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Retention of propanal in protein-stabilised tuna oil-in-water emulsions

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Abstract

Propanal concentrations in the static headspace (HS) above water, aqueous protein solutions and freshly made tuna oil-in-water emulsions spiked with propanal (an indicator of omega-3 fatty acid oxidation) were compared. In the presence of proteins, HS propanal concentration was reduced and its decrease above aqueous hydrolysed whey protein isolate (HWPI) solutions was significantly greater than that above whey protein isolate (WPI) solutions. Similar trends were found for emulsions stabilised by HWPI and WPI. The results suggested that there was stronger binding of propanal to HWPI compared to WPI. Emulsification decreased the HS propanal concentration even further for both the WPI and HWPI matrices, but its effect was less in comparison to the protein type. Phosphate buffer decreased the HS propanal concentration, but this effect was minor. The difference in the release of propanal from protein stabilised tuna oil-in-water emulsions was interpreted in terms of the chemical interaction between propanal and protein.

Keywords: Whey protein isolate (WPI); Hydrolysed whey protein isolate (HWPI); Tuna oil-in-water emulsion; Headspace analysis (HS); Propanal; Interaction

1. Introduction

The interest in the incorporation of omega-3 fatty acids in foods is due to their health effects (De Deckere, Korver, Verschuren, & Kantan, 1998). A convenient method for delivery of omega-3 fatty acids is the use of oil-in-water emulsions (McClements & Decker, 2000). However, the omega-3 fatty acids susceptible to oxidation present a major challenge for their delivery (Augustin & Sanguanri, 2003). Milk proteins have been used to stabilise omega-3 oil-in-water emulsions because of their emulsifying and antioxidative properties (Djordjevic, McClements, & Decker, 2004; Faraji, McClements, & Decker, 2004; Hu, McClements, & Decker, 2003; McClements & Decker, 2000). Propanal, a very volatile aliphatic short chain aldehyde and secondary oxidation product of omega-3 oil, is commonly used as an indicator of oxidation of omega-3 oil and has been directly measured by static headspace (HS) analysis (Djordjevic et al., 2004; Faraji et al., 2004; Hu et al., 2003; McClements & Decker, 2000). Aldehydes are known to react with proteins and this affects the flavour perception of aldehyde in aqueous protein solutions or emulsions stabilised by proteins. Both hydrophobic and covalent interactions have been implicated in the binding of flavour compounds to protein – the nature of the interaction depending on the type of compound and protein. In addition, the microstructure of the food matrix also affects release of aldehydes and other flavours (Druaux & Voilley, 1997; Guichard & Langouriex, 2000; Hansen & Heinis, 1991; Hansen & Heinis, 1992; Leaver, Law, Brechany, & McCrae, 1999; Meynier, Rampon, Dalgalarrondo, & Genot, 2004; Meynier, Lecoq, & Genot, 2005; Stapelfeldt & Skibsted, 1994; Weel et al., 2003).

The flavour persistence during the tasting of food products has been shown to be increased by the presence of proteins (Druaux & Voilley, 1997), which might accord with the flavour compounds forming reversible hydrophobic bonds with proteins and gradually being released during food consumption and flavour perception. The decrease of volatility of some hydrophobic flavour

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compounds (e.g., aldehydes, ketones, esters and lactones) in aqueous solutions or emulsions containing β-lactoglobulin has been ascribed to hydrophobic interactions, as evidenced by the higher retention of the same class of compounds with increasing chain length. However, benzaldehyde was considered to be partially covalently bound to β-lactoglobulin (Guichard & Langouriex, 2000). Others have shown that in the presence of casein, the flavour intensity of benzaldehyde was not significantly decreased, but whey proteins reduced the flavour intensity of benzaldehyde, with higher concentrations of whey proteins having a greater effect (Hansen & Heinis, 1992). Weel et al. (2003) confirmed that there was increased interaction between whey protein (3%, w/v) and aldehydes (5 ppm) with increased length of the aldehyde carbon chain, which is evidence for hydrophobic interactions. These authors further showed binding dramatically increased with increasing pH from 6 to 9 (adjusting pH by buffer) for short carbon chain aldehydes (e.g., butanal and hexanal), an effect ascribed to the increased flexibility of the whey protein at higher pH, giving flavour compounds better accessibility to hydrophobic binding sites.

That covalent binding of some aldehydes to proteins also occurs has been supported by a number of studies. With the addition of either hexanal or *trans*-2-hexenal at various concentrations to solutions of whey proteins or sodium caseinate, the maximum intensity of fluorescence of the tryptophanyl residues of both milk proteins was reduced. Some browning was visible after adding *trans*-2-hexenal. The changes to protein physical and chemical properties were attributed to the formation of covalent compounds (Meynier et al., 2004). The secondary oxidation products of lipid, pentanal, hexanal and heptanal, can modify β-lactoglobulin in a two-phase model system (continuously stirred aqueous phosphate buffer with 1-octanol). A change in protein fluorescence was attributed to condensation caused by the covalent reaction with aldehydes (Stapelfeldt & Skibsted, 1994). Analysis of soy oil emulsion stabilised by β-lactoglobulin showed that in stored emulsions there was an increase in protein molecular weight of approximately 300–400 Da, indicating a change due to covalent modification of protein by volatile products from polyunsaturated fatty acid autoxidation, especially aldehydes (Leaver et al., 1999). Decline of vanillin (4-hydroxy-3-methoxybenzaldehyde) flavour perception in the presence of casein and whey proteins has been attributed to cysteine–aldehyde condensation-covalent binding (Hansen & Heinis, 1991).

With the diversity of aldehydes, proteins and media possible, the interaction between protein and aldehydes has to be investigated individually, according to one's particular research aim. In this study, the release of propanal from aqueous solutions of protein (whey protein isolate (WPI) and hydrolysed whey protein isolate (HWPI)) and tuna oil-in-water emulsions stabilised by these proteins was examined. In addition, the effects of buffer components (phosphate) in the aqueous phase were investigated. Our aim was to determine the effects of protein and emulsion matrices on HS analysis of propanal, in order to understand the interactions between proteins and propanal.

2. Materials and methods

2.1. Materials

Tuna oil HiDHA™ 25S5 was purchased from Nu-Mega Ingredients (Brisbane, Australia). Two commercial WPI products containing 90.0% w/w protein and 3.9% w/w moisture (WPI 1) and 89.2% w/w protein and 3.4% w/w moisture (WPI 2) were purchased from different suppliers. HWPI (80.0% w/w protein) with a degree of hydrolysis of 17% was from Myopure (Australia). Propanal (>98% purity) was purchased from Sigma–Aldrich Pvt. Ltd. (Sydney, Australia).

2.2. Preparation of aqueous protein solution

WPI or HWPI were dispersed in deionized water or in 0.05 M phosphate buffer (pH 7.0), to make 3% or 5% w/w total solids (TS) solutions, by stirring the proteins for 30 min at 60 °C. The unadjusted pH values for WPI 1, WPI 2 and HWPI water solutions were 6.7, 6.3 and 7.0, respectively. All protein solutions were allowed to hydrate for 3 h and homogenised at 14 MPa (one pass), using a homogenizer (Foss Electric Miko Tester). All protein solutions were stored at 4 °C before analysis within 3 days of preparation.

2.3. Preparation of tuna oil-in-water emulsions

An appropriate amount of protein powder was dispersed in deionized water or 0.05 M phosphate buffer (pH 7.0), as outlined above. Tuna oil was removed from storage (−18 °C) and completely melted in a water bath at 60 °C. The oil was dispersed at 60 °C in the protein solution using a Silverson stirrer, to make a tuna oil-in-water coarse emulsion (10% TS, comprising 5% w/w tuna oil and 5% w/w protein ingredient). The coarse emulsions were homogenised at 14 MPa (2 passes) using a homogeniser (Foss Electric Milko Tester). The pH values of all emulsions were the same as their corresponding protein aqueous solutions. The emulsions were stored at 4 °C and analysed within 3 days of preparation.

2.4. Propanal analysis

Aqueous protein solutions and tuna oil-in-water emulsions were spiked with propanal to obtain final concentrations of 0.1, 0.25 (aqueous protein solution only) or 0.5 (emulsion only), 1, 2 and 5 μg propanal/g solution on the testing day. These concentrations correspond to 0.002, 0.004 or 0.009, 0.017, 0.034 and 0.086 mM propanal. Aliquots of standard solutions (5 ml) were pipetted into

20 ml HS vials. The vials were sealed and equilibrated at 60 °C for 30 min in the HS auto-sampler. Approximately 0.6 ml of the HS vapour was directly transferred into the GC column (DB1, 30 m × 0.25 mm i.d., 5 μm film thickness, Agilent Technologies, CA, USA). The GC column was programmed from 40 °C (hold for 5 min) increasing at a rate of 10 °C/min to 120 °C and then increasing at 30 °C/min to 200 °C. The column head pressure was maintained at 120 kPa and the flame ionization detector temperature was 240 °C. Triplicate sample analyses were carried out. The retention time of standard propanal was used for qualitative identification and data analysis by Turbochrom software. Excel was used to generate standard deviations (SD) of the GC area counts of measured propanal in the HS.

3. Results and discussion

3.1. Effect of proteins on HS propanal in aqueous solutions

There was no detectable HS propanal above the unspiked two WPI solutions, while there were traces of HS propanal (corresponding to less than 0.002 mM in solution) above the unspiked HWPI solutions. These values have been subtracted during subsequent calculations. HS propanal was decreased in propanal spiked protein solutions (Figs. 1 and 2), relative to that in water, suggesting that propanal interacted with the proteins. The decrease in HS propanal was significantly greater above HWPI than WPI solutions, irrespective of whether the solutions were unbuffered or buffered at pH 7.0 (Figs. 1 and 2). This

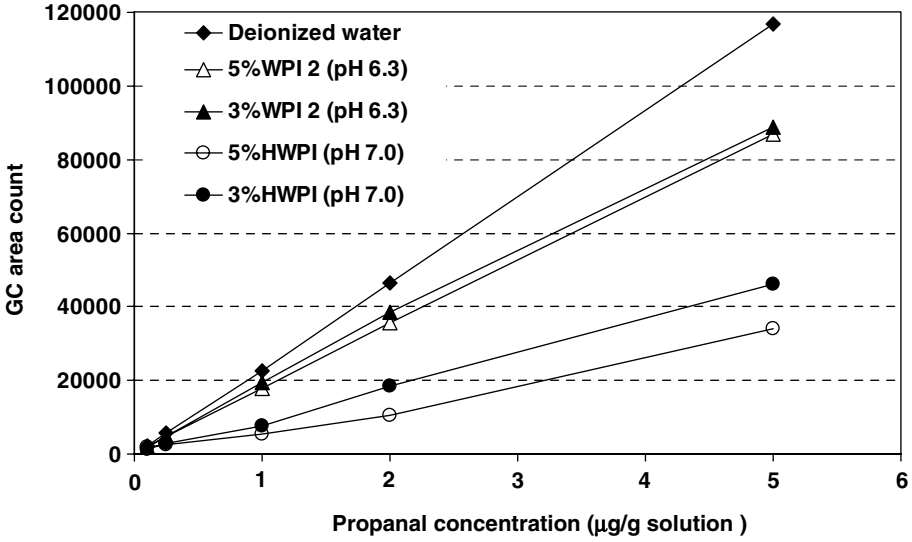

Fig. 1. HS propanal above water and aqueous protein solutions spiked with propanal at their unadjusted pHs, as a function of protein concentration.

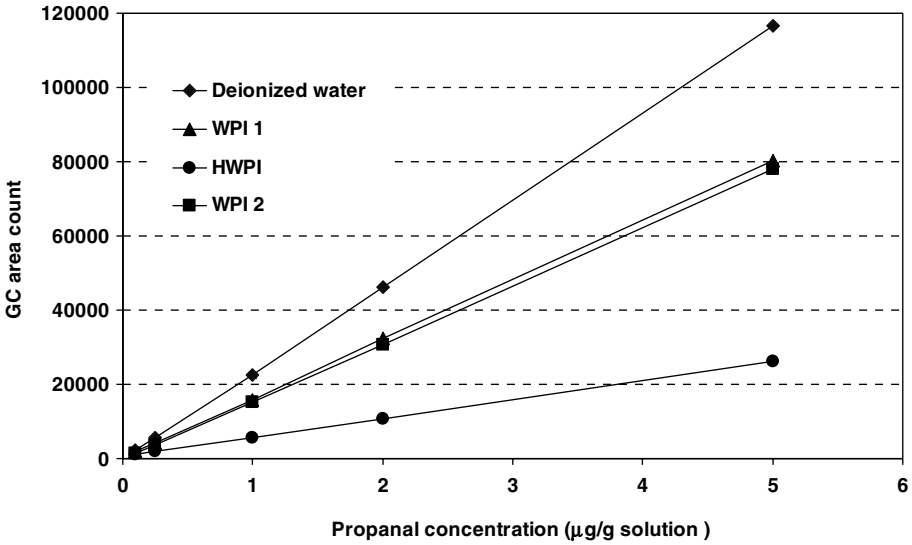

Fig. 2. HS propanal above 5% total solids solutions of WPI and HWPI spiked with propanal in phosphate buffer at pH 7.0.

agrees with suggestions in the literature quoted in the introduction that aldehydes react with free amine groups in milk proteins. In the present study, the increased reaction of propanal with HWPI compared with WPI can be attributed to the greater accessibility of amine group residues and the production of new N-terminal amine groups, as a result of protein fragmentation, due to hydrolysis of the whey protein.

HS propanal concentrations above the 5% solutions were slightly reduced relative to the 3% solutions for WPI 2 water solutions and for HWPI solutions at lower propanal concentrations (0.004 mM propanal) (Table 1). At higher propanal concentrations (0.017–0.086 mM) in HWPI solutions, the difference between the two HWPI solutions was more pronounced (Table 1). Increasing HWPI concentration from 3% to 5% caused propanal HS concentration to decrease to 70% and 80% of that in 3% protein solutions for propanal at 0.017 and 0.086 mM, respectively (Table 1). The effects of increasing HWPI concentration on the interaction between protein and propanal were due to the increased number of available binding sites for propanal. It is not clear why increasing the concentration of HWPI had more effect on HS propanal than increasing the concentration of WPI.

The extent of propanal reaction, as a proportion of initial spiked concentration, was higher at higher propanal concentrations (Tables 1 and 2). This is especially true for propanal–HWPI solutions (for concentrations at or higher than 0.004 mM). The other protein solutions showed smaller increases in extent of reaction with increased propanal concentration. In this study, there was no direct determination of whether propanal in samples heated at 60° for 30 min had reached equilibrium between HS and protein matrix. However, the reaction time for some replicate samples sealed in the auto-sampler waiting for analysis was increased by up to 20 h at room temperature. It was invariably observed that each replicate had a slightly lower HS propanal concentration than the previous replicate, the effect being more pronounced for HWPI solutions. Analysis errors have been ruled out by triplicate analysis of propanal standard samples in pure water solution over a period of 20 h, with less than 1% coefficient of variation (CV) and no significant variation with time.

Table 2
Percentage of propanal concentration remaining in HS above propanal spiked buffered protein aqueous solution (5% TS protein ingredient) at pH 7.0

Propanal (mM)	Percentage of propanal remaining in the headspace[a] (CV%)		
	WPI 1	WPI 2	HWPI
0.002	90 (12.1)	71 (17.1)	56 (5.2)
0.004	74 (5.1)	68 (11.1)	36 (6.9)
0.017	70 (3.9)	68 (11.5)	24 (11.0)
0.034	70 (6.5)	66 (10.6)	22 (10.4)
0.086	69 (6.3)	67 (9.9)	21 (14.5)

[a] Normalised against concentration of propanal above HS of water.

HS propanal concentrations above buffered protein solutions were lower than in their corresponding unbuffered solutions (Table 3). A possible explanation could be an interaction between the phosphate in the buffer and possible protein–calcium complexes, through which calcium could be removed, thereby changing the conformation of the protein, exposing more amine groups to react with propanal. In this study, the concentrations of calcium for WPI 1, 2 and HWPI solutions were about 0.03, 0.04 and 0.06 M, respectively, which were similar to 0.05 M for phosphate buffer.

A larger coefficient of variance (CV%) (Tables 1 and 2) resulted from consistently lower HS propanal concentrations in the later replicates. This indicates that, especially for unbuffered HWPI, the reactions were not complete at the time of analysis. A possible explanation for this is that the Maillard reaction between aldehydes and the amine groups of protein continued during analysis waiting time. This is probably the result of the slow reaction between propanal and proteins demonstrated previously (Meynier et al., 2004). These authors used a much higher aldehyde concentration range (5–40 mM) and their reaction time at 20 °C continued for up to 2 days. Thus, the CV% of the results in Tables 1 and 2 may be related to the degree of incomplete reaction. For most solutions, the low CV% indicates virtually complete reaction, but the high CV% for unbuffered HWPI solutions at propanal concentrations of 0.004, 0.017 and 0.034 mM indicates that the reaction was still appreciably incomplete after 30 min at 60 °C. Despite the possibility that some reactions were not com-

Table 1
Percentage of propanal concentration remaining in HS above propanal spiked protein solutions (3% and 5% TS protein ingredient) at their unadjusted pHs

Propanal (mM)	% Propanal remaining in the headspace[a] (CV%)			
	3% WPI 2 (pH 6.3)	5% WPI 2 (pH 6.3)	3% HWPI (pH 7.0)	5% HWPI (pH 7.0)
0.002	93 (1.7)	92 (7.5)	72 (3.2)	85 (4.6)
0.004	87 (1.7)	85 (2.4)	49 (1.6)	46 (20.2)
0.017	86 (3.1)	80 (5.1)	43 (6.2)	30 (41.3)
0.034	83 (5.4)	77 (7.1)	40 (7.0)	30 (51.7)
0.086	76 (18.1)	75 (8.0)	40 (5.4)	32 (14.6)

[a] Normalised against concentration of propanal above HS of water.

Table 3
Percentage of propanal concentration remaining in HS above propanal spiked tuna oil-in-water emulsions (10% TS: 5% oil and 5% protein ingredient), in comparison with protein solutions (5% TS protein ingredient)

Propanal (mM)	Matrix type	Percentage of propanal remaining in the headspace[a] (CV%)					
		WPI 1 H_2O	WPI 1 buffer	WPI 2 H_2O	WPI 2 buffer	HWPI H_2O	HWPI buffer
0.002	Protein	104	90	92	71	85	56
	Emulsion	84 (10.9)	72 (7.7)	95 (17.6)	75 (19.6)	27 (62.1)	21 (68.3)
0.017	Protein	78	70	80	68	30	24
	Emulsion	60 (11.5)	56 (8.5)	60 (9.4)	60 (11.3)	23 (37.0)	14 (65.2)
0.034	Protein	72	70	77	66	30	22
	Emulsion	56 (3.2)	56 (7.3)	56 (8.2)	57 (9.7)	23 (14.9)	15 (39.0)
0.086	Protein	77	69	75	67	32	21
	Emulsion	62 (7.9)	58 (7.1)	57 (8.0)	54 (3.8)	25 (7.9)	20 (16.1)

[a] Normalised against concentration of propanal above HS of water.

plete, spiking with the same propanal concentration into all the different solutions and heating at the same temperature for the same time did allow for a reasonable comparison in a practical time period.

3.2. Effect of protein matrices on HS propanal in freshly made propanal spiked tuna oil-in-water emulsions in comparison with aqueous protein solutions

There was no detectable HS propanal above both WPI emulsion blank samples and there was only a trace of HS propanal above HWPI emulsion blank samples. These trace amounts produced the same GC response as their corresponding pure protein solutions, equivalent to less than 0.002 mM propanal. Beltran, Aguilera, and Gordon (2005) suggested that lipid oxidation in an oil-in-water emulsion (10% oxidised sunflower oil and 0.1% surfactant) in a headspace GC vial could take place after 15 min heating at 60 °C, based on increasing HS-GC responses of oxidation compounds. The results of this study are not in line with that suggestion, possibly because of differences in emulsifying agents used. HS propanal concentrations of freshly made tuna oil-in-water emulsions were lower, compared with their corresponding pure protein solutions in all cases (Figs. 3 and 4). HS concentrations in 70% of emulsions were reduced to 58–82%, compared with their corresponding pure protein solutions (Table 3).

Hu et al. (2003) reported that the interface of the emulsion droplets becomes saturated with 0.2% w/w WPI in an oil-in-water emulsion containing 5% w/w oil and from our study, there were no significant reductions of propanal in HS when the WPI concentration in the aqueous phase increased from 3% to 5%. Therefore, the lower HS concentration in the WPI emulsions, compared with their corresponding pure protein solutions, cannot be attributed to more interaction between propanal and the protein

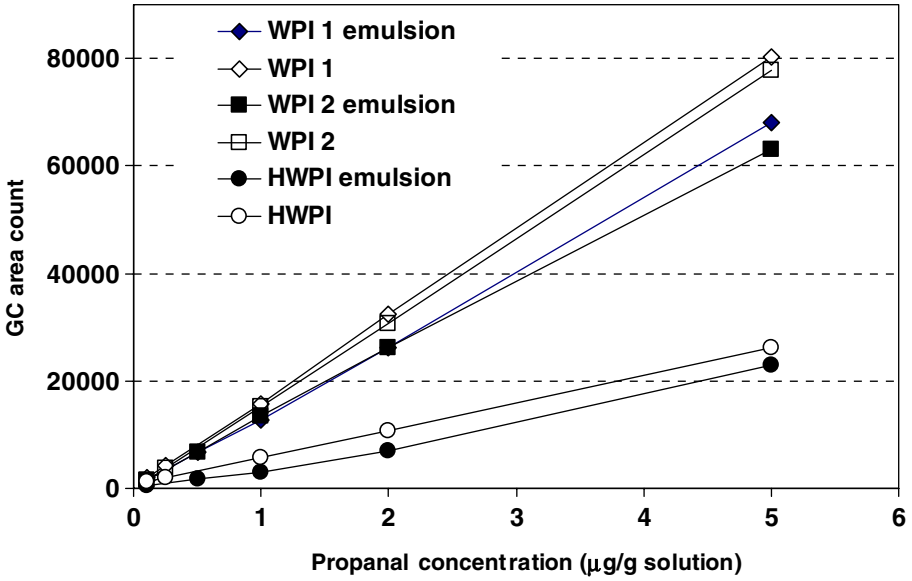

Fig. 3. Comparison of HS propanal above buffered emulsions spiked with propanal (10% TS: 5% tuna oil and 5% protein) with aqueous protein solutions (5% protein) at pH 7.0.

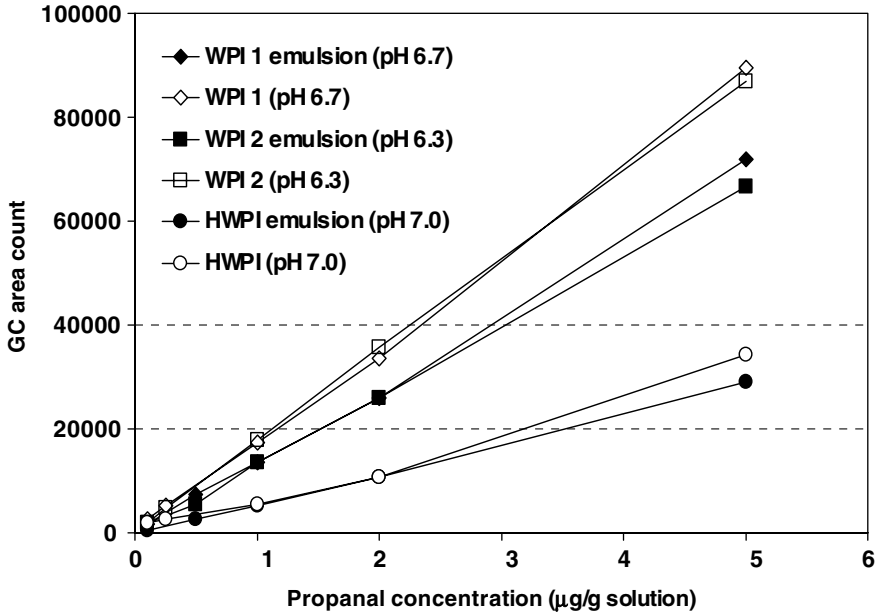

Fig. 4. Comparison of HS propanal above propanal spiked emulsions (10% TS: 5% tuna oil, 5% protein) with aqueous protein solutions (5% protein) at their unadjusted pHs.

aqueous phase in emulsions. It is more likely that the reductions in HS propanal are a result of the added tuna oil dissolving more propanal.

Meynier et al. (2005) reported that the partition coefficients of aroma compounds such as esters and aldehydes over a protein stabilised emulsion could not be calculated from the partition coefficients of their corresponding constitutive phases and were significantly lower for aldehyde compounds, suggesting that the interface barrier formed around the oil droplets was a major governing factor, whereas modification of the emulsion particle size distribution was a minor factor. Their study, to a certain extent, corroborates our observation that emulsification decreased the HS propanal concentration still further over both WPI and HWPI matrices. The differences in the HS propanal above unbuffered and buffered WPI emulsions (Table 3) were smaller than the differences between their corresponding protein solutions (Tables 1 and 2). As stated previously, Meynier et al. (2005) noted that the partition coefficients of aldehydes were drastically reduced for small droplet sizes, a situation where the amount of interfacial protein would be greatly increased. The two types of WPI behaved very similarly in terms of reaction with propanal in all buffered and unbuffered protein solutions, as well as in their corresponding emulsions, although the pH values of their unbuffered solutions were slightly different (Table 3). It seems that there were no noticeable effects on the interaction rates between proteins and propanal within unadjusted pH values from 6.3 to 6.7.

In this study, it was found that, especially for HWPI emulsions, later replicate measurements of HS propanal were invariably lower than earlier replicates. In Table 3, this is shown by the larger values of CV% for HWPI emulsions. This behaviour is similar to that observed with pure protein solutions. Again this suggested that, especially for HWPI emulsions, the interaction between the proteins and propanal was slow and likely to be covalent binding. Such a strong interaction between proteins and propanal is in accord with the results of Meynier et al. (2005).

Because the extent of interaction of HWPI with propanal could be two or three times higher than the extent of interaction shown by WPI, the intensity of rancid odour produced by propanal in the presence of HWPI would be much lower than in WPI solution. Therefore, the flavour perception from tuna oil oxidation might be improved for tuna oil–HWPI, emulsions compared to tuna oil–WPI emulsions for the same amount of propanal produced.

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