

# Analytical method to model human mouth-level transfer of ingredients from Swedish pouched snus

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## Objective

- To support the development of a mouth-level transfer model, levels of target compounds (nicotine and the ingredients menthol and 1,8 cineole) from Swedish pouched snus were compared following human use and *in vitro* extraction. Unused pouched snus were extracted with 3 different media (1%  $\beta$ -cyclodextrin solution, artificial saliva and water) and with 3 different immersion times for each media (5, 10 or 30 min).
- The aim was to rank analytically the products with an *in vitro* extraction in a similar order than the human extraction average levels.



### Consumer study protocol

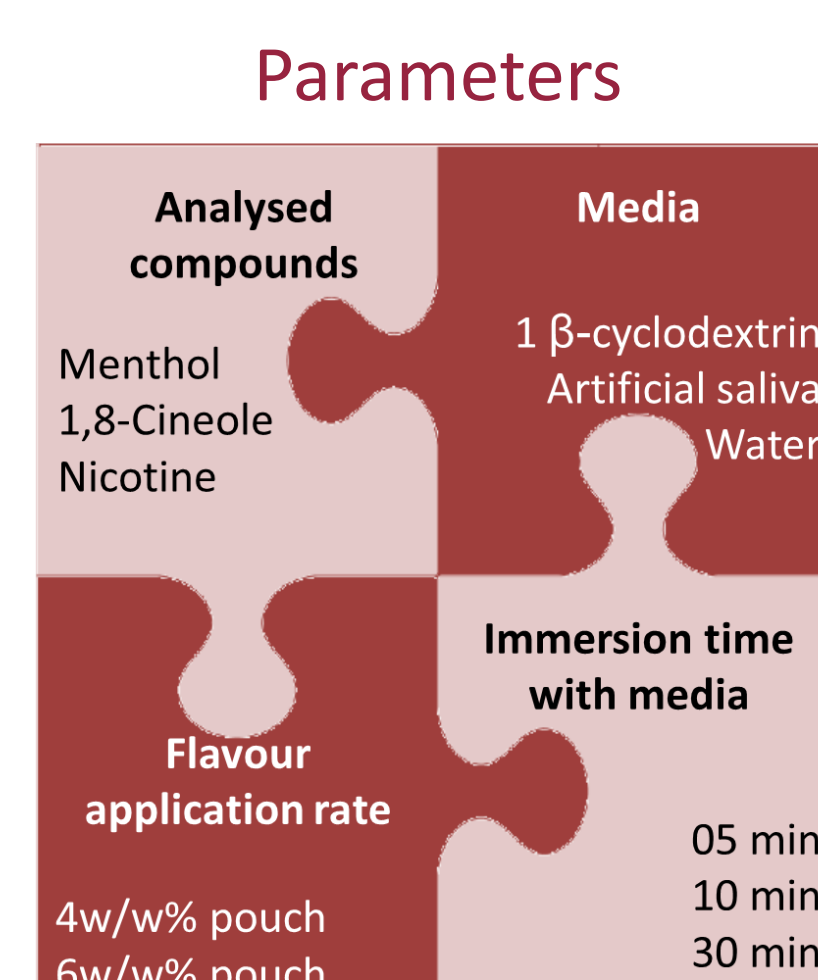
- 2 flavour application rates (4 and 6w/w%)
- Panel: 200 Swedish consenting adults
- 3 pouches (~0,8g) per volunteer (600 samples)
- Time in mouth = 30 minutes
- Snus transferred into a flask for analysis

## Introduction

- The first step of the study** was based on panel consumption of 2 pouched snus samples with 2 different flavour application rates. The aim was to calculate the human mouth-level transfer rate of nicotine and the ingredients menthol and 1,8-cineole. The transfer rate (TR%) was calculated from residual levels of target compound remaining in snus after consumption versus the amount present in unused snus. Human mouth-level extraction transfer rates were then compared with the results from the *in vitro* extraction study.

## Analytical approach

### Extraction with the media



### Protocol

**Snus sample:** 1 pouch weighed

**Media:** pre-heated at 37°C for 3 hours

***In vitro* extraction:**

- volume: 30mL in a 50mL falcon tube
- stirred at 150 rpm in a water bath at 37°C
- immersion time: 5, 10 or 30 minutes

**After *in vitro* extraction,** pouches are isolated from media and transferred into an other tube

### Solvent extraction step

- Pouch was slit open and 25mL of dichloromethane/methanol (1:1 v/v) containing 10 $\mu$ L/L tetradecane (ISTD) was added.
- Extraction for 24 hours (lab shaker = 220 rpm).
- Supernatant filtered into a vial with a syringe filter (0,45 $\mu$ m PTFE).
- Extraction efficiency confirmed with a second extraction (< LOQ).

### Analytical method

**Gas Chromatography** Agilent GC 7890A: Solgel Wax column (30m x 0,25mm x 0,25 $\mu$ m) - 1 $\mu$ L injected in hot needle (250°C) - Split mode (1:100).

**Mass Spectrometry:** Electronic impact *EI* source - Single Ion Monitoring *SIM* detection mode.

## Results

$$\text{Transfer rate TR (\%)} = 100 \times \frac{\text{Total extractable amount} - \text{Residual amount found after } in vitro \text{ extraction}}{\text{Total extractable amount}}$$

### Total extractable results and water solubility

Application rate	Menthol <sup>a</sup>		1,8-Cineole <sup>a</sup>		Nicotine <sup>a</sup>	
	Mean amount [mg/pouch]	CV [%]	Mean amount [mg/pouch]	CV [%]	Mean amount [mg/pouch]	CV [%]
Water Solubility (mg/L)	490 @25°C		3500 @21°C		1000000	
4%	1,75	5%	0,238	8%	9,2	6%
6%	2,99	3%	0,399	13%	10,3	4%

<sup>a</sup> Values based on n=6 individual replicates  
Results on wet weight based of unused snus

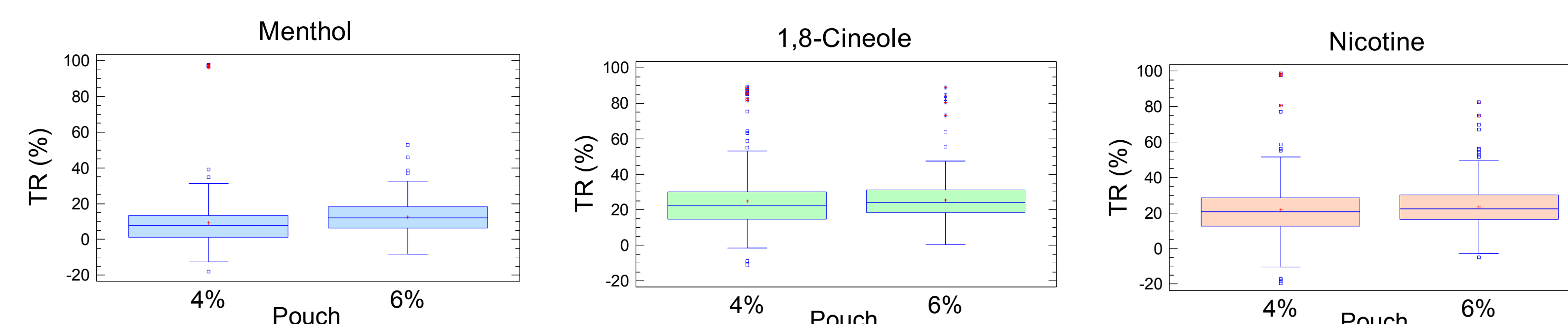
### Consumer panel results

- Figures obtained from the consumer study

Application rate	n replicates	Menthol <sup>a</sup>			1,8-Cineole <sup>a</sup>			Nicotine <sup>a</sup>		
		Mean amount [mg/pouch]	CV [%]	Mean TR <sup>b</sup> [%]	Mean amount [mg/pouch]	CV [%]	Mean TR <sup>b</sup> [%]	Mean amount [mg/pouch]	CV [%]	Mean TR <sup>b</sup> [%]
4%	294	Mean	1,50	16%	0,195	18%	25%	6,8	19%	22%
		90th Percentile	1,66	16%	0,224	16%	35%	8,0	13%	37%
6%	300	Mean	2,61	6%	0,346	11%	25%	7,9	13%	23%
		90th Percentile	2,81	18%	0,389	34%	33%	9,0	33%	33%

<sup>a</sup> Results on wet weight based of used snus  
<sup>b</sup> TR calculated with the total extractable amount from the panel study

- Box plot



### Artificial media results (*in vitro*)

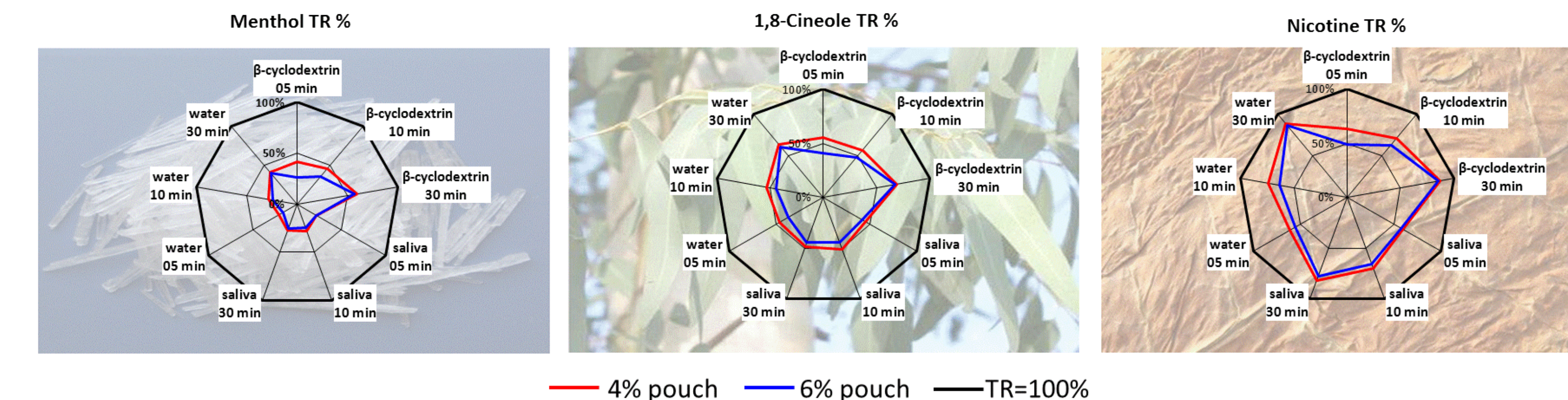
- Figures obtained from the transfer in artificial media

Application rate	Artificial Media	Contact Time [min]	Menthol <sup>a</sup>			1,8-Cineole <sup>a</sup>			Nicotine <sup>a</sup>		
			Mean amount [mg/pouch]	CV [%]	Mean TR [%]	Mean amount [mg/pouch]	CV [%]	Mean TR [%]	Mean amount [mg/pouch]	CV [%]	Mean TR [%]
4%	1% $\beta$ -Cyclodextrin	5	1,02	11%	42%	0,107	12%	55%	3,3	14%	64%
		10	0,95	8%	45%	0,102	10%	57%	2,6	16%	71%
		30	0,71	18%	60%	0,073	26%	69%	1,2	26%	87%
	Artificial Saliva	5	1,38	5%	21%	0,130	18%	45%	3,7	23%	60%
		10	1,26	12%	28%	0,117	19%	51%	2,7	26%	70%
		30	1,28	11%	27%	0,122	16%	49%	1,7	20%	82%
6%	Water	5	1,39	7%	20%	0,127	10%	47%	3,6	13%	61%
		10	1,24	7%	29%	0,112	10%	53%	2,4	20%	74%
		30	1,03	8%	41%	0,086	12%	64%	1,0	14%	89%
	1% $\beta$ -Cyclodextrin	5	2,19	12%	27%	0,235	17%	41%	5,3	20%	49%
		10	1,94	13%	35%	0,207	16%	48%	3,8	26%	63%
		30	1,30	29%	57%	0,131	35%	67%	1,5	45%	85%
6%	Artificial Saliva	5	2,35	11%	21%	0,235	18%	41%	4,4	27%	57%
		10	2,26	12%	24%	0,223	19%	44%	3,5	23%	66%
		30	2,24	6%	25%	0,223	9%	44%	2,3	18%	78%
	Water	5	2,50	8%	16%	0,249	12%	38%	4,6	21%	55%
		10	2,26	9%	24%	0,224	13%	44%	3,8	23%	64%
		30	1,78	12%	40%	0,155	26%	61%	1,4	58%	86%

<sup>a</sup> Values based on n=6 individual replicates, except for 5 and 10 min with 1%  $\beta$ -Cyclodextrin where n=3  
Results on wet weight based

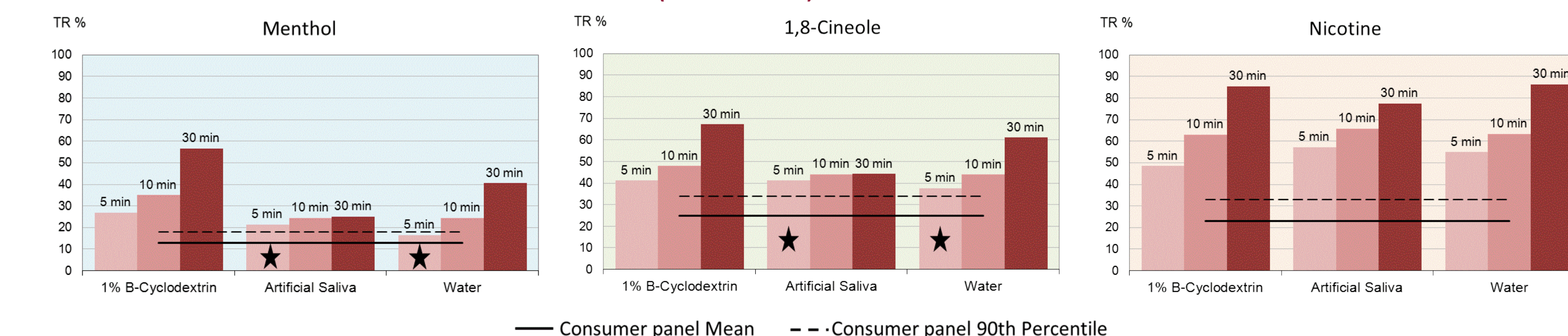
## Discussion

### Transfer rate vs. Flavour application rate



- The transfer rates of nicotine and the ingredients menthol and 1,8-cineole were similar, independent of application rates used (no statistically significant difference between 4% and 6%,  $p \geq 0,05$ ).

### Transfer rate vs. Immersion time in media (6% Pouch)



- The *in vitro* transfer rate of nicotine and the ingredients menthol and 1,8-cineole after 30 min extraction in all 3 media overestimated the potential mouth-level transfer rate in consenting adults using the product for 30 min. Nevertheless, artificial saliva provides the result closest to that of the consumer panel.
- The *in vitro* transfer rate of nicotine increased with increasing immersion time with all 3 media.
- The *in vitro* transfer rate of menthol and 1,8-cineole increased with increasing immersion time in water and 1%- $\beta$ -cyclodextrin, whereas the transfer rate with artificial saliva was largely independent of immersion time.
- The human-mouth level transfer rate of menthol and 1,8-cineole (after 30 min of use) was the closest to the results obtained with *in vitro* extraction of 5 min in water or artificial saliva.

## Conclusions

- Nicotine transfer was overestimated for all media and all immersion times. Closest results to the human data for a immersion time of 5 minutes in 1%  $\beta$ -cyclodextrin solution.
- In vitro* transfer rates for menthol and 1,8-cineole: Closest results to the human data for a immersion time of 5 minutes in water or artificial saliva (★).
- A similar order for flavour compounds (menthol and 1,8-cineole) in human extraction and *in vitro* extraction.

## Perspectives

- Additional tests to be performed to confirm the *in vitro* results with an enlarged panel of flavours and the specificity of the Nicotine.
- Determination of a multi-analyte approach to analyse several tobacco constituents from different kinds of pouched snus (such as different recipes, flavours, flavour application rates...).

**Reference** Saskia van Ruth: "Evaluation of the influence of mastication on temporal aroma release of ripe and unripe bananas, using a model mouth system and gas chromatography-olfactometry"; European Food Res. Technol. 217, 291-295, 2003.