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Aroma-retention capacities of functional whey protein aggregates: Study of a strawberry aroma in solutions and in fat-free yogurts

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ABSTRACT

The aroma-retention capacity of functional whey protein aggregates (WPA) was compared to that of native whey protein isolate (WPI) in aqueous solutions and in fat-free yogurts. The retention of aroma compounds, constituting a model strawberry aroma, was evaluated by calculating gas-matrix partition coefficients using headspace gas chromatography (HS-GC).

The retention capacity of WPA differed from the one of WPI for three out of seven aroma compounds detected in HS-GC. Incorporating WPA in fat-free yogurts tended to decrease the release of hydrophobic aroma compounds such as 2-nonanone or methyl-cinnamate. The magnitude of the differences between the partition coefficients of yogurts enriched in WPI or WPA was lower than in aqueous solutions, which is likely to be due to the higher complexity of the food matrix and potential interactions with other ingredients. Overall, the different aroma-retention capacities of native WPI and functional WPA are likely to lead to unbalanced aroma, especially in fat-free dairy products.

1. Introduction

Aroma release has a strong influence on the organoleptic qualities, and hence on the acceptability of food by consumers. Among the most common ingredients, it is well known that proteins interact with aroma compounds. Several studies focused on dairy proteins and evidenced a retention of aroma compounds with the increase in protein concentration (Guichard, 2006; Kopjar, Andriot, Saint-Eve, Souchon, & Guichard, 2010; Landy, Druaux, & Voilley, 1995). The main whey protein, β -lactoglobulin, was particularly studied. The retention of most aroma compounds was increased by increasing the β -lactoglobulin content in a solution, with a stronger effect for compounds with a larger carbon chain length (van Ruth & Villeneuve, 2002). The type of interaction and the strength of binding highly depend on the intrinsic properties of the aroma compounds and of the proteins, as well as on external conditions (pH, temperature, ionic strength...) (Kühn, Considine, & Singh, 2006).

Aroma perception is governed by the distribution of aroma compounds between gas and matrix phases, which can be influenced by the formulation of the product. The release of aroma compounds from a matrix can be characterized by the partition coefficient between the

matrix and the headspace (Kühn et al., 2006). This coefficient represents the ratio between the concentration of volatile compounds in the matrix and in the gas phase. Gas-matrix partition coefficients can be calculated using static headspace methods, which are based on measurements performed at equilibrium between matrix and gas. The Phase Ratio Variation (PRV) method is widely used for the determination of partition coefficient values by static headspace gas chromatography (Ettre, Welter, & Kolb, 1993). It is an indirect method that does not require the use of external calibration, nor the exact knowledge of the concentration of the volatile compound in the solution, and it can be applied to mixes of several volatile molecules (Atlan, Trelea, Saint-Eve, Souchon, & Latrille, 2006). Complementary results can be obtained by using other methodologies, such as gas chromatography-ion mobility spectrometry, which monitor the release of aroma compounds during food consumption (Pu et al., 2020).

The studies on aroma retention abilities of milk proteins are of great importance, because milk proteins are used in a wide range of food products, including dairy products. This is especially true with the increasing demand of “light” and natural products. Indeed, dairy ingredients are considered as promising natural ingredients to be used as fat replacers and to achieve desirable texture properties (Ipsen, 2017).

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Whey protein ingredients are of interest, and research has been done to further improve their functional properties. In this context, whey protein aggregates (WPA) with specific and novel texture properties were produced in controlled conditions (temperature, pH, ionic force). These functional WPA have been shown to be promising natural ingredients to obtain desirable texture in fat-free yogurts (Gélébart et al., 2019; Lesme et al., 2019).

Besides texture modifications, the use of WPA in fat-free products is likely to change the aroma profile of dairy products, even at very low concentration (Kühn, Zhu, Considine, & Singh, 2007). Indeed, whey proteins were found to bind aroma compounds more than caseins (Fabre, Aubry, & Guichard, 2002). However, the aroma-retention ability of functional WPA has never been studied. Further insights are thus needed to be able to develop low-fat dairy products with desirable organoleptic qualities.

Therefore, the first objective of this work is to investigate the aroma-retention capacities of WPA in comparison to native WPI in order to assess if they are likely to have an impact on aroma release in fat-free dairy products. We hypothesized that the change of structure of WPA impacted their aroma binding abilities in addition to their already known new textural functionalities (Lesme et al., 2019).

Moreover, up to now, most systems that have been studied regarding aroma retention capacities of proteins, involved one protein and one aroma compound in model aqueous solutions (Jouenne & Cruzet, 2000; Kühn et al., 2007). To go further and complement the results obtained in model systems, our study was performed on a mix of twenty aroma compounds, constituting a model strawberry aroma, instead of only one aroma compound as traditionally done. Using a complex aroma is thus closer to a real situation when aromatizing yogurts and allow to consider the competition phenomenon between aroma compounds to link with protein. To determine the binding capacities of WPA, partition coefficients were thus measured in aqueous protein suspensions flavored with the model strawberry aroma.

As the functional WPA are meant to be used in fat-free yogurts, the second aim of this study focused on their impact on aroma release in fat-free strawberry yogurts. Fat free yogurts were chosen as more complex food matrices to get in-depth knowledge on the impact of the retention-capacity of proteins in real food matrices. By this way, this study attempted to study the aroma retention capacities of proteins in model systems but also in more complex conditions to fill the gap between model matrix and real products.

2. Materials and methods

2.1. Raw materials

Low-heat spray dried skimmed milk powder (34.00% proteins, < 1.50% fat, 8.50% ash) and whey protein isolate (WPI) (86.51% (w/w) proteins, 1.98% caseins, 0.40% fat, 1.92% ash) were kindly supplied by local dairy companies (confidential origin). The composition of the powders is given according to manufacturer's information. Food grade sodium chloride (NaCl, $\geq 99\%$) and sodium hydroxide (1 M) were bought at Sigma Aldrich (Saint-Louis, USA). To produce fat-free yogurts, YFL-812 (Chr Hansen, France) was used as starter culture because of its low ability to produce exopolysaccharides. Milli Q (Merck Millipore, Burlington, USA) water was used to produce WPI solutions and functional WPA. All ingredients were food-grade.

2.2. Production of whey protein aggregates

Two types of whey protein aggregates, MFA (Monodisperse Fractal Aggregate) and PFA (Polydisperse Fractal Aggregate) were produced by heat-treatment of a solution of WPI (80 °C for 2 h) at pH 7 according to Lesme et al. (2019). The two different type of aggregates were obtained by varying the ionic strength of a WPI solution (50.00 g.L⁻¹). A low ionic strength (15.00 mM) led to MFA (one single population with a

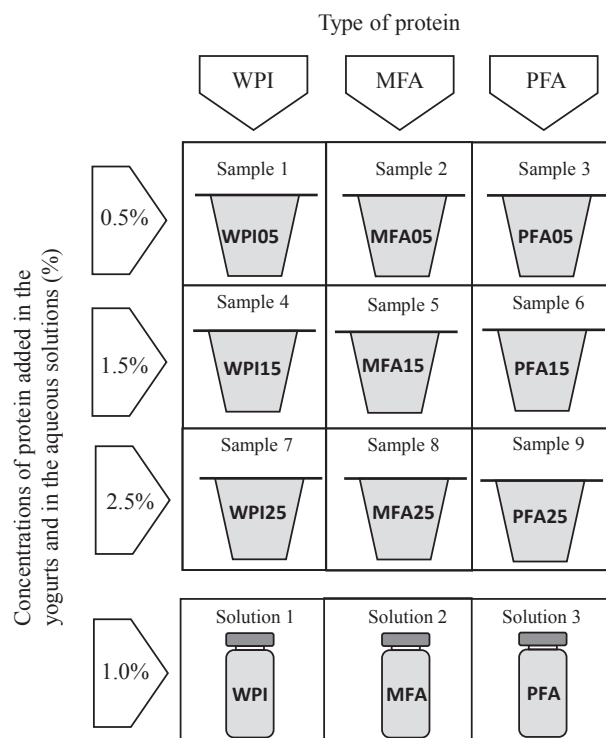


Fig. 1. Experimental design of the samples studied. Yogurts were enriched with either Whey Protein Isolate (WPI), Monodisperse Functional Aggregates (MFA) or Polydisperse Functional Aggregates (PFA). The two types of aggregates were obtained in controlled conditions from a WPI solution. Three different concentrations (0.5%, 1.5%, 2.5%) were used to modify the yogurt texture. Aqueous solutions were enriched with WPI, MFA and PFA in a single concentration (1.0%).

diameter of 200 nm), whereas a higher salt concentration (45.00 mM) led to PFA (two distinct populations with a diameter of 200 and 1000 nm).

2.3. Experimental design

In the following text, the term “yogurt” will be used to mean “fat-free set-type strawberry yogurt”. The experimental design used in the study is depicted in Fig. 1. Yogurt samples or protein suspensions in water were either enriched with native whey protein isolate (WPI), MFA or PFA. WPI, MFA and PFA were incorporated in the yogurts at three different concentration levels: 0.50%, 1.50%, 2.50% (w/w). The aroma retention abilities of protein solutions were investigated using a single protein concentration of 1.00% (w/w).

2.4. Strawberry aroma

The strawberry aroma contained 20 odorant compounds mixed with propylene glycol (Mane R&D, France) (Table 1). Among the 20 compounds, 7 compounds were selected with special attention. These compounds belong to different chemical classes with different physico-chemical properties and were commonly used in aromas formulated for dairy products. Consequently, they were of importance for the study and their concentrations in the strawberry aroma were boosted to ease their detection in headspace. These concentrations were chosen thanks to preliminary studies to determine the limit of quantification in the yogurt matrix employing HS-GC. The other aroma compounds were selected between the main key food odorants of strawberry and were important to obtain a balanced strawberry aroma (Du, Plotto, Baldwin, & Rouseff, 2011).

Table 1

Flavor compounds of the strawberry aroma, their concentrations in protein solutions and yogurts and their physicochemical characteristics. *("The Good Scents Company - Search for product information,"2019). The compounds in bold are the compounds detected and quantified with the PRV method.

	n°CAS	Concentration in solutions and in yogurts (ppm)	M (g/mol)	Vapor pressure (mm/Hg @ 25 °C)	logP ^a	Flavor note
Furanone strawberry	3658-77-3	27.0	128.13	0.03	-0.08	Caramelic, strawberry
Vanillin	121-33-5	6.0	152.15	0.002	1.21	Sweet vanilla
Pyrazine tetramethyl	290-37-9	30.0	80.10	19.74	-0.26	Pungent, roasted hazelnut
Diacetyl	431-03-8	25.0	96.09	56.80	-1.34	Butter
Methyl cinnamate	103-26-4	25.0	162.18	0.011	2.62	Balsamic strawberry
Ethyl acetate	141-78-6	15.0	88.11	111.72	0.73	Ethereal, fruity, sweet
Maltol	118-71-8	12.5	126.11	0.000326	0.09	Sweet, caramelic
Cis-3-hexenol	928-96-1	10.0	100.16	1.04	1.04	Fresh, green, grassy
Ethyl butyrate	105-54-4	10.0	116.60	12.80	1.80	Fruity, pineapple
Benzaldehyde	100-52-7	5.0	106.20	1.27	1.48	Almond
2-Nonanone	821-55-6	3.0	142.24	0.55	3.14	Fresh, sweet, green
Limonene	5989-27-5	3.0	136.24	1.54	4.38	Lemon
γ-decalactone	706-14-9	1.5	179.25	0.005	2.72	Fruity, peach
γ-undecalactone	104-67-6	1.5	184.28	0.003	2.96	Fruity, peach, creamy
Acid butyric	107-92-6	1.0	88.11	1.65	0.79	Acetic, cheesy, dairy
Ethyl isovalerate	108-64-5	0.1	130.19	7.85	2.16	Fruity, sweet, apple
Linalool	78-70-6	1.0	154.25	0.02	2.97	Citrus, floral, sweet
Ethyl-2-methyl-2-butyrate	7452-79-1	1.0	130.19	7.85	2.16	Fruity, fresh, berry
Hexenyl-cis-3-acetate	3681-71-8	1.0	142.20	1.22	2.42	Green, fruity, apple
Trans-2-hexenal	6728-28-3	0.7	98.15	4.62	1.79	Fresh, green, leafy

^a Value of the logarithm of the partition coefficient between n-octanol and water

2.5. Preparation of the protein solutions

Protein solutions were made using WPI, MFA or PFA at a concentration of 1.00% (w/w) in Milli Q water (Fig. 1). The protein solutions had a neutral pH. Strawberry aroma was added to the diluted protein solutions to reach a final aroma concentration of 0.05% (w/w) as recommended by the aroma manufacturer. The concentration of the volatile compounds in the final suspensions ranged from 0.1 to 30 mg.kg⁻¹ (Table 1). The solutions were stored 24 h at 4 °C before headspace analysis.

2.6. Preparation of the strawberry flavored yogurts

Skimmed milk was reconstituted to 100 g.kg⁻¹ milk solids using low-heat spray-dried skimmed milk powder. The reconstituted milk was stored overnight at 4 °C to allow hydration of the powders. After being heated at 90 °C for 5 min, the milk was cooled to the fermentation temperature (43 °C) and inoculated with the yogurt starter culture. At this point, 5.00% sugar (w/w) was added to the milk. Yogurts were also flavored with 0.05% (w/w) strawberry aroma. The concentration of the odorous compounds ranged from 0.1 mg.kg⁻¹ to 30 mg.kg⁻¹ of yogurt (Table 1).

During yogurt manufacture, the milk was enriched either with WPI or whey protein aggregates (MFA or PFA) which were added respectively before or after the heat treatment of the milk. The mix was conditioned in glass cups of 40 mL and put in an incubator for fermentation at 43 °C during about 6 h until the pH reached 4.6. Yogurts were stored at 4 °C during 5 days prior to analysis to ensure an equilibration of the aroma release from the matrix.

2.7. Chromatographic analysis of aroma compounds

The samples were placed in an agitator/incubator of an automatic headspace sampler (GERSTEL MPS, Linthicum, MD, USA). Preliminary analysis showed that equilibrium was reached after 90 min at 30 °C without stirring (results not shown). After equilibration, a 1 mL sample of headspace was automatically taken off by a 2.5 mL gas-tight syringe pre-heated at 35 °C (GERSTEL MPS, Linthicum, MD, USA). The analysis was done using a gas chromatograph (Agilent 7890A, Wilmington, DE, USA) equipped with a Flame Ionisation Detector (FID) and paired to a mass spectrometer (Agilent 5975C, Wilmington, DE, USA). The inlet

temperature was 260 °C and the FID detector temperature was 250 °C. Helium was used as carrier gas in splitless mode at a flow of 2 mL.min⁻¹. The capillary column was DB-WAX (30 m, 0.32 mm, 0.5 μm, Agilent). The program of temperature used was 40 °C for 5 min, ramp to 230 °C at 12 °C.min⁻¹ and 230 °C for 10 min.

2.8. Calculation of partition coefficients

The retention of aroma compounds in protein solutions was compared to equally concentrated aqueous solutions according to the following relationship:

$$R(\%) = \frac{K_{G/W} - K_{G/S}}{K_{G/W}} * 100 \quad (1)$$

where R is the percentage of retention in protein solutions compared to aqueous solutions, $K_{G/S}$ and $K_{G/W}$ are the gas/solution and gas/water partition coefficients of aroma compounds respectively. These partition coefficients were determined by the Phase Ratio Variation (PRV) method. Likewise, the partition coefficients of yogurt samples were named $K_{G/Y}$.

The PRV method, described by Ettre et al. (1993), was used to determine the gas/matrix partition coefficient of the aroma compounds detected by headspace analysis, in water $K_{G/W}$, in protein solutions $K_{G/S}$, and in yogurts $K_{G/Y}$.

The PRV method consists in studying the aroma release in the headspace for different phase ratios (β) inside the headspace vial, according to:

$$\beta = \frac{V_G}{V_S} \quad (2)$$

with V_G the gas volume and V_S the sample volume.

The phase ratio is the ratio of the volumes of the headspace and the sample solution.

By plotting the reciprocal of the peak area ($1/A$) against the phase ratio (β), a linear equation is obtained:

$$\frac{1}{A} = a\beta + b \quad (3)$$

The partition coefficient K is defined as the ratio between the slope (a) and the intercept (b) (Savary, Hucher, Petibon, & Grisel, 2014).

Increasing volumes (50 μL, 100 μL, 250 μL, 500 μL, 1000 μL) of each matrix (water, protein solutions or yogurts) were poured into

headspace vials (22 mL). Each vial represented a gas/liquid phase ratio β of 439, 219, 87, 43, 21 respectively. Therefore, a high gas/matrix partition coefficient indicates that the aroma compound is more released in the gas phase at thermodynamic equilibrium.

2.9. Statistical analysis

Measurements were performed in triplicate on different productions for yogurts and for protein suspensions. Statistical analysis was performed using the XLStat software from Addinsoft (version 2018.5). A one-way ANOVA analysis was performed on the gas/water ($K_{G/W}$), gas/protein solutions ($K_{G/S}$) and gas/yogurts ($K_{G/Y}$) partition coefficients. A one-way ANOVA was also performed on the retention (R) of protein solutions for each aroma compound. A two-way ANOVA with interaction (protein type + protein concentration) was performed on the gas/yogurts partition coefficients ($K_{G/Y}$) to study the effect of the protein type (WPI, MFA, PFA) and the protein concentration (0.50%, 1.50%, 2.50%) on the release of aroma compounds.

When a significant effect was present ($p < 0.05$), the differences between protein solutions were tested using a Fisher test of Least Significant Difference (LSD) for multiple comparisons of means for each aroma compound.

3. Results and discussion

3.1. Detection and quantification of aroma compounds

Seven aroma compounds out of the 22 aroma compounds of the strawberry aroma were detected and quantified by using the PRV method in both protein solutions and yogurts (Table 2). The detected compounds corresponded to the most volatile ones and to the ones present in high concentrations in the strawberry aroma (Table 1). This is in line with Fabre et al. (2002), who quantified only three out of ten aroma compounds by static headspace measurement, and this is comparable to the 8 aroma compounds out of 17 detected by Saint-Eve, Juteau, Atlan, Martin, and Souchon (2006). As already reported, the poor sensitivity for compounds with low volatility constitutes a drawback of the static headspace method (Kühn et al., 2006). For the detected compounds, correlation coefficients R^2 higher than 0.98 were

Table 2

Mean partition coefficients ($K_{G/S} \times 10^2$) of aroma compounds in water and in protein solutions (WPI, MFA, PFA) flavored with the strawberry aroma. Different letters in the columns indicate significant differences among solutions ($p < 0.05$). Air/Water partition coefficients ($K_{G/W} \times 10^2$) of flavor compounds at 30 °C are compared with literature values ($K_{G/M}$ in water Lit).

	$K_{G/W}$ in Water Lit	$K_{G/S}$ in Water	$K_{G/S}$ in WPI	$K_{G/S}$ in MFA	$K_{G/S}$ in PFA
Diacetyl	0.13 ⁽¹⁾ 0.07 ⁽²⁾ 0.19 ⁽³⁾	0.21 ^{bc}	0.19 ^c	0.29 ^a	0.26 ^{ab}
Ethyl butyrate	1.80 ⁽¹⁾ 2.31 ⁽²⁾ 1.80 ⁽³⁾ 2.40 ⁽⁴⁾ 2.80 ⁽⁶⁾	3.10	2.03	2.51	2.02
Limonene	25.10 ⁽¹⁾ 1.04 ⁽²⁾	7.63 ^a	3.23 ^b	3.25 ^b	5.42 ^{ab}
Cis-3-hexenol	0.05 ⁽¹⁾ 0.05 ⁽²⁾	0.07 ^a	0.06 ^{ab}	0.05 ^{ab}	0.05 ^b
2-Nonanone	2.0 ⁽⁵⁾ 1.5 ⁽⁶⁾	1.55 ^a	1.21 ^{ab}	0.79 ^c	1.04 ^{bc}
Benzaldehyde	–	0.15	0.11	0.13	0.13
Methyl cinnamate	0.024 ⁽²⁾	0.10 ^a	0.08 ^b	0.06 ^c	0.05 ^c

⁽¹⁾ Savary et al., 2006 ; ⁽²⁾ Atlan et al., 2006 ; ⁽³⁾ van Ruth et al., 2002 ; ⁽⁴⁾ Gierczynski, Labouré, Sémon, & Guichard, 2007; ⁽⁵⁾ Philippe et al., 2003; ⁽⁶⁾ Buttery, Ling, & Guadagni, 1969.

found for the linear regression between $1/A$ and the phase ratio β . The detected aroma compounds had different physico-chemical properties. This was especially true regarding hydrophobicity with a very hydrophilic compound (diacetyl, logP of -1.34) and a very hydrophobic one (limonene, logP of 4.38) (Table 1). These differences could imply different binding behaviors between proteins and aroma compounds. Indeed, previous studies already reported the importance of hydrophobic interactions regarding aroma compounds retention by proteins (Kühn et al., 2006).

3.2. Comparison of $K_{G/W}$ with the results of the literature

Partition coefficients of aroma compounds were first measured in water. The obtained values of the partition coefficients measured were compared to those already reported by other authors (Table 2). The partition coefficients tended to be slightly higher than the ones of the literature, but there was an overall good similarity. It is interesting to notice that having a combination of aroma compounds in a water solution did not impact their individual partition coefficients in comparison with more simple systems made of one aroma compound. In addition, there is an overall good agreement for the partition coefficients among studies, apart for the ones of limonene which go from $1.04E$ to 3 to 0.25 depending on the study.

As expected, limonene, which is very hydrophobic with a very low solubility in water (Table 1), was the most released compound (Table 2). The release of limonene from water was much higher than the other compounds, which is in line with the results of Savary, Doublier, and Cayot (2006). Ethyl butyrate and 2-nonanone were the two other aroma compounds detected that had a partition coefficient greater than 1, meaning that they were released from water at thermodynamic equilibrium. On the contrary, diacetyl, cis-3-hexenol, benzaldehyde and methyl cinnamate had partition coefficients lower than 1, indicating that they were less released and more water-soluble at thermodynamic equilibrium.

3.3. Aroma retention in MFA and PFA versus WPI aqueous protein solutions

The comparison of $K_{G/S}$ and $K_{G/W}$ values showed that the addition of whey proteins in aqueous solutions decreased the partition coefficients of the aroma compounds. However, the observed changes depended on the aroma compounds considered, and on the type of protein added in the solution (WPI, MFA, PFA). A significant decrease of the partition coefficients with the addition of proteins in the aqueous solution was observed for limonene, cis-3-hexenol, 2-nonanone and methyl cinnamate. The same trend was found for ethyl butyrate and benzaldehyde, without being significant. These results indicated a retention of these aroma compounds by the WPI and WPA, probably due to interactions between aroma compounds and proteins. This result coincided with results obtained by van Ruth and Villeneuve (2002) on the aroma retention properties of pure β -lactoglobulin, or by Viry, Boom, Avison, Pascu, and Bodnár (2018) on the partitioning of aroma compounds in a more complex system where WPI and sodium caseinate were mixed. Differences in aroma partition coefficients exist between native proteins WPI and functional aggregates MFA and PFA (Table 2). These aroma-dependent differences were further highlighted throughout the retention (R) of the aroma compounds by the proteins (Fig. 2.). Aside from diacetyl, the retention goes from 8.60% for benzaldehyde with MFA to 81.90% for limonene with MFA. The retention ability of WPI, MFA and PFA were significantly different for three compounds: diacetyl, 2-nonanone and methyl cinnamate. Aroma binding ability of proteins is highly dependent on the conformational state of the protein, and more particularly on the sites available to bind aroma compounds. It is well known that β -lactoglobulin is readily denatured by heat treatment (Wijayanti, Brodkorb, Hogan, & Murphy, 2019), and the resulting change of conformation is likely to influence its retention abilities. Few studies have been performed on the aroma retention abilities of

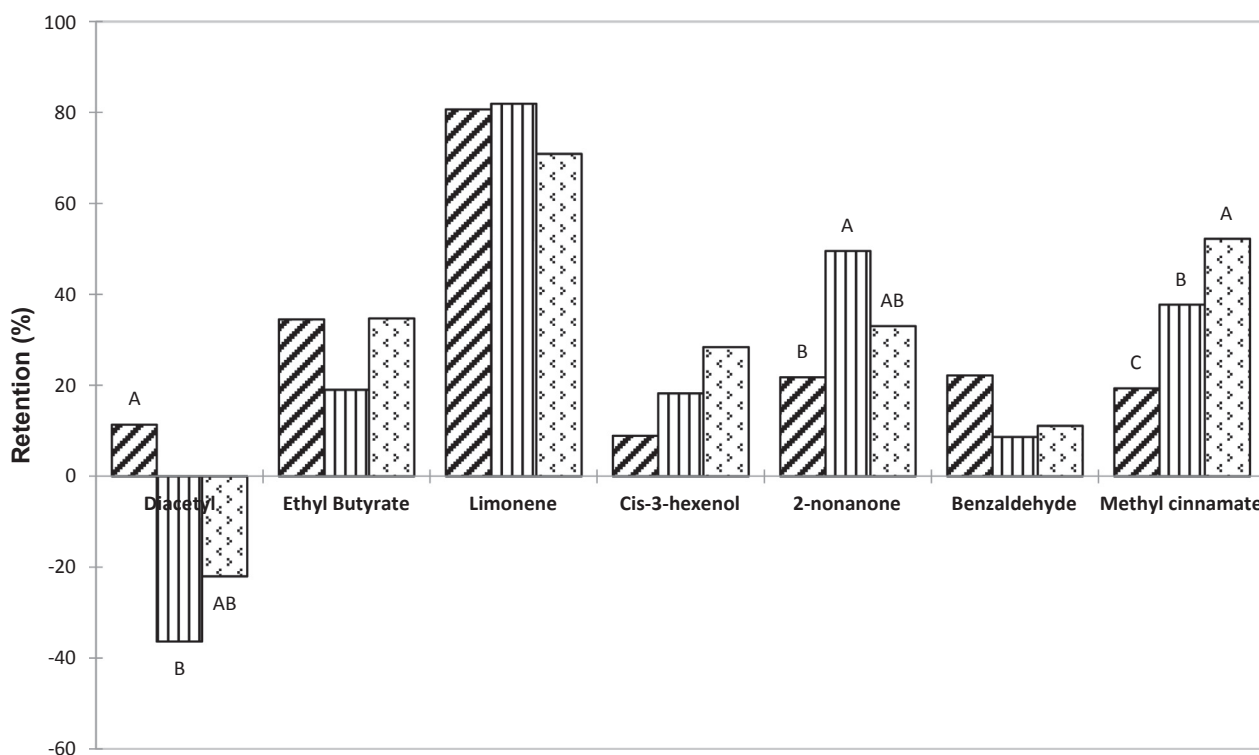





Fig. 2. Retention (%) of the seven aroma compounds detected by headspace measurements in aqueous solutions containing the strawberry flavor and the different proteins studied:  WPI,  MFA,  PFA. The letters indicate a significant effect of the type of protein added in the aqueous solution ($p < 0.05$)

denatured whey proteins, and the results are contradictory between studies. Increased affinity for β -ionone and guaiacol was achieved after partial unfolding of β -lactoglobulin (Tavel, Moreau, Bouhallab, Li-Chan, & Guichard, 2010) while a decreased binding ability with the denaturation of the proteins was evidenced by Kühn et al. (2006). This contradiction between the results evidences that there is no general trend and that the effect depends on the type of the considered aroma compounds (Table 2).

For 2-nonanone and methyl cinnamate, the retention was significantly higher in the aqueous solutions containing whey protein aggregates compared to WPI solutions while the reverse trend was observed for diacetyl (Fig. 2.). To our knowledge, no studies have been performed on binding abilities of denatured whey proteins on methyl cinnamate or diacetyl, and a single study was performed on denatured β -lactoglobulin (heated at 75 °C for 10 and 20 min) and 2-nonanone (O'Neill & Kinsella, 1988). These authors evidenced a decreased binding affinity of 2-nonanone for denatured β -lactoglobulin. However, the denaturation rate of the β -lactoglobulin in this study might be far less important than in our experiment, and the aggregates formed are likely to have a different shape than MFA and PFA, which were obtained by a more intense heat treatment (80 °C for 2 h). Indeed, the heating time was six times shorter and the concentration of the heated β -lactoglobulin solution was five times smaller than in the current protocol. It can be noted that the aroma compounds more retained by whey protein aggregates than that of WPI (2-nonanone and methyl cinnamate) were the 2 compounds with the higher molecular weight, in contrast to diacetyl which had the lowest one. We assume that because of the unfolding of native WPI and subsequent formation of branched aggregates, previously buried binding sites (i.e. hydrophobic residues) become accessible for interaction with non-polar aroma compounds in MFA and PFA, resulting in a lower release of methyl cinnamate and 2-nonanone. The increased number of hydrophobic residues present at the surface of MFA and PFA could in the same way limit the interactions with a highly hydrophilic compound such as diacetyl. This could be the reason why diacetyl is much more released in the presence of MFA and

PA.

The importance of the structure of WPA was evidenced by the significant difference existing in the retention ability of PFA and MFA regarding methyl cinnamate. The polydispersity of MFA and PFA was varied by changing the ionic strength of the WPI solution. Nicolai, Britten, and Schmitt (2011) reported that increasing the ionic strength led to higher denaturation rates, which might correspond to more available binding sites, and therefore inducing a higher retention of hydrophobic compounds such as methyl cinnamate. It was evidenced in a previous study that PFA and MFA have different structures (Lesme et al., 2019). PFA have a more branched and expanded structure while MFA are denser aggregates. Moreover, this change of structure might induce a rise in total area of PFA compared to MFA, which could favor the binding of aroma compounds such as methyl cinnamate. Indeed, Tavel et al. (2010) evidenced that a less tightly packed structure induced an easier access to the binding sites and favored aroma binding.

MFA or PFA did not have a significant impact on the retention of ethyl butyrate, limonene, *cis*-3-hexenol and benzaldehyde (Table 2.). These molecules were retained by whey proteins, regardless of the state of the whey proteins. Benzaldehyde was the only aroma compound for which the retention ability of MFA and PFA tended to be inferior to the one of WPI, even if the difference was not significant. Hansen & Booker (1996) evidenced that heat-treated β -lactoglobulin (30 min at 70 °C) has a higher binding ability for benzaldehyde. However, the authors added the aroma compound before heat treatment, and the elevated temperature might have favored covalent bindings between the aldehyde compound of benzaldehyde with amino groups of β -lactoglobulin. The particularly high retention of limonene might be explained by a preferential binding in the hydrophobic pocket of native β -lactoglobulin that makes up WPI, and by the multiple hydrophobic sites available on MFA and PFA.

The use of a mixture of aroma compounds in a protein solution can also influence the retention and then the partition coefficient of individual aroma components. van Ruth and Villeneuve (2002) explained the increased partition coefficients of some esters and aldehydes in the

presence of other aroma compounds by a competition for the binding sites on the proteins. For instance, the authors found that the air/liquid partition coefficient of ethyl butyrate was increased when present with others. In our study, ethyl butyrate was not significantly more retained by WPI compared to MFA and PFA (Table 2) while previous studies evidenced a high affinity between ethyl butyrate and WPI (Pelletier, Sostmann, & Guichard, 1998; van Ruth & Villeneuve, 2002). We can hypothesize that a form of competition between aroma compounds of the strawberry aroma for the binding sites of the proteins impacted the retention of ethyl butyrate. Moreover, Jouenne and Crouzet (2000) evidenced that this competition for the binding sites was concentration and pH dependent, which is probably related to the accessibility of the binding sites.

Heat-denatured whey proteins are of great importance for the dairy industry because heat treatment is an important production step of dairy products. According to the results obtained in the first part of the article, MFA and PFA have different aroma binding abilities than native WPI, which evidenced that a modification of the conformation of whey proteins has an impact on their interactions with aroma compounds. However, the aroma binding abilities of WPA were not necessarily reduced, as documented by several studies (Chobpattana, Jeon, Smith, & Loughin, 2002; Mcneill & Schmidt, 1993; O'Neill & Kinsella, 1988). These modifications are likely to influence the balance of the overall strawberry aroma. Complex food matrixes involve interactions with other food components (proteins, carbohydrates, lipids) which could further modify the impact of functional whey protein aggregates on aroma release.

3.4. Impact of WPA on the release of aroma compounds in fat-free yogurts

Fat-free strawberry yogurts were chosen as a model complex food. The yogurts studied are presented in Fig. 1. The PRV method enabled to detect and quantify the same 7 out of the 22 aroma compounds in the strawberry flavored yogurts than in protein aqueous solutions. Measured partition coefficients over yogurts ranged from 2.5×10^{-4} for methyl cinnamate to 3.1×10^{-2} for ethyl butyrate (Table 3). The partition coefficients were of the same order of magnitude for the different yogurts. However, the partition coefficients of some compounds such as limonene and methyl cinnamate are very inferior compared to the ones measured in water. Saint-Eve et al. (2006) evidenced the same behavior for the most hydrophobic aroma compounds and explained it by the presence of fat in their yogurts and by a temperature difference between the measurements performed on solutions and on yogurts. As the yogurts investigated in the study were fat-free and all the measurements were performed at 4 °C, it can be hypothesized that hydrophobic interactions between proteins and aroma compounds were responsible for their lower volatility in yogurts.

A significant product effect was evidenced for four compounds out of the seven detected, namely limonene, *cis*-3-hexenol, 2-nonanone and methyl cinnamate. Aside from *cis*-3-hexenol, these compounds were the

most hydrophobic molecules that make up the strawberry aroma. Interactions between these compounds and the whey proteins were evidenced in the first part of this study as they were the only compounds significantly more retained in protein solutions compared to water solutions (Table 2). Consequently, physico-chemical interactions evidenced between aroma compounds and proteins appeared to also impact the aroma release in more complex matrixes.

Two factors (protein type and concentration) were varied in the fat-free yogurts and a two-way ANOVA with interaction (type of protein and concentration of protein) was applied on the partition coefficients. The main effects of the ANOVA are depicted in Fig. 3. The concentration of proteins had a significant impact on the release of two aroma compounds out of the seven detected (Fig. 3. (A)). The increase of protein concentration led to a significant increased retention of methyl cinnamate (F-value = 5.75 and p-value = 0.009) and limonene (F-value = 4.35 and p-value = 0.025) in the yogurts. The impact of the protein concentration was particularly important on the release of methyl cinnamate, with a retention increased by 40.00% in yogurts enriched with 0.50% compared to yogurts enriched with 2.50% proteins.

The type of protein also had an impact on the release of two aroma compounds (Fig. 3. (B)). *Cis*-3-hexenol (F-value = 3.14 and p-value = 0.05) and 2-nonanone (F-value = 3.72 and p-value = 0.05) were significantly more retained by MFA and PFA than native WPI. A similar trend was observed, without being significant, for methyl cinnamate and limonene. However, the magnitude of the differences between native WPI and functional WPA partitioning of *cis*-3-hexenol and 2-nonanone was rather small, as the partition coefficients were only decreased by 12% and 16% respectively. Moreover, the interaction between the type of protein and the protein concentration was significant for 2-nonanone (F-value = 8.93 and p-value = 0.00). This result implied that the impact of the type of protein depended on the concentration considered and vice versa, indicating that the two factors, should be considered an integrated phenomenon.

In a complex matrix such as yogurt, other parameters in addition to direct protein-aroma interactions may be at stake and influence the release of aroma compounds in a different way than in model aqueous solutions. For instance, the aroma release can be impacted by interactions with other food components such as carbohydrates, fat, or by the existence of a three-dimensional protein network (Seuvre, Philippe, Rochard, & Voilley, 2006). Moreover, the differences in pH between the protein solutions (neutral pH) and the yogurts (pH 4.6) could induce different binding behaviors of the proteins. Knowledge on the structure of the protein network and on the textural properties of protein-enriched yogurts appear to be useful to explain the differences observed. The impact of MFA and PFA on the texture and the protein network of fat-free yogurts was evidenced in a previous study (Lesme et al., 2019). While yogurts enriched in MFA had a firmness close to the one of yogurts enriched with WPI, yogurts enriched with PFA were much softer. However, the impact of MFA and PFA on the protein network structure

Table 3

Mean partition coefficients ($K_{G/Y} \times 10^2$) of aroma compounds in fat-free strawberry yogurts enriched with different whey proteins (WPI, MFA, PFA) at different concentrations (0.5%, 1.5%, 2.5%). Different letters in the columns indicate significant differences among yogurts ($p < 0.005$).

	Aroma compounds ($K_{G/M} \times 10^2$)						
	Diacetyl	Ethyl butyrate	Limonene	Cis-3-hexenol	2-nonanone	Benzaldehyde	Methyl cinnamate
WPI05	0.15	2.81	1.08 ^{ab}	0.068 ^{ab}	0.60 ^{bc}	0.17	0.059 ^{ab}
WPI15	0.19	2.83	1.06 ^{abc}	0.074 ^{ab}	0.93 ^a	0.20	0.049 ^a
WPI25	0.17	2.66	0.94 ^{abcd}	0.072 ^{ab}	0.49 ^c	0.14	0.045 ^c
M_FA05	0.15	2.93	1.20 ^a	0.064 ^a	0.59 ^{bc}	0.15	0.025 ^{ab}
M_FA15	0.18	3.08	0.73 ^d	0.066 ^{ab}	0.46 ^c	0.15	0.038 ^{bc}
M_FA25	0.17	2.45	0.86 ^{abcd}	0.049 ^c	0.59 ^{bc}	0.26	0.041 ^{abc}
P_FA05	0.17	2.54	0.92 ^{abcd}	0.069 ^{abc}	0.49 ^c	0.14	0.024 ^{ab}
P_FA15	0.15	2.32	1.02 ^{abc}	0.060 ^{bc}	0.54 ^{bc}	0.16	0.048 ^{bc}
P_FA25	0.18	2.84	0.85 ^{cd}	0.059 ^{ab}	0.69 ^b	0.23	0.033 ^c

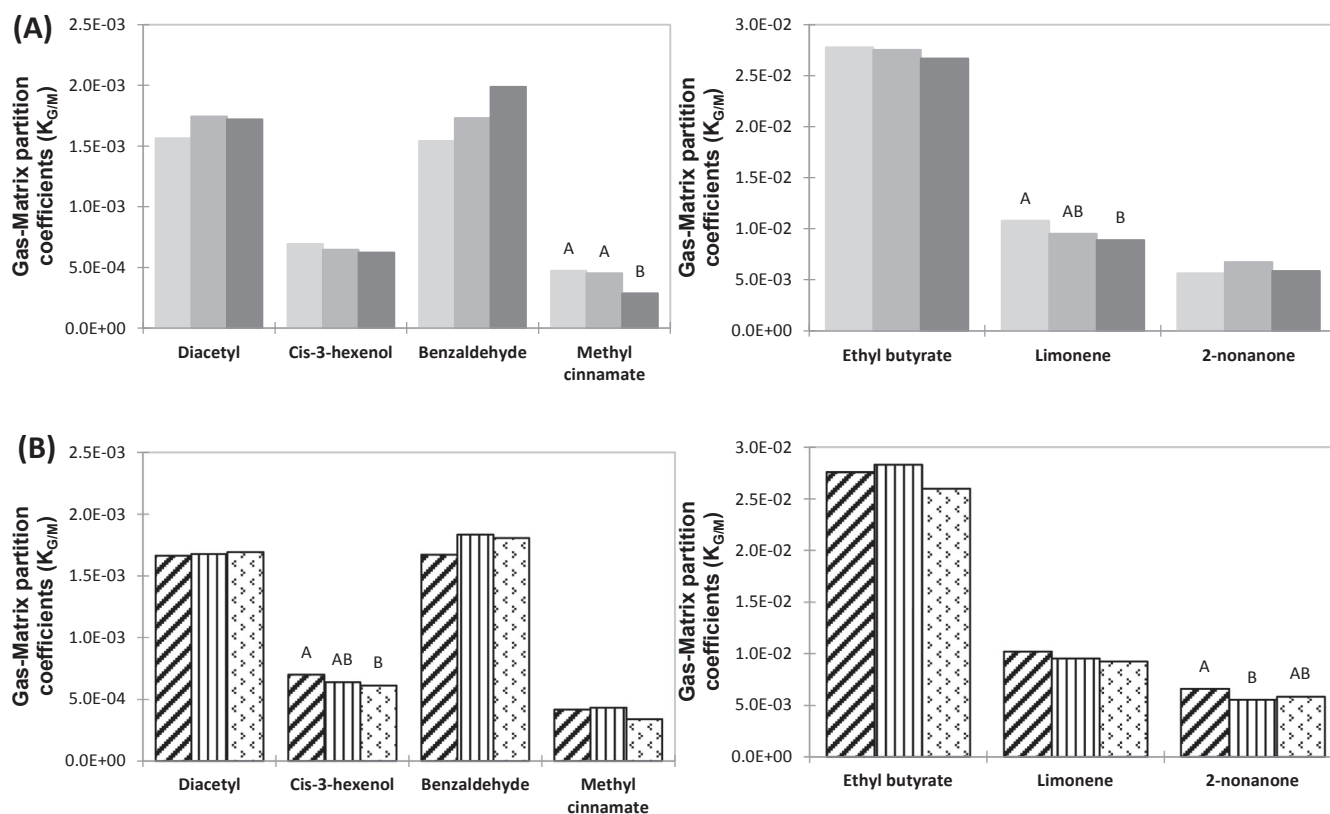


Fig. 3. (A) Impact of the concentration of protein on the gas-matrix partition coefficients (KG/M) for the seven aroma compounds detected by headspace measurements in fat-free strawberryflavored yogurts studied enriched with different types of proteins in different concentrations: 0.5%, 1.5%, 2.5% (B) Impact of the type of protein on the gas-matrix partition coefficients (KG/M) for the seven aroma compounds detected by headspace measurements in fatfree strawberryflavored yogurts studied enriched with the different types of proteins: WPI, MFA, PFA. Different letters indicate a significant protein effect ($p < 0.005$)

was limited to a slight increase of porosity in yogurts enriched with PFA. The protein concentration had a greater impact on texture properties, and Gélébart et al. (2019) evidenced that the increase of protein concentration led to a clear increase of the protein network density. These textural modifications can impact the aroma release from yogurts.

The denser protein network of yogurts enriched with high concentrations of proteins might explain the higher retention of hydrophobic compounds such as limonene and methyl cinnamate while less hydrophobic compounds such as diacetyl and benzaldehyde tend to be more released when the concentration of protein was increased. However, the modifications of the structure of the protein network between yogurts enriched with the same concentration of either WPI, MFA and PFA was subtle and might not be enough to induce important variations of aroma release. It is interesting to notice that there is a tendency for limonene, 2-nonanone, methyl cinnamate and *cis*-3-hexenol to be more retained by WPA whereas diacetyl and benzaldehyde follow the opposite trend. It can be hypothesized that thank to their branched structure, MFA and PFA can both interact with the protein network to strengthen it and with aroma compounds. At the opposite, WPI denatured by the heat treatment applied to the yogurt milk base, are preferentially bound to the protein network, which might explain the lower aroma retention.

Important texture modifications and subtle variations of aroma release were also evidenced in the few studies performed to investigate the impact of the protein content of yogurts on the aroma release (Chua, Deeth, Oh, & Bansal, 2017; A. Saint-Eve, Lévy, Martin, & Souchon, 2006; Anne Saint-Eve, Juteau, Atlan, Martin, & Souchon, 2006). Chua et al., 2017 investigated the effect of the casein to whey protein ratio on the release of key volatile compounds of yogurt aroma, i.e. acetaldehyde, butyric acid and diacetyl. They evidenced that addition of

WPI significantly decreased the release of diacetyl and butyric acid, compared to yogurts enriched with casein, showing that WPI can hold volatile aroma compounds in yogurts containing 5.00% total protein content. However, the variations were rather small and the authors concluded that WPI was unlikely to lead to an unbalanced aroma in plain yogurts. Saint-Eve et al. (2006a, 2006b) performed two studies varying the casein to whey protein content of stirred strawberry flavored yogurts and evidenced that the modification of the protein content led to small differences in aroma partitioning (inferior to 30%) and to important texture modifications. They showed that yogurts enriched with casein had a heterogeneous structure with large pores and showed that most of aroma compounds detected had a greater affinity for casein than for whey proteins. The authors hypothesized that the coarse network structure of the yogurts enriched with caseinate could limit the diffusion of aroma compounds in static conditions. Despite the small variations in aroma release, yogurts enriched with caseins were perceived as being the less intense in aroma (Saint-Eve et al., 2006). However, the authors wondered if the difference of aroma perception was due to direct physico-chemical interactions between aroma compounds and proteins or to sensory interactions between texture and aroma perception.

4. Conclusions

PRV method gave absolute results regarding retention ability of functional whey protein aggregates compared to native WPI for a limited number of aroma compounds present in the strawberry aroma studied. The calculation of partition coefficients confirmed that WPA had different aroma-binding abilities compared to native WPI. The retention ability of MFA and PFA was dependent on the aroma compound considered, but contrary to previous studies performed on denatured

they proteins, a significant higher retention by MFA and PFA was evidenced for some hydrophobic compounds. Moreover, performing the study on a combination of aroma compounds forming a strawberry aroma was relevant to supplement the existing data as a form of competition for the binding sites can influence the release of some compounds.

The impact of functional WPA in a real food such as yogurt was more difficult and complex to assess as the proteins and the aroma compounds can interact with other ingredients. However, the shift from aqueous solutions to real food products is essential to complement the knowledge gained on model systems and gives important information for the formulation of fat-free dairy products.

Obtaining a complete aroma profile of yogurts enriched with functional WPA would be interesting to confirm the results evidenced with the PRV method on a greater number of aroma compounds. It would also allow to investigate the impact of aroma retention by proteins on strawberry aroma perception by correlating the instrumental data with sensory profiles of yogurts.

CRedit authorship contribution statement

Hanna Lesme: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Visualization. **Clémence Alleaume:** Resources, Investigation, Formal analysis. **Saïd Bouhallab:** Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Marie-Hélène Famelart:** Conceptualization, Writing - review & editing. **Cécile Marzin:** Resources, Investigation. **Lizeth Lopez-Torres:** Methodology, Resources. **Carole Prost:** Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Cécile Rannou:** Conceptualization, Methodology, Supervision, Validation, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodres.2020.109491>.

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