefficient (K^f_{wg}) at equilibrium between flavor concentration in the water phase (c^e_{fw}) and gas phase (c^e_{fg}) is defined as:

$$K^f_{wg} = \frac{c^e_{fg}}{c^e_{fw}} \tag{1}$$

When protein is added to the water phase, part of the flavors could interact with the protein. When we consider that the flavor-protein interaction is a reversible, first-order reaction, the global interaction constant (K^f_p) between protein P and flavor F is defined as:

$$K^f_p = \frac{c^e_{fp}}{c^e_p c^e_{fw}} \tag{2}$$

in which c^e_{fp} and c^e_p are the concentrations of protein-retained flavor in the dispersion at equilibrium, and protein. Since in the experimental set-up, protein concentration exceeds the flavor concentration largely, c_p is simplified as the total concentration of protein in the dispersion that thus remains constant during the experiment. Now, the effective partition coefficient K^{eff}_{wg} between flavor in the gas phase and the water-protein phase becomes:

$$K^{eff}_{wg} = \frac{c^e_{fg}}{c^e_{ft}} \tag{3}$$

in which c^e_{ft} is the total flavor in the water system. The mass balance reads:

$$c_{ft} = c_{fp} + c_{fw} \tag{4}$$

In which c_{ft} equals c_{fw} when no protein is present in the water phase. Using the mass balance and eq. (1) and eq. (2), we can describe K^{eff}_{wg} as:

$$K^{eff}_{wg} = \frac{K^f_{wg}}{1 + K^f_p c^e_p} \tag{5}$$

When flavor retention is dominated by hydrophobic interactions, we could approach the interaction constant with:

$$K^f_p = a_p P^f_{ow} \tag{6}$$

in which a_p and P^f_{ow} are the hydrophobic interaction parameter and the octanol-water partition coefficient. For aldehydes, the covalent interaction has to be taken into account, leading to (Viry et al., 2018):

$$K^f_p = a_p P^f_{ow} + K_{Ald} \tag{7}$$

in which K_{Ald} is the covalent interaction parameter between aldehydes and proteins. For alkenals, this parameter becomes K_{alk}. Aldehydes can interact with proteins through a condensation reaction (Schiff base adduct), and alkenals can have an additional conjugate addition (Michael adduct, Fig. 1) (Anantharamkrishnan et al., 2020a). The total contribution of both reactions is captured in the parameters K_{ald} or K_{alk}.

3. Methods and materials

3.1. Materials

Soy protein isolate (SPI, Supro® 500E A) was obtained from Solae (St. Louis, United States). Pea protein isolate (PPI, Nutralys® F85M) was obtained from Roquette Frères S.A. (Lestrem, France). Fava bean protein isolate (FBPI, FFBP-90-C-EU) and chickpea protein isolate (CPPI, FCPP-70) were both obtained from AGT Foods (Regina, Canada). Whey protein isolate (WPI, BiPRO) was obtained from Davisco Foods International (Minnesota, USA). Amino acid content was measured in a

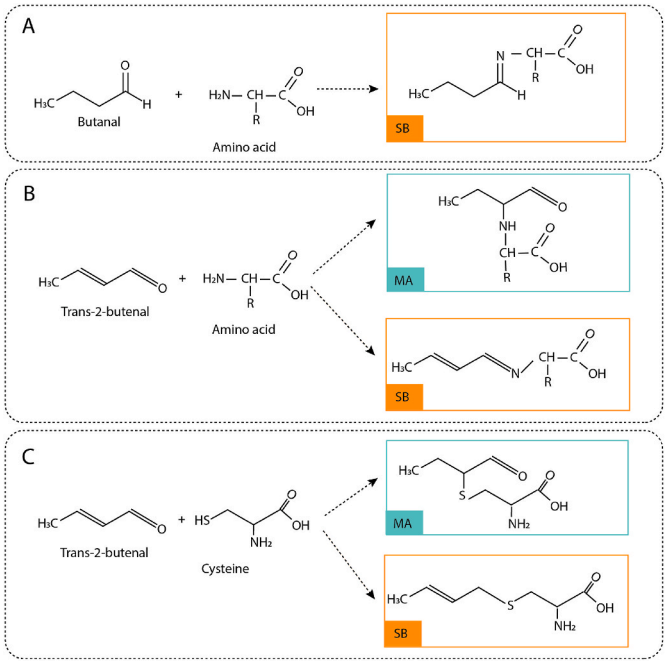


Fig. 1. Schematical representation of the chemical reactions possible between the amine group of amino acids and butanal (A), and *trans*-2-butenal (B), and the thiol group of cysteine with *trans*-2-butenal (C) (Anantharamkrishnan & Reineccius, 2020b). Butanal and *trans*-2-butenal are chosen in this example to represent aldehydes and alkenals respectively. SB = schiff base, MA = michael adduct.

Table 1
Weighted hydrophobic indexes (WHI), protein content, pH, solubility, and moisture content of soy, pea, faba bean, chickpea, and whey protein isolates. Values are means ± standard deviations, and letters indicate significant groups ($p \leq 0.05$). Amino acids were measured in duplicate and the protein content, pH, solubility, and moisture content in triplicate. WHI's are a summation of the amino acid weight multiplied by the hydrophobic index.

	Soy	Yellow pea	Faba bean	Chickpea	Whey[a]
WHI	147.35 ± 0.63^{b}	149.41 ± 0.09^{ab}	143.22 ± 1.69^{c}	152.28 ± 0.91^{a}	236.18
Protein (g/100g)	83.25 ± 2.77^{a}	77.11 ± 0.29^{b}	80.42 ± 2.99^{ab}	67.03 ± 1.83^{c}	89.7
pH	7.1 ± 0.01^{c}	7.5 ± 0.01^{a}	6.4 ± 0.02^{e}	6.6 ± 0.01^{d}	7.1 ± 0.02^{b}
Solubility (g/g)	0.59 ± 0.01^{b}	0.41 ± 0.03^{c}	0.12 ± 0.01^{d}	0.12 ± 0.00^{d}	1.02 ± 0.00^{a}
Moisture content (g/100g)	8.8 ± 0.0	8.1 ± 0.0	8.0 ± 0.1	6.6 ± 0.1	5.2 ± 0.1

[a] WHI calculated with amino acid composition taken from (Amagliani et al., 2017).

previous study with the same protein isolates and used to measure protein content (Snel et al., 2023). Furthermore, solubility, pH, and moisture content were measured (Table 1).

Trans-2-butenal and ethanol were purchased from Sigma-Aldrich (St. Louis, USA). The other flavors listed in Table 2 were provided by Firmenich S.A. (Geneva, Switzerland).

3.2. Preparation flavored protein dispersions

Stock solutions of 50 g/kg were made for each protein and subsequently diluted to obtain dispersions of 5, 10, 20, 30, and 50 g/kg for each protein isolate (in demineralized water). The protein content in the dispersion was then corrected using the protein content and dry matter content. Each flavor compound was first diluted in ethanol, to ensure solubility, and then added to the protein dispersion to reach a concentration in a range from 0.05 to 1.00 mg/kg, depending on their spectra intensity. The flavored dispersions were then vortexed for 30 s. The presence of ethanol in the final solution (1 g/kg) did not affect the MS signal. The mixture was allowed to reach equilibrium for 24 h at 21 °C.

3.3. Static headspace measurements

The headspace concentrations were measured as independent triplicates with a G2-XS Q-TOF high-definition mass spectrometer (XEVO, Waters, Milford, United States) coupled to a patented Venturi interface (Linforth & Taylor, 1999). An automated PAL system (CTC Analytics AG, Zwingen, Switzerland) injected 5 mL of the headspace into the mass spectrometer. Mass spectra were collected in centroid mode over the range m/z 20–400 every 1 s. APCI-MS was performed in positive ionization mode with a cone voltage of 4.0 kV, source temperature of 105 °C, heated sample transfer line temperature of 130 °C and auxiliary gas flow of 600 L/h. Lock spray (on-the-fly mass calibration, Waters, Milford, United States) was used to apply a mass correction to measured m/z values during the analysis. All the signal intensities were corrected for the background intensities of the protein isolate dispersions. The relative headspace concentration (RHC) was approximated as:

$$\mathrm{RHC\%} = \frac{\mathrm{Peak\ \ area_{flavored\ \ dispersion}} - \mathrm{Peak\ \ area_{dispersion}}}{\mathrm{Peak\ \ area_{flavor\ \ in\ \ water}}} * 100\% \quad (8)$$

3.4. Model fitting

The experimental results were described with the flavor partitioning model (eq (5)). The relative headspace can be interpreted as $\frac{c_{fg}}{c_{fg}^{p}}$, since we can assume that $c_{ft} = c_{fw}$ when no protein is added to the dispersion. Equations (5) and (7) can be combined and rewritten to obtain a linear relation:

$$RHC = \frac{K_{wg}^{eff}}{K_{wg}^{f}} = \frac{c_{fg}}{c_{fg}^{p}} = 1 + (a_p P_{ow}^{f} + K_{ald})c_p \quad (9)$$

For the alkenals, K_{ald} becomes K_{alk}. The calculated octanol-water partition coefficient was obtained from EPIWEB. The c_p was taken as the concentration of protein, thus corrected with the dry matter and protein content for each isolate. Since in eq (9) the slope parameters a_p and K_{ald} can not be extracted as independent parameters, it was chosen to only fit K_{ald}. The a_p was based on previously obtained fitting parameters for esters, for which only hydrophobic interactions were assumed (Snel et al., 2023). These a_p values were 4.8E-5, 1.1E-4, 8.6E-5, 1.7E-4, and 7.2E-5 g/L for SPI, PPI, FBPI, CPPI, and WPI respectively. The fitting was done for each protein and each aldehyde separately. The fitting was performed with Python and the SciPy package. This resulted in a prediction for K_{ald} or K_{alk} and the uncertainties for these predictions. Residuals squared were calculated as the squared Pearson correlation coefficient, which was calculated with the sciPy.stats package. A ratio between K_{ald} or K_{alk} and the hydrophobic contribution was calculated as:

$$Ratio = \frac{K_{ald}}{a_p P_{ow}^{f}} \quad (10)$$

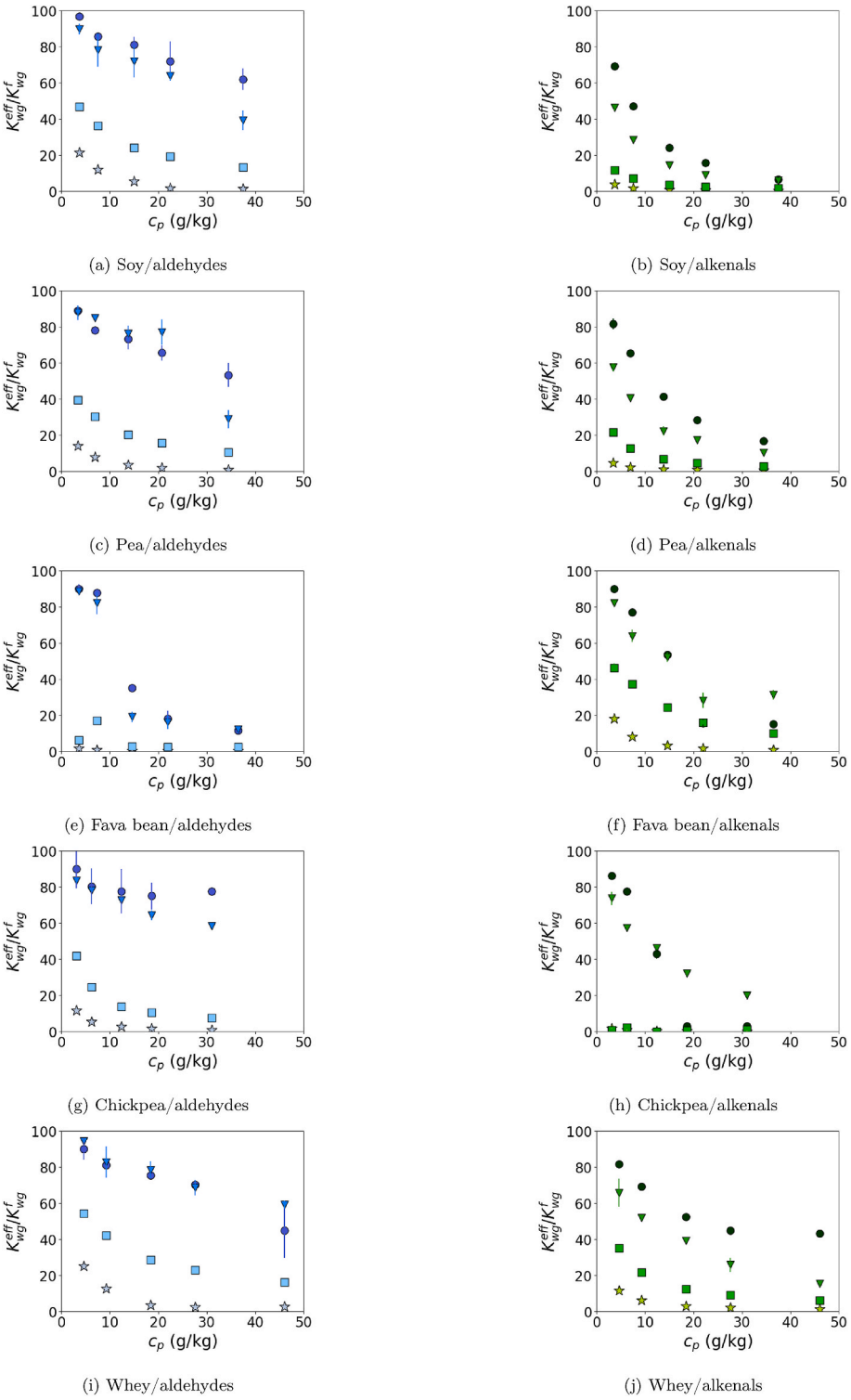
3.5. Statistical analysis

Statistical analysis was performed with R. Normality was tested with descriptive statistics. When the data were normally distributed, a one-way analysis of variance (ANOVA) was done to test if the observed differences between samples were significant. Multiple comparison Tukey tests were done to indicate which treatments were significantly different from each other.

Table 2
Aldehydes studied with their molecular weight (MW), solubility and octanol-water partition coefficient (Log P), and the concentration in the flavored protein dispersions (mg/kg).

Name	MW (g/mol)[a]	Solubility (mg/kg)[a]	Log P[a]	Concentration (mg/kg)
Butanal	72.11	23850	0.60	1.00
Hexanal	100.16	3527	1.80	0.75
Octanal	128.22	394	2.78	0.25
Decanal	156.27	43.5	3.76	0.05
Trans-2-butenal	70.09	41480	0.60	5.00
Trans-2-hexenal	98.15	5261	1.58	1.00
Trans-2-octenal	126.2	612	2.57	0.75
Trans-2-decenal	154.25	67.8	3.55	0.50

[a] Software EPIWEB v4.1, KOWWIN v1.68.



(a) Soy/aldehydes (b) Soy/alkenals

(c) Pea/aldehydes (d) Pea/alkenals

(e) Fava bean/aldehydes (f) Fava bean/alkenals

(g) Chickpea/aldehydes (h) Chickpea/alkenals

(i) Whey/aldehydes (j) Whey/alkenals

Fig. 2. K^{eff}_{wg}/K^{f}_{wg} (peak area flavor in dispersion/peak area flavor in water) measured with atmospheric pressure chemical ionization time-of-flight mass spectroscopy of aldehydes (a, c, e, g, blue), and alkenals (b, d, f, h, green) as a function of SPI (a, b), PPI (c, d), FBPI (e, f), CPPI (g, h), and WPI (i, j) concentration. Protein concentration, c_p is the concentration of the isolate corrected for its protein and moisture content. Values are averages for butanal/butenal (round), hexanal/hexenal (triangle), octanal/octenal (square), and decanal/decenal (star). Colored bars represent the standard error, n = 3. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Results

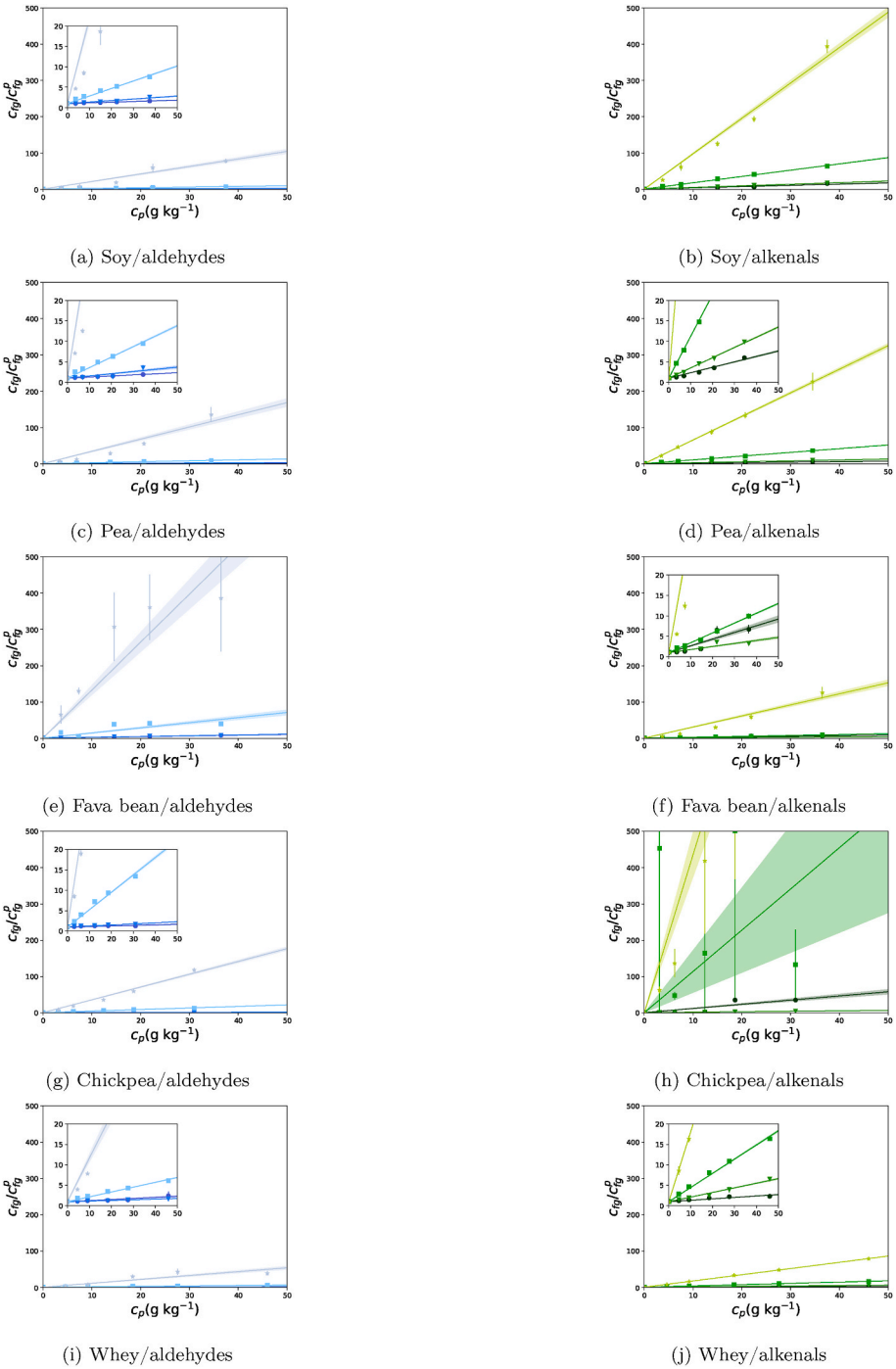
4.1. Influence of protein concentration on flavor retention

The relative headspace concentration (RHC) of aldehydes was measured as a function of protein concentration for SPI, PPI, FBPI, CPPI, and WPI (Fig. 2). A decrease in RHC is interpreted as an increase in flavor retention. Protein concentrations were corrected with the known protein and moisture content of each protein isolate. Furthermore, the background signal intensities of the protein isolates were subtracted (Equation (8)). The background signal of the protein dispersion can be seen in the supplementary materials. Most aldehydes had low signal intensities in the protein isolates, with all background intensities below 10% of the added aldehyde concentration in water, which ranged from 0.05 to 1.00 g/kg. When the signal of added aldehyde in the protein dispersion is directly compared to the blank protein dispersion (supplementary materials) it is observed that in most cases the signal of the blank protein dispersion is less than 20%, with some higher values found

for the longer chain aldehydes. Since the added aldehyde concentration was very low (Table 2), subtracting the background signal intensities was assumed to be sufficient to account for the matrix effects on flavor retention. Moreover, in this study, considering the low molar ratio of flavor compounds to protein isolate, it is unlikely that saturation of binding sites or protein conformational changes occur, as demonstrated in the case of SPI (Guo et al., 2020). The residual polysaccharides are assumed to have minimal nonspecific molecular interactions, but it is important to note that certain polysaccharides, such as starch, can potentially interact with flavor compounds (Guichard, 2002).

The RHC of the aldehydes decreased with increasing protein concentration. Furthermore, the RHC decreased when the molecular weight of the aldehydes increased (Table 2). The RHCs of butanal and hexanal were around 90% at low protein concentrations but decreased with increasing protein concentration. Butanal was more retained by FBPI and WPI. Hexanal was also highly retained by FBPI, but less by WPI, and more by CPPI. The RHCs of octanal were around 40–50% at the lowest protein concentrations tested (3–4 g/kg) and decreased with increasing protein concentration. The exception was FBPI, which had an RHC as low as 6.1% at 4 g/kg protein, which first increased slightly at 7 g/kg, followed by a decrease. Decanal had RHCs around 10–20% at low protein concentration, with a lower value for FBPI (1.7%). Both octanal and decanal were more retained by the plant proteins than by WPI.

The RHC of alkenals also depended on protein concentration and octanol-water partition coefficient, and retention was larger compared to the aldehydes. Butenal was more retained than butanal, especially at



(a) Soy/aldehydes (b) Soy/alkenals

(c) Pea/aldehydes (d) Pea/alkenals

(e) Fava bean/aldehydes (f) Fava bean/alkenals

(g) Chickpea/aldehydes (h) Chickpea/alkenals

(i) Whey/aldehydes (j) Whey/alkenals

Fig. 3. c_{fg}/c^p_{fg} (headspace concentration of flavor in water/headspace concentration flavor in dispersion) measured with atmospheric pressure chemical ionization time-of-flight mass spectroscopy and model fits of aldehydes (a, c, e, g, blue), and alkenals (b, d, f, h, green) as a function of soy (a, b), yellow pea (c, d), fava bean (e, f), chickpea (g,h), and whey (i,j) protein isolate concentrations. Lines represent the model fits of the experimental points for butanal/butenal (round), hexanal/hexenal (triangle), octanal/octenal (square), and decanal/decenal (star). Colored areas represent uncertainties of the fitted parameters. Colored bars represent the standard error, n = 3. Zoomed-in graphs are included for some graphs. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

higher protein concentrations. Butenal was retained the most by SPI. The retention of hexenal and octenal was again higher compared to hexanal and octanal. Hexenal was again more retained by SPI. Octenal was especially highly retained by CPPI, based on the fact that RHC approached 0. Lastly, RHCs of decenal were around 10% and decreased to approximately 0% when protein concentration was increased. Again for CPPI the RHC of decenal was lower compared to the other proteins. Decenal was least retained by FBPI and WPI.

The retention of the alkenals was higher than the retention of aldehydes. Furthermore, the retention increased with the molecular weight of the aldehydes. Differences between flavors were more important than differences between proteins, though differences were observed. Both classes of aldehydes were more retained than the esters and ketones tested in a previous study (Snel et al., 2023).

4.2. Flavor partitioning models

Experimental data obtained for aldehydes and alkenals were fitted with eq (9) (Fig. 3). This resulted in fitted values for the chemical interaction parameters K_{ald} and K_{alk} (Table 3). It was found that each combination of flavor and protein led to different values for the fitting parameter. It was observed that the fitted values for K_{ald} increased with increasing chain length. The fitted values for K_{ald} for decanal were even a factor of 100 higher than butanal for the plant proteins. For WPI, a factor of 20 was observed. The fitted values for K_{ald} for WPI were the lowest, followed by SPI, PPI/CPPI, and FBPI. The fitted K_{ald} for FBPI was around 10x higher compared to the other proteins. The ratio between K_{ald} and the hydrophobic interactions ($a_p P^f_{ow}$) was calculated to illustrate their relative contribution (Table 3). Butanal had the highest ratio of aldehydes for each protein. It seemed that for the aldehydes with a chain length longer than 4, the ratio between the binding effect of hydrophobic interactions ($a_p P^f_{ow}$) and the covalent binding (K_{ald} or K_{alk}) remained in the same order of magnitude. For WPI, this ratio even remained constant at 2, meaning that the ratio between covalent and hydrophobic interactions was 2:1.

The fitted values for K_{alk} were higher than the values found for K_{ald}, except for FBPI (Table 3). As seen for the aldehydes, an increase was seen for K_{alk} with increasing chain length, except for CPPI and FBPI with hexenal, which decreased compared to butenal. WPI had the lowest fit values for K_{alk} overall, followed by FBPI, PPI, SPI, and CPPI. The ratio between covalent and hydrophobic interactions was highest for butenal, and higher compared to the ratio for the aldehydes. For the alkenals with longer chain lengths, the ratio remained in the same order of magnitude. The high retention of octenal and decenal by CPPI resulted in very low headspace concentrations (Fig. 2h). This concentration was close to the detection limit of the APCI-TOF-MS, which might explain the high uncertainties for CPPI (Table 3, Fig. 3h).

4.3. Correlation between amino acid contents and covalent interaction parameters

In a previous study, a complete amino acid profile for the plant proteins was obtained (Snel et al., 2023). To assess whether the amino acid composition could be linked to the retention of aldehydes by proteins, a Pearson test of correlation was performed (supplementary materials). The fitted parameter for alkenals, K_{alk} correlated positively with methionine content: 75, 43, 73, and 75% for butenal, hexenal, octenal, and decenal respectively. A smaller positive correlation was found with cysteine content, 30, 84, 26, and 29%. Cysteine and methionine contain a thiol group that can react covalently (Fig. 1). The thiol group of cysteine has been shown previously to react with aldehydes by forming both Schiff bases and Michael adducts (Hamzalıoğlu & Gökmen, 2018). Methionine can react with 4-hydroxy-trans-2-nonenal (Baker et al., 1998). Fitted values for K_{ald} strongly correlated negatively with cysteine and methionine content (around −80% correlation). This could indicate that other amino acids are more likely to form Schiff bases.

Meynier et al. (2004) studied hexanal and hexenal interactions with WPI and caseinate and showed that lysine residues decreased upon both hexanal and hexenal addition, while histidine residues reduced only with hexenal addition. Cysteine and methionine content were not measured, due to limitations of using reversed-phase chromatography to measure amino acids (Meynier et al., 2004). The fitted parameter K_{ald} showed a slight correlation with histidine content (59, 58, 57, 55%), but no clear correlation with lysine content. Apart from histidine, K_{ald} correlated with arginine (61–73%), leucine (86–89%), and valine content (51–78%). When excluding the hexenal correlations, K_{alk} correlated with alanine (92–95%), aspartic acid (84–90%), phenylalanine

Table 3
Fitting results of aldehydes and alkenals for soy (SPI), yellow pea (PPI), faba bean (FBPI), chickpea (CPPI), and whey (WPI) protein isolates, using the flavor partitioning model. Model parameters are the covalent interaction parameters K_{ald} and K_{alk} for aldehydes and alkenals respectively, and their corresponding residuals squared R^2. Values are reported as fit value ± uncertainty of the fitted parameter (n = 3). The ratio between the covalent interaction parameter and hydrophobic interactions is calculated as $\frac{K}{a_p P_{ow}}$ in which a_p is the hydrophobic interaction parameter per protein and P_{ow} the octanol-water partition coefficient of the flavor.

Protein	Aldehyde	K_{ald} (10^{-2} L/g)	R^2	Ratio	Alkenal	K_{alk} (10^{-2} L/g)	R^2	Ratio
SPI	Butanal	0.17 ± 0.01	0.78	87	Butenal	3.40 ± 0.30	0.88	1755
	Hexanal	0.34 ± 0.03	0.84	11	Hexenal	4.40 ± 0.23	0.91	240
	Octanal	1.55 ± 0.05	1.00	5	Octenal	17.13 ± 0.25	0.99	96
	Decanal	17.88 ± 1.29	0.91	6	Decenal	95.64 ± 2.87	0.98	56
PPI	Butanal	0.26 ± 0.02	0.86	58	Butenal	1.32 ± 0.05	0.99	294
	Hexanal	0.46 ± 0.08	0.73	7	Hexenal	2.46 ± 0.05	0.99	58
	Octanal	1.88 ± 0.06	1.00	3	Octenal	9.81 ± 0.12	0.99	24
	Decanal	27.03 ± 2.28	0.93	4	Decenal	60.95 ± 1.36	0.98	15
FBPI	Butanal	1.96 ± 0.13	0.94	568	Butenal	1.64 ± 0.17	0.78	475
	Hexanal	2.12 ± 0.16	0.85	39	Hexenal	0.71 ± 0.07	0.69	22
	Octanal	13.56 ± 1.56	0.55	26	Octenal	2.09 ± 0.05	0.98	7
	Decanal	126.94 ± 13.68	0.60	25	Decenal	27.40 ± 1.70	0.96	9
CPPI	Butanal	0.13 ± 0.03	0.10	20	Butenal	11.46 ± 1.39	0.76	1706
	Hexanal	0.16 ± 0.02	0.96	1	Hexenal	1.15 ± 0.03	0.98	18
	Octanal	3.24 ± 0.10	0.98	3	Octenal	112.86 ± 58.56	0.01	183
	Decanal	25.45 ± 1.04	0.98	3	Decenal	429.36 ± 62.14	0.64	72
WPI	Butanal	0.26 ± 0.04	0.55	91	Butenal	0.35 ± 0.02	0.84	121
	Hexanal	0.11 ± 0.01	0.88	2	Hexenal	1.10 ± 0.04	0.96	41
	Octanal	0.75 ± 0.03	1.00	2	Octenal	3.21 ± 0.06	0.99	12
	Decanal	6.51 ± 1.01	0.68	2	Decenal	14.56 ± 0.15	1.00	6

(91–95%), and serine content (66–69%). K_{alk} for hexenal did not show the same correlations. This might be a result of the lower value for K_{alk} for CPPI-hexenal and FBPI-hexenal, compared to the K_{alk} for CPPI-butenal and FBPI-butenal. Although not conclusive, these correlations could be used as starting points for future research to investigate if these amino acids are related to higher flavor retention indeed.

5. Discussion

The headspace concentrations of aldehydes and alkenals with different chain lengths were measured at varying protein concentrations for four different plant proteins and whey. These results were then described with a flavor retention model, using the octanol-water partition coefficients of the flavors, the hydrophobic interaction parameter of the esters, and fitting a covalent interaction parameter for SPI, PPI, FBPI, CPPI, and WPI. Most of the interactions between aldehydes and alkenals with the tested proteins were well described and thus the obtained fitted parameters could be used as a predictive tool.

An increased protein concentration resulted in increased flavor retention. Furthermore, an increase in chain length, related to hydrophobicity, of the aldehydes also increased flavor retention. Lastly, alkenals were more bound than aldehydes for the proteins tested in this study. Wang and Arntfield (2014) found that octanal was 68% bound to a 10 g/kg PPI dispersion. We found a similar value based on headspace concentrations of 70% for octanal in a 7 g/kg PPI dispersion. Gremli (1974) showed that hexenal and decenal were more strongly retained than hexanal and decanal, which is in line with our findings. We observed stronger retention of the alkenals compared to the aldehydes, which can be possibly related to the type of covalent interactions that these chemical classes can undergo. Apart from a Schiff base reaction, alkenals can furthermore form a Michael adduct with nucleophilic sites in proteins (Fig. 1).

The different proteins tested showed some differences in their retention of aldehydes and alkenals. The smaller flavors, butanal, and butenal, were retained to a similar extent by WPI compared to the plant proteins at low protein concentrations. However, with increasing protein concentration, butenal was more retained by the plant proteins. For the other flavors, higher retention was observed for the plant proteins, especially for chain lengths of 8 and 10. However, in comparison, the effects of flavor on flavor retention were more pronounced than the effect of protein source, although both had an effect. This was observed before for aldehydes when comparing canola, pea, and wheat gluten isolates (Wang & Arntfield, 2014). Wang and Arntfield (2014) did see differences between the protein sources, but the effect of the flavor was more pronounced.

A flavor-partition model was used to describe the data. In the model, both the hydrophobic and covalent interaction parameters scale linearly with the protein concentration according to eq. (9). It was thus decided to only model the covalent interaction parameter, and keep the hydrophobic parameter constant. The hydrophobic parameter was set with obtained fits for the same proteins with esters, which are considered to be retained by hydrophobic interactions only (Snel et al., 2023).

The increase in retention of aldehydes with increasing chain length is often attributed to an increase in hydrophobic interactions (Tan & Siebert, 2008; Weel et al., 2003). The observed increase in the covalent interaction parameter in this study suggests that chain length also affects the degree of covalent interactions. However, when comparing the ratio of hydrophobic interactions to covalent interactions, it was clear that the covalent interactions determine to a larger extent the retention of smaller flavor molecules. Hydrophobic interactions for butenal and butanal seem less important when assuming a constant hydrophobic interaction parameter. For larger flavor molecules, hydrophobic interactions became more important, but still, covalent interactions contributed to a high extent, as seen as the ratio between the reactions remained above 1. Our results, therefore, align well with previous studies, such as Wang and Arntfield (2016) who found that for octanal, both non-covalent and covalent interactions were important for flavor retention, but non-covalent interactions were only responsible for a small part of the flavor retention.

The values for the fitting parameters for alkenals were in general higher compared to the fitting parameters for aldehydes. Except for FBPI, all K_{alk} fits were around 2-10x higher than K_{ald}. This was attributed to the Michael adduct formation in addition to the Schiff base adduct. Michael adducts are expected to have higher reactivity than Schiff bases (Zou et al., 2016).

The degree of retention, and fits for the covalent interaction parameters differed between the proteins. Most notably, WPI retained the aldehydes and alkenals to a lower extent compared to the plant proteins. This effect is especially noticeable for the larger flavor molecules. WPI had the lowest parameter estimates for both the aldehydes and alkenals, with the fit for butanal as the only exception. FBPI had the highest parameter estimates for the aldehydes and CPPI for the alkenals. It is known that the protein source and extraction method influence the degree of flavor retention (Viry et al., 2018; Wang & Arntfield, 2014). The difference in retention for the different plant proteins could be related to their amino acid profile. It has been suggested that specific amino acids are more likely to interact with aldehydes (Meynier et al., 2004; Zou et al., 2016). To test this, a simple correlation assay was performed on the fitted results and amino acid contents. Interestingly, both cysteine and methionine content correlated with K_{alk}. Cysteine has been shown to have a higher reactivity compared to lysine, and arginine, which was attributed to its thiol group (Hamzalıoğlu & Gökmen, 2018). The correlations found in this study thus suggest that protein isolates with higher contents of sulfur-containing amino acids react more strongly with alkenals. Furthermore, other correlations with amino acids were identified, which might serve as handles for future studies.

In this study, concentrations up to 50 g/kg protein isolate were tested. With the predictive models, it can be calculated that higher protein contents will lead to even higher flavor losses, especially for plant proteins and large flavor molecules. For example, a 100x reduction of the headspace concentration of decanal is already reached at a protein concentration of 48, 30, 8, 28, and 93 g/kg for SPI, PPI, FBPI, CPPI, and WPI respectively. For decenal, these concentrations drop to 10, 15, 33, 2, and 58 g/kg. At 400 kg/kg protein, a relevant concentration for meat analogues, the headspace reduction for decanal is roughly 800, 1300, 5300, 1400, and 400x for SPI, PPI, FBPI, CPPI, and WPI respectively. For decenal, the headspace reduction is roughly 3900, 2600, 1200, 17400, and 700x respectively. These examples illustrate that plant-protein products might be harder to flavor than milk-protein products and this thus causes further challenges in their design.

6. Conclusion

This study investigated the flavor retention of aldehydes in protein dispersions, finding that alkenals bound stronger than aldehydes. Aldehydes can undergo a condensation reaction (Schiff base), whereas alkenals can have a further conjugate addition (Michael reaction). The protein source had a secondary effect on the retention. Aldehydes were more retained by FBPI and PPI, whereas alkenals were more retained by SPI and CPPI. The experimental data were fitted with a flavor partitioning model that revealed covalent interaction parameters differed between protein sources and increased with aldehyde chain lengths. The ratio between covalent and hydrophobic interactions was highest for the small aldehydes. This led to the conclusion that butanal and butenal were mostly retained by covalent interactions. For the other aldehydes, the outcomes of the fits suggested that hydrophobic interactions became increasingly important. WPI retained the aldehydes and alkenals to a less extent compared to the plant proteins, except for butanal. Additionally, we found that the fitting parameters for alkenals correlated with the cysteine and methionine content of the plant proteins, suggesting thiol-containing amino acids were more reactive and contributed to higher retention of alkenals. Our results provide important

insights into the mechanisms of flavor retention in protein dispersions.

CRediT authorship contribution statement

Silvia J.E. Snel: Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Mirela Pascu:** Investigation, Methodology. **Igor Bodnár:** Conceptualization, Writing – review & editing, Supervision. **Shane Avison:** Methodology. **Atze Jan van der Goot:** Writing – review & editing, Supervision. **Michael Beyrer:** Project administration, Writing – review & editing, Funding acquisition, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Michael Beyrer reports financial support was provided by Innosuisse Swiss Innovation Agency.

Data availability

Data will be made available on request.

Acknowledgements

This work is part of the project GreenXProt, co-financed by the Swiss Innovation Agency 'Innosuisse' - under contract number 36440.1 IP-LS.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2023.115177.

References

Aiking, H., & de Boer, J. (2018). The next protein transition. *Trends in Food Science and Technology*, (May), 0–1.

Amagliani, L., O'Regan, J., Kelly, A. L., & O'Mahony, J. A. (2017). Composition and protein profile analysis of rice protein ingredients. *Journal of Food Composition and Analysis, 59*, 18–26.

Anantharamkrishnan, V., Hoye, T., & Reineccius, G. A. (2020a). Covalent adduct formation between flavor compounds of various functional group classes and the model protein β-lactoglobulin. *Journal of Agricultural and Food Chemistry, 68*(23), 6395–6402.

Anantharamkrishnan, V., & Reineccius, G. A. (2020b). Method to characterize and monitor covalent interactions of flavor compounds with β-lactoglobulin using mass spectrometry and proteomics. *Journal of Agricultural and Food Chemistry, 68*(46), 13121–13130.

Baker, A., Žídek, L., Wiesler, D., Chmelík, J., Pagel, M., & Novotny, M. V. (1998). Reaction of N-acetylglycyllysine methyl ester with 2-alkenals: An alternative model for covalent modification of proteins. *Chemical Research in Toxicology, 11*(7), 730–740.

Broekema, R., Tyszler, M., van 't Veer, P., Kok, F. J., Martin, A., Lluch, A., & Blonk, H. T. (2020). Future-proof and sustainable healthy diets based on current eating patterns in The Netherlands. *The American Journal of Clinical Nutrition, 112*(5), 1338–1347.

Gremli, H. A. (1974). Interaction of flavor compounds with soy protein. *Journal of the American Oil Chemists' Society, 51*(1), 95–97.

Guichard, E. (2002). Interactions between flavor compounds and food ingredients and their influence on flavor perception. *Food Reviews International, 18*(1), 49–70.

Guo, J., He, Z., Wu, S., Zeng, M., & Chen, J. (2020). Effects of concentration of flavor compounds on interaction between soy protein isolate and flavor compounds. *Food Hydrocolloids, 100*(July 2019), Article 105388.

Hamzalıoğlu, A., & Gökmen, V. (2018). Investigation and kinetic evaluation of the reactions of hydroxymethylfurfural with amino and thiol groups of amino acids. *Food Chemistry, 240*(May 2017), 354–360.

Harrison, M., & Hills, B. P. (1997). Mathematical model of flavor release from liquids containing aroma-binding macromolecules. *Journal of Agricultural and Food Chemistry, 45*(5), 1883–1890.

Kale, P., Mishra, A., & Annapure, U. S. (2022). Development of vegan meat flavour: A review on sources and techniques. *Future Foods, 5*.

Linforth, R. S. T., & Taylor, A. J. (1999). Apparatus and methods for the analysis of trace constituents in gases. *United States Patent, 5*, 869 344.

Meynier, A., Rampon, V., Dalgalarrondo, M., & Genot, C. (2004). Hexanal and t-2-hexenal form covalent bonds with whey proteins and sodium caseinate in aqueous solution. *International Dairy Journal, 14*(8), 681–690.

Michel, F., Hartmann, C., & Siegrist, M. (2021). Consumers' associations, perceptions and acceptance of meat and plant-based meat alternatives. *Food Quality and Preference, 87*(April 2020), Article 104063.

Ramalingam, V., Song, Z., & Hwang, I. (2019). The potential role of secondary metabolites in modulating the flavor and taste of the meat. *Food Research International, 122*, 174–182.

Snel, S. J., Pascu, M., Bodnár, I., Avison, S., van der Goot, A. J., & Beyrer, M. (2023). Flavor-protein interactions for four plant proteins with ketones and esters. *Heliyon, 9*(6), Article e16503.

Tan, Y., & Siebert, K. J. (2008). Modeling bovine serum albumin binding of flavor compounds (alcohols, aldehydes, esters, and ketones) as a function of molecular properties. *Journal of Food Science, 73*(1).

Viry, O., Boom, R., Avison, S., Pascu, M., & Bodnár, I. (2018). A predictive model for flavor partitioning and protein-flavor interactions in fat-free dairy protein solutions. *Food Research International, 109*(December 2017), 52–58.

Wang, K., & Arntfield, S. D. (2014). Binding of carbonyl flavours to canola, pea and wheat proteins using GC/MS approach. *Food Chemistry, 157*, 364–372.

Wang, K., & Arntfield, S. D. (2015). Binding of selected volatile flavour mixture to salt-extracted canola and pea proteins and effect of heat treatment on flavour binding. *Food Hydrocolloids, 43*, 410–417.

Wang, K., & Arntfield, S. D. (2016). Probing the molecular forces involved in binding of selected volatile flavour compounds to salt-extracted pea proteins. *Food Chemistry, 211*, 235–242.

Wang, Y., Tuccillo, F., Lampi, A. M., Knaapila, A., Pulkkinen, M., Kariluoto, S., Coda, R., Edelmann, M., Jouppila, K., Sandell, M., Piironen, V., & Katina, K. (2022). Flavor challenges in extruded plant-based meat alternatives: A review. *Comprehensive Reviews in Food Science and Food Safety*, 2898–2929. March).

Weel, K. G., Boelrijk, A. E., Burger, J. J., Claassen, N. E., Gruppen, H., Voragen, A. G., & Smit, G. (2003). Effect of whey protein on the in vivo release of aldehydes. *Journal of Agricultural and Food Chemistry, 51*(16), 4746–4752.

Zhou, Q., & Cadwallader, K. R. (2006). Effect of flavor compound chemical structure and environmental relative humidity on the binding of volatile flavor compounds to dehydrated soy protein isolates. *Journal of Agricultural and Food Chemistry, 54*(5), 1838–1843.

Zou, Y., Pei, K., Peng, X., Bai, W., Huang, C., & Ou, S. (2016). Possible adducts formed between hydroxymethylfurfural and selected amino acids, and their release in simulated gastric model. *International Journal of Food Science and Technology, 51*(4), 1002–1009.