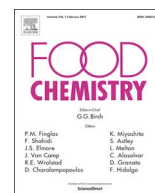




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Understanding fat, proteins and saliva impact on aroma release from flavoured ice creams

Charfedinne Ayed^{a,d}, Sara I.F.S. Martins^b, Ann-Marie Williamson^c, Elisabeth Guichard^{a,*}^a Centre des Sciences du Goût et de l'Alimentation, AgroSupDijon, CNRS, INRA, Université Bourgogne Franche-Comté, F-21000 Dijon, France^b Unilever R&D Vlaardingen, Olivier van Noortlaan 120, 3133 AT Vlaardingen, The Netherlands^c Unilever R&D Colworth, Colworth Science Park, Bedford MK44 1LQ, United Kingdom^d Flavour group, Division of Food Sciences, University of Nottingham, Sutton Bonington Campus, Sutton Bonington, Loughborough, Leicestershire LE12 5RD, United Kingdom¹.

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ABSTRACT

The release profile of fourteen aroma compounds was studied in ice cream samples varying in fat and protein, both in level and type. *In vitro* aroma release was monitored by solid phase micro-extraction gas chromatography using an innovative saliva reactor, which imitated human chewing under temperature control. The results showed that the effect of the fat type on aroma release was smaller than that of fat level. Ice creams with low fat level released more hydrophobic aroma compounds than ice creams with high fat level. At low fat level more aroma compounds were released from ice creams with lower protein content. At high fat level a small increase of aroma release was observed by the addition of saliva, which was explained by a salting out effect, due to the presence of proteins and salts in the saliva. These findings confirmed that the interactions between salivary proteins and aroma compounds occurring in aqueous solutions are not observed in emulsions.

1. Introduction

Ice cream is a complex aerated emulsion composed of protein and milk fat which play an important role in the stabilisation of its structure. In order to answer to nutritional recommendations, ice cream manufacturers have been reconsidering the formula of their products using less fat or different sources of fat and protein. It has been shown that modifications in the process conditions leading to different diameters of ice crystals and air bubbles induce differences in ice cream microstructure which thus impact sensory perception and more specifically mouthfeel (Inoue et al., 2012). Considering ice cream as an oil-in-water emulsion, its microstructure also impacts the physico-chemical properties and as an example, fat droplet size was found to have a significant impact on emulsion destabilisation, meltdown behaviour and creamy mouthfeel (Koxholt, Eisenmann, & Hinrichs, 2001). Moreover, the consumption of ice cream is highly determined by its overall sensory acceptability, mainly flavour perception, which justifies the need to better understand the impact of food reformulation on the release of aroma compounds in conditions as close as possible as in-mouth consumption.

The effect of fat type and fat level on either aroma release or sensory perception in ice creams has been the subject of different studies. Frost,

Heymann, Bredie, Dijksterhuis, and Martens (2005) demonstrated that a modification of fat type and fat level induced differences in the perceived rate of melting of ice cream and also in flavour perception, because an increase in fat level delayed the perceived ice cream melting measured by time intensity and the time to reach maximum flavour intensity. The authors also noticed different effects according to the different aroma compounds used, which were partly explained by the boiling point and hydrophobicity. Prindiville, Marshall, and Heymann (1999) showed that chocolate flavour was perceived differently in ice creams manufactured with milk fat or with cocoa butter. The impact of the fat type on the headspace composition of chocolate ice cream was then studied by Welty, Marshall, Grün, and Ellersieck (2001) who demonstrated that the release in the vapour phase of two important aroma compounds in chocolate, 3,5-diethyl-2-methylpyrazine and 2-methyl-5-propyl pyrazines, was higher in ice creams containing milk fat than in ice creams containing cocoa butter. Another study on strawberry ice creams showed that the strawberry flavour was perceived faster and more intensely in ice creams realised with a higher level of unsaturated vegetable fat (Hyvonen, Linna, Tuorila, & Dijksterhuis, 2003). Similar results were observed by other authors on model food emulsions realised with animal or vegetal oils flavoured with a fruity aroma (Fabre, Guichard, Aubry, & Hugé, 2003). Emulsions realised with a vegetal oil

* Corresponding author.

E-mail address: elisabeth.guichard@inra.fr (E. Guichard).¹ Present address.<https://doi.org/10.1016/j.foodchem.2017.10.127>Received 21 February 2017; Received in revised form 4 August 2017; Accepted 25 October 2017
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presented the highest maximal perceived intensity and the longer duration, which was explained by the fact that the vegetable fats used in the experiment had a lower liquid proportion upon heating which increased the release of hydrophobic compounds such as ethyl hexanoate, (Relkin, Fabre, & Guichard, 2004). This could be explained by a lower solubility of this hydrophobic compound in solid fat as was observed in model systems composed of oils differing in their melting points (Roudnitsky, Irl, Roudaut, & Guichard, 2003). The opposite behaviour was observed for diacetyl, which was more released from emulsions containing anhydrous milk fat (Relkin et al., 2004). The authors explained the higher release by the higher triacylglycerol content of anhydrous milk fat in comparison with vegetal fat and also by the greater droplet size of the emulsions which favoured the release of this hydrophilic compound (Charles, Rosselin, Beck, Sauvageot, & Guichard, 2000). However the effect of emulsion droplet size on the release of hydrophilic aroma compounds seems to highly depend on the nature of the emulsion, because in another study no effect of droplet size was observed on the release of diacetyl from ice creams (Miettinen, Tuorila, Piironen, Vehkalahti, & Hyvönen, 2002). There seems to be a better consensus for the behaviour of hydrophobic compounds, because both studies showed that the smaller the droplet size, the more intense was the release of the most hydrophobic compounds. Besides fat, ice cream is also composed of proteins, mainly milk proteins which are known to interact with aroma compounds according to both the nature of aroma and the nature of the protein (Guichard, 2002; Tromelin, Andriot, & Guichard, 2006). The nature and amount of protein in the ice cream will change the structure of the emulsion by modifying the interfacial properties and the fat droplet agglomeration in the emulsion (Sourdet, Relkin, & Cesar, 2003). This will impact the rate of transfer of aroma compounds from oil to water and then from the emulsion to the gas phase (Druaux & Voilley, 1997).

During consumption, even if the role of chewing can be considered negligible in the case of emulsions, ice cream undergoes phase changes from semi-solid to liquid, due to the combined actions of temperature increase and dilution with saliva, before swallowing (Salles et al., 2011). In water and oil model systems, the addition of artificial saliva modifies the air/liquid partitioning of aroma compounds (van Ruth, Grossmann, Geary, & Delahunty, 2001), inducing either a retention or a salting out effect. This effect has not been explored yet in real food emulsions.

Even if some general trends of flavour release from ice cream during eating have already been reviewed (Chung, 2007) there is still a need for a better understanding of the relative impacts of fat level, fat type and protein content on aroma release from ice creams, taking into account thermal exchanges occurring in the mouth and the effect of human saliva. A salivary reactor has been previously developed within the research group to mimic the in-mouth breakdown of fat spreads (Poette et al., 2010), which highlighted the impact of human saliva on aroma release. This device reproduces as faithfully as possible the principal phenomena occurring in the mouth during eating (i.e. stirring, saliva flow, and temperature) by using data from real measurements on subjects consuming the same products.

Our aim was therefore to determine the combined effects of food composition and human saliva on the release of a wide range of aroma compounds from ice creams, using the saliva reactor in conditions reproducing as close as possible the in-mouth process. We will thus design our experimental protocol to reproduce the thermal exchanges occurring in the mouth during ice cream consumption and work with a pool of human saliva. The results will allow us to determine the respective impacts of saliva, fat and protein on aroma release. This work will provide innovative tools to guide food industries in the reformulation of low fat ice creams with a limited effect on aroma release.

2. Materials and methods

2.1. Samples composition

The study was done with different samples of ice creams realised with two fat types (A and B) varying in their solid fat content (SFC). Fat A had respectively 83.3% and 39.6% SFC and fat B, 0.9% and 0.1% SFC at the temperatures of 10°C and 20°C. Each fat type was added at two different fat levels (L for low = 3%; H for high = 9%). The ice creams contained two different levels of skimmed milk powder enriched with whey protein (level 1: standard – SMP: 6.4% Whey: 2.3%; level 2: low – SMP: 3.2% Whey: 1.15%). They were flavoured with a mixture of 14 aroma compounds (acetoin: 450 mg/kg ice cream; 2,5-dimethylpyrazine, 33.3 mg/kg; vanillin: 550 mg/kg; 2-methoxy phenol: 45 mg/kg; benzaldehyde: 18 mg/kg; phenyl ethyl alcohol: 18 mg/kg; 2-ethyl-3,5-dimethylpyrazine: 54 mg/kg; 2-methoxy-4-methylphenol: 18 mg/kg; hexanal: 54.9 mg/kg; *p*-anisaldehyde: 300 mg/kg; ethyl butyrate: 18 mg/kg; butyl propionate: 64.8 mg/kg; *cis*-3-Hexenyl acetate: 3.6 mg/kg; ethyl octanoate: 18 mg/kg). To study the impact of human saliva on aroma release the experiments were realised after diluting the samples in either ultra-pure water (MilliQ®, Bedford, MA) (W) or human saliva (S). Thus a total of 16 samples were analysed (Table 1).

2.2. Human saliva collection

Resting human saliva was collected from 20 volunteers as already described (Poette et al., 2014). Participants were not allowed to eat or drink one hour before sampling. Resting saliva was collected by instructing the subjects to spit out the saliva into a pre-weighed plastic bottle until it was full. To obtain the most representative salivary composition, the different saliva samples were pooled, mixed and centrifuged at 15000g for 15 min. The salivary pool was then sampled into aliquots of 10 mL stored at –80 °C until use. In a previous study, no effect of saliva storage was observed on the retention of 2-heptanone and ethyl heptanoate by human saliva in aqueous solution (Pagès-Hélary, Andriot, Guichard, & Canon, 2014).

2.3. Saliva reactor

A saliva reactor cell was used to reproduce ice cream breakdown as closely as possible to in mouth conditions (Fig. 1). This device was specifically designed to evaluate the particular role of saliva during liquid and semi-solid food consumption (Poette et al., 2010). It was composed of a water-jacketed glass flask (250 ml), which allowed a temperature control of the sample, equipped with four orifices, one for the temperature sensor, two others to introduce the sample and the SPME fibers and the last one was equipped with a 3-blade marine propeller with digital speed control.

The amount of water/saliva to be added in the reactor and the temperature changes of the ice cream was estimated from preliminary tests with a panel of 10 volunteers. As an average of 1.6 g of saliva was produced by consuming 8 g of ice cream and considering that 50 g was the minimum amount of ice cream in the reactor, 10 ml of water/saliva

Table 1
Samples compositions and codes after dilution in water or saliva.

code in water	code in saliva	fat type	fat level	Protein level
WAH1	SAH1	A	High	1
WAH2	SAH2	A	High	2
WBH1	SBH1	B	High	1
WBH2	SBH2	B	High	2
WAL1	SAL1	A	Low	1
WAL2	SAL2	A	Low	2
WBL1	SBL1	B	Low	1
WBL2	SBL2	B	Low	2

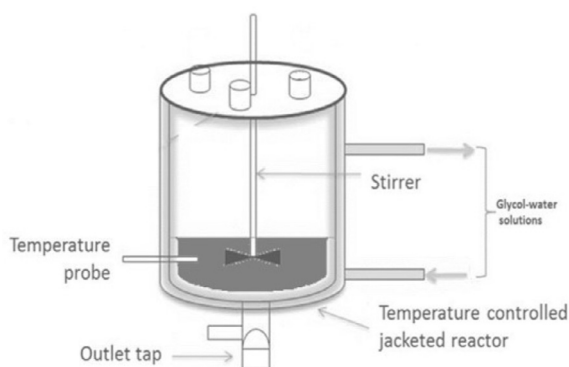


Fig. 1. Schematic diagram of the Saliva reactor.

were transferred into the reactor (250 ml), which was kept at 37°C, and then 50 g of ice cream (at -20°C) were added and the mixture stirred (400 rpm; maximum available speed in this device). The temperature of the mixture in the reactor decreased from 37°C to -20°C after 25 s which follows the temperature decrease in the mouth after the introduction of the sample (oral-phase) then the jacket of the reactor was warmed-up in order to increase to 15°C which corresponds to the swallowing temperature of the mixture after 80 s.

2.4. Solid phase micro-extraction – gas chromatography – mass spectrometry (SPME–GC–MS) analysis

As two fibers were introduced into the reactor (each in one orifice) to follow aroma release, a preliminary inter-fiber repeatability study was performed in order to select the most similar fibers. Nine SPME fibers were tested to recover the aroma compounds of the ice creams, and the two SPME fibers exhibiting the lowest variation (less than 10% RSDs for the extraction of the same aroma compound) were selected. The two SPME fibers were exposed 25 s after the introduction of the ice cream, which corresponds to the time at which the mixture reaches the minimum temperature (-20°C). Extraction was then performed for 1 minute. All experiments were realised in triplicate.

SPME fibers were injected in splitless mode (250 °C, 5 min) in a Gas Chromatograph (Agilent 6890N) coupled to a quadrupole Mass Detector (Agilent 5973N). After desorption of the SPME fiber, volatile compounds were separated on a DB-Wax polar capillary column (30 m × 0.25 mm i.d. × 0.50 µm film thickness) from Agilent (J&W Scientific, Folsom, USA). Helium was the carrier gas at a flow rate of 1 mL/min. The oven temperature was initially held at 40 °C, then increased at a rate of 5 °C/min until 240 °C and held for 10 min. The fibers were regenerated 15 min at 240 °C before novel use.

For the MS system, the temperatures of the transfer line, quadrupole and ion source were respectively 250 °C, 150 °C and 230 °C. Electron impact mass spectra were recorded at 70 eV ionization voltages and the ionization current was 10 µA. The acquisitions were performed in Scan mode (from 29 to 350 amu). The presence of aroma compounds was validated by their retention indexes (RIs) and mass spectra. RIs were calculated from the retention times of *n*-alkanes (C5–C30) on the same column. RIs values were compared with RIs from literature (Volatile Compounds in Food 16.3, <http://www.vcf-online.nl/VcfHome.cfm>). The mass spectra were compared with those from three databases: NIST 2.0, WILEY 138 and INRA-MASS (internal database achieved using standard compounds).

The comparisons of the relative amounts of aroma compounds was done using the total ion current (TIC) except for 2,5-dimethylpyrazine and vanillin for which the specific ions area (*m/z* respectively ion 108 and ion 152) was calculated. As the aim of the project was to compare the relative amounts of aroma compounds released in the vapour phase in different samples containing the same amount of aroma compounds,

there was no need a real quantification. However, in order to verify that there was no saturation of the fiber for any of the compounds and no competition between the compounds, we realised a calibration of the amount of each aroma compounds in the vapour phase after SPME extraction. The calibration curve was done using 7 concentrations of the 14 aroma compounds diluted in a model emulsion composed of 3% sunflower oil (retailer own-brand products – Carrefour France) and 0.15% β-lactoglobulin (analytical grade, purchased from Sigma – St Louis, MO, USA) in ultra-pure water. The concentrations were chosen as to cover the range of concentrations in the headspace for the different ice creams samples.

2.5. Kinetic study

This study was carried out to investigate the effect of saliva or of the ice-cream composition on the initial rate of aroma release. It was realised with the samples SAH1, WAH1, WAL1, WAH2 in order to test the effect of saliva (SAH1 vs WAH1), fat level (WAL1 vs WAH1), and protein level (WAH2 vs WAH1). Aroma release was measured as a function of time (10, 20, 30, 50, 60, 80, 100 s). After plotting the area (A) of the GC peak for each aroma compound as a function of time ($A = a \cdot t + b$), it was possible to calculate the slope of the curve between 10 and 100 s, indicator of the rate of release and the area under the curve, indicator of the total amount of aroma release in 100 s.

2.6. Statistical analysis

The statistical analyses were done on the GC peak areas for each aroma compound after headspace SPME–GC–MS in the different ice cream samples. Data were subjected to univariate analysis of variance (ANOVA – $\alpha = 0.05$) and the [Student]-Newman-Keuls Procedure (SNK) mean comparison test was performed separately in water and saliva, to determine for each aroma compound the significant differences between the foods matrices. Principal component analysis (PCA) was applied to examine the relationship between aroma release data and ice-cream composition. PCA (Principal Component Analysis) is a mathematical method used to reduce the dimensionality of multivariate data into linearly uncorrelated variables (called principal components). This transformation is defined in such a way that the first principal component (F1) has the largest possible variance (that is, accounts for as much of the variability in the data as possible), and each succeeding component (F2, F3...) in turn, has the highest variance possible under the constraint that it is orthogonal to the preceding components. This allowed trends in aroma differences to be observed. Each principal component has an eigenvalue which shows the weighting of the variance for that component. Microsoft® Excel 2010/XLSTAT®-Pro (2013.4.03, Addinsoft, Inc., Brooklyn, NY, USA); was used for statistical evaluations. The significance level was set at $P < .05$.

3. Results and discussion

Results of calibration curves realised for the 14 aroma compounds are presented in Table 2. The compounds are presented in increasing order of log P value. The good coefficient of determination ($R^2 > 0.9$) obtained for each aroma compound using the calibration curve after SPME–GC–MS analysis allowed us to verify that there was no saturation of the fiber and no competition between molecules in this range of concentrations in the headspace (linearity zone of the measurement).

3.1. Analysis of total amount of aroma compounds released from the different ice creams

Table 3 presents the results of the analysis of variance using 4 factors (medium, fat type, fat level and protein level). Their interactions (medium fat type, medium * fat level and medium protein level) are not presented because none was statistically significant. The raw data are

Table 2

Equation of the linear regression between absolute peak area (y: arbitrary unit) and aroma compounds concentration in model emulsion (x: mg/kg).

N°	Molecules	RI ^a	RI ^b	CAS Number	logP	Equations	R ²
1	Acetoin	1297	1287	513-86-0	-0.66	y = 5E + 08x + 945767	0.989
2	2,5-Dimethylpyrazine	1330	1320	123-32-0	0.63	y = 3E + 08x + 257463	0.988
3	Vanillin	2579	2577	121-33-5	1.21	y = 1E + 07x + 15381	0.992
4	2-Methoxy phenol	1872	1861	90-05-1	1.34	y = 4E + 07x - 2186.7	0.998
5	Benzaldehyde	1529	1520	100-52-7	1.5	y = 1E + 09x + 2E + 06	0.957
6	Phenyl ethyl alcohol	1917	1909	60-12-8	1.5	y = 1E + 08x - 214585	0.996
7	2-Ethyl-3,5-dimethylpyrazine	1471	1464	13925-07-0	1.63	y = 2E + 09x + 1E + 06	0.998
8	2-Methoxy-4-methylphenol	1968	1952	93-51-6	1.65	y = 3E + 08x - 26474	0.997
9	Hexanal	1086	1079	66-25-1	1.78	y = 1E + 10x + 6E + 06	0.969
10	<i>p</i> -Anisaldehyde	2046	2025	123-11-5	1.79	y = 2E + 09x + 2E + 06	0.997
11	Ethyl butyrate	1040	1034	105-54-4	1.8	y = 3E + 10x + 7E + 06	0.998
12	Butyl propionate	1143	1144	590-01-2	2.3	y = 5E + 10x + 2E + 07	0.906
13	<i>Cis</i> -3-Hexenyl acetate	1309	1316	3681-71-8	2.4	y = 1E + 09x + 2E + 06	0.991
14	Ethyl octanoate	1443	1434	106-32-1	3.8	y = 1E + 09x + 912141	0.998

^a Retention index measured on a DB-Wax column.^b Retention index from the literature (<http://www.vcf-online.nl/VcfHome.cfm>).presented as histograms in [supplementary Fig. 1](#).

No significant effect of the medium (water versus saliva) was observed for any of the compounds. Previous results already showed a retention of the most hydrophobic ketones (2-octanone, 2-nonanone) by salivary proteins through non-covalent interactions and hydrolysis of ethyl esters with human saliva in aqueous media (Pagès-Hélary et al., 2014). The effect of human saliva seems negligible in comparison with that of fat type, fat content and protein content. Our work was conducted on clarified saliva and a recent paper showed that the effect of human saliva on the metabolism of aroma compounds, mainly aliphatic aldehydes and di-ketones, was reduced after centrifugation (Munoz-Gonzalez, Feron, Brulé, & Canon, 2017). However, in that study, no

such effect was observed for alcohols, aliphatic ketones and benzaldehyde and the other aroma compounds present in our ice cream, which allows us to conclude that our results are fairly representative of the mechanisms in the mouth.

A significant effect of fat level was observed for 11 compounds. The 10 compounds with a log P higher than 1 (vanillin, benzaldehyde, phenyl ethyl alcohol, 2-methoxy-4-methyl phenol, hexanal, *p*-anisaldehyde, ethyl butyrate, butyl propionate, *cis*-3-hexenyl acetate, ethyl octanoate) were more released from samples with the low fat level, which can be easily explained by a higher emulsion/air partition coefficient of these hydrophobic compounds which are more soluble in oil than in water (van Ruth, King, & Giannouli, 2002). This effect was

Table 3

Statistical analysis (4 factors ANOVA – NSK test).

		Acetoin	2,5-dimethyl pyrazine(ion 108)	Vanillin (ion 152)	2-methoxy phenol	Benzaldehyde	Phenyl ethyl alcohol	2-ethyl-3,5-dimethyl pyrazine
Medium	F	1.127	0.736	1.719	0.626	0.026	1.014	0.848
	p-value	0.298	0.398	0.201	0.436	0.874	0.323	0.365
	Effect	-	-	-	-	-	-	-
Fat type	F	0.029	0.056	8.69	0.969	0.033	2.059	0.006
	p-value	0.866	0.815	0.007	0.334	0.856	0.163	0.939
	effect	-	-	A > B	-	-	-	-
Fat level	F	7.147	4.059	46.401	3.602	25.951	17.509	0.699
	p-value	0.013	0.054	< 0.0001	0.068	< 0.0001	< 0.001	0.41
	effect	H > L	-	L > H	-	L > H	L > H	-
Protein	F	6.095	0.257	5.268	0.009	4.319	5.243	1.677
	p-value	0.02	0.617	0.03	0.927	0.047	0.03	0.206
	effect	2 > 1	-	2 > 1	-	2 > 1	2 > 1	-
		2-methoxy -4-methyl phenol	Hexanal	<i>p</i> -anis aldehyde	Ethyl butyrate	Butyl propionate	<i>Cis</i> -3-Hexenyl acetate	Ethyl octanoate
Medium	F	0.618	1.129	0.258	0.191	0.028	0.004	0.018
	p-value	0.439	0.297	0.615	0.665	0.869	0.947	0.895
	Effect	-	-	-	-	-	-	-
Fat type	F	0.027	328.378	21.303	6.364	13.294	12.936	17.892
	p-value	0.872	< 0.0001	< 0.0001	0.018	0.001	0.001	< 0.001
	effect	-	A > B	A > B	A > B	A > B	A > B	A > B
Fat level	F	57.468	160.721	34.306	30.707	29.448	60.841	122.377
	p-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	effect	L > H	L > H	L > H	L > H	L > H	L > H	L > H
Protein	F	6.627	0.246	0.198	0.753	0.76	2.014	9.617
	p-value	0.016	0.624	0.66	0.393	0.391	0.167	0.004
	effect	2 > 1	-	-	-	-	-	2 > 1

Bold = significant (p-value < .05).

observed by other authors in emulsions or model cheeses (Doyen, Carey, Linforth, Marin, & Taylor, 2001; Lauverjat, Deleris, Trelea, Salles, & Souchon, 2009; van Ruth et al., 2002). However this effect was stronger for *cis*-3-hexenyl acetate and ethyl octanoate, the most hydrophobic compounds ($\log P$ value > 2.3), reaching up to 150% increase from AH2 to AL2, that is for fat A at the low protein level. The most hydrophilic compound acetoin ($\log P = -0.66$) was more released from samples with a high fat level due to its low solubility in oil. The release of 2-methoxy phenol was higher in the samples with a low fat level except in the sample with fat A and the standard protein level for which the opposite effect was observed.

A significant effect of fat type was observed for 7 compounds (vanillin, hexanal, *p*-anisaldehyde, ethyl butyrate, butyl propionate, *cis*-3-hexenyl acetate, ethyl octanoate) with a higher release from samples realised with fat A which has a higher solid fat content at eating temperature than fat B (Table 3). The effect was stronger for hexanal and *p*-anisaldehyde at the two fat levels and for ethyl octanoate at the lower fat level. This effect of solid fat content was also observed in emulsions varying in fat composition (Relkin et al., 2004), due to the lower solubility of aroma compounds in fat which contains a high proportion of solids at the studied temperature (Roudnitzky et al., 2003). As the solid fat fraction cannot solubilise the aroma compounds, the solid fat fraction in the ice cream at the temperatures below 15 °C (swallowing temperature) has to be taken into account to predict in-mouth aroma release.

A significant effect of protein level was observed for 6 compounds of different chemical class and hydrophobicity (acetoin, vanillin, benzaldehyde, phenyl ethyl alcohol, 2-methoxy-4-methyl phenol, ethyl octanoate). A lower amount of aroma was released from samples with a higher level of proteins. This could be explained by a decrease in the transfer of aroma compounds from oil to water phase, due to the presence of protein at the oil/water interface (Landy, Courthaudon, Dubois, & Voilley, 1996; Rogacheva, Espinosa-Diaz, & Voilley, 1999) and then a greater retention of hydrophobic aroma compounds due to the higher amount of proteins in the aqueous phase (Andriot, Harrison, Fournier, & Guichard, 2000; Pelletier, Sostmann, & Guichard, 1998). For two medium hydrophobic compounds, *cis*-3-hexenyl acetate and butyl propionate, the protein effect was not significant considering all the samples, however this effect was only significant at the low fat level for fat type A (between AL1 and AL2). At the low fat level, a greater amount of proteins is present in the aqueous phase which will increase the retention of aroma compounds. The difference of the effect according to the fat type has also been observed on the release of ethyl hexanoate from emulsions (Fabre et al., 2003; Ghosh, Peterson, & Coupland, 2007). We can hypothesise that the nature and composition of the interface is different between fat A and fat B.

A PCA has been done on the 8 ice creams diluted in water or in saliva (a total of 16 samples) and the 14 aroma compounds (variables). The principal plan contains 81.74 % of the information with 65.66% on the first axis (Fig. 2, a and b). On Fig. 2a representing the samples, the first axis allowed the separation of the samples with a low (L) fat level on its positive part from the samples with a high (H) fat level on its negative part. Looking at the representation of the variables (Fig. 2b), the most hydrophobic compounds ($\log P > 1.8$) such as esters (ethyl butyrate, butyl propionate, *cis*-3-hexenyl acetate, ethyl octanoate) have a positive contribution to this axis, which means that they are more released from ice creams with a low fat content, which confirms the results of the ANOVA. On the positive part of axis 1, the samples with a low fat level are separated on the second axis according to the protein level, the samples with the lower protein level (level 2) being on the positive part of this axis. Considering the variables which have a positive contribution to this second axis, we can conclude that this separation can be explained by a higher release of 2-methoxy phenol, acetoin, 2,5-dimethyl pyrazine and 2-ethyl-3,5-dimethylpyrazine in samples with the lower level of protein at the low fat level for the two fat types, with and without saliva (AL2 and BL2). However this effect is

not significant which justifies that the information supported by this axis is low (16%). Concerning the samples with the high fat level located on the negative part of axis 1, those with a low protein level are grouped together with no or few contributions to axis 2, whereas those with the higher protein level are separated along axis 1 according to the fat type, explained by a higher release of most of the aroma compounds from fat A.

In order to better highlight the effect of fat type, protein level and saliva effect, two other PCA were performed for each fat level separately.

Concerning the PCA realised on samples with a high fat level (Fig. 2, c and d), the principal plan contains 74.98% of the information. The samples (Fig. 2c) are separated according to the fat type on axis 1, with fat A on the positive part of the axis and fat B on the negative part. Considering the variables (Fig. 2d), the most hydrophobic compounds have a positive contribution to axis 1. This means that the fat with the higher solid fat content (fat A) allows a better release of hydrophobic compounds at the high fat level. Axis 2 seems to separate samples realised with fat A regarding the medium, with the samples realised in water more represented on the positive part of axis 2 (Fig. 2c), however the observed effects for vanillin, benzaldehyde and phenyl ethyl alcohol are not significant. No effect of protein level can be observed on this representation.

Concerning the PCA realised on the samples with a low fat level (Fig. 2, e and f), the principal plan contains 91.62% of the information. The samples are separated according to the protein level on axis 1 (Fig. 2e), with the higher level (level 1) on the positive part of the axis, which is represented by the most hydrophobic compounds (Fig. 2f), which are less released in the vapour phase due to their retention by whey proteins (Pelletier et al., 1998). A small effect of the fat type is also observed, with a lower release of the most hydrophobic compounds from ice creams realised with fat type B, these compounds are positioned on the positive part of both axis 1 and axis 2.

As a conclusion, a representation of the results in a space of two dimensions using PCA allowed us to highlight the different effects observed by ANOVA. The main effect is that of fat level (from 3 to 9%), then the effect of fat type at the higher fat level. The effect of protein level is more significant at the lower fat level. The effect of saliva is relatively low and only observed at the higher fat level.

3.2. Analysis of the rate of release of aroma compounds from the different ice creams

The aim of this part is to determine if the modifications observed on the total amount of aroma release during the eating process are initiated at the beginning of the eating process. This study was conducted on selected samples (WAH1, SAH1, WAL1, WAH2).

Fig. 3a represents the percentages of increase/decrease in the rate of release (between 10 and 100 s) as a function of WAH1 for the 3 other samples. Fig. 3b represents the percentages of increase/decrease in the total amount of aroma released in the headspace in 100 s.

Considering the comparison between SAH1 and WAH1, the effect of the addition of human saliva on the amount of aroma released was not significant (Fig. 3b) but a significant increase in the rate of release (Fig. 3a) was observed for vanillin, *p*-anisaldehyde and ethyl butyrate with the addition of saliva instead of water and a significant decrease for 2,5-dimethylpyrazine. This increase could be explained by a salting out effect of aroma compounds present in the aqueous phase, due to the presence of proteins and salts in saliva (Mao, Roos, O'Callaghan, & Miao, 2013; van Ruth et al., 2001). No explanation can be given for the decrease observed for 2,5-dimethylpyrazine. Considering the small effect of saliva on aroma release from ice cream, we can hypothesise that the interactions between salivary proteins and aroma compounds observed in aqueous solutions (Pagès-Hélary et al., 2014; van Ruth et al., 2001) are modified in emulsions containing fat and other proteins.

As the effect of saliva is relatively small, the effect of fat level (WAL1

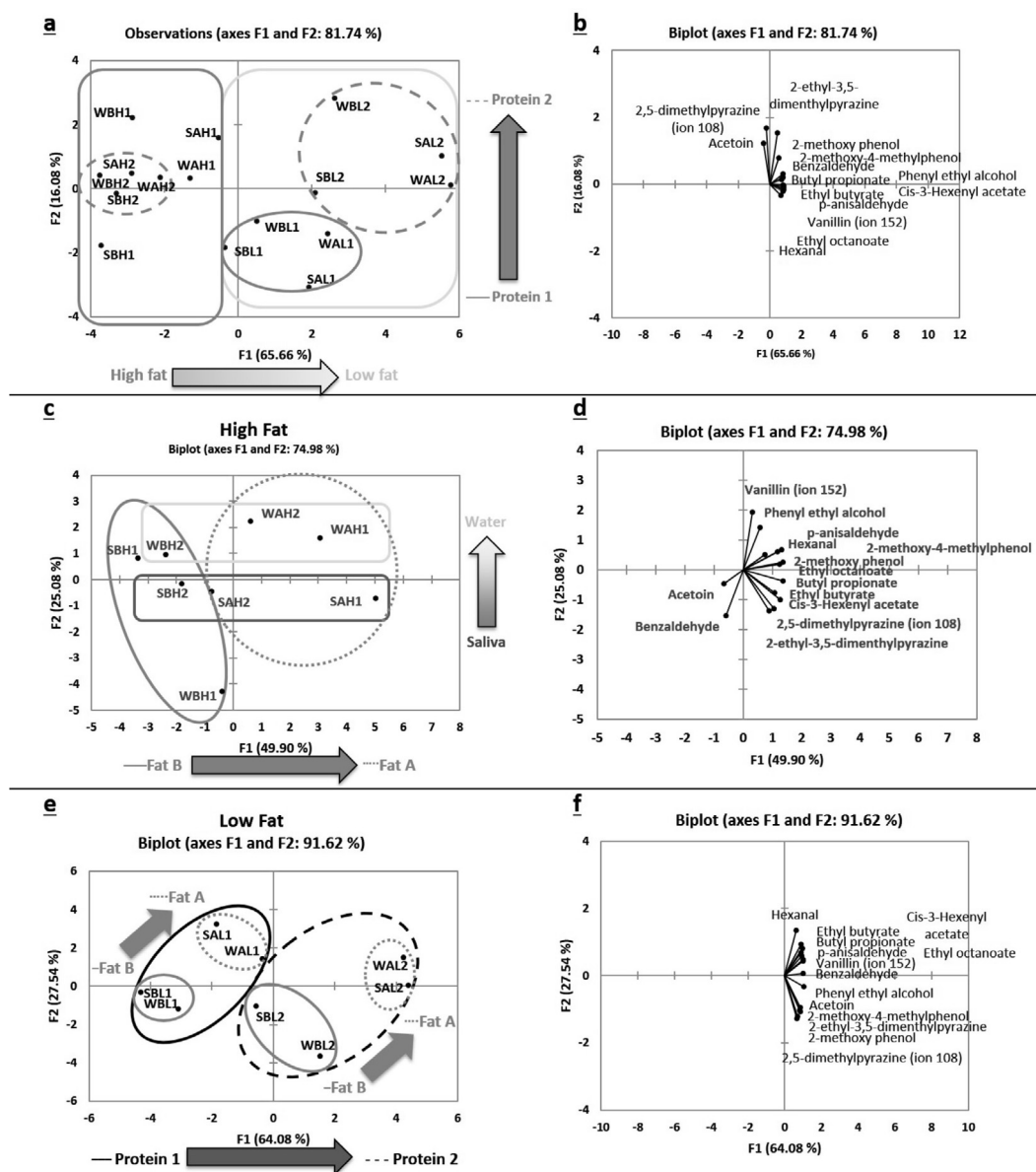


Fig. 2. Principal component analysis (PCA) realised on the 16 samples (a, b); the 8 high fat samples (c, d); the 8 low fat samples (e, f); on the left (a, c, e): representation of the samples, on the right (b, d, f): representation of the variables (aroma compounds).

vs WAH1) and that of protein level (WAH2 vs WAH1) on the rate of release have been tested in ice-creams diluted in water.

A decrease in fat level (WAL1 vs WAH1) induced a significant increase in the rate of release (Fig. 3a) for eight hydrophobic aroma compounds (vanillin, benzaldehyde, 2-methoxy-4-methylphenol, hexanal, ethyl butyrate, butyl propionate, *cis*-3-hexenyl acetate, ethyl octanoate). This observation is in agreement with the results obtained for the total amount of aroma released (Fig. 3b), confirming that fat can be considered as a reservoir of hydrophobic aroma compounds. The reverse effect was observed for acetoin, 2,5-dimethyl pyrazine, 2-methoxyphenol, which are the most hydrophilic compounds.

A decrease in protein level (WAH2 vs WAH1) induced a significant increase in the rate of release (Fig. 3a) for four aroma compounds, acetoin, vanillin, hexanal and ethyl butyrate. These compounds ($\log P = -0.66; 1.21; 1.78; 1.8$ respectively) interact with whey proteins and thus a higher amount of proteins limits their rate of release from the aqueous to the vapour phase. No significant effect was observed on the total amount of aroma released in the headspace for these compounds in the same samples (Fig. 3b), suggesting that transfer of aroma

compounds from water to air at the beginning of the release is quicker than the transfer of these compounds from oil to water then to air phase, which is driven by the transfer at the oil/water interface (Landy et al., 1996). Thus the fraction of protein which impacts more aroma release seems to be the proteins in the aqueous phase and not the proteins at the oil/water interface. For 2-methoxyphenol ($\log P = 1.34$) and 2,5-dimethylpyrazine ($\log P = 0.63$) the opposite phenomenon was observed on the rate of release.

4. Conclusion

The results obtained in this study offered deeper insight into the modifications of aroma release, under simulated in-mouth conditions, from ice creams varying in composition. The saliva reactor was a simple and useful tool to mimic the thermal exchanges during the in-mouth process. The main result is that the effect of human saliva is very low in comparison to the effect of the ice cream composition. The effect of saliva on aroma release was only observed in ice creams with a higher fat level, in particular on the initial rate of release. This could be

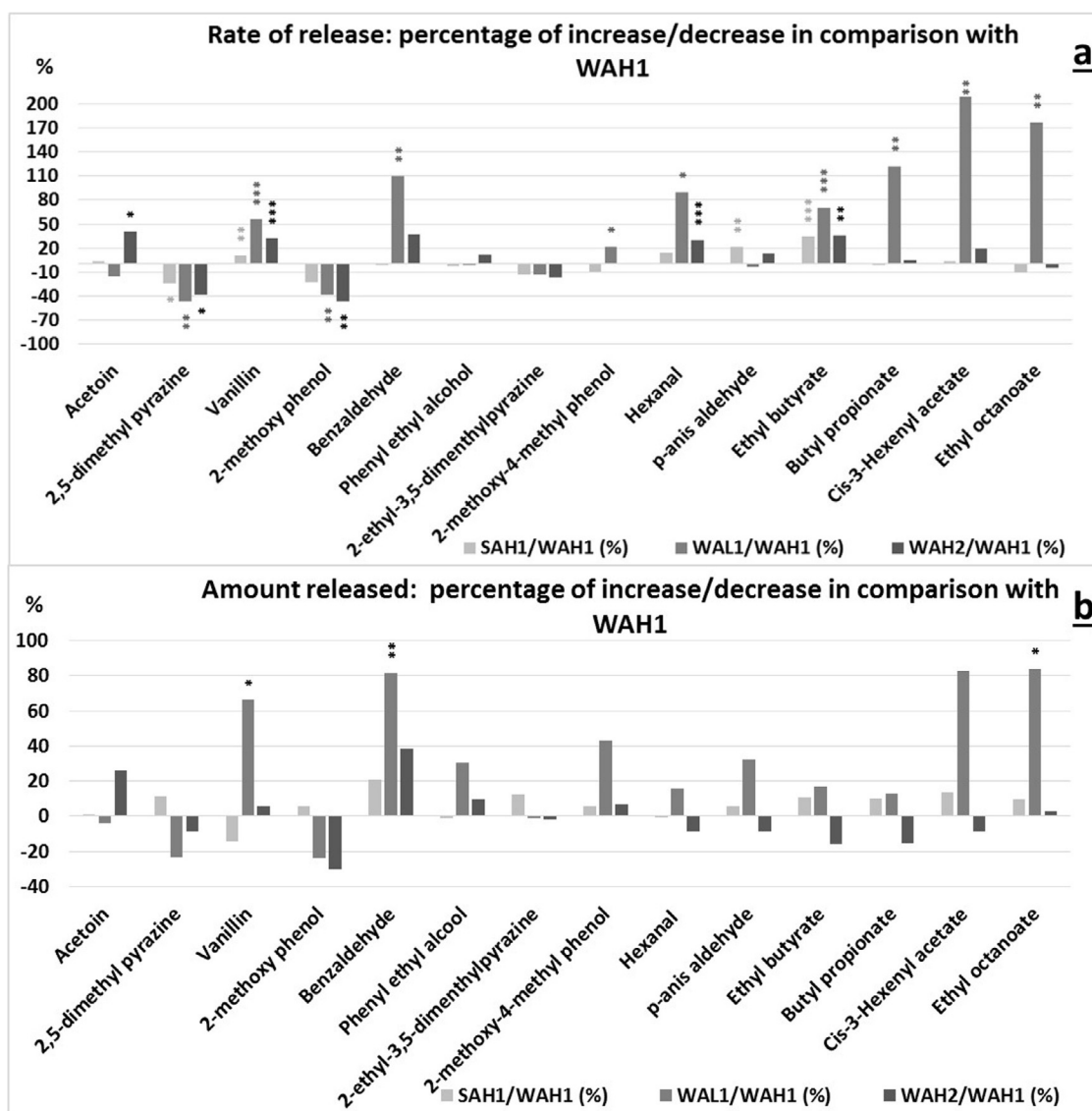


Fig. 3. Percentage of increase/decrease in the rate of release (a) and total amount of aroma release (b) from ice creams realised with fat A in comparison with WAH1 for the following samples, SAH1: addition of human saliva, WAL1: a lower level of fat, WAH2: a lower level of protein. Aroma compounds are ranked in ascending logP values. The increase/decrease is significant at: ***p-value < .0001; **p-value < .001; *p-value < .05.

explained by a salting out effect due to the presence of proteins and salts in the saliva, confirming that the interactions between salivary proteins and aroma compounds occurring in aqueous solutions are not observed in emulsions. Decreasing fat content in ice cream led to a higher rate and higher total amount of release for hydrophobic aroma compounds. Changing the nature of fat also modified the release profile, with a higher release of the most hydrophobic compounds from fat with a greater percentage of solid fat at the temperature of eating. The effect of protein level was dependent on both fat type and fat level. The level of whey proteins impacted more the aroma release at lower fat level, with a higher amount of released aroma from samples with a low level of protein. The obtained results showed that the reformulation of ice creams impacts aroma release as a function of fat type, fat level and protein level and also depending on the nature of the aroma compound. Also, the combined effects of fat and protein have to be taken into consideration. These results could help food industry in the reformulation of ice creams following nutritional recommendations with reduced changes in aroma release.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2017.10.127>.

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