A saliva reactor to mimic *in-vivo* aroma release from flavoured ice-creams

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Abstract

The aim of the present study was to develop a saliva reactor allowing temperature control and the addition of human saliva in order to follow the release of five aroma compounds from different ice creams. The developed method was a useful tool to mimic the in-mouth process by taking into account sample quantity, mouth volume, temperature, salivary flux, and mastication. The reactor was fit with solid phase micro-extraction for gas chromatography allowing data collection similar to nose-space sampling. Different ice creams were assessed, with varying fat type and level, and protein level. The results showed that the effect of saliva is relatively low and only observed at the higher fat level. Also the effect of the fat type was smaller than that of the fat level. The ice cream with a low fat level released more hydrophobic aroma compounds than the one with a high fat level. The ice creams with both low fat level and low protein level, showed the highest release of aroma compounds. Less added proteins led to less interaction with the aroma compounds and increased their rate of release from the aqueous to the vapour phase. Overall, an innovative tool was provided to guide food industries to reformulate ice creams answering nutritional recommendations in line with consumer demands.

Introduction

The consumption of ice cream is highly determined by its overall sensory acceptability, mainly flavour perception.

During consumption, ice cream undergoes phase changes from semi-solid to liquid, due to the combined actions of temperature increase and dilution with saliva, before swallowing [1]. In water and oil model systems, the addition of artificial saliva modifies the air/liquid partitioning of aroma compounds [2], 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 [3] there is still a need for a better understanding of the relative impact 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 device simulating the retronasal aroma release was developed by Robert and Acree (1995) [4] in order to mimic *in vivo* aroma release of a model wine with artificial saliva. Later in 2001, Deibler *et al.* showed that the ratios of aroma compounds from this device were closely related to those from the subjects' breath [5]. More recently, a saliva reactor has been developed within our research group to mimic the in-mouth breakdown of fat spreads [6], which highlighted the impact of human saliva on aroma release. The aim of the present paper is to adapt the saliva reactor to mimic ice cream consumption in order to determine the effect of fat type, fat level and protein level on aroma release in conditions as close as possible to human

B. Siegmund & E. Leitner (Eds): Flavour Sci., 2018, Verlag der Technischen Universität Graz
DOI: 10.3217/978-3-85125-593-5-6, CC BY-NC-ND 4.0

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, realised under *in vitro* conditions without addition of human saliva, showing an effect of fat type [7], fat level [8] or protein type and level [9] on aroma release. However, none of these studies combined these effects with that of saliva and they were not realised on the same aroma compounds which renders the comparison of the results difficult even if some general trends are common. An increase in fat level decreases the release of hydrophobic aroma compounds [10]. 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 [11]. and thus impacting the rate of transfer of aroma compounds from oil to water and then from the emulsion to the gas phase [12].

Our aim was therefore to design an experimental protocol with the saliva reactor to reproduce the thermal exchanges occurring in the mouth during ice cream consumption and worked with a pool of human saliva. The reactor was then used to determine the combined effects of food composition and human saliva on the release of aroma compounds from ice creams. This work will provide innovative tools to guide food industries to reformulate ice creams answering nutritional recommendations such as less fat, more sustainable fat and protein type with a limited effect on aroma release and, thus, on perception.

Experimental

Saliva reactor

A saliva reactor cell was used to reproduce ice cream breakdown in the mouth as close as possible (Figure 1). This device was specifically designed to evaluate the particular role of saliva during liquid and semi-solid food consumption [6]. 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 fibres and the last one equipped with a 3-blade marine propeller with digital speed control.

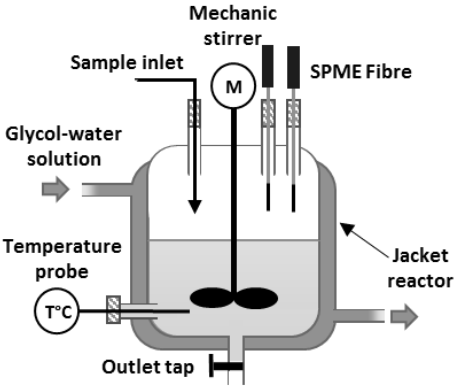

Figure 1: Schematic diagram of the saliva reactor

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 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 5 aroma compounds (acetoin: 450 mg/Kg ice cream; vanillin: 550 mg/Kg; benzaldehyde: 18 mg/Kg; hexanal: 54.9 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.

Human saliva collection

Resting human saliva was collected, centrifuged and stored from 20 volunteers as already described by Poette *et al.* [13]. It should be noted, however, that in a previous study, no effect of saliva storage was observed on the retention of 2-heptanone and ethyl heptanoate by human saliva [14].

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

Two fibres were introduced into the reactor (each in one orifice) to follow the aroma release, and were exposed 25 sec. after the introduction of the ice cream, which corresponds to the time at which the mixture reaches the minimum temperature (-22°C). Extraction was then performed for 1 minute. All experiments were realised in triplicate.

SPME fibres were injected in splitless mode (250ºC, 5 min) in a Gas Chromatograph (Agilent 6890N) coupled to a quadrupole Mass Detector (Agilent 5973N). After desorption of the SPME fibre, 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 40ºC, then increased at a rate of 5ºC/min until 240ºC and held for 10 min. The fibres 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 250ºC, 150ºC and 230ºC, respectively. Electron impact mass spectra were recorded at 70 eV ionization voltage and the ionization current was 10 µA. The acquisitions were performed in Scan mode (from 29 to 350 amu). The semi-quantification was done on the peak areas. However, the linearity of the peak area as a function of aroma concentration was previously verified by doing a calibration curve using 7 concentrations of the 5 aroma compounds diluted in a model emulsion.

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 – α=0.05) and the [Student]-Newman-Keuls Procedure (SNK) mean comparison test was performed separately in water and saliva, to determine significant differences between the foods matrices for each aroma compound. Microsoft® Excel 2010/XLSTAT©-Pro (2013.4.03, Addinsoft, Inc., Brooklyn, NY, USA); was used for statistical evaluation.

Results and discussion

Experimental protocol design in the saliva reactor

The amount of water/saliva to be added to 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 needed in the reactor for a good stirring, 10 mL of water/saliva were transferred into the reactor (250 mL), which was kept at 37ºC, and then 50 g of ice cream (at -22°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 37ºC to 15ºC after 25 sec. 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 sec. (Figure 2).

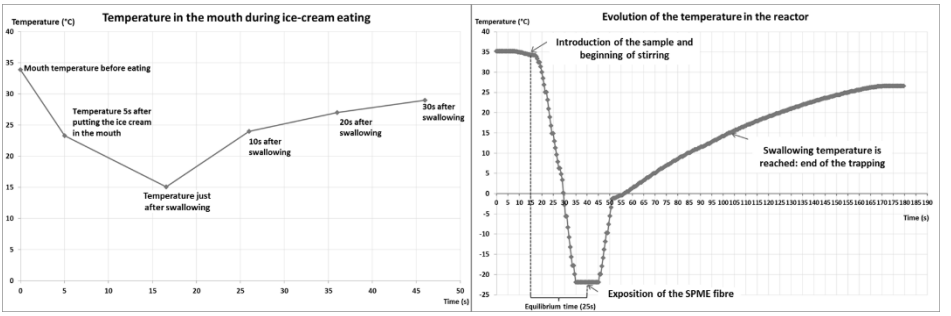

Figure 2: Temperature evolution in the mouth (left) and in the reactor (right)

Effect of ice cream composition and saliva addition on total amount of aroma release

An analysis of variance was performed (Table 1) with 4 factors (medium, fat type, fat level and protein level).

Table 1: ANOVA test on the effect of saliva, fat type fat and protein level on the total amount of release for 5 aroma compounds (univariate tests of Significance - α=0.05)

		Acetoin	Vanillin	Benz aldehyde	Hexanal	Ethyl octanoate
	logP	-0.66	1.21	1.5	1.78	3.8
Medium	F	0.779	1.572	0.028	1.042	0.034
	P-value	0.390	0.228	0.870	0.323	0.856
	Factor effect	-	-	-	-	-
Fat type	F	0.020	7.945	0.036	302.876	34.394
	P-value	0.889	0.012	0.852	< 0.0001	< 0.0001
	Factor effect	-	**B<A**	-	**B<A**	**B<A**
Fat level	F	4.942	42.422	28.083	148.240	235.247
	P-value	0.041	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	Factor effect	**L<H**	**H<L**	**H<L**	**H<L**	**H<L**
Protein level	F	4.215	4.816	4.674	0.227	18.486
	P-value	0.057	0.043	0.046	0.640	0.001
	Factor effect	**1<2**	**1<2**	**1<2**	-	**1<2**

The 5 aromas are sorted by increasing logP and three parameters are presented: F-test, P-value and factor effect highlighting composition impact on each aroma.

The effect of human saliva seems negligible in comparison to that of the fat type, fat content and protein content. This might be explained by the fact that 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 [15]. 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. Interactions between salivary proteins and aroma compounds in water was observed in previous studies and it might be modified here in emulsions containing fat and other proteins [2, 14].

Changing the nature of fat modified the release profile. Fat type is significant for the most hydrophobic aroma compounds (logP > 1.7 hexanal and ethyl octanoate; p-value < 0.001). This might be explained by a higher release of hydrophobic compounds from matrices with a greater percentage of SFC at 15°C (cannot solubilise the aroma compounds) [16]. A significant effect of the fat level (p-value < 0.001) was observed for the majority of the aroma compounds. Decreasing fat content led to a higher release for hydrophobic aroma compounds (logP > 1). This is probably due to a high solubility of hydrophobic aroma compounds in fat (more retained) [9b]. A significant effect of protein level (p-value: < 0.05) was observed for 3 volatiles and they are less released from ice creams with a high protein content (protein level 1).

Effect of ice cream composition and saliva addition on the initial rate of aroma release

The aim of this part was to determine if the modifications observed on the total amount of aroma release during the eating process were initiated at the beginning of the eating process. This study was conducted on four selected samples (WAH2, SAH1, WAL1, and WAH1 as the reference). Figure 3 represents the percentages of increase/decrease in the rate of release (between 0 and 100 seconds) as a function of WAH1 for the 3 other samples.

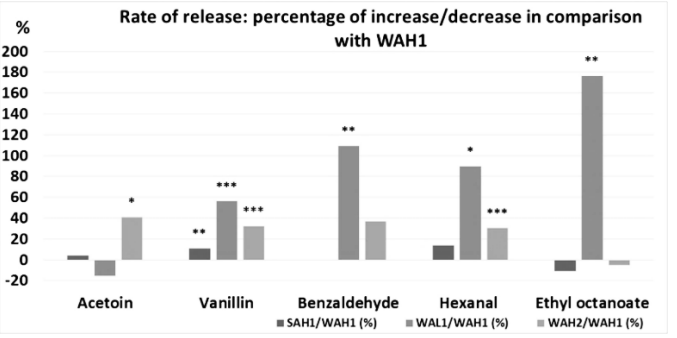

Figure 3: Impact of medium, fat type, fat level and protein level on the rate of release (The increase/decrease is significant at: *** p-value < 0.0001 ; ** p-value < 0.001 ; * p-value < 0.05)

A significant impact of saliva was observed for vanillin leading to an increase of the rate of release with saliva (SAH1/WAH1; p-value < 0.001). This compound might be more sensitive to a salting-out effect of salivary proteins [2]. Less fat (WAL1/WAH1) led to a significant better rate of release for hydrophobic (logP > 1) aroma compounds. Confirming that hydrophobic aroma compounds are more retained in fat. A decrease in protein level (WAH2 vs WAH1) induced a significant increase in the rate of release. Less added proteins lead to less interaction with the aromas and increase their rate of release from the aqueous to the vapour phase.

Discussion and conclusion

As a conclusion, the saliva reactor was a simple and useful tool to mimic the in-mouth process by taking into account sample quantity, mouth volume, temperature,

salivary flux, and mastication. Connecting the reactor with the use of SPME makes the technique easy to use, and provides data similar to nose-space sampling.

An ANOVA test on the collected data highlighted the different effects of composition on aroma release. The effect of saliva is relatively low and only observed at the higher fat level. 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. Decreasing fat content in ice cream led to a 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 more hydrophobic compounds from fat with a greater percentage of solid fat at the temperature of eating ($\leq 15°C$). The effect of protein level depends on both fat type and fat level. The level of whey proteins impacted more the aroma release at a low fat level, with a higher amount of aroma released at a low level of protein. However, a small effect was also evidenced at the high level of the fat type with the higher solid fat content. 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. The combined effects of fat and protein have also to be taken into consideration.

This *in-vitro* study using a saliva reactor could be easily applied now to study the impact of saliva or reformulation on aroma release. It can potentially provide a big amount of data allowing the computation of a flavour behaviour model in complex liquid or semi-liquid matrices.

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