

Elucidation of the combined effect of vacuum-assisted HS and salting-out in the profiling of dark chocolate by HS-SPME/GC×GC-MS



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DEGLI STUDI
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INTRODUCTION

Among Belgium's renowned specialties, chocolate is globally enjoyed for its exquisite taste, delicate texture, and complex aroma profiles. As a cornerstone of the confectionery industry, this popular snack continues to see consistent market growth, driven largely by increasing consumer awareness of the health benefits associated with dark chocolate's high phenolic and antioxidant content. The aroma of dark chocolate is a primary quality driver, resulting from a synergy of over 600 volatile organic compounds (VOCs). Traditional headspace solid-phase microextraction (HS-SPME) typically requires elevated temperatures and prolonged sampling times to capture these analytes. However, these conditions risk altering the original volatile profile through artifact formation, failing to accurately reflect the aroma effectively perceived by consumers.

This study explored the alternative and complementary use of vacuum-assisted HS-SPME (Vac-HS-SPME) and salting out effect determined by the use of a salty solution simulating saliva. By reducing pressure within the sampling vial, Vac-HS-SPME accelerates the volatilization of semi-volatile compounds at milder temperature, thereby maintaining the thermodynamic integrity of the sample. On the other hand, the addition of a saliva simulant allowed the simulation of retro-nasal aroma release. To simulate the in vivo sensory experience, extractions were performed at physiological temperature (37 °C). Comparative investigations between Vac-HS-SPME and classic HS-SPME, both with and without salty solution addition, demonstrate that vacuum conditions significantly enhance the extraction of less volatile and more polar compounds compared to atmospheric sampling (Atm-HS-SPME). The addition of simulated saliva improved VOC release, particularly for aldehydes and ketones. The relative advantage of vacuum conditions was most pronounced in the absence of the salty solution. The use of comprehensive two-dimensional gas chromatography-mass spectrometry (GC × GC-MS) for the final characterization provides superior sensitivity and a more representative volatile "fingerprint".

MATERIALS AND METHODS

(Vac-)HS-SPME: SPME DVB/CAR/PDMS 1 cm

- 1 g of chocolate (+ 1 mL salt solution) in 20 mL vial
- For Vac-HS-SPME only:
 - Use of specific vac-SPME arrow cap (ExtraTech), Thermogreen LB-1 septa
 - Vial was let to freeze before 1 min vacuuming.
- **Extraction:** 15 min equilibration, 25 min extraction at 37 °C
- **Desorption:** 5 min at 280 °C, re-focus on cooled trap at 0 °C, splitless injection at 300 °C for 3 min.

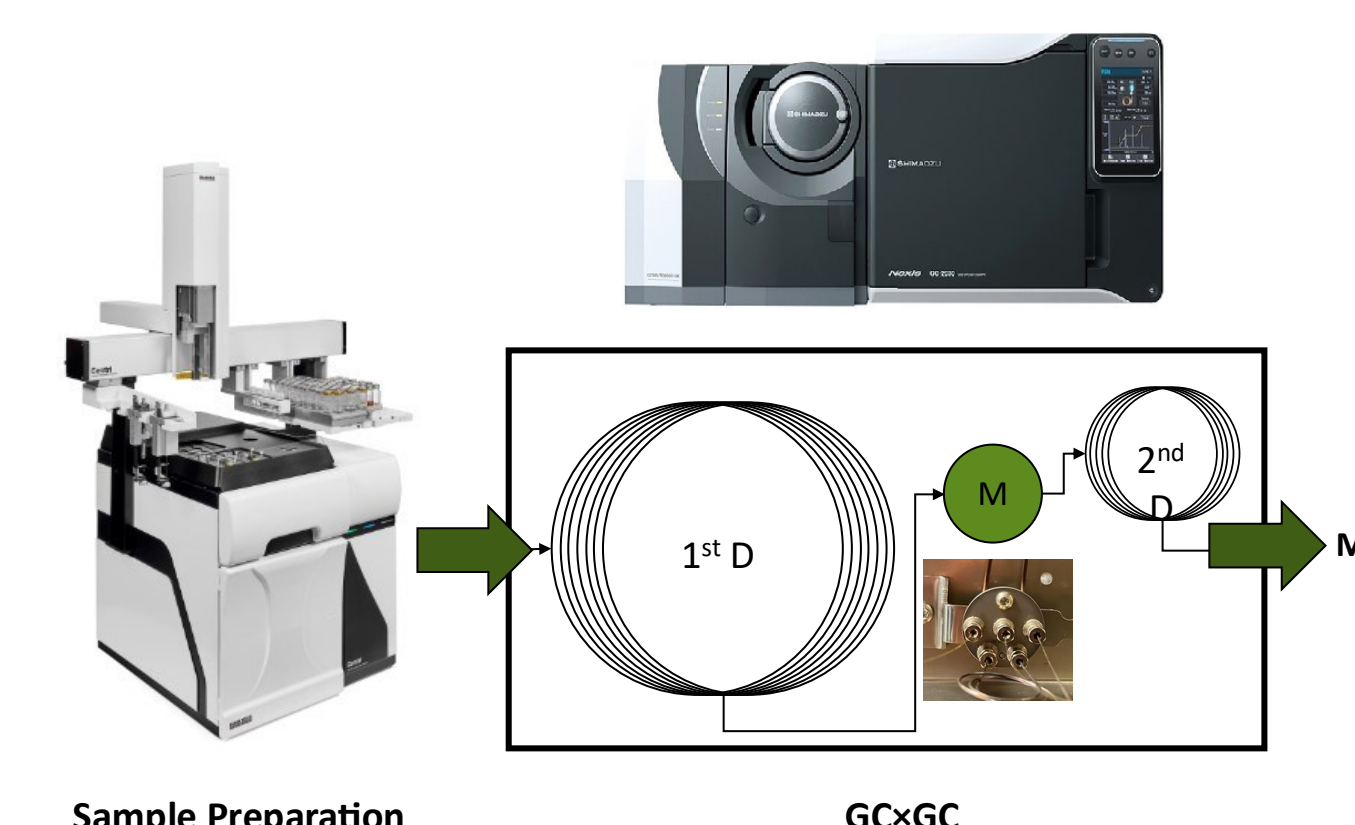


Salt solution [1]:

- KH₂PO₄ 12 mM
- NaCl 40 mM
- CaCl₂ 1.5 mM
- NaOH to pH 7.4

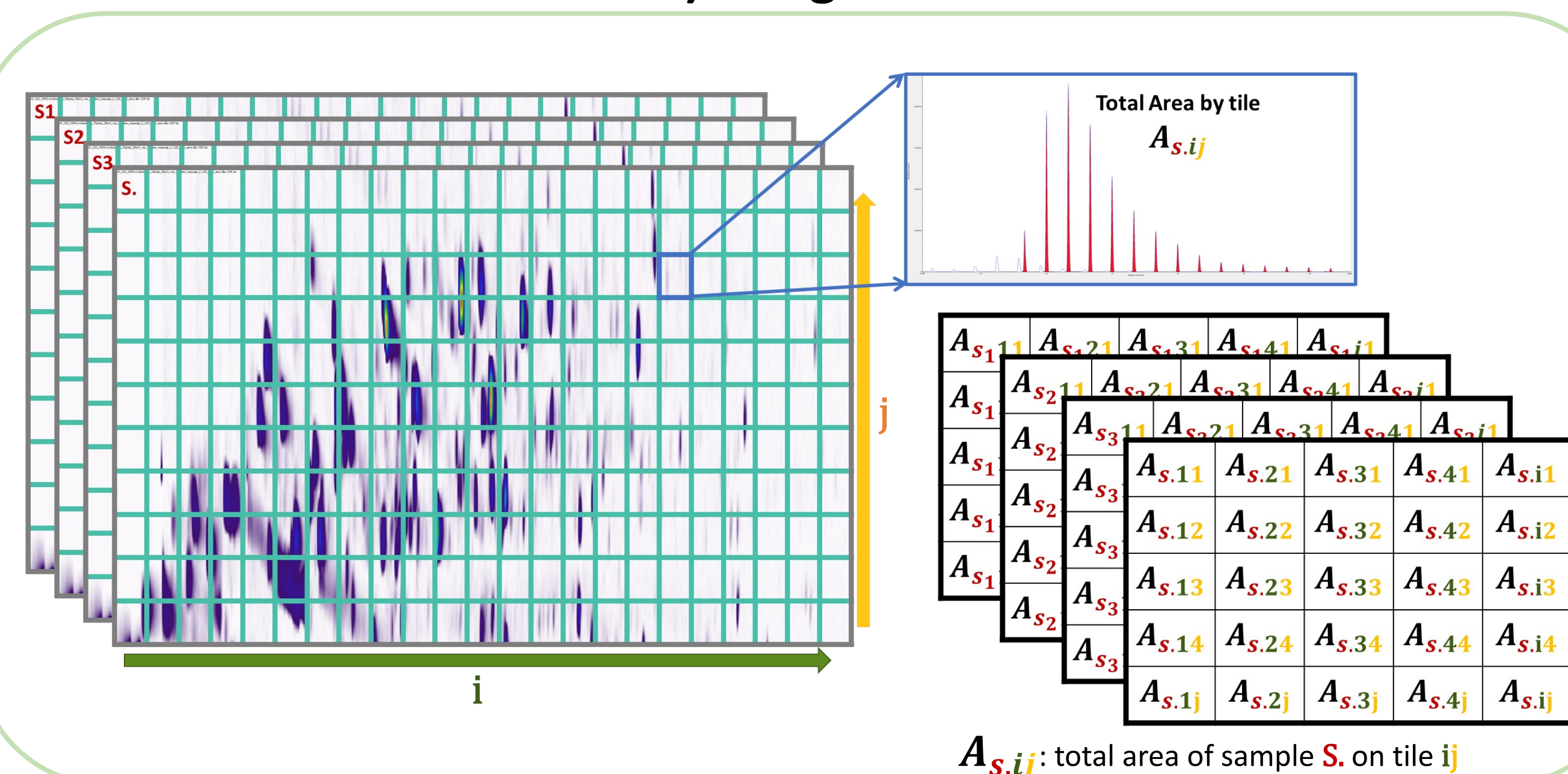
GC×GC-qMS: Shimadzu GCMS-TQ8050 NX;

Columns: 1D: BPX-5 20m × 0.18mm i.d. × 0.18 μm df, 0.5 mLmin⁻¹
2D: BPX-50 3 m × 0.25 mm i.d. × 0.25 μm df, 15 mLmin⁻¹
Oven program: 35 °C (1 min) to 220 °C at 6 °C/min;
Flow modulator: INSIGHT (SepSolve Analytical Ltd), Pm: 2.5 s;
qMS: Scan (45-300 m/z) for 50 Hz.



DATA PROCESSING

1) Integration



2) Operations

Class average Area by tile:

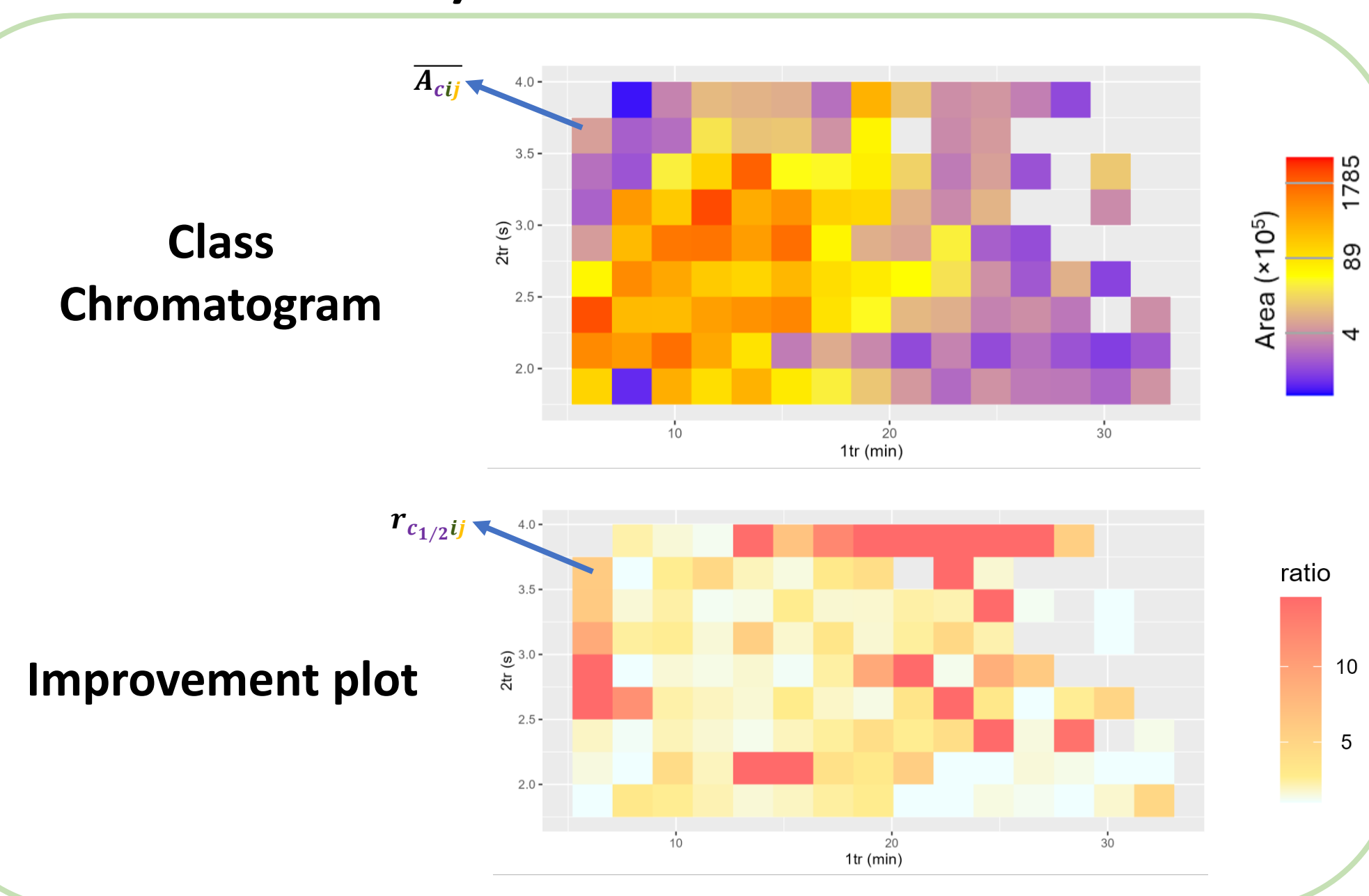
$$\overline{A_{cij}} = \frac{\sum_{s_c}^{n_{s_c}} A_{s_cij}}{n_{s_c}}$$

Improvement calculation (ratio of tile):

$$r_{c1/2ij} = \frac{\overline{A_{c1ij}}}{\overline{A_{c2ij}}}$$

$\overline{A_{cij}}$: Average area of all samples of class C on tile ij
 n_{s_c} : Number of samples of class C

3) Visualisation



OPTIMISATION AND COMPARISON

Fig. 1 illustrates how extraction time, pressure, and addition of salt solution impact different chromatographic regions. When heated, chocolate melts and creates a viscous slurry. Adding salt solution to the chocolate powder reduce the viscosity and enhance the diffusion and solubility of compounds which impact their volatile release in the headspace. This effect in synergy with the vacuum multiplies the extraction yield especially for the late eluting compounds (low volatile). While both modifications accelerate early-stage extraction, Vac extraction yields plateau after 25 min. Longer duration provides no additional benefit and trigger a displacement effect for certain compounds, such as benzaldehyde; thus, 25 min was selected as the optimal extraction time.

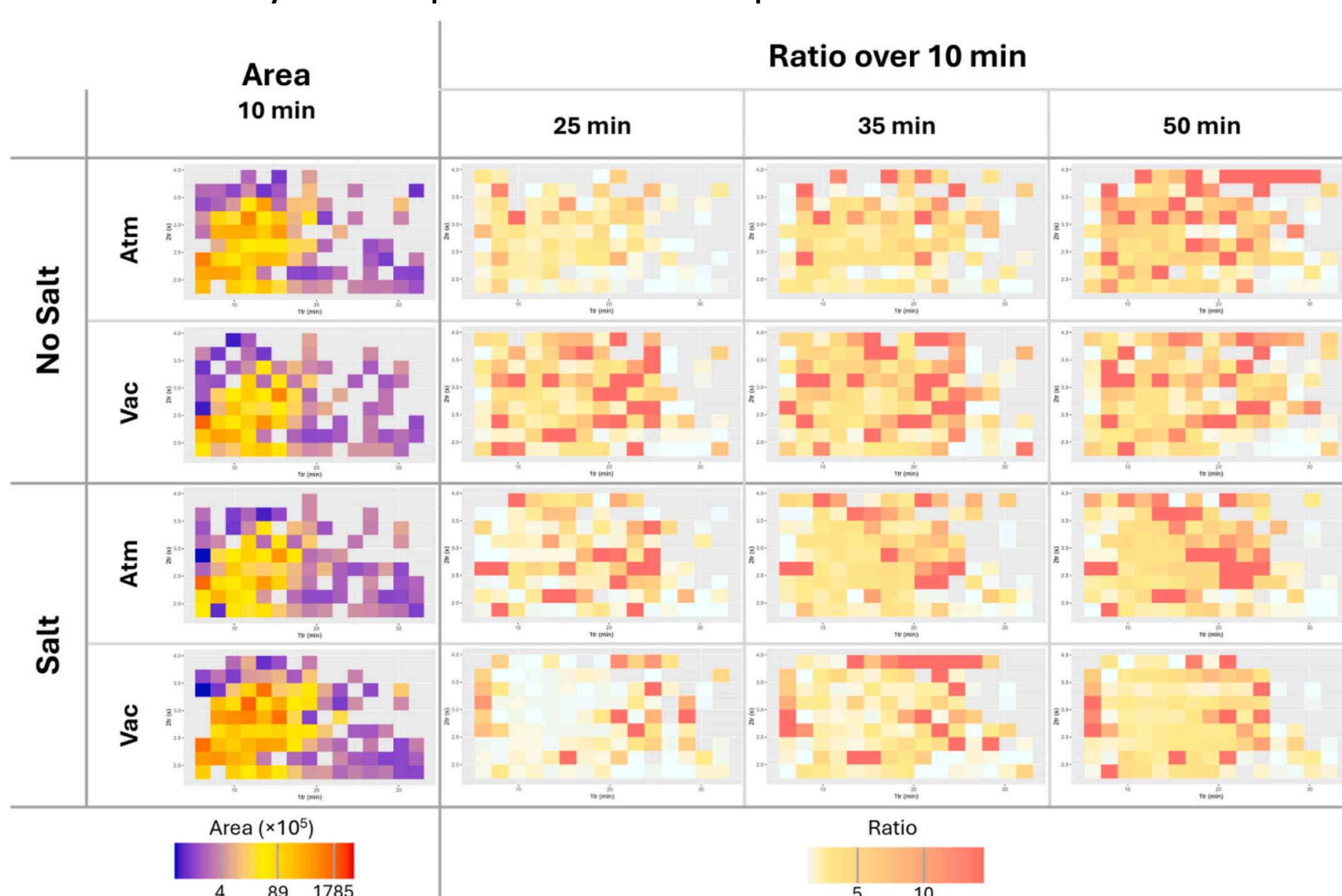


Fig.1: Improvement plot of different conditions (Atm/Vac, Salt/noSalt) for different extraction times over 10 min of extraction

Comparing 25 min extractions under varying conditions highlights the individual and interactive effects of pressure and addition of salt solution (**Fig. 2**). Consistent with literature [2], vacuum extraction improves yields, most notably for low-volatility compounds in dry samples (A), this effect is less pronounced upon saline addition where more volatile and polar compounds are preferentially extracted (B). Indeed, the addition of salt solution markedly improves the yield of mid-to-high volatility polar compounds (C, D). This effect is particularly strong under atmospheric pressure. By comparing B and D, Vac and Salty solution give a global similar effect although highest extraction rate have been observed with the combination of both (**Fig.1**)

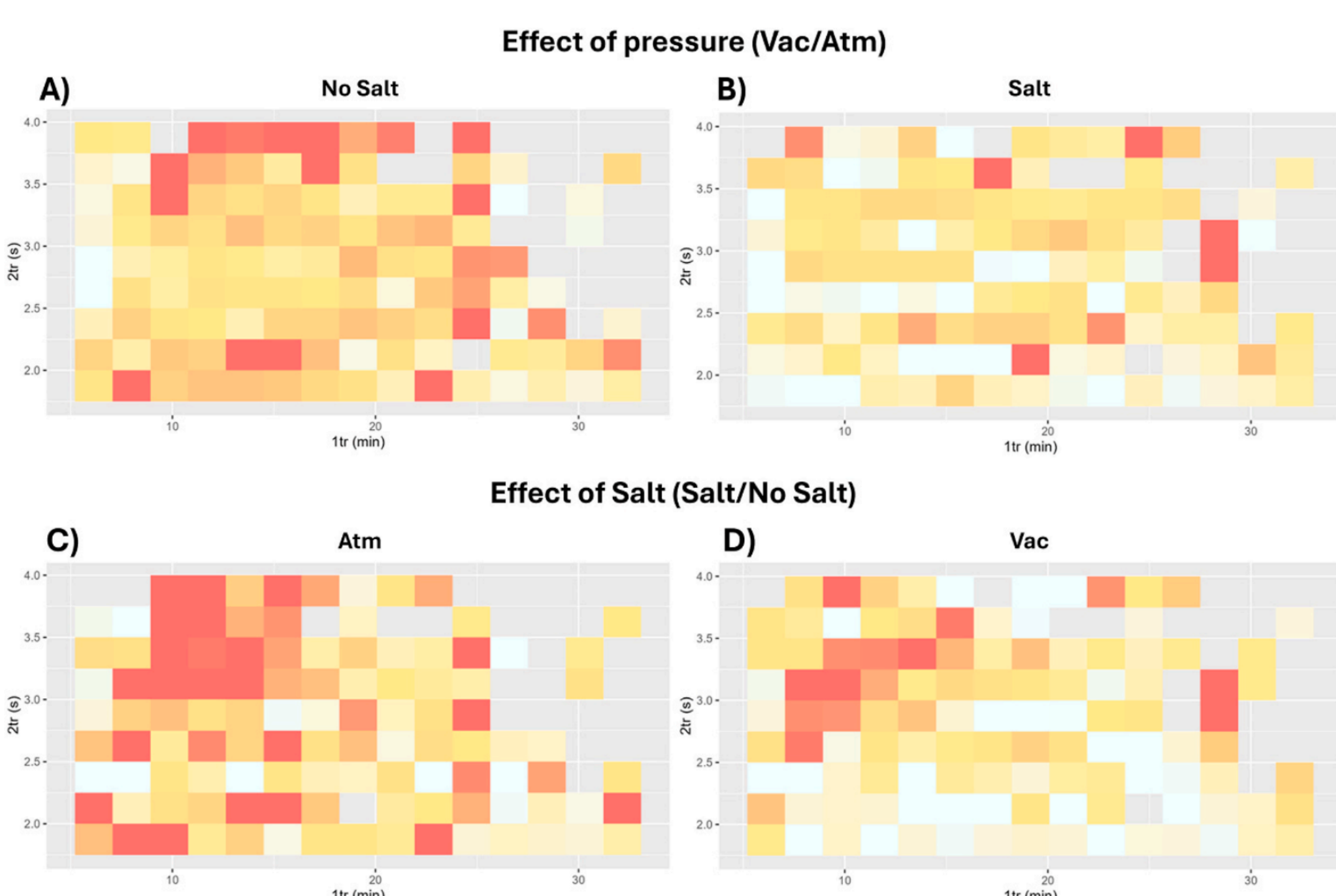


Fig.2: Improvement plot of the impact of pressure or salt solution addition of 25 min extraction

Beyond these physical mechanisms, water addition chemically triggers the production of Strecker aldehydes (e.g., benzeneacetaldehyde), driving the most substantial differences between treatments [3].

CONCLUSION

In this study, we presented a novel, more informative approach to process and compare different chromatograms to highlight and locate differences between conditions and/or samples. The study compares both the impact of vac-HS-SPME extraction and the addition of salt solution on the extraction of volatile from chocolate. The use of GC×GC-MS with the improvement plot approach indicates that Vac-HS-SPME increases the extraction of semi-volatile while salty solution increase the mid-to-high volatility polar one. Largely, both Vac and the addition of salt solution similarly increase the global extraction yield and synergetic effect is observed with both conditions. These observations underscore how significantly extraction conditions affect the selectivity. Further investigation will be performed to investigate the influence of salty water, its composition and the vacuum on the extraction performances.