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VACUUM-ASSISTED HS-SPME/GC×GC-MS TO ENHANCE THE VOLATILE CHROMATOGRAPHIC FINGERPRINT OF COMMERCIAL DARK CHOCOLATE

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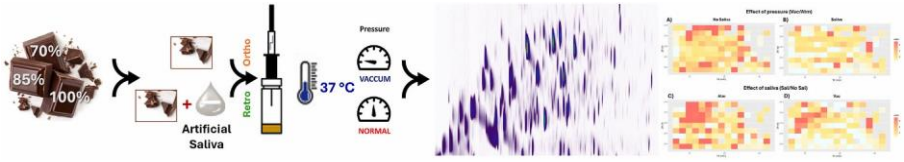
KEYWORDS: SOLID-PHASE MICROEXTRACTION (SPME) -- VACUUM-ASSISTED HEADSPACE (VAC–HS)–SPME -- COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY (GC×GC) -- VOLATILE ORGANIC COMPOUNDS (VOCS) -- CHOCOLATE

HIGHLIGHTS :

- Comparison of HS-SPME and Vac–HS–SPME for dark chocolate volatile analysis.
- Combine the Vacuum-assisted and salting-out effect.
- Chromatographic fingerprinting evaluation of the different variables tested.

GRAPHICAL ABSTRACT :



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ABSTRACT

Background: This study explored the potential of vacuum-assisted headspace solid-phase microextraction (Vac–HS–SPME) coupled with two-dimensional gas chromatography mass spectrometry for the evaluation of the volatile profiles of dark chocolate samples. Comparative analyses between Vac- and classic HS-SPME sampling were performed at different sampling times. To simulate the release of volatile compounds during human consumption and the possible synergistic effect with Vac, experiments were carried out at the physiological temperature (37 ◦C) on powdered chocolate alone and with the addition of salty water as surrogate saliva.

Results: Overall, Vac–HS–SPME showed higher extraction efficiencies of the overall profile, and based on the area of nine key odorant volatiles compared to classic HS-SPME. The addition of salty water showed an increased extraction yield comparable to the effect of vacuum. When the addition of salty water was then under Vac–HS–SPME conditions, the increase in extraction yield was proportionally higher. The different combined conditions, i.e., salty water or not, and HS-SPME and Vac–HS–SPME were compared in a complete untargeted way, exploiting the structured information provided by the two-dimensional plot generated using comprehensive two-dimensional gas chromatography (GC × GC) coupled to a quadrupole mass spectrometer. Additionally, the volatile profiles of the dark chocolates tested were compared selecting nine key aroma compounds.

Novelty: This work explored for the first time the combined effect of vacuum and the addition of salty water simulating saliva on the overall volatile profile. The two-dimensional plots generated by GC × GC were treated as chromatographic fingerprints, building for the first time what we called an "improvement plot" showing the zone of increment corresponding to specific chemical classes.

1. Introduction

Chocolate is among the most widely consumed and appreciated food delicacies worldwide, thanks to its pleasant sensory properties related to texture, aroma, and flavor [1]. Dark chocolate is a rich natural source of phenolic compounds, which can provide a range of health benefits, mainly due to their antioxidant properties. Recently, both epidemiological and clinical trials have demonstrated that a daily moderate intake of dark chocolate with high cocoa content can contribute to the prevention and treatment of cardiovascular and metabolic diseases [1, 2]. Therefore, consumers' awareness of the beneficial effects on health has recently led to an increased consumption of dark chocolate [3].

The chocolate aroma is a key quality parameter that drives the consumers' acceptance and results from the synergy of a broad array of volatile and non-volatile compounds [1,4]. So far, over 600 volatile organic compounds (VOCs) have been detected in cocoa and cocoa-derived products. These

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compounds belong to multiple and heterogeneous chemical classes, including acids, alcohols, aldehydes, ketones, esters, phenols, and pyrazines [4].

Solid-phase microextraction (SPME) is a well-established solvent- free sampling method widely used for the extraction of volatile and semi-volatile compounds, thanks to its inherent advantages of being rapid, relatively low-priced, selective, sensitive, and automated [5]. The headspace SPME (HS-SPME) extraction mode, in which the SPME fiber is exposed to the vapor phase above the sample, is commonly adopted for complex food matrices, especially to obtain an overview of the volatile profile, which can be correlated to different characteristics, such as the geographical origin, process characteristics, and the aroma perception [4].

The theory of HS-SPME sampling approach relies on a three-system partition equilibrium among the sample, the headspace, and the fiber coating. The time required to reach this equilibrium is influenced by several parameters, including the type of sample and the features of the target analytes [6]. To maximize and accelerate the release of semi-volatile compounds in the headspace allowing them to sorb into the SPME coating, thermodynamic and/or kinetic parameters can be tuned. The former encompasses the optimization of parameters that affect the distribution constant (K), which, besides the selection of the fiber coating and the nature of the sample matrix, includes temperature, salting, and pH optimization. However, the use of high temperatures may reduce the affinity of volatile analytes for the SPME fiber, lowering sensitivity and potentially inducing the formation of artifacts [7,8]. The adjustment of pH is essential for the extraction of analytes that can dissociate in the sample, as only undissociated forms can be extracted by SPME. In contrast, the effect of salting is highly dependent on the type of analyte and particularly on their K value. Compounds with already low K are generally little impacted by the addition of salt, while polar volatile solutes will benefit the most by the addition of salt, as their solubility is reduced, favoring the transfer into the HS. From a kinetic viewpoint, stirring is the most common practice, increasing mass transfer and thus accelerating the extraction process. However, in solid samples, stirring is not always efficient [9,10].

An alternative and less explored strategy to enhance the HS-SPME comprehensive extraction is to perform the sampling step under reduced-pressure conditions. This method, known as vacuum-assisted HS-SPME (Vac–HS–SPME), involves an additional step in which the pressure in the sample vial is lowered by applying a vacuum before the equilibration stage. Reducing the total pressure in the sampling vial increases the volatilization rates of analytes with low affinity for the headspace (low K), thereby improving their extraction efficiency. In the meantime, the release of analytes that rapidly reach equilibrium is not affected, as the thermodynamic parameters are independent of the pressure settings in the sampling vial [10–12]. Indeed, reduced pressure conditions affects the extraction kinetics without impacting the process's thermodynamics. Initially used for aqueous samples, Vac–HS–SPME has been successfully applied to a variety of complex food matrices, including dairy products [12,13], wine [14], extra virgin olive oil [15,16], fish [7], honey [17] and

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tomato [18], proving improved extraction efficiency and high sensitivity with shorter times of extraction and milder temperatures of sampling compared to classical HS-SPME.

The enrichment of the volatile profile, thanks to the use of Vac–HS–SPME, largely benefits from an enhanced separation that can be obtained using comprehensive two-dimensional gas chromatography (GC × GC). GC × GC has been demonstrated to be one of the most powerful analytical tools to achieve effective separations of very complex mixtures of volatile analytes in a single step [19]. The hugely enhanced separation power of this technique compared to conventional monodimensional GC relies on the fact that GC × GC involves the use of two columns, with different stationary phases, serially connected through a suitable interface (i.e., the modulator) that allows the transfer of the compounds eluted from the first column onto the second column for an additional separation. Moreover, this method has been reported to narrow the peak width, increasing the peak height and, hence, sensitivity. Specifically, HS-SPME combined with GC × GC has proven to be a consistent yet simple procedure for the comprehensive analysis of volatile and semi-volatile analytes in food analysis [19].

Dark chocolate is a solid, complex food. The volatiles are usually extracted by HS-SPME sampling at relatively high temperatures and/or for long times (45 min sampling 50 °C; 30 min sampling 60 °C) [1,4,20, 21] with the consequent risk of altering the VOCs profile of the original sample, thus not providing reliable information on the profile effectively perceived by the consumer. Generally, in papers that analyze VOCs in chocolate water with salts is not added, while they are in cocoa-based products [22–27], although with results varying accordingly to the targeted analytes. Generally, aldehydes and ketones are favored by the addition of water and even more by the presence of salt. Pyrazines are little impacted by the addition of salty water compared to the dry sample, while acids, alcohols, and terpenes are negatively impacted by the addition of water and salty water compared to the dry sample [24].

The aim of this study was to explore for the first time the potential of Vac–HS–SPME for the assessment of the volatile profiles in dark chocolate and the possible synergism with the addition of salt. To investigate the possible combined effect a comparative investigation between Vac–HS–SPME and classic HS-SPME sampling, with and without the addition of a salty solutions was performed on chocolate samples at different sampling times. The experiments were carried out at the physiological temperature (37 °C) and, as a salty solution, a simple saliva simulant was chosen, based on the literature [28]. The mixing with saliva plays a crucial role in the aroma release and transport to the olfactory receptors via the retro-nasal route [28,29], therefore the addition of model saliva could be correlated to the VOCs amount that would be extracted in the mouth cavity *in vivo* (retro-nasal VOCs release).

To study the selectivity of the different conditions tested and to maximize the information the final analysis was performed by GC × GC- MS.

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2. Materials and methods

2.1. CHEMICALS AND MATERIALS

Sodium hydroxide and calcium chloride were purchased from Sigma- Aldrich (Steinheim, Germany), whereas monopotassium phosphate and sodium chloride were obtained respectively from Acros Organics (Geel, Belgium) and Fisher Chemicals (Loughborough, UK). Normal alkanes (C_7–C_{30}) and divinylbenzene/carboxen/polydimethylsiloxane (DVB/ CAR/PDMS) df 50/30 μm/1 cm length fiber were from MilliporeSigma (Bellefonte, PA, USA). Each SPME fiber was conditioned as recommended by the manufacturer.

2.2. CHOCOLATE SAMPLE PREPARATION

In this study, three commercial chocolate samples bought at a local supermarket (Gembloux, Belgium) were analyzed, including 70, 85 and 100 % cocoa dark chocolate. Commercial samples of 70 % cocoa dark chocolate were used for the preliminary evaluations of the effects of different sampling times. All chocolate samples were homogenized in a blender (VEO Home, KWG-130B, France), weighted in 20 mL SPME vials (Restek, Bellefonte, USA) to 1.00 ± 0.05 g, capped with metallic screw caps with a hole and polytetrafluoroethylene (PTFE)/silicone septa (Restek), and stored in a freezer (− 18 °C) until analysis. The same cocoa dark chocolate bar samples were used for the entire set of experiments. Prior to use, vials and caps were maintained at 65 °C to remove any contaminants.

2.3. VAC- AND CLASSICAL HS-SPME EXTRACTION PROCEDURES

The specifically designed vial closures used for Vac–HS–SPME sampling were kindly supplied by ExtraTech (Chania, Greece). Each closure was provided with a cylindrical Thermogreen® LB-1 septum (Supelco) with half-hole (6 mm diameter × 9 mm length) and could ensure gastight fit to the 20 mL screw top vials and the automation of the procedure. For Vac–HS–SPME experiments, the samples were stored in the freezer before the air inside the sampling vial was evacuated for 1 min using an MD 4C diaphragm vacuum pump (7 mbar = 0.007 atm ultimate vacuum without gas ballast, by Vacuubrand GmbH & Co. KZ, Wertheim, Germany), keeping the sample in an iced water bath during air evacuation to minimize volatile losses. For HS-SPME analyses at atmospheric pressure, the metallic screw caps and vials containing the samples were used as stored, omitting the air-evacuation step. The sealed vials were put in the autosampler (Centri®, Markes International Ltd) and allowed to equilibrate at 37 °C for 15 min. Extraction was performed at physiological temperature, *i.e.*, 37 °C, at four different extraction times, namely 10, 25, 35, and 50 min, for Vac- and atmospheric HS-SPME sampling. Moreover, chocolate samples were prepared according to two different workflows: 1 g of powdered dark chocolate alone and 1 g of powdered dark chocolate with

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the addition of 1 mL of salty water (hereafter called "Salt"). This solution was formulated according to Guhmann et al. [28]. Briefly, phosphate buffer solution at pH 7.4 with the addition of sodium hydroxide, sodium chloride and calcium chloride. All samples were prepared in triplicate.

Following the sampling procedure, the fiber was thermal desorbed for 5 min at 280 °C (splitless mode) and the released volatiles were focused on a general-purpose trap (U-T12ME-2S, Markes International Ltd) at 0 °C before fast desorption (splitless) during 3 min at 300 °C and injection in the GC × GC-MS system.

Before any analytical sequence, the SPME fiber was conditioned for 5 min at 280 °C. Blanks were also regularly run to check for carryover between runs. Each experiment was performed in triplicate.

2.4. GC × GC-MS ANALYSIS

The VOCs separation was performed on Shimadzu GCMS-TQ8050 NX system (Shimadzu), comprising a GC-2030 and triple-quadrupole mass spectrometer detector (TQ-MS), hyphenated to the Centri platform (Markes International Ltd). The system was equipped with an INSIGHT differential reverse-fill/flush flow modulator (SepSolve Analytical Ltd). The primary column used (1D) was a 20 m × 0.18 mm i. d. × 0.18 μm SLB-5MS silphenylene polymer capillary column from MilliporeSigma (USA), while the secondary column (2D) was a BPX-50 (Trajan Scientific, 3 m × 0.25 mm i.d. × 0.25 μm) equivalent to 50 % phenylpolysilphenylene/50 % siloxane. The outlet of the 2D column was linked through a Y-union (Restek) to a 1.1 m × 0.18 mm i.d. uncoated capillary joined to the MS and a 0.5 cm × 0.2 mm i.d. uncoated capillary free in the oven, resulting in about 45 % of the flow (at initial temperature) reaching the MS detector. The GC oven temperature program was initially set at 35 °C for 1 min and then increased at 6 °C/min to 220 °C and then to 280 °C at 30 °C/min for 1 min. Helium was employed as the carrier gas in a programmed pressure mode at both the inlet and the auxiliary pressure-controlled module to control the 2D column flow. The pressure was set to achieve a flow of 0.5 mL/min in the 1D column and 15 mL/min in the 2D column. Additionally, a 1 m × 0.1 mm i.d. bleed line was mounted and linked to an auxiliary pressure control to easily adjust the bleed flow during the method optimization. The equivalent flow of the bleed line was regulated at 0.5 mL/min. The modulation time was 2.5 s, with 200 ms of reinjection time. The MS operated in single- quadrupole mode, using electron ionization (EI) at 70 eV. The ion source and transfer line temperatures were set at 200 °C and 250 °C, respectively. The scan range was between 45 and 350 *m/z* with an acquisition frequency of 50 Hz.

Data were acquired using GCMS Solution v4.53 (Shimadzu, Japan), then converted to AIA format for import and processing in ChromSpace software v2.1.7 (SepSolve Analytical Ltd., UK). Compounds tentative identification was performed using the NIST17 and FFNSC 3.0 mass spectral libraries based on a similarity match ≥85 % and a linear retention index (LRI) match of ±20 units.

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2.5. DATA ELABORATION AND STATISTICAL ANALYSIS

Statistical analyses were conducted using RStudio 2025.04.0 + 496 (Posit Software, PBC) with R version 4.3.2 (The R Foundation for Statistical Computing), and Microsoft Excel® from Office 2016 (Microsoft). EPI suite v4.1 (US Environmental Protection Agency) and its modules were used to calculate the theoretical partitioning parameters.

3. Results and discussion

The optimization of the extraction time was performed using a 70 % commercial chocolate sample. Analyses were conducted both with atmopsheric HS-SPME (Atm) and Vac–HS–SPME (Vac) and samples were prepared according to two different procedures: chocolate just grounded with and without the addition of salty water (Salt) in the sample vial to form a slurry simulating the mixture generated when chocolate is introduced in the mouth. The salty water as surrogate saliva composition was taken from the literature [28]. It is a simple model, but it was deliberately chosen to establish a first repeatable study. Indeed, as the first study in this direction, coupled with Vac–HS–SPME, we aimed to control for variability. More complex models (e.g., in enzyme activity and protein content) will be studied in future work.

Unlike the usual optimization procedure, where at least the combination of time and temperature is studied, it was decided to set the temperature at body temperature (37 °C) to simulate the human perception of the aroma released by the chocolate once melted in the mouth (simulated by the addition of the salty water). Therefore, to simplify the interpretation of the results and isolate the variable investigated (vacuum-assisted extraction and salt addition), all the experiments were performed at 37 °C, even though the aroma of chocolate before consumption is typically perceived at 20–25 °C. Fixing the temperature, only the time profile was studied at four different sampling times, *i.e.*, 10, 25, 35, and 50 min. Longer times were not applied to avoid instrument idle time and increase the throughput. Chromatograms are shown in Supplementary Materials Fig. S1.

3.1. COMPREHENSIVE AND TARGETED TIME PROFILE AT ATMOSPHERIC AND VACUUM CONDITIONS, AND WITH AND WITHOUT SALTY WATER

Based on the theory [10], the vacuum speeds up the kinetics of volatiles from condensate phases by increasing the mass transfer at the sample/gas interface in the gas-phase boundary layer. This is true for compounds exhibiting very low Henry's constant value (K_H). This leads to a higher overall extraction yield at shorter extraction times compared to atmospheric extraction. However, when analyzing highly viscous or solid samples, limited mass transfer within the matrix becomes a restricting factor, reducing the added value of vacuum-assisted extraction.

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In the present study, despite the mild temperature used (i.e., 37 °C), the chocolate still melted and formed a viscous semi-liquid matrix, which impacted the VOCs release and sample homogeneity.

Dark chocolate is a dispersion of solid particles (e.g., cocoa powder, sugar) maintained in a continuous solid fat phase thanks to lecithin [30]. Upon melting, the fat phase liquifies and can solubilize lipophilic content, such as milk fat or phospholipid-coated particles. Meanwhile, most solid particles remain undissolved and may be available for adsorption interactions. The high viscosity of the melted chocolate restricts compound diffusion in the liquid/solid phase, a limitation that vacuum conditions alone cannot improve. While this statement is true for the overall extraction efficiency across the full chromatogram, notable differences between Atm and Vac extractions can still be observed in the middle and latter part of the chromatograms, suggesting that vacuum may influence the release of specific compounds in such a viscous system. To better visualize such elution zone variations and the impact of extraction modalities (Atm vs Vac and No-Salt vs Salt) as well as their interaction and evolution throughout the extraction process, a 2D map of the averaged chromatographic intensity and a "map of improvement" were created (Fig. 1). Each 2D chromatogram was arbitrarily split into a grid of 15 (first-dimension) by 9 (second-dimension) cells. Data reduction was performed by summing the areas of all peaks falling inside each tile. Tile-wise sums from the three replicate injections were then averaged, obtaining a 2D map of the average intensity for each condition (reported in Supplementary Material Figs. S2 and S3). An improvement ratio for each cell was calculated by dividing each tile area by the area of the corresponding tile at 10 min of extraction for the same modality (i.e., Vac, Atm, Vac + Salt, and Atm + Salt); if the denominator was zero, half of the smallest non-zero cell area observed was used instead. These "maps of improvement" were generated by plotting the ratio of all tiles using *geom_tile()* function of the ggplot2 package (v3.5.2). Finally, and as suggested by Maesen and Salingros [31], the color scale was adapted using the 10th, 50th, and 90th percentiles as minimum, medium and maximum values to avoid distortion by extreme values. Values outside this range were mapped to the corresponding boundary colors. Fig. 1 displays, in its first column, the average-area map for each extraction modality at 10 min of extraction, followed by three columns showing the 2D maps of improvement at longer times for each of the extraction conditions tested (*i.e.*, Vac, Atm, Vac + Salt, and Atm + Salt). The 2D maps represent the distribution of compounds on the 2D chromatogram, which means that the x-axis (which corresponds to the first column elution time) accounts for a decrease in volatility of the compounds. In contrast, the y-axis (corresponding to the second dimension elution time) accounts for an increment of polarity of the compounds eluted.

Evaluating these results, it can be easily noticed that, when no salty water is added, vacuum conditions increase the extraction kinetics for heavier (i.e., mid-to late-eluting part of the maps) and more polar compounds (upper part of the maps) compared to atmospheric extraction. Indeed, under vacuum conditions, no further improvement is observed after 25 min; whereas atmospheric extraction continues to show gradual increases along the studied extraction time range.

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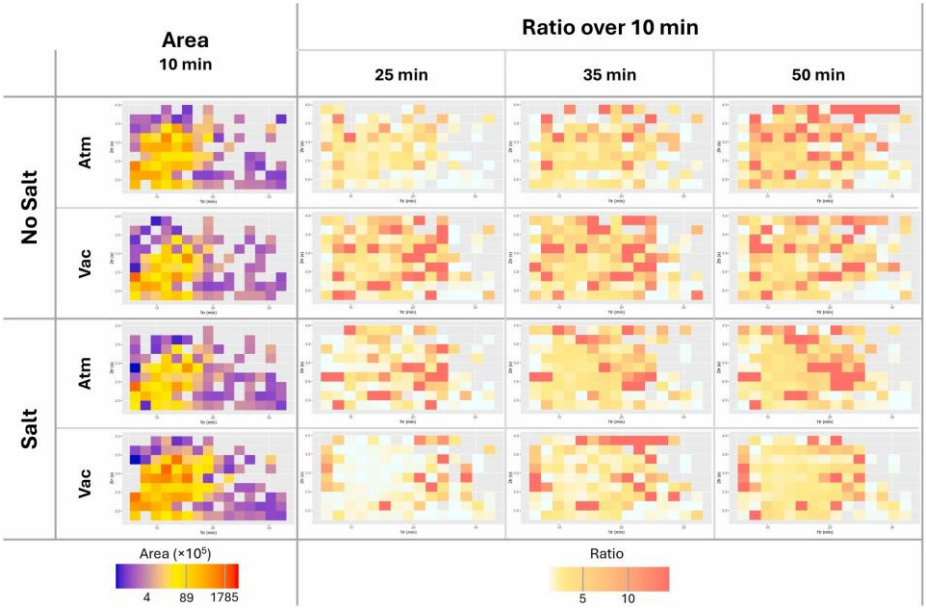
The salting-out effect can be isolated by comparing pair-wise Atm and Atm + Salt and the two Vac conditions in Fig. 1. In both cases (Atm and Vac), the presence of salty water significantly increases the extraction yields of VOCs. Based on the above considerations, it seems that for the aqueous slurry, composed of the chocolate and the salty water, a synergy of reduced viscosity and the salting out effect can explain the high extraction efficiency observed for atmopsheric HS- SPME (Atm + Salt), which is further enhanced by the use of vacuum. Both salty water addition and vacuum application enhance the extraction at short extraction times. Examination of the time profiles reveals that Atm extractions exhibit a gradual increase in signal intensity (*i.e.,* improvement ratio) over time, whereas Vac–HS–SPME extractions maintain an essentially constant ratio across all extraction durations, with negligible improvement beyond 25 min. At 25 min, the Vac–HS–SPME map looks essentially grey as no significant improvement is observed compared to 10 min (grey area corresponds to about a 2-fold improvement).

Figure 1. *Maps of improvement (area ratio) for the different conditions tested (i.e., Salt or No Salt and Atm or Vac) for 25, 35, and 50 min in comparison with their relative 10 min extraction for 70 % chocolate. Values outside the 10–90th percentiles range were mapped to the corresponding boundary colors. Grey tiles correspond to null value. Maps of area of 10 min extraction are displayed in the first column. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)*



A further target comparison was performed on 9 volatile compounds selected based on the following conditions: i) observed in all samples; ii) covering the volatility range of different chemical classes; iii) previously reported as active odor compounds for dark chocolate [32]. The selected compounds are listed in Table 1 and they include 3-methylbutanoic acid, benzaldehyde, octanal, benzeneacetaldehyde, ethylphenyl acetate, 2-ethyl-3,5-dimethylpyrazine, tetramethyl pyrazine, linalool and 2-phenylethanol. Fig. 2 shows the extraction time profiles of the 9 selected volatiles in

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70 % dark chocolate obtained under reduced and atmospheric pressure conditions, as well as with and without salty water, at the four different sampling times. The results confirmed the general trends reported in Fig. 1, with a significant impact of both the Vac and the salty water addition on the overall extraction profile. 3-Methylbutanoic acid was an exception, with a similar extraction efficiency of Vac (grey curve) and Atm + Salt (orange curve) at both 25 and 35 min. At longer extraction times, the Atm + Salt decreased while the Vac continued increasing (Fig. 2). In general, the addition of salty water showed higher extraction yield for all volatile markers compared to the relative pressure conditions (Fig. 2, orange and yellow lines).

Based on these results, an extraction time of 25 min was chosen to analyze the remaining samples. In addition to the conclusion drawn above, a displacement effect is observed for certain compounds beyond this extraction time (e.g., benzaldehyde and benzenacetaldehyde, see Fig. 2).

3.2. INVESTIGATION ON THE EFFECT OF VACUUM AND SALTING ADDITION

To isolate the effect of vacuum, the maps of the ratio of Vac–HS–SPME on HS-SPME were created without and with the addition of salty water (Fig. 3A–B). A general improvement was observed throughout the 2D maps, regardless of the addition of salty water; however, the benefits of Vac–HS–SPME are more pronounced in dry samples for the most volatile compounds (up to ~25 min). The less volatile and mid-polar compounds benefit from the combination of vacuum conditions and salt addition (Fig. 3B and D).

As for the pressure, the effect of salty water addition can be isolated and better visualized by plotting the Salt/No Salt ratio maps for both Vac and Atm extraction (Fig. 3C and D). The use of salty water generally showed an extraction increase of early to mid-eluting (*i.e.*, high-mid volatile) and more polar compounds (higher 2D retention time) for the vacuum conditions (Fig. 3D), while a more general improvement is observed for the atmospheric conditions (Fig. 3A). By comparing Fig. 3D with 3B, it can be concluded that vacuum and salty water provide similar effects in terms of enhanced extraction of mid-volatile compounds (lighter colored tiles on the right part of the map). While, the addition of salty water improves the extraction of more polar volatile compounds (red tiles on the upper left part of the improvement map in Fig. 3C and D) compared to the dry sample. When salty water is added, the enhancement obtained with vacuum is overall more balanced in the volatility and polarity range, and generally limited to about a 2-fold increment. However, significant incremental results were obtained from the combination of the two (Vac and salty water) for the less- volatile compounds (red tiles in the middle-right of the improvement maps in Fig. 3B and D).

Mixing chocolate with a water-based salty solution considerably alters the physico-chemical properties of the melted sample. The addition of a less viscous solvent, such as water, reduces overall viscosity, thereby enhancing diffusion through the liquid boundary layer, in accordance with the Wilke-Chang equation [38]. However, this addition also increases system complexity by generating a triphasic system composed of a hydrophobic phase (from melted fat), a hydrophilic

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phase (from the added salty solution), and undissolved solid particles. Lecithins may facilitate the formation of an emulsion between the two liquid phases. Consequently, the migration and release of volatile compounds depend on several factors, including the chocolate composition (dry matter, fat, and lecithin content) and the amount of aqueous solution introduced. Notably, the identity of the continuous phase, whether water or fat, can shift depending on the relative proportions of these components and may even dynamically change during the extraction. Indeed, in a water-continuous system, Henry's law constant is typically used to describe mass transfer resistance in the gas boundary layer, whereas in a fat-continuous system, the octanol–air partition coefficient may be more appropriate [15]. This distinction significantly impacts the release dynamics of various volatile compounds, depending on their physicochemical properties. Additionally, the presence of salts in the aqueous phase increases the ionic strength of the solution, thereby promoting the salting-out effect, further enhancing the release of polar volatile compounds (left and top part of the map in Fig. 3C and D; compounds with generally higher K_H) [10].

The global effect of the salty water can prevent the chocolate from forming a viscous slime, thereby increasing mass transfer into the gas phase and enhancing the extraction efficiencies of the analytes. Moreover, salts contained in the salty water modified the solubility of the solutes, changing their partition in the headspace.

A targeted comparison was also performed, based on the 9 selected volatile compounds reported in Table 1. In particular, Fig. 4 reports the data regarding the VOCs total area and the area of the nine selected compounds in 70 % dark chocolate. The differences in extraction yield obtained with the different conditions were all significant for each selected compound ($p < 0.05$).

Overall, the Vac method enhanced the differences between the dry sample and salty water addition. Similar results were obtained for 85 and 100 % dark chocolate samples, as reported in Figs. S4 and S5. The raw data are reported in Supplementary Tables S2–S4.

To better visualize the effect of the use of vacuum and the addition of salty water, the ratios between the areas obtained with the addition of salty water and dry sample under vacuum and atmospheric conditions, and the ratio between Vac–HS–SPME and HS-SPME with and without the addition of salty water is ploted in Fig. 5A and B, respectively. Apart from compounds 3 (linalool) and 6 (benzeneacetaldehyde) that showed a peculiar behaviour, the others showed a similar increment profile in all the conditions tested, although the extent of the increment is different. An increment of 2.3 and 2.9 on average was observed when the salty water is added under atmospheric or vacuum conditions, respectively (Fig. 5A). More important is the increment that was obtained observing the results using Vac–HS–SPME and HS-SPME (Fig. 5B). An average increment of 3.6 and 5.1 (not considering benzeneacetaldehyde) was observed not adding salty water and adding it, respectively. The increment is coherent with the Vac–HS–SPME theory [39]. In particular an increment was observed for all the compounds showing a log $K_{ow} > 2$ (Table 1). It is known that more apolar solutes (high log K_{ow}) are "squeeze out" from water system, either in the HS or in the more lipid solution;

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nevertheless, at the same time, the addition of water reduced the viscosity of the sample favoring the mass transfer. As described above, chocolate is a complex matrix and the addition of salty water forms even more complex triphasic system. More investigations on simplified models should be performed to evaluate idependently better the effect of reduced viscosity, salt addition and vacuum conditions. Anyway, it can be generally concluded that the profile remains comparable, although with different overall intensity.

The case of benzeneacetaldehyde presents a notable difference. This Strecker aldehyde, derived from the amino acid phenylalanine and reacting with an α-dicarbonyl (from carbohydrate degradation), a deoxyosone (from the Maillard reaction), or a quinone compound (from enzymatic polyphenol oxidation) [40]. Indeed, the extraction of benzeneacetaldehyde using salty water is significantly increased by approximately 175 times compared to an equivalent dry extraction (Supplementary Material, Table S5). As previously observed by Schieberle's team, a similar scale of Strecker aldehyde production occurs when chocolate reacts with hot water without any additional reagent [41,42]. This leads to the hypothesis that the observed increase is due to enhanced production rather than simply improved extraction, although the addition of salt may have a secondary impact on the release of this compound. It is worth noting that 2- and 3-methylbutanal (other Strecker aldehydes derived from isoleucine and leucine, respectively) were observed only with the addition of water, corroborating the findings of Schieberle et al. [41,42]. Regarding pressure-related aspects, vacuum does not significantly improve the extraction of these volatile compounds. This is likely because they exhibit relatively high K_H values, resulting in a moderated improvement.

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Table 1. *List of the 9 selected compounds, their Chemical Abstracts Service (CAS) number, molecular weight (MW), boiling point (BP), odor thresholds in water and in oil, logarithmic values of the octanol-water (logK_{OW}) and octanol-air partition coefficient (logK_{OA}), henry constant partition coefficient (K_H), experimentally calculated linear retention index (LRI Exp) and literature reported (LRI Lit), mass spectra similarity (MS%), the ion used for area calculation (Quantifier).*

n°NAME	CAS	MW (g mol^{-1})	BP (°C)	Odor threshold in water[a] (ppb)	Odor threshold in oil[b] (ppb)	LogKOW Predi[c]	Log*K*OA Predi[d]	KH Predi[e] (atm·m^3/ mol)	LRI Exp	LRI Lit	MS %	Quantifier (*m/z*)
1 3-Methylbutanoic acid	503-74-2	102.13	175	490	11	1.49	5.63	1.28E-06	702	875	98	60
2 Benzaldehyde	100-52-7	106.12	180	751	60	1.71	4.44	1.34E-05	955	961	96	77
3 Linalool	78-70-6	154.25	199	6	3.4	3.38	6.03	4.23E-05	1103	1098	90	71
4 2-Phenylethaol	60-12-8	122.16	219	1880	490	1.57	6.34	2.89E-07	1133	1139	85	91
5 Octanal	124-13-0	128.21	171	0.7	140	2.78	4.46	3.71E-04	1009	1007	92	43
6 Benzeneacetaldehyde	122-78-1	120.15	195	4	37	1.54	5.43	5.48E-06	1062	1051	97	91
7 Ethyl phenylacetate	101-97-3	164.20	227	650	300	2.57	5.39	1.88E-05	1273	1253	93	91
8 2-Ethyl-3,5-dimethylpyrazine	13925-07-0	136.19	181	1	1.7	2.07	5.74	5.21E-06	1087	1088	93	135
9 Tetramethyl pyrazine	1124-11-4	136.19	190	1000	9.4	2.13	5.03	4.33E-06	1095	1096	88	54

[a] Czerny 2008 [33]; Lin 2024 [34]; http://www.leffingwell.com/odorthre.htm [35].
[b] Seyfried and Granvogl, 2019 [36]; Fricke and Schieberle, 2020 [37]; Quelal et al., 2023 [32].
[c] Computed from KOWWIN v1.69.
[d] Computed from KOAWIN v1.10.
[e] Computed from HENRYWIN v3.20 (bond method).

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Fig. 2. *Extraction time profiles (15–50 min of sampling) of the selected volatiles in 70 % dark chocolate performed under atmospheric (HS-SPME; Atm) and reduced total pressure (Vac–HS–SPME; Vac) with and without the addition of the salty water (Atm + Salt and Vac + Salt). Coding of compounds as in Table 1. Relative standard deviations are given in Supplementary Table S2.*

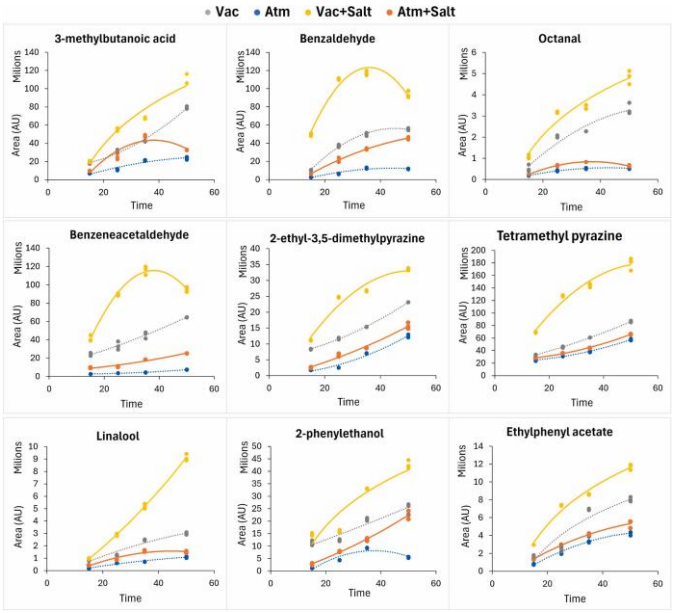
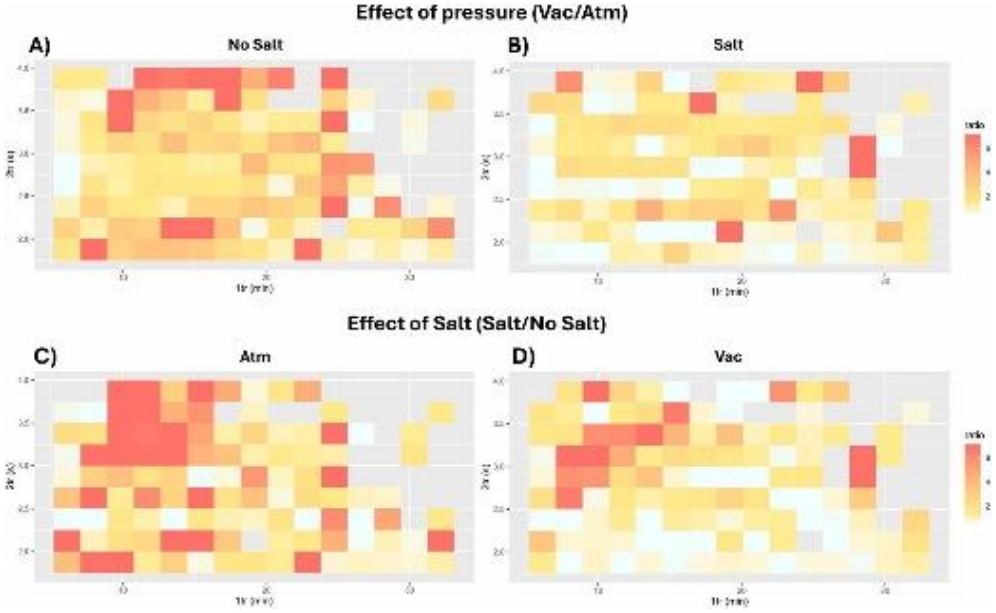


Fig. 3. *Maps of improvement relative to the effect of pressure (top, area ratio of vacuum on atmospheric extraction without (A) or with (B) salty water) and salty water addition (bottom, area ratio of Salt on No Salt extraction at atmospheric pressure (C) or under vacuum (D)) of 25 min of extraction of 70 % chocolate. Grey tiles correspond to null value.*



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3.3. COMPARISON ON THE VOLATILE PROFILE OF CHOCOLATE WITH DIFFERENT COCOA CONTENT

Finally three samples of chocolate with different content of cocoa, namely 70, 85, and 100 %, were analyzed using the different conditions tested (but keeping 25 min as extraction time). The total number of extracted compounds for the three different chocolates confirms the general trend previously discussed. Indeed, Vac + Salt and Atm give the highest and lowest extraction performance, yielding an average of 647 and 415 compounds, respectively, for the 100 % chocolate. Meanwhile, Atm + Salt and Vac show similar numbers of extracted compounds for the 100 % chocolate (approximately 440). The complete compound count data for the other chocolate types are provided in the Supplementary Material (Fig. S6). Additionnaly, a detailed investigation was conducted regarding the profiles of the nine targeted compounds. Their descriptors (odor/flavour) may change depending on the way of sampling, dry (which can be correlated with the ortho nasal perception) or with the addition of salty water (correlated to the retro-nasal perception). To simplify the discussion, their identifying number will be used instead of descriptors. The associated descriptors are available in Supplementary Table S1. The volatile profiles in terms of the content of individual selected volatiles were visualized using radar plots. Profiles obtained by atmospheric HS-SPME without salty water (Fig. 6A) resulted quite similar for the chocolate samples with 85 and 100 % of cocoa, for which the VOCs profile was dominated by tetramethyl pyrazine (9) followed by 3-methylbutanoic acid (1). For the sample 70 %, on the contrary, tetramethyl pyrazine (9) was the most predominant VOC. Applying vacuum (Fig. 6B), the profile of the sample 85 % changed only in intensity, but not in the distribution of compounds, while the profile of the sample 100 % was slightly modified, in particular for a more intense release of benzaldehyde (2) and. Conversely, the profile of the sample 70 % showed a significantly higher release of benzaldehyde (2) and benzeneacetaldehyde (6). With the addition of the salty water during the sample preparation at ambient pressure (Fig. 6C), only the VOCs profile of 85 % dark chocolate showed a significant variation, as a higher amount of 3-methylbutanoic acid (1), benzaldehyde (2) and benzeneacetaldehyde (6) was released (Fig. 6C) with respect to the Atm profile (Fig. 6A). Possible benzenacetaldehyde production has previously been discussed. Regarding benzaldehyde, this compound may also have been formed during the extraction through oxidative cleavage of benzeneacetaldehyde. This reaction is facilitated by the aqueous solution, as observed by Chu and Yaylayan [43], and could be catalyzed by metal ions present in the solution. This result suggests that for 85 % dark chocolate, almond, nutty and chocolate with a slight earthy notes, particularly conferred by enhanced contents of benzaldehyde (2) and benzeneacetaldehyde (6), respectively, could be perceived with the addition of salty water simulating saliva, thus it can be correlated with the retro-nasal aroma. Notably, these notes were missing in the other two chocolate samples.

In contrast, the volatile profile obtained by the vacuum-assisted approach dry in salty water addition condition showed several differences (Fig. 6B and D). In 70 % dark chocolate the most abundant

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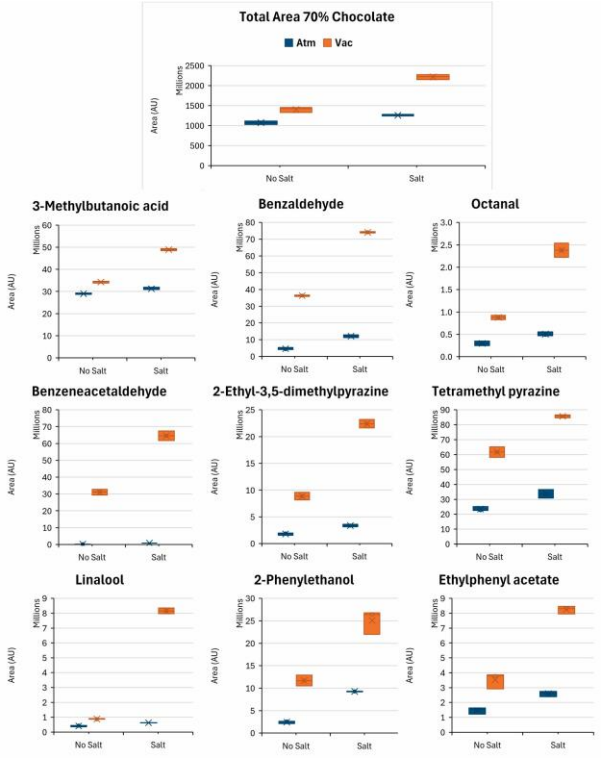
volatiles in the dry sampling were tetramethyl pyrazine (9), benzaldehydes (2), and benzenacetaldehydes (6), (blue line, Fig. 6B), while the profile using vacuum and salty water addition reduced significantly the content of benzenacetaldehydes (6)(blue line, Fig. 6D).

The volatile profile of 85 % dark chocolate under vacuum in the dry sample was mainly characterized by 3-methylbutanoic acid (1) and tetramethyl pyrazine (9) (orange line, Fig. 6B), while in 100 % dark chocolate, also benzaldehydes (2) was more abundant (grey line, Fig. 6B). The aroma patterns obtained by Vac–HS–SPME in the presence of salty water (Vac + Salt), reported in Fig. 6D, highlighted slight differences among the samples. The profiles of all chocolate samples showed higher amounts of both benzaldehydes (2), and benzenacetaldehydes (6). On the contrary, tetramethyl pyrazine (9) showed slightly lower intensity in the profile of both 70 and 100 % chocolate samples in the two conditions (blue and grey line for 70 and 100 %, respectively, Fig. 6D).

Figure 4. *Comparison of extraction without and with addition of salty water evaluated on the total area and on the area of the nine selected compounds in 70 %dark chocolate.*



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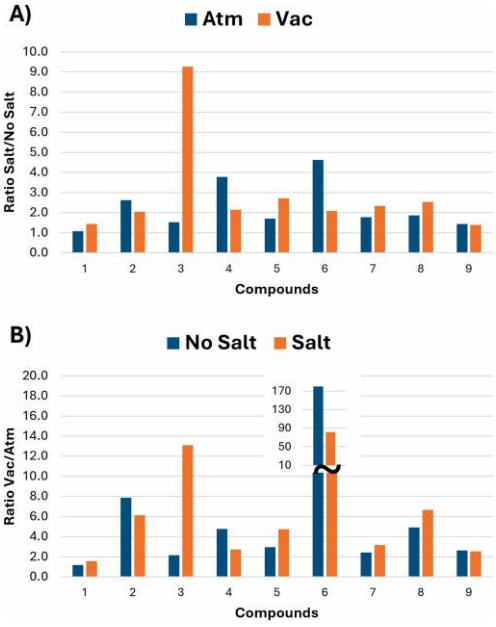
4. Conclusion

Figure 5. *Comparison of improvement ratio relative to the effect of salty water addition for Atm and Vac extraction (A) and effect or pressure for No Salt and Salt (B) evaluated on the 9 selected compounds. Numbers refer to compounds as reported in Table 1.*



This work investigated, for the first time, the application of Vac–HS–SPME/GC × GC-MS for the assessment of the volatile profiles in dark chocolate samples at 70, 80, and 100 % cocoa content. The impact of vacuum was evaluated, combined with the addition of a salty water solution simulating the saliva, thus investigating release of VOCs when the chocolate is melting in the mouth. To simulate the VOCs perception, experiments were conducted at the physiological temperature (37 °C) on chocolate alone and chocolate with the addition of salty water, using both Vac- and classic HS-SPME sampling. These conditions also allowed to compare the application of vacuum versus the salting-out effect and their combined effect.

The overall effect of the conditions tested was explored using a so- called "improvement map", which simplifies the comparison of the overall chromatograms, enhancing the differences between specific conditions and maintaining the 2D distribution, which correlates with volatility and polarity of the extracted compounds. Further, 9 selected compounds among the chocolate key odorants were used for a more targeted comparison.

As expected, Vac–HS–SPME proved superior extraction of less volatile and more polar compounds compared to Atm–HS–SPME. Nevertheless, when the salty water was added, the benefits of vacuum appeared reduced, showing a more moderate improvement compared to Atm–HS–SPME with salty

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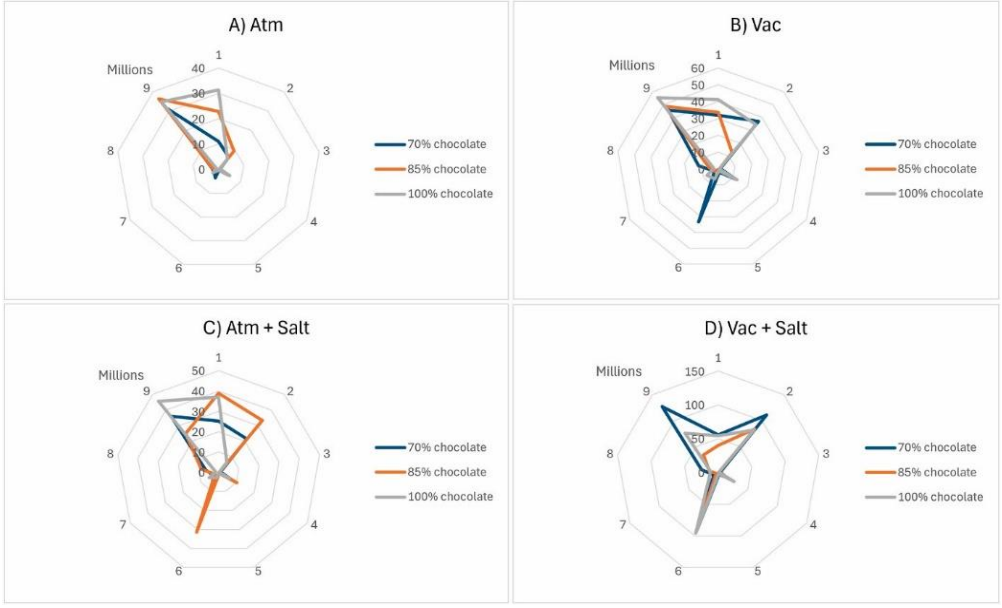
water as well. Future research will be oriented to isolate the effect of reduced viscosity by the addition of only water in comparison to salty water.

Finally, the comparison of the volatile profiles in the different conditions, evaluated by the 9 selected key aroma compounds, showed that in the vacuum condition the chocolate samples displayed similar profiles both profiles with and without the addition of satly water. Additionally, different simulated saliva's receipts will be tested to get closer to the real composition and understand further its impact on the sensory perception.

The enhanced sensitivity obtained under vacuum-assisted conditions surely can benefit studies related to quality and authenticity. Nevertheless, the sensory perception of a food sample not only relies on the presence or absence of specific volatile compounds, but also depends on their odor strength and the resulting content in the final product. In addition, the sensory perception is occurring at atmospheric pressure; therefore, to evaluate whether the results obtained using vacuum- assisted conditions can be used for evaluating the sensory perception of chocolate (considering that they are impacting only the kinetics of extraction and not the thermodynamics) should be further verified and supported by sensory evaluation in parallel to the volatile characterization.

Figure 6. *Comparison of extraction profile evaluated on the nine key odorants (see Table 1) in 70, 85 and 100 % dark chocolate analyzed by classical HS-SPME and Vac–HS–SPME without (Atm and Vac) and with salty water (Atm + Salt; Vac + Salt). Numbers refer to compounds as reported in Table 1*



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CRediT authorship contribution statement

Rosaria Cozzolino: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Data curation. **Damien Eggermont:** Writing – review & editing, Writing – original draft, Visualization, Software, Investigation, Formal analysis, Data curation. **Livia Malorni:** Writing – review & editing, Writing – original draft. **Giorgia Purcaro:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2025.345015.

Data availability

Data will be made available on request.

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References

[1] S.J. Calva-Estrada, M. Utrilla-Vazquez, A. Vallejo-Cardona, D.B. Roblero-Perez, ´ E. Lugo-Cervantes, Thermal properties and volatile compounds profile of commercial dark-chocolates from different genotypes of cocoa beans (Theobroma cacao L.) from Latin America, Food Res. Int. 136 (2020) 109594, https://doi.org/ 10.1016/j.foodres.2020.109594.

[2] J. Cartas, N. Alvarenga, A. Partidario, M. Lageiro, C. Roseiro, H. Gonçalves, A. ´ E. Leitao, C.M. Ribeiro, J. Dias, Influence of geographical origin in the physical and ˜ bioactive parameters of single origin dark chocolate, Eur. Food Res. Technol. 250 (2024) 2569–2580, https://doi.org/10.1007/s00217-024-04558-0.

[3] E. Bartkiene, E. Mockus, E. Mozuriene, J. Klementaviciute, E. Monstaviciute, V. Starkute, P. Zavistanaviciute, E. Zokaityte, D. Cernauskas, D. Klupsaite, The evaluation of dark chocolate-elicited emotions and their relation with physico chemical attributes of chocolate, Foods 10 (2021) 642, https://doi.org/10.3390/ foods10030642.

[4] S. Guzman Penella, R. Boulanger, I. Maraval, G. Kopp, M. Corno, B. Fontez, ´ A. Fontana, Link between flavor perception and volatile compound composition of dark chocolates derived from trinitario cocoa beans from Dominican Republic, Molecules 28 (2023) 3805, https://doi.org/10.3390/molecules28093805.

[5] M.A. Mottaleb, M.J. Meziani, M.R. Islam, Solid-phase microextraction and its application to natural products and biological samples, in: Encycl. Anal. Chem., Wiley, 2019, pp. 1–28, https://doi.org/10.1002/9780470027318.a9905.pub2.

[6] E. Psillakis, The effect of vacuum: an emerging experimental parameter to consider during headspace microextraction sampling, Anal. Bioanal. Chem. 412 (2020) 5989–5997, https://doi.org/10.1007/s00216-020-02738-x.

[7] N. Delbecque, S. Mascrez, E. Psillakis, G. Purcaro, Sub-ambient temperature sampling of fish volatiles using vacuum-assisted headspace solid phase microextraction: theoretical considerations and proof of concept, Anal. Chim. Acta 1192 (2022) 339365, https://doi.org/10.1016/j.aca.2021.339365.

[8] E. Yiantzi, K. Murtada, K. Terzidis, J. Pawliszyn, E. Psillakis, Vacuum-assisted headspace thin-film microextraction: theoretical formulation and method optimization for the extraction of polycyclic aromatic hydrocarbons from water samples, Anal. Chim. Acta 1189 (2022) 339217, https://doi.org/10.1016/j. aca.2021.339217.

[9] S. Risticevic, H. Lord, T. Gorecki, C.L. Arthur, J. Pawliszyn, Protocol for solid-phase ´ microextraction method development, Nat. Protoc. 5 (2010) 122–139, https://doi. org/10.1038/nprot.2009.179.

[10] E. Psillakis, Vacuum-assisted headspace solid-phase microextraction: a tutorial review, Anal. Chim. Acta 986 (2017) 12–24, https://doi.org/10.1016/j. aca.2017.06.033.

[11] E. Psillakis, E. Yiantzi, L. Sanchez-Prado, N. Kalogerakis, Vacuum-assisted headspace solid phase microextraction: improved extraction of semivolatiles by non-equilibrium headspace sampling under

reduced pressure conditions, Anal. Chim. Acta 742 (2012) 30–36, https://doi.org/10.1016/j.aca.2012.01.019.

[12] M.J. Trujillo-Rodríguez, V. Pino, E. Psillakis, J.L. Anderson, J.H. Ayala, E. Yiantzi, A.M. Afonso, Vacuum-assisted headspace-solid phase microextraction for determining volatile free fatty acids and phenols. Investigations on the effect of pressure on competitive adsorption phenomena in a multicomponent system, Anal. Chim. Acta 962 (2017) 41–51, https://doi.org/10.1016/j.aca.2017.01.056.

[13] M. Sýkora, E. Vítova, H.H. Jeleń, Application of vacuum solid-phase microextraction for the analysis of semi-hard cheese volatiles, Eur. Food Res. Technol. 246 (2020) 573–580, https://doi.org/10.1007/s00217-020-03426-x.

[14] M. Vakinti, S.-M. Mela, E. Fernandez, E. Psillakis, Room temperature and sensitive ´ determination of haloanisoles in wine using vacuum-assisted headspace solid- phase microextraction, J. Chromatogr. A 1602 (2019) 142–149, https://doi.org/ 10.1016/j.chroma.2019.03.047.

[15] S. Mascrez, E. Psillakis, G. Purcaro, Multifaceted investigation on the effect of vacuum on the headspace solid-phase microextraction of extra-virgin olive oil, Anal. Chim. Acta 1103 (2020) 106–114, https://doi.org/10.1016/j. aca.2019.12.053.

[16] S. Mascrez, J. Aspromonte, N.D. Spadafora, G. Purcaro, Vacuum-assisted and multi- cumulative trapping in headspace solid-phase microextraction combined with comprehensive multidimensional chromatography-mass spectrometry for profiling virgin olive oil aroma, Food Chem. 442 (2024) 138409, https://doi.org/10.1016/j. foodchem.2024.138409.

[17] D. Eggermont, F. Pardi, G. Purcaro, Enhancing the targeted and untargeted analysis of honey by vacuum-assisted SPME-GC × GC-MS. A green, practical, and highly informative approach, Green Anal. Chem. 12 (2025) 100207, https://doi.org/ 10.1016/j.greeac.2025.100207.

[18] Pateraki, E. Psillakis, Vacuum-assisted headspace solid phase microextraction for monitoring ripening-induced changes in tomato volatile profile, J. Chromatogr. A 1740 (2025) 465556, https://doi.org/10.1016/j.chroma.2024.465556.

[19] J. Aspromonte, S. Mascrez, D. Eggermont, G. Purcaro, Solid-phase microextraction coupled to comprehensive multidimensional gas chromatography for food analysis, Anal. Bioanal. Chem. 416 (2024) 2221–2246, https://doi.org/10.1007/s00216- 023-05048-0.

[20] B.J. Assi-Clair, M.K. Koné, K. Kouamé, M.C. Lahon, L. Berthiot, N. Durand, M. Lebrun, A. Julien-Ortiz, I. Maraval, R. Boulanger, T.S. Guéhi, Effect of aroma potential of Saccharomyces cerevisiae fermentation on the volatile profile of raw cocoa and sensory attributes of chocolate produced thereof, Eur. Food Res. Technol. 245 (2019) 1459–1471, https://doi.org/10.1007/s00217-018-3181-6.

[21] T.S. Ooi, A.S.Y. Ting, L.F. Siow, Volatile organic compounds and sensory profile of dark chocolates made with cocoa beans fermented with Pichia kudriavzevii and Hanseniaspora thailandica, J. Food Sci. Technol. 59 (2022) 2714–2723, https:// doi.org/10.1007/s13197-021-05292-1.

[22] D. Suzuki, Y. Sato, H. Nishiura, R. Harada, H. Kamasaka, T. Kuriki, H. Tamura, A novel extraction method for aroma isolation from dark chocolate based on the oiling-out effect, Food Anal. Methods 12 (2019) 2857–2869, https://doi.org/ 10.1007/s12161-019-01642-0.

Published in : Analytica Chimica Acta (2026), vol 1385, art n°345015
DOI: 10.1016/j.aca.2025.345015
Status : Postprint (Author's version)

[23] B. Kruszewski, M.W. Obiedziński, Impact of raw materials and production processes on furan and acrylamide contents in dark chocolate, J. Agric. Food Chem. 68 (2020) 2562–2569, https://doi.org/10.1021/acs.jafc.0c00412.

[24] S. Ducki, J. Miralles-Garcia, A. Zumbe, A. Tornero, D.M. Storey, Evaluation of solid-phase micro-extraction coupled to gas chromatography-mass spectrometry for the headspace analysis of volatile compounds in cocoa products, Talanta 74 (2008) 1166–1174, https://doi.org/10.1016/j.talanta.2007.08.034.

[25] L. Godočiková, E. Ivanišová, G. Zagula, L. Noguera-Artiaga, Á.A. Carbonell-Barrachina, P.Ł. Kowalczewski, M. Kačániová, Antioxidant activities and volatile ´ flavor components of selected single-origin and blend chocolates, Molecules 25 (2020) 3648, https://doi.org/10.3390/molecules25163648.

[26] Z. Ayazi, A.A. Matin, N. Mohammadi-Harib, Determination of alkylpyrazines in cocoa samples applying head-space hollow fiber protected-liquid phase microextraction followed by gas chromatography-flame ionization detection, J. Food Meas. Char. 14 (2020) 322–332, https://doi.org/10.1007/s11694-019- 00294-2.

[27] G.F. Pini, E.S. de Brito, N.H.P. García, A.L.P. Valente, F. Augusto, A Headspace Solid Phase Microextraction (HS-SPME) method for the chromatographic determination of alkylpyrazines in cocoa samples, J. Braz. Chem. Soc. 15 (2004) 267–271, https://doi.org/10.1590/S0103-50532004000200017.

[28] M. Guhmann, M. Preis, F. Gerber, N. Pöllinger, J. Breitkreutz, W. Weitschies, Development of oral taste masked diclofenac formulations using a taste sensing system, Int. J. Pharm. 438 (2012) 81–90, https://doi.org/10.1016/j. ijpharm.2012.08.047.

[29] S. Ployon, M. Morzel, F. Canon, The role of saliva in aroma release and perception, Food Chem. 226 (2017) 212–220, https://doi.org/10.1016/j. foodchem.2017.01.055.

[30] E.O. Afoakwa, Chocolate Science and Technology, Wiley, 2016, https://doi.org/ 10.1002/9781118913758.

[31] P. Maesen, E. Salingros, Introduction to reproducible geospatial analysis and figures in R: a tutorial article, data 9, 58, https://doi.org/10.3390/data9040058, 2024.

[32] O.M. Quelal, D.P. Hurtado, A.A. Benavides, P.V. Alanes, N.V. Alanes, Key aromatic volatile compounds from roasted cocoa beans, cocoa liquor, and chocolate, Fermentation 9 (2023) 166, https://doi.org/10.3390/fermentation9020166.

[33] M. Czerny, M. Christlbauer, M. Christlbauer, A. Fischer, M. Granvogl, M. Hammer, C. Hartl, N.M. Hernandez, P. Schieberle, Re-investigation on odour thresholds of key food aroma compounds and development of an aroma language based on odour qualities of defined aqueous odorant solutions, Eur. Food Res. Technol. 228 (2008) 265–273, https://doi.org/10.1007/s00217-008-0931-x.

[34] S. Lin, N. Li, X. Zhou, S. Li, A. Yang, J. Zhou, P. Liu, Evaluation of perceptual interactions between key aldehydes in Kung Pao Chicken, Food Chem. X 21 (2024) 101183, https://doi.org/10.1016/j.fochx.2024.101183.

Published in : Analytica Chimica Acta (2026), vol 1385, art n°345015
DOI: 10.1016/j.aca.2025.345015
Status : Postprint (Author's version)

[35] Leffingwell and Associates, Odor thresholds database, (n.d.). http://www.leffingwell.com/odorthre.htm (accessed March 20, 2025).

[36] C. Seyfried, M. Granvogl, Characterization of the key aroma compounds in two commercial dark chocolates with high cocoa contents by means of the sensomics approach, J. Agric. Food Chem. 67 (2019) 5827–5837, https://doi.org/10.1021/ acs.jafc.8b06183.

[37] K. Fricke, P. Schieberle, Characterization of the key aroma compounds in a commercial milk chocolate by application of the sensomics approach, J. Agric. Food Chem. 68 (2020) 12086–12095, https://doi.org/10.1021/acs.jafc.0c05787.

[38] C.R. Wilke, P. Chang, Correlation of diffusion coefficients in dilute solutions, AIChE J. 1 (1955) 264–270, https://doi.org/10.1002/aic.690010222.

[39] E. Yiantzi, N. Kalogerakis, E. Psillakis, Vacuum-assisted headspace solid phase microextraction of polycyclic aromatic hydrocarbons in solid samples, Anal. Chim. Acta 890 (2015) 108–116, https://doi.org/10.1016/j.aca.2015.05.047.

[40] G.P. Rizzi, The strecker degradation of amino acids: newer avenues for flavor formation, Food Rev. Int. 24 (2008) 416–435, https://doi.org/10.1080/ 87559120802306058.

[41] P. Schieberle, P. Pfnuer, Characterization of key odorants in chocolate, in: Flavor Chem., Springer US, Boston, MA, 1999, pp. 147–153, https://doi.org/10.1007/ 978-1-4615-4693-1_13.

[42] K. Buhr, C. Pammer, P. Schieberle, Influence of water on the generation of strecker aldehydes from dry processed foods, Eur. Food Res. Technol. 230 (2010) 375–381, https://doi.org/10.1007/s00217-009-1169-y.

[43] F.L. Chu, V.A. Yaylayan, Model studies on the oxygen-induced formation of benzaldehyde from phenylacetaldehyde using pyrolysis GC-MS and FTIR, J. Agric. Food Chem. 56 (2008) 10697–10704, https://doi.org/10.1021/jf8022468.