Chemical messages in 170-year-old champagne bottles from the Baltic Sea: Revealing tastes from the past

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Edited by Jerrold Meinwald, Cornell University, Ithaca, NY, and approved March 24, 2015 (received for review January 13, 2015)

Archaeochemistry as the application of the most recent analytical techniques to ancient samples now provides an unprecedented understanding of human culture throughout history. In this paper, we report on a multiplatform analytical investigation of 170-y-old champagne bottles found in a shipwreck at the bottom of the Baltic Sea, which provides insight into winemaking practices used at the time. Organic spectroscopy-based nontargeted metabolomics and metallomics give access to the detailed composition of these wines, revealing, for instance, unexpected chemical characteristics in terms of small ion, sugar, and acid contents as well as markers of barrel aging and Maillard reaction products. The distinct aroma composition of these ancient champagne samples, first revealed during tasting sessions, was later confirmed using state-of-the-art aroma analysis techniques. After 170 y of deep sea aging in close-to-perfect conditions, these sleeping champagne bottles awoke to tell us a chapter of the story of winemaking and to reveal their extraordinary archaeometabolome and elemental diversity in the form of chemical signatures related to each individual step of champagne production.

metabolomics | archaeochemistry | champagne | wine

Discovering ancient objects from excavation sites or simply at the back of a cellar has always piqued human interest because of the messages from the past they may contain. Unsurprisingly, our interest increases even more when exhuming old bottles or even jars that seem to have contained grapes or wine (1–3), giving a glimpse into the little-known history of winemaking. When divers first discovered bottles in a shipwreck off the Finnish Åland archipelago in the Baltic Sea (4) in July 2010 (Fig. 1) and tasted one of them on site, they realized that they were most likely drinking a century-old champagne. The numerous echoes that have since resounded in the international media highlight the worldwide interest in such stories.

A total of 168 bottles were retrieved from the shipwreck (Fig. 1). None of the labels remained, but bottles were later identified as champagnes from the Veuve Clicquot Ponsardin (VCP), Heidsieck, and Juglar (known as Jacquesson since 1832) champagne houses thanks to branded engravings on the surface of the cork that is in contact with the wine. A few of the recovered bottles lay horizontally in close-to-perfect slow-aging conditions, kept in total darkness at a fairly constant temperature (2–4 °C) and in conditions of low salinity (<10 g/kg NaCl), typical of the depth of about 50 m where they were found.

These bottles of champagne, while perhaps not the oldest to have made it to our time, most likely contain the oldest champagne to ever have been tasted. However, in the midst of the initial excitement raised by this discovery, it became clear that many questions remained: When were these wines produced, and what winemaking processes were in use at the time? Were they traveling on a regular trade route, and what was their final destination? One could expect to begin to answer these questions and many others by simply comparing the current champagne elaboration process to historical records in conjunction with straightforward basic analyses of these 170-y-old champagne samples. However, it proved difficult to draw any conclusions about these decisively enigmatic bottles. Fewer than 200 bottles were found in the wreck, which raises questions as to their intended use and/or destination. Based on the site of discovery, one could suppose that they were en route for the Russian Empire. Indeed, Madame Clicquot did strive to please the Russian taste for sweet wines from as early as 1814. Nevertheless, the frequent correspondence with her agent in Saint Petersburg testifies to the customers' distinctive request for a very specific sugar dosage of nearly 300 g/L: "Here they always have some sugar on any table close to their wine glass, for they add sugar not only to red wine but also to champagne" (5). Thus, the relatively low sugar levels of the shipwrecked bottles, less than 150 g/L, suggest that they might instead have been intended for the customers in the Germanic Confederation. Even dating these bottles proved difficult, as no information could be inferred from the shipwreck itself, and the bottles gave contradictory clues. Based on historical records, the characteristic branding found on the bottom face of the corks, known in French as the "mirroir," indicated that the VCP bottles had been sealed

Significance

The composition of 170-y-old champagne samples found in a shipwreck in the Baltic Sea constitutes a remarkable and unprecedented example of long-term combinatorial chemistry, which can occur in such sealed 750-mL microlaboratories. Multiple analytical tools, including metabolomics, metallomics, and sensory analysis, were combined to characterize the molecular diversity of these champagnes having aged in close-to-perfect conditions at the bottom of the sea. The analyzed champagnes retained intrinsic features allowing us to shed light on the winemaking practices in use in the middle of the 19th century. Therefore, this archeochemistry approach enabled us to rewrite a piece of our cultural heritage.

Author contributions: P.J., R.D.G., and P.S.-K. designed research; S.S.H., C.R.-G., F. Moritz, B.M., M.W., M.L., and P.S.-K. performed research; P.J., S.S.H., C.R.-G., C.C., D.D., C.B., F. Moreau, D.S., R.D.G., and P.S.-K. analyzed data; and P.J., S.S.H., C.R.-G., C.C., A.A., M.A.D., T.K., G.L.-B., M.L., D.S., R.D.G., and P.S.-K. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1500783112/-/DCSupplemental.

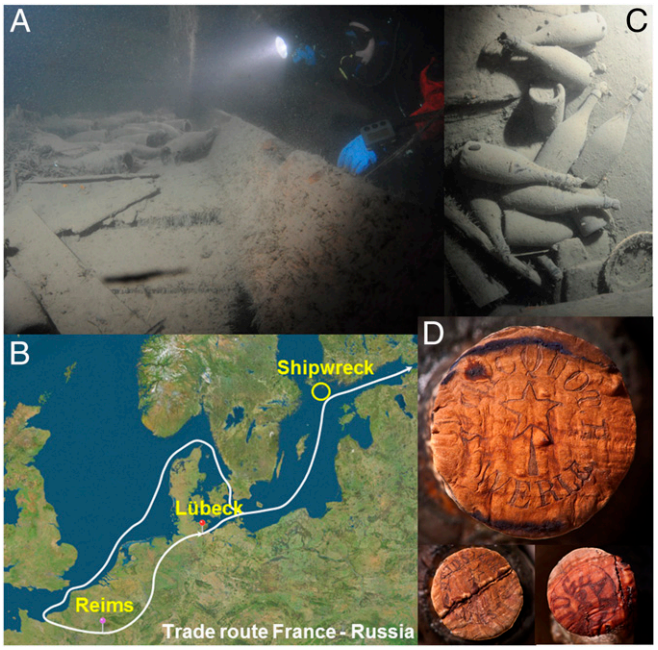

Fig. 1. (*A*) Discovery of champagne bottles in a shipwreck off the Finnish Åland archipelago, in July 2010. The ship was a two-masted schooner (21.5 m long × 6.5 m broad) around 200 y old (location: south of the municipality of Föglö, Åland). Copyright, Anders Näsman/The Government of Åland. (*B*) One possible planned itinerary for the boat. (*C*) A few of the 168 bottles found in the Baltic Sea at a depth of about 50 m, in nearly ideal slow-aging conditions in terms of temperature (2–4 °C), darkness, low salinity, and high pressure. (*D*) Branded engravings on the cork surface that is in contact with the wine (*mirroir* in French). The representation of the 1811 great meteorite attests to the bottles having been corked after 1811. (*C* and *D*) Courtesy of Visit Åland.

after 1841, but the Juglar bottles must have been produced before 1832, when the house was renamed Jacquesson. Interestingly, bottles of beer were also found in the same shipwreck that did not bring any clue as to either the destination or the age of the champagne bottles (6). Therefore, various questions remain, the answers to which could shed light not only on the history of the commerce of champagne in the beginning of the 19th century but also on the winemaking practices of the time.

Archaeochemistry, as the application of the most recent analytical techniques to ancient samples, now provides an unprecedented understanding of human culture throughout history (1–3). Indeed, these methods recently demonstrated the introduction of viniculture in France (7) and have identified the earliest known winemaking activities as having occurred in the Middle East, thousands of years B.C.E. Similarly, a combination of various chromatographic techniques and antioxidant activity measurements applied to 600-y-old fermented fruit juice found in amphorae from the vestiges of a ship discovered off the Sicilian coast in 2000 (8) demonstrated that under such storage conditions, this juice retained significant health-protecting properties. The application of a combination of targeted and nontargeted modern chemical analytical approaches to such historical samples can now provide unique characterization in terms of chemical diversity and molecular resolution, therefore elucidating various aspects of the winemaking practices of their time.

Using such an approach, three of the "Baltic champagne" samples (named A11, A33, and B17) were tasted and subsequently analyzed in comparison with three modern champagne samples from VCP (see *Materials and Methods* and Table S1). Various straightforward observations based on expert sensory analyses confirmed that these Baltic samples exhibited characteristics of very old champagnes. Furthermore, elemental analyses, alongside metabolomics (9, 10) and aroma analyses (11), provided a significant amount of data, which, once compiled and analyzed, shed light on the champagne making process used in the 19th century and defined its most characteristic steps (12).

For instance, the Baltic champagne samples displayed unusually high metallic cation concentrations. Iron and copper reached unexpectedly high levels compared with those commonly found in modern champagne samples. Iron levels in the Baltic champagnes reached up to 13–118 mg/L compared with 1–4.6 mg/L in modern samples, and copper levels attained 100–1,400 μg/L in the recovered champagnes vs. 27–78 μg/L in the modern analogs (Table S2). At the estimated time of production of these ∼170-y-old bottles of champagne, the use of proper picking and pressing methods (13) ensured high-quality musts (the extracted grape juice) that would allow for the subsequent production of light and delicate still white wines to be converted into champagne during the second fermentation (12). An example of an important practice to ensure this quality is the fractionation of the juice during pressing of the grapes between the *cuvée*, considered to be the finest fraction, and the following *taille*. Compared with the *cuvée*, the *taille* is characterized by a lower total acidity and increased pH as well as mineral and phenolic concentrations (14, 15). Therefore, one could speculate that the high concentrations in metallic cations in the Baltic champagnes may be the result of the use of a significant proportion of *taille* having been incorporated into the blend. However, such concentrations could just as easily be explained by a combination of increased cation extraction from the grapes (16) together with the use of metal-containing vessels during the winemaking process. Indeed, consistent with our knowledge of the pinot noir phenology at the time (17), the smaller berries harvested in the 19th century would have exhibited a higher skin-to-pulp mass ratio, hence a higher ratio of dry matter to grape juice volume (18), and thus a greater concentration of both endogenous cations extracted from the skin and exogenous cations from treatment residues present on the grape skin. In fact, although the term "bouillie bordelaise" or "Bordeaux mixture" was probably coined later (around 1883–1885), copper sulfate was already used at the time to protect the grapevine against fungal pathogens. Furthermore, the use of iron nails in the assembly of barrels and iron rods to hold sulfur wicks during the sulfurization of these barrels could most certainly have contributed to the increased iron concentration, since these iron elements would easily have been oxidized during winemaking and/or attacked by sulfuric acid-containing vapors.

Following the harvesting and pressing of the grapes, the must would have undergone its first alcoholic fermentation (AF). Historical records indicate that in the late 1830s, this step took place later in the year than it does now, hence under colder temperatures, and it was carried out by native yeast (17), which, it is reasonable to assume, would have been less efficient than modern selected yeast. Therefore, incomplete AFs may have been a common occurrence (17). Furthermore, one could suppose that the slightly colder climate of the 19th century would have retarded grape maturation, leading to an overall reduced sugar content than is seen today. All of these elements are likely to have contributed to the significantly lower alcohol level of the Baltic specimens (9.34–9.84% alcohol by volume) compared with the modern ones (12.33% alcohol by volume) (Tables S1 and S3) as determined by conventional analyses and mouthfeel, and further confirmed by NMR. Moreover, the low alcohol content would suggest that these wines did not undergo chaptalization, that is, the addition of sugar to must to increase the alcohol level of the finished wine. Nonetheless, it is difficult to ascertain this point, as the Baltic champagnes may have undergone a subsequent dilution when the *liqueur d'expédition* was added, as is later discussed herein. Finally, wood markers such as 5-carboxyvanillic acid and

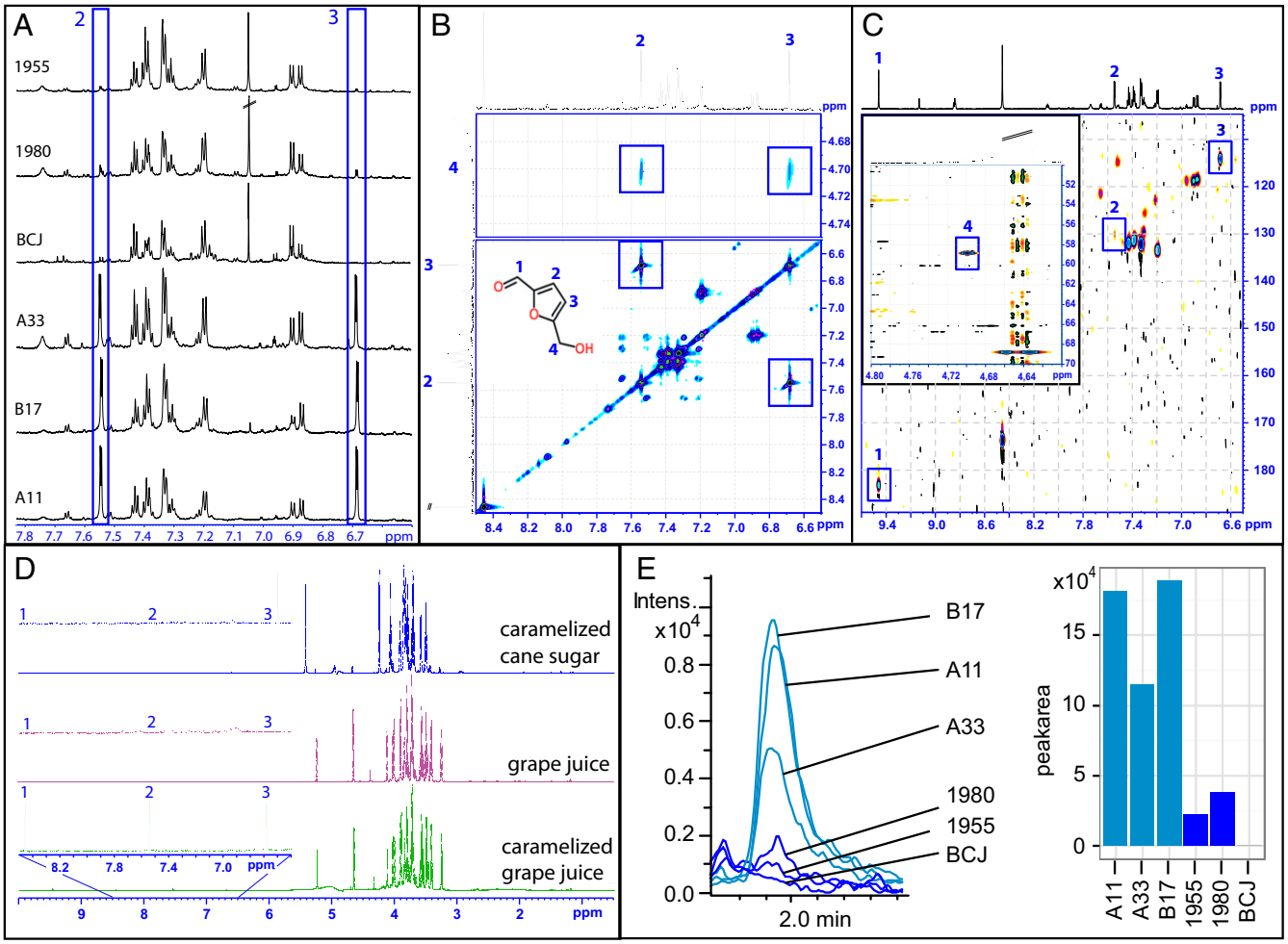

Fig. 2. Characterization of 5-hydroxymethylfurfural (HMF) in the Baltic Sea champagne samples. (*A*) ^{1}H NMR spectra (spectral region 7.8–6.6 ppm) illustrating the presence or absence of HMF in the six different champagne samples (blue boxes). (*B*) Molecular structure of HMF and carbon annotation. (*B and C*) Identification of HMF via (*B*) 2D-^{1}H-^{1}H DIPSI and (*C*) 2D-^{1}H-^{13}C-NMR (blue boxes) (*D*) Determination of the possible origin of HMF in the Baltic champagne samples. Absence of HMF (with focus on carbons 1, 2, and 3) in caramelized cane sugar and unheated grape juice vs. presence of HMF in heat-treated grape juice. (*E*) Quantification of HMF by UPLC-MS. High-resolution–extracted ion chromatogram of mass 125.024418 ± 0. 005 Da corresponding to [M-H]– ion of hydroxymethylfurfural in different champagne samples analyzed under reversed-phase conditions and peak area of the chromatographic peak of hydroxymethylfurfural.

castalin (a wood ellagitannin) (19) were systematically found in the Baltic samples using Fourier transform ion cyclotron resonance/mass spectrometry (FTICR/MS) (Fig. S1) and suggest that AF took place in barrels.

Another striking difference between the Baltic and modern champagne specimens can be seen in their Na^{+}, Cl^{-}, and Br^{-} concentrations. In fact, with 0.4–1 g/L of Na^{+}, 0.92–1.5 g/L of Cl^{-}, and 2–4 mg/L of Br^{-}, the Baltic samples displayed significantly higher concentrations than their modern analogs that contained 8–12 mg/L of Na^{+}, 6–12 mg/L of Cl^{-}, and no detectable Br^{-} (Table S2). Given the circumstances, seawater contamination was initially suspected but immediately ruled out. In fact, the A33 sample, which was identified as contaminated by seawater upon tasting (Table S2), provided standard values for some major sea-derived compound ratios in the case of contamination by the Baltic Sea, the composition of which is known to be stable throughout open seas and oceans. Consequently, if all of the samples had been contaminated, one would have expected to find the same relative proportion of Cl and Br in A11 and B17 as in A33. However, the Cl/Br ratios for the former samples, respectively 444 and 240, were significantly different from that of A33 (Cl/Br = 645) (see Table S2 for further discussion). The aforementioned high sodium and chlorine concentrations in the champagne samples from the Baltic Sea are thus more likely to be related to enological practices used at the time of their production. Wine clarification to control colloidal instability can be carried out using fining agents such as sodium chloride or sodium chloride-containing gelatin. Although VCP's archives do not report the use of sodium chloride as a clarifying agent, Madame Clicquot's correspondence does mention the use of gelatin, the preparation of which required sodium chloride to facilitate protein dissolution (20). Moreover, Etienne (17) states that, at the time, clarification used to be done two or three times over the course of the winemaking process, hence possibly contributing to an accumulation of Na^{+} and Cl^{-} in the Baltic samples.

Today, tartaric stabilization of champagne (to avoid precipitation of tartaric acid salts inside the bottle) is done after blending by chilling the wine to −4 °C (21). Based on historical records, including the writings of Dom Pérignon, we know that the practice of blending wines, that is, the mixing of wines from different grape varieties, origins, and years (*assemblage*), which is essential to maintaining the quality and style of each house, predates the 19th century. Even so, it is likely that at the time the Baltic samples were produced, tartaric acid salt stabilization occurred naturally during winter and thus before blending, which normally takes place just before spring. Indeed, the similarity

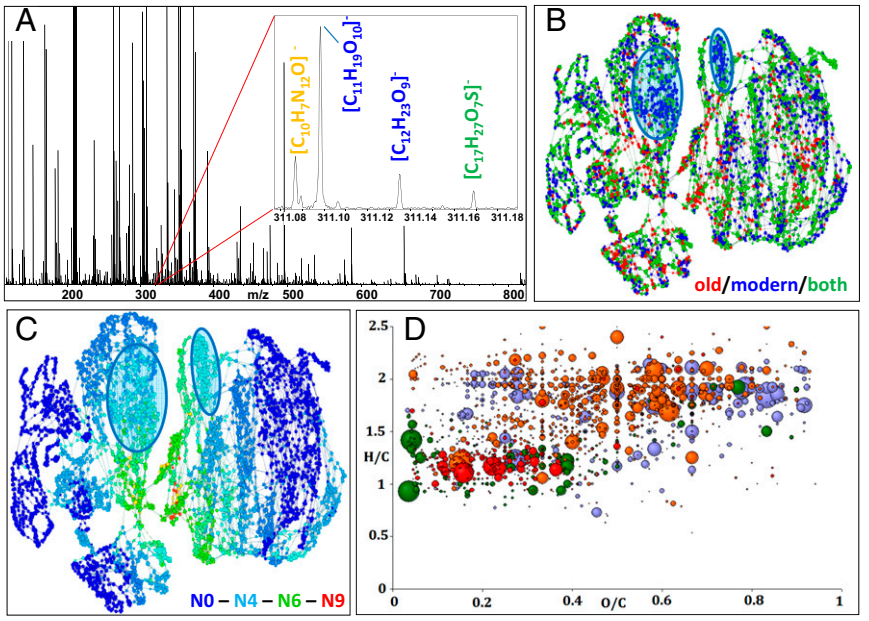

Fig. 3. Compositional space of champagne samples as analyzed with FTICR/MS. (*A*) Mass spectrum of sample A33 in the *m/z* range from 150 to 800; 10-millimass-range spectrum details (331.08–311.18) with elemental composition assignment are shown. (*B*) Compositional similarity network (CSN) of all 4,196 features highlighting the *m/z* specific of old (Baltic Sea) (red) and modern (blue) champagne samples; two zones specific to the modern champagne specimens are highlighted in blue. (*C*) Nitrogen compositional space showing the distribution of compounds according to the number of nitrogen atoms they contain, ranging from none (N0, dark blue) to 9 (color gradient). (*D*) Typical electrospray ionization (+)-FTICR/MS van Krevelen diagram of sample A33 with CHO (blue), CHNO (orange), CHOS (green), and CHNOS (red) compounds; circle size is proportional to signal intensity.

between the Baltic and modern champagne samples regarding the average concentrations of the two cations central to tartaric acid salt precipitation, Ca^{2+} and K^{+} (Table S2), indicates that the Baltic samples did undergo proper tartaric stabilization. It is thus reasonable to assume that the wine experienced a slow cold precipitation while stored in wooden barrels exposed to naturally low wintertime temperatures. Tartaric acid salt precipitates were most likely removed during subsequent racking and filtration operations. This would explain the slight haze observed inside the bottles but the lack of proper precipitates even after the 170 y that this wine remained under the sea. By the beginning of spring, increasing temperatures would have allowed for completion of AF, in cases where it had been stopped prematurely by the winter cold. Concomitantly with the end of AF, uncontrolled malolactic fermentation (MLF) might have occurred in the barrels. MLF consists of a biological deacidification of wines resulting in the transformation of sharp, green-tasting malic acid into the softer lactic acid. Nowadays, this process usually occurs after AF and is closely monitored to complete malic acid consumption by the lactic acid bacteria *Oenococcus oeni* (21), as illustrated by the NMR measurements obtained for the modern samples (Table S3). Conversely, champagne samples from the Baltic Sea exhibited high malic acid contents with malic acid/lactic acid ratios in the range of 0.46–0.81. These results underscore the fact that, although now widely used in champagne production, MLF was left uncontrolled during the 19th century, leading to partial MLF probably occurring in the bottle.

According to VCP historical archives, the second AF (or *prise de mousse*) took place in April, in capped bottles, after the addition of sugar in the *liqueur de tirage* (the use of this name came into practice by the middle of the century) and carried out by indigenous yeast, since the use of prepared cultures was not introduced until the end of the 19th century. During the second AF, carbon dioxide is produced, leading to the effervescence characteristic of champagne. In the early 1840s, the second AF would have enabled the production and dissolution of about 10 g of CO_2 per liter of wine. Nevertheless, cork being a porous material, over 170 y, the CO_2 slowly diffused (along its inverse pressure gradient) out of the bottleneck and into the sea, so much so that even with undamaged corks, the retrieved bottles contained far less dissolved CO_2 than the characteristic 10 g/L of their youth. Expert tasters noticed that, whereas no bubbles were observed upon pouring, a slight tingling effect was felt upon tasting. This is a clear indication that dissolved CO_2 fell below the critical concentration required to enable heterogeneous bubble nucleation (on the order of 2.5 g/L at 10 °C) (22) but nevertheless still exceeded the several tenths of a milligram per liter needed to stimulate both trigeminal receptors and gustatory receptors, via the conversion of dissolved CO_2 into carbonic acid (23).

Possibly the most striking feature of the Baltic champagne samples is their extraordinarily high sugar content (over 140 g/L) (Table S1), when nowadays the amount of sugar added before corking via the *liqueur d'expédition* (a mixture of mature wine, sugar, and antioxidants) generally varies from none at all for some *extra brut* wines to 50 g/L for *demi-sec*, with the *champagne doux* (greater than 50 g/L) having been more or less abandoned. From the location of the shipwreck, one is naturally tempted to suggest that the champagne bottles from the Baltic Sea were being shipped to Russia. However, the *dosage* (amount of added sugar) usually practiced by VCP for Russia in the middle of the 19th century was considerably higher, with levels approaching 300 g/L, and thus warranting the creation of a specific category of champagne known as *Champagne à la Russe*. According to VCP's archives, a *dosage* of around 150 g/L was desired by the French and the German markets, while British and Americans preferred a lower sugar content (22–66 g/L).

The *liqueur d'expédition* can be prepared from sugar beet, sugar cane, or grape syrup. Thanks to her correspondence, we know that Madame Clicquot was reluctant to use sugar beets for the preparation of her *liqueur d'expédition*, and recommended sugar cane instead (24). Interestingly, the NMR profile of the Baltic samples reveals the presence of pentoses such as ribose,

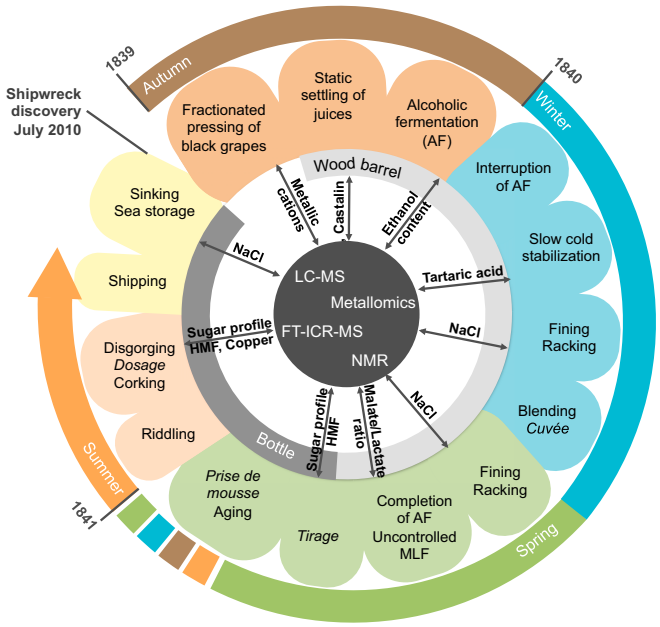

Fig. 4. Representation of the putative champagne elaboration process in place at the beginning of the 19th century.

which were also seen in grape juice but neither in cane syrup nor in modern champagne specimens. This observation suggests that grape syrup may have been used for the liquor preparation, albeit possibly in combination with sugar cane syrup (Fig. S2).

Nevertheless, the question of how winemakers could achieve such high sugar concentrations remains. In the 19th century and for the aforementioned reasons, it is unlikely that champagne grapes reached a sugar content of more than 180 g per liter of juice; thus the addition of a disproportionate volume of this grape juice to the wine would have been necessary to reach the final sugar concentration of around 150 g/L observed in the Baltic samples. Although it is difficult to evaluate to what extent, such an addition would have undoubtedly caused a significant dilution of the alcohol content of the wine, hence concealing a potential earlier chaptalization of the must. In addition, the Baltic champagne samples exhibited relatively high concentrations of furfural derivatives such as 5-hydroxymethylfurfural (5-[hydroxymethyl]-2-furaldehyde) (25), which originates from fructose during the caramelization that occurs via Maillard reactions in acidic conditions (Fig. 2). Similarly, the presence of difructose dianhydrides, which are key markers of caramelization processes, was proven by their detection as chlorine adducts by FTICR/MS (26, 27) (Fig. S3). Consequently, the evidence seems to point toward the use of grape juice concentrated by heating to produce grape syrup with over 700 g of sugar per liter as the base for the *liqueur d'expédition*. This hypothesis was nicely confirmed by the detection of the same furfural derivatives in heated grape juice, but not in heated cane syrup or unheated grape juice (Fig. 2 and Table S3).

FTICR/MS further provided comprehensive chemical signatures for each of the samples, ancient and modern. The Baltic champagne fingerprints were typical of wines that have undergone slow molecular diagenesis over time, with molecular masses that are, on average, significantly elevated and a lower chemical diversity. Indeed, the Baltic champagne specimens seem to have undergone a greater loss of peptidic fractions, as illustrated in the compositional networks by an overall lower number of nitrogen-containing compounds compared with the modern samples (Fig. 3 and Figs. S4 and S5).

In today's context of growing concerns about food safety, wine producers take particular care to ensure that their wines are free of any bacterial contamination, through the use of antimicrobial compounds such as sulfites and good hygiene practices. We are often led to believe that hygiene is a modern concept, so it was inspiring to realize that the 170-y-old champagne samples presented very low concentrations of acetic acid, a sign of wine spoilage (21). Acetic acid levels were similar to those found in the modern specimens (Tables S1 and S3). Moreover, the concentrations of anions such as SO_4^{2-} were also similar between the Baltic and modern champagnes. These anions are oxidation products of sulfites (Table S2), and the similar levels attest to the likelihood of microbiological stabilization already being in use in the 19th century.

In addition to the scientific and historical value of the chemical analysis of these ancient samples, there remains also the crucial question of their taste. Here we attempted to compare the information collected during expert tastings with the analytical data. Of the 40 aromas identified by GC/MS, 26 of these compounds showed concentrations higher than their known detection thresholds (Tables S4 and S5), and the correlation between the compiled descriptors and the aromas considered detectable is compelling.

At first, the Baltic samples were described using terms such as "animal notes," "wet hair," "reduction," and sometimes "cheesy." Animal notes are unequivocally related to the presence of volatile phenols, particularly 3-ethyl- and 3-propyl-phenol as identified by GC/MS. FTICR/MS analyses also revealed the presence of *p*-coumaroyltartaric acid and feruloyltartaric acid (Fig. S6), the respective precursors of 4-vinylphenol, which is responsible for empyreumatic notes, and 4-ethylphenol, which would contribute to the animal notes (28). Production of volatile phenols is classically attributed to *Brettanomyces* contamination (28). However, acid phenol decarboxylases and the corresponding vinylphenol reductases involved in volatile phenol biogenesis could also derive from *Saccharomyces cerevisiae* and lactic acid bacterial activity, albeit limited, resulting in the significant production of these compounds over time. "Reduction" and "wet hair" descriptors were to be expected for a wine that had spent such a long time sheltered from any oxygen source, and they were justified by the presence of light sulfurous compounds such as hydrogen sulfide, methanethiol, and dimethyldisulfide (29). Finally, the term

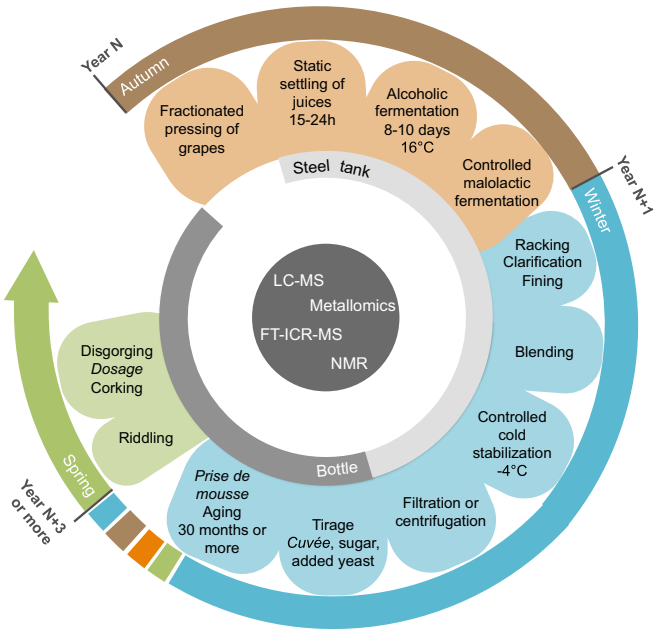

Fig. 5. Representation of the modern champagne-making process.

"cheesy" is related to butanoic and octanoic acids (21) as well as, to a lesser extent, to isoamyl lactate (30) (creamy character), all present in the tasted wine, the latter being a sign of an incomplete malolactic fermentation having taken place inside the bottle. Upon swirling the wine in the glass to oxygenate it, the aroma became far more pleasant, with the main aromas described as empyreumatic, grilled, spicy, smoky, and leathery, together with fruity and floral notes. The ferulic acid released after hydrolysis of the corresponding ester (see above) would most likely have generated 4-vinylguaiacol and 4-ethylguaiacol, which are indeed described as spicy, smoky, and grilled (28). The "fruity" character of the Baltic champagne samples can unambiguously be attributed to the presence of some ethyl esters of fatty acids (31) (ethyl hexanoate/octanoate), which are the main aroma compounds derived from fermentation in white wines, but also to isoamyl acetate (30) and diethyl succinate (32), while ethyl dihydrocinnamate (33), octan-1-ol (21), and 2-phenylethanol (34) are clearly responsible for the floral notes. Finally, *cis*-oak and *trans*-oak lactones were also detected, and their presence further confirms the use of wooden barrels in the production of the Baltic Sea champagnes.

Owing to molecular insights acquired by comparing the Baltic champagnes to modern ones, we managed to decipher a clearer view of the winemaking practices used in Champagne at the beginning of the 19th century (Fig. 4) and to compare it to the modern winemaking process (Fig. 5). In addition, our nontargeted enolomic analysis shows a good conservation of the organoleptic quality of the champagne over almost two centuries, raising the question of the intrinsic qualities of a vibration-free and isothermal marine environment for long-term wine conservation.

Materials and Methods

Six different champagne samples were used for this set of experiments: three discovered in the Baltic Sea (A11, A33, and B17) and three modern ones dating from 1980 (1980), 1955 (1955), and 2011 (BCJ) and provided by the Veuve Clicquot Ponsardin (VCP) house. A11 and A33 were two VCP champagnes, with the latter being contaminated by seawater, whereas the B17 sample was a Juglar champagne. Quantification of elements was done using inductively coupled plasma atomic emission spectroscopy (ICP-AES). In case of elements close to- or below their detection limit, samples were reanalyzed by inductively coupled plasma sector field mass spectrometry (ICP-sf-MS). Metabolomics data were obtained using FTICR/MS (9, 10). From the resulting datasets, up to thousands of formulas containing C, H, O, N, P, and S elements were calculated and then represented using 2D van Krevelen diagram projections, which sort them along two axes according, for instance, to H/C and O/C atomic ratios. Metabolites were also separated and analyzed using reversed phase (RP) and hydrophilic interaction (HILIC)–ultrahigh-performance liquid chromatography coupled to quadrupole time of flight mass spectrometry (RP- and HILIC-UPLC-Q-ToF-MS) (10). For a quantitative nontargeted overview of abundant metabolites, one-dimensional and multidimensional NMR spectra were generated. One-dimensional spectra (^{1}H) served for quantitative comparison of metabolites between the samples, and 2D experiments [^{1}H-^{1}H-DIPSI (decoupling in the presence of scalar interactions), ^{1}H-^{1}H-J-resolved, ^{1}H-^{13}C-heteronuclear single quantum coherence, ^{1}H-^{13}C-heteronuclear multiple bond correlation] helped with metabolite identification. Aroma analyses were performed through stir bar sorptive extraction-liquid desorption-gas chromatography-mass spectrometry (SBSE-LD-GC-MS) (11, 35–37).

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Supporting Information

Jeandet et al. 10.1073/pnas.1500783112

SI Materials and Methods

Champagne Sampling. Six different wines were used for this set of experiments: three old champagnes discovered in Baltic Sea (A11, A33, and B17) and three modern champagnes from 1980 (1980), 1955 (1955), and 2011 (BCJ) from VCP House. A11 and A33 were two VCP champagnes, with the latter contaminated by seawater. B17 was a champagne from the producer Juglar. Data concerning those champagnes (Table S1) were obtained according to current enological analyses by VCP.

Elemental Analysis. For the analysis, 250-μL aliquots of the samples were introduced into quartz vessels, and 1 mL of suprapure, subboiling distilled HNO_3 (Merck) was added. The vessels were closed and introduced into a pressure digestion system (Seif) for 10 h at 170 °C. The resulting clear solution was diluted to exactly 5 mL with Milli-Q H_2O and was then ready for element determination. Since only a small sample volume was available, the resulting 1:20 dilution of the samples (250 /5,000 μL) provided enough sample volume for measurements and the digestion eliminated matrix effects and sensitivity changes due to ethanol from samples.

ICP-AES. An ICP-AES SPECTRO CIROS Vision system (SPECTRO Analytical Instruments GmbH & Co. KG) was used for a complete elemental screening. The measured spectral lines were: Al, 167.078 nm; As, 189.042 nm; B, 249.773 nm; Ba, 455.404 nm; Bi, 223.061 nm; Ca, 396.847 nm; Cd, 214.438 nm; Co, 228.616 nm; Cr, 267.716 nm; Cu, 324.754 nm; Fe, 259.941 nm; Hg, 184.950 nm; K, 766.490 nm; Mg, 279.553 nm; Mn, 257.611 nm; Mo, 202.030 nm; Na, 589.592 nm; Ni, 231.604 nm; P, 177.495 nm; Pb, 220.353 nm; S, 180.731 nm; Sb, 206.833 nm; Se, 196.090 nm; Sn, 189.991 nm; Sr, 407,771 nm; V, 292.464 nm; W, 207.922 nm; and Zn, 213.856 nm. Samples were introduced using a peristaltic pump equipped with an antipulse head (SPETEC), connected to a Meinhard nebulizer with a cyclon spray chamber.

The RF power was set to 1,000 W, and the plasma gas flow rate was 15 L Ar/min, whereas the nebulizer gas flow rate was 0.6 L Ar/min.

ICP-sf-MS. In cases where element analysis by ICP-AES resulted in readings close to or below the detection limit, samples were reanalyzed by an ELEMENT 2, ICP-sf-MS instrument (Thermo). Analyzed element isotopes were ^{111}Cd, ^{208}Pb, ^{202}Hg, ^{95}Mo, ^{121}Sb, ^{182}W in low-resolution mode, ^{59}Co, ^{52}Cr, ^{63}Cu, ^{56}Fe, ^{55}Mn, ^{60}Ni, ^{64}Zn in medium-resolution mode, ^{75}As, ^{77}Se, and ^{78}Se in high-resolution mode. Samples were introduced using a peristaltic pump equipped with an antipulse head (SPETEC), connected to a Meinhard nebulizer with a cyclon spray chamber. The RF power was set to 1,200 W, and the plasma gas flow rate was 15 L Ar/min, whereas the nebulizer gas flow rate was 0.9 L Ar/min.

Quality Control for ICP-AES and ICP-sf-MS. Every 10 measurements, three blank samples and a control sample of certified standards (SPEX CertiPrep) for all mentioned elements were run. Results were calculated on a computerized laboratory data management system, relating the sample measurements to calibration curves, blank determinations, control standards, and the weight of the digested sample.

FTICR/MS. High-resolution mass spectra (1) were acquired on a Bruker (Bruker Daltonics GmbH) solariX FTICR/MS equipped with a 12-Tesla superconducting magnet (Magnex Scientific Inc.) and an APOLO II electrospray ionization (ESI) source (Bruker Daltonics GmbH) in negative and positive ionization modes. Spectra were first externally calibrated using arginine clusters (10 mg/L in methanol), and the accuracy was attained. Spectra were acquired with a time domain of four megawords and a mass range of *m/z* 100–1,000. Five hundred scans were accumulated in negative mode and 300 scans in positive mode for each sample. FTICR spectra were internally recalibrated to fatty acids with mass errors below 0.05 ppm and exported to peak lists at signal-to-noise ratio (S/N) of 4 and higher. After calibration and peak alignment, the *m/z* values were annotated with unambiguous elemental formulas by in-house written software based on exact mass difference of 0.1 ppm and considering the elements C, H, N, O, P, and S and their corresponding isotopes as described in ref. 2. It is noteworthy for readers not familiar with ultrahigh mass spectrometry that this exceptional mass accuracy and mass resolution allows assignment of the exact mass of molecular ions and the respective elemental composition directly out of mixtures with precision greater than the mass of an electron (which amounts to $9.10938215 \times 10^{-31}$ kg, or 1/1836.2 of the mass of a proton). Accordingly, two molecular compositions with mass differences smaller than that of a single electron mass can be differentiated, and elementary compositions can be conclusively assigned to their isotopologues. As many as thousands of such compositions containing C, H, O, N, and S elements can be calculated and then be represented using 2D van Krevelen diagrams, which sort them onto two axes according for instance to H/C and O/C atomic ratios (Fig. S5).

RP and HILIC-UPLC-Q-ToF-MS. Metabolites were separated using a Waters Acquity UPLC system coupled to a Bruker maXis UHR-ToF-MS. The RP method was used (1). Detection was carried out in negative ionization mode. Bruker raw data files were imported into Genedata Expressionist for MS 7.6 and noise substracted, aligned, and peak picked using three workflows to split workflow.

Hierarchical Clustering Analysis. Hierarchical clustering analysis (HCA) was performed on the normalized data using the Hierarchical Clustering Explorer 3.5 software. This method was able to group the samples into homogeneous and distinct clusters without imposing preliminary hypotheses on the data. Principal Component Analysis (PCA) also disclosed the principal groups of data. PCA is another unsupervised method with the capacity to reduce the complexity of a multivariate dataset. The masses discriminative for the two groups were calculated with an Orthogonal PLS model (OPLS). PCA and OPLS were performed with the SIMCA 13.0.3 software (Umetrics) for FTICR/MS data. Genedata Expressionist has been applied for MS Analyst 7.6 for LC-MS data. The 2D van Krevelen diagrams were constructed using compositional networks (based on elemental compositions) and functional networks based on selected functional group equivalents, enabling improved assignment option of elemental composition and classification of organic complexity with tunable validation windows.

Network Analysis of FTICR/MS Data. The matrix that resulted from the six unified mass spectra in ESI(-)-FTICR/MS was filtered for missingness (features with less than two nonzero intensities were excluded), which resulted in a matrix of 11,242 *m/z* signals. Mass defect filtering and correlation based deisotoping excluded 3,269 features. Network reconstruction was performed on the remaining 7,973 features, first with an edge error of 0.1 ppm and then with an edge error of 0.2 ppm (100 ppb and 200 ppb, respectively). Before network-based feature annotation as presented in ref. 2, all given

m/z signals were converted into the carbon, hydrogen, nitrogen, oxygen, phosphorus, sulfur space using the following parameters: C100, O80, N10, S3, P3, and ±0.5 ppm error, generating 12,915 sum formulas after filtering the Senior rules. Consequent isotopic pattern matching enabled the definition of six very high confidence formulas for the initiation of network annotation. Then 4,196 *m/z* features were annotated using 0.1-ppm edge error (Fig. 3).

CSN. The given formulas were deconvoluted into vectors that contained all element/C and element/H ratios for each formula (multiplex van Krevelen vectors). On the basis of these vectors, it was possible to construct CSNs.

The CSN was colored for nonsignificant features (green), specific modern champagne features (blue), and specific old champagne features (red). The network contains 4,196 nodes (*m/z* features) and 20,880 edges with a modularity of 0.91 (ranging from −1 and 1). This modularity score is high in comparison with other values in the literature (3, 4). This network comprises the framework for all network visualizations in Fig. 3 and Fig. S4.

Compound Class Assignment. The neutral formulas of the dataset were matched against compound databases. Only 8% of the features could be matched with KNAPSACK, which is in the range of usual annotations in our mass translator into pathways Kyoto Encyclopedia of Genes and Genomes-based database and which made a colored network diagram for annotation obsolete. To gain insights into possible ontological compound classes, another database was used [MeSH (www.ncbi.nlm.nih.gov/mesh)/IDEOM database (5)] including 18,159 formulas with known compound class distribution (72 classes). Each champagne formula was attributed to the most abundant compound class assignment of its compositionally most similar MeSH/IDEOM entry, and the node coloring was performed according to these compound classes. It can be seen in Fig. S4 that the compound class labels (colors) form a multitude of modules (clusters).

Compound Class Enrichment Analysis for Modern and Baltic Champagnes. Since it is tedious to present all interesting areas of the CSN, a compound class enrichment analysis for markers for modern and Baltic champagnes was performed. The enrichment analysis is performed assuming a discontinuous hypergeometric distribution, which is tested with the Fisher exact test.

Sample Preparation for NMR Analysis. An aliquot of 120 μL of each champagne was mixed with 60 μL D_2O buffer [90% D_2O, 500 mM PO_4, 0.1% trimethylsilyl-tetradeuteropropionic acid (TSP), pH 7.4] and transferred to 3-mm-outer-diameter NMR Bruker Match tubes (Hilgenberg GmbH).

NMR Analysis. NMR spectra were acquired on a Bruker 800-MHz spectrometer (Bruker Biospin) operating at 800.35 MHz with a quadruple inverse cryoprobe. For an overview of molecules present in the sample, a standard 1D pulse sequence [recycle delay (RD)-90°-*t1*-90°-*tm*-90°-acquire free induction decay (FID)] was acquired, with water suppression irradiation during RD of 2 s, mixing time (*tm*) set on 200 ms, and a 90° pulse set to 8 μs, collecting 1,024 scans into 64,000 data points with a spectral width of 12 ppm. A representative sample of one Baltic Sea champagne and one modern champagne underwent a series of 2D analyses [*J*-resolved (JRES), total correlation spectroscopy (TOCSY), and heteronuclear single quantum coherence (HSQC)] for detailed metabolite analysis and metabolite identification. The 1H NMR spectra were acquired with the pulse sequence [*d*1-90°-τ-180°-τ-acquire FID], with suppression of the water resonance during *d*1 (2 s) into 4,000 data points in F2 and 16 transients using 64 increments of τ; the spectral widths in F2 and F1 were 12 ppm and 0.08 ppm, respectively. For the 2D 1H-13C HSQC spectra, phase-sensitive ge-2D-HSQC using preservation of equivalent pathways and adiabatic pulses for inversion and refocusing with gradients were used. For each 2D spectrum, 4,096 × 2,048 data points were collected using 32 scans per increment, an acquisition time of 0.25 s, and 16 dummy scans. The spectral widths were set to 12 ppm and 230 ppm in the proton and carbon dimensions, respectively. For 2D 1H-1H TOCSY spectra, phase-sensitive sensitivity-improved 2D TOCSY with water suppression by gradient tailored excitation (3-9-19) and using DIPSI-2 were acquired. For each 2D spectrum, 19,228 × 1,024 data points were collected using 32 scans per increment, an acquisition time of 1 s, and 16 dummy scans. The spectral widths were set to 12 ppm in the F2 and F1 dimensions. Processing of the spectra was performed using TopSpin 3.2 (Bruker BioSpin). FIDs were multiplied by an exponential decaying function corresponding to a line broadening of 0.3 Hz before Fourier transformation. All spectra were manually phased, baseline corrected, and calibrated to TSP (δ 0.00). Data were imported to Matlab (Mathworks) and normalized to the TSP signal (area under the curve of TSP).

Quantification of Selected Metabolites. Each champagne sample was prepared and its spectra acquired under identical laboratory and experimental conditions. The spectra were normalized to the internal standard TSP. The area under the curve of the NMR peak from metabolites of interest was calculated in Matlab and stoichiometrically corrected by dividing by the number of protons that give rise to the peak. The obtained value is an arbitrary unit.

Aroma Analyses Through SBSE-LD-GC-MS. Volatile compounds were extracted by SBSE-LD (6). A stir bar (0.5 mm thick; 10 mm long) coated with a polydimethylsiloxane film (Twister; Gerstel) was immersed in 3 × 5 mL of a Baltic Sea champagne sample and stirred for 180 min at 1,250 rpm at room temperature. For reverse extraction purposes, the stir bars were rinsed with deionized water and gently dried, then placed into 250-μL glass flat-bottom inserts filled with 100 μL of acetonitrile inside a glass vial. The reverse extraction was performed by ultrasonic treatment for 30 min. Volatile compounds were analyzed with 1 μL of the extracted material in an Agilent 6890N gas chromatograph equipped with an Agilent 7683 automatic liquid sampler coupled to an Agilent 5975B inert mass selective detector (MSD) (Agilent Technologies). The gas chromatograph was fitted with a DB-Wax capillary column (60 m × 0.32 mm i.d. × 0.50 μm film thickness; J & W Scientific), and helium was used as carrier gas (1 mL/min constant flow). The GC oven temperature was programmed without initial hold time at a rate of 2.7 °C/min from 70 °C to 235 °C (hold 10 min). The injector was set to 250 °C and used in pulsed splitless mode (25 psi for 0.50 min). The temperatures of the interface, MS ion source, and quadrupole were 270 °C, 230 °C, and 150 °C, respectively. The mass spectrometer was operated in electron impact ionization mode (70 eV), and the masses were scanned over an *m/z* range of 29–300 atomic mass units. Agilent MSD chemStation software (G1701DA, Rev D.03.00) was used for instrument control and data processing. All compounds were identified by mass spectrometry data, their linear retention index (KOVATS indexes) (7–9), and/or comparison with a standard (Table S5).

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8. Pino JA, Tolle S, Gök R, Winterhalter P (2012) Characterisation of odour-active compounds in aged rum. *Food Chem* 132(3):1436–1444.
9. Csoka M, Amtmann M, Sardy DN, Kallay M, Korany K (2013) GC-MS description of the primary aroma structure of two Kadarka wines considered indigenous in Hungary. *J Appl Bot Food Qual* 86:104–112.

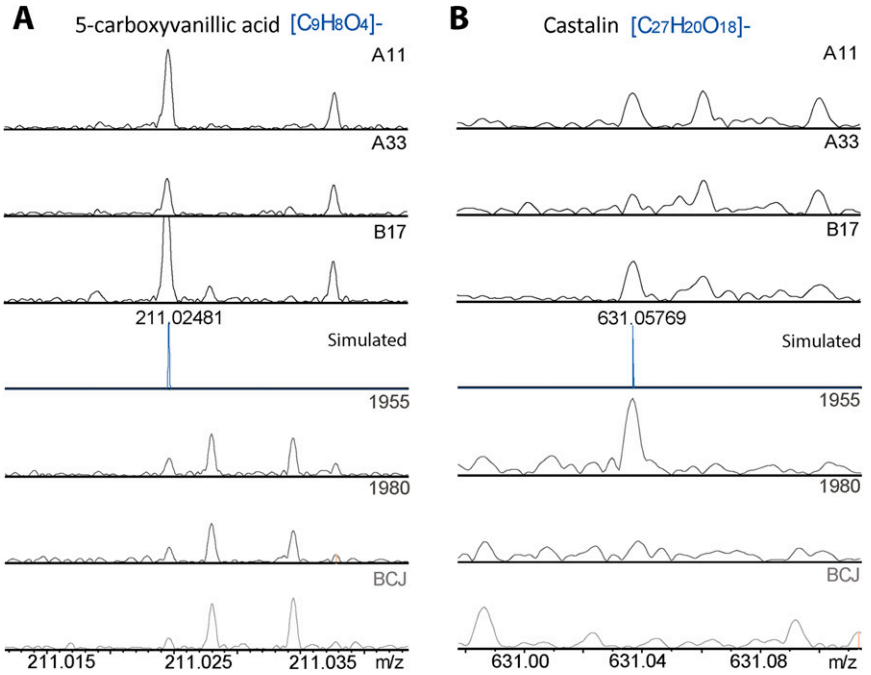

Fig. S1. FTICR/MS characterization of wood markers (5-carboxyvanilic acid and castalin). (*A*) Details of the (–) FTICR/MS mass spectra *m/z* 211 showing the presence of the *m/z* 211.02481 signal in all champagnes, assigned to $[C_9H_8O_4]^-$. (*B*) The *m/z* 631 showing the presence of the *m/z* 631.05769 signal in Baltic Sea and 1955 champagnes, assigned to $[C_{27}H_{20}O_{18}]^-$.

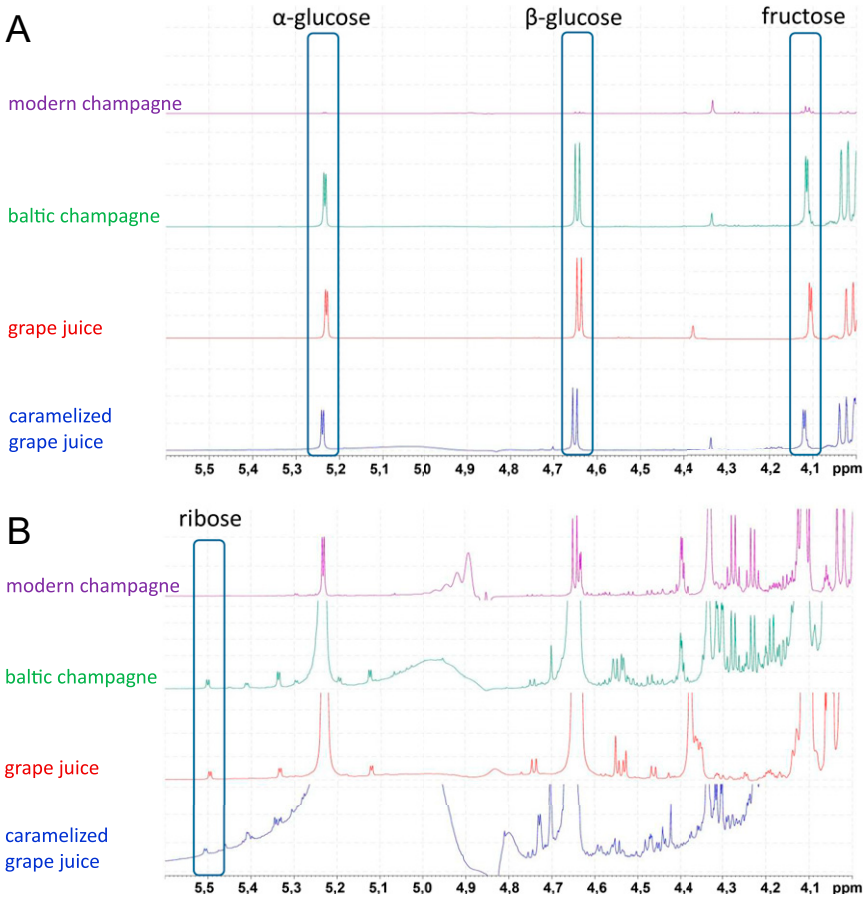

Fig. S2. The ^{1}H NMR spectroscopy-generated sugar profiles. (*A*) Profiles including α-glucose, β-glucose, and fructose in one representative sample each of modern champagne, Baltic Sea champagne, grape juice, and caramelized grape juice. (*B*) Profiles including ribose in one representative sample each of modern champagne, Baltic Sea champagne, grape juice, and caramelized grape juice. All sugars are present in Baltic Sea champagnes and both grape juices. Modern champagnes only contain very low amounts of glucose and fructose, and ribose was not detected.

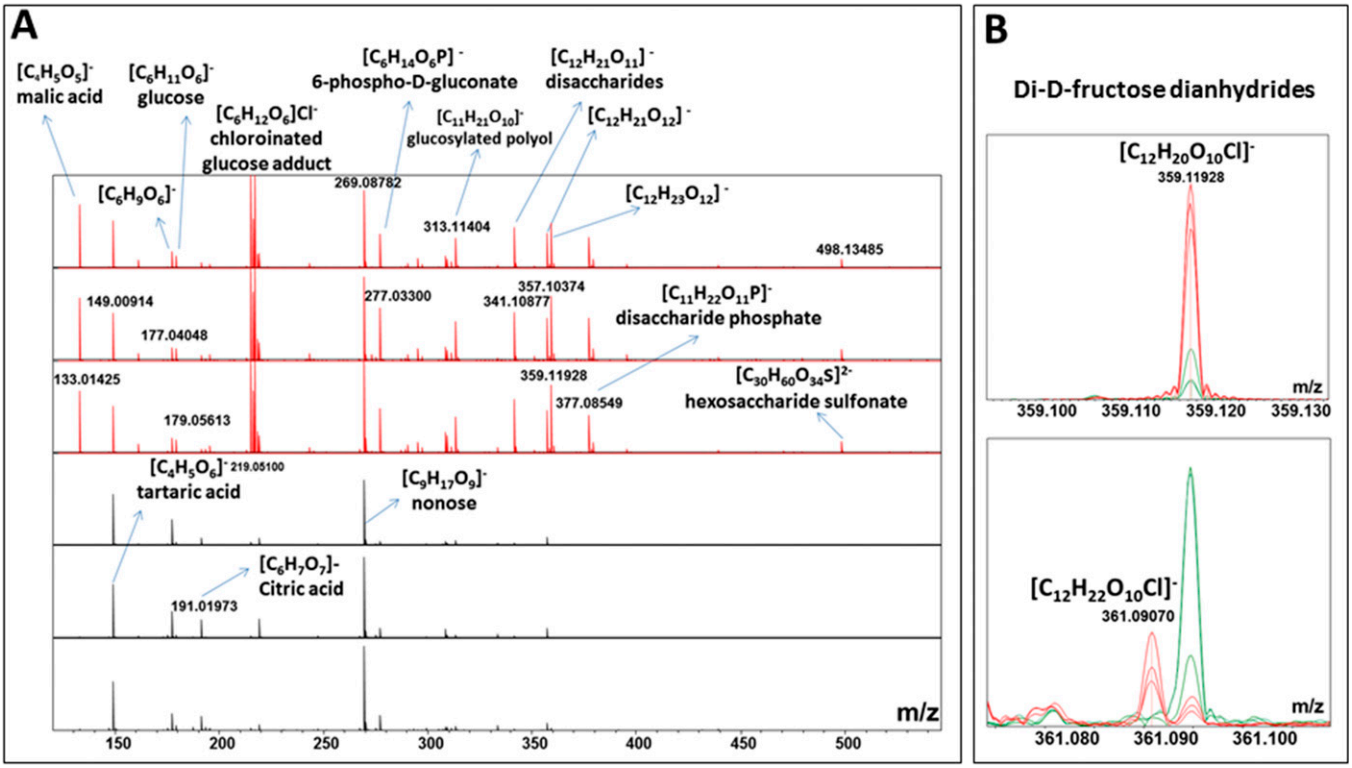

Fig. S3. Mass spectra of all champagnes. (*A*) In the *m/z* range from 120 to 550 normalized to tartaric acid signal intensity and showing the main signal differences. The most intense signals occur in Baltic Sea champagnes and can be assigned to malic acid, citric acid, tartaric acid, monosaccharides, and disaccharides; many of these signals correspond to chlorine adducts produced in the electrospray source due to the presence of chlorine ions. (*B*) In Baltic champagne, an increased intensity of chlorine adducts of Di-D-fructose dianhydrides $[C_{12}H_{20}O_{10}Cl]^-$ and $[C_{12}H_{22}O_{10}Cl]^-$ is noted. These are well-known markers of caramelization and thus give strong evidence of the addition of heated grape juice, in addition to the presence of HMF as shown in Fig. 2.

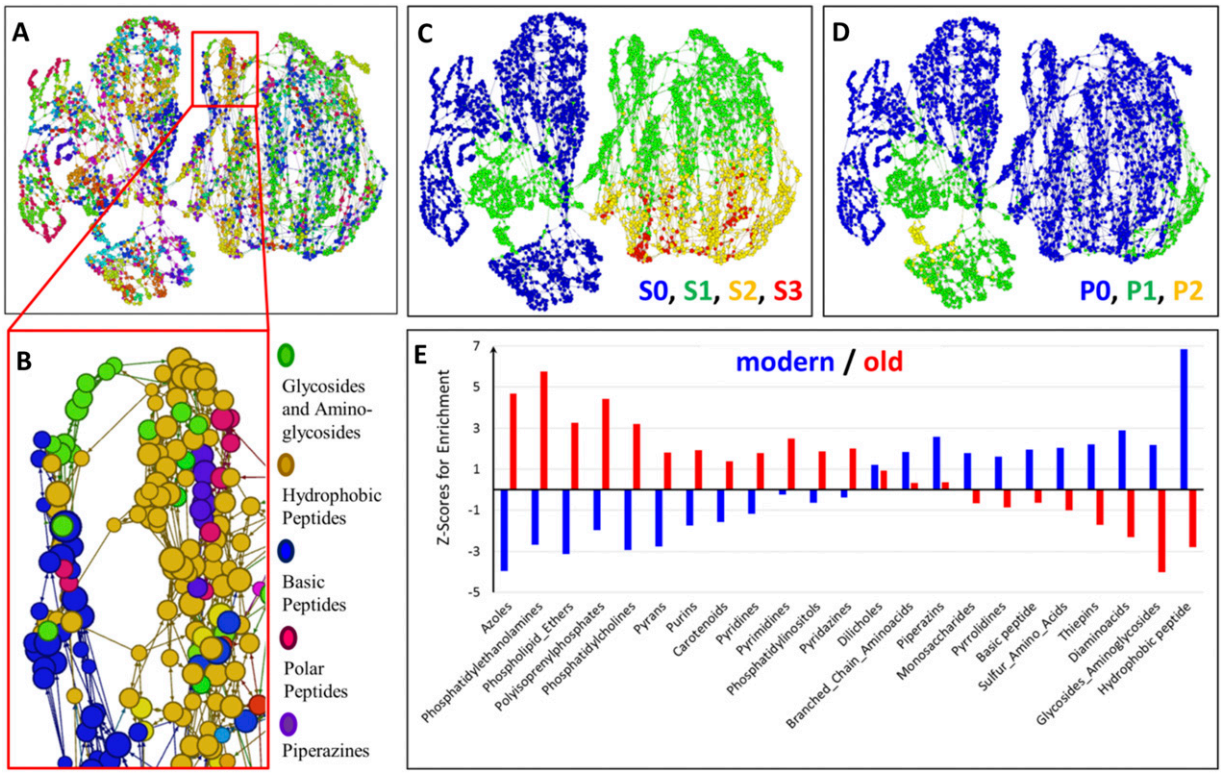

Fig. S4. Compositional network of 4,196 *m/z* features as obtained by FTICR/MS analysis of Baltic Sea and modern champagnes. (*A*) Color-differentiated representation of chemical classes highlighting, in *B*, one of the nitrogen-rich zones involving peptidic structures enriched in the modern champagne samples. (*C*) CHNO–sulfur compositional space. (*D*) CHNO–phosphorus compositional space. (*E*) Compound class enrichment analysis for modern and old champagnes showing the relative enriched structural characteristics in old (phosphorus) and modern champagnes (nitrogen compounds).

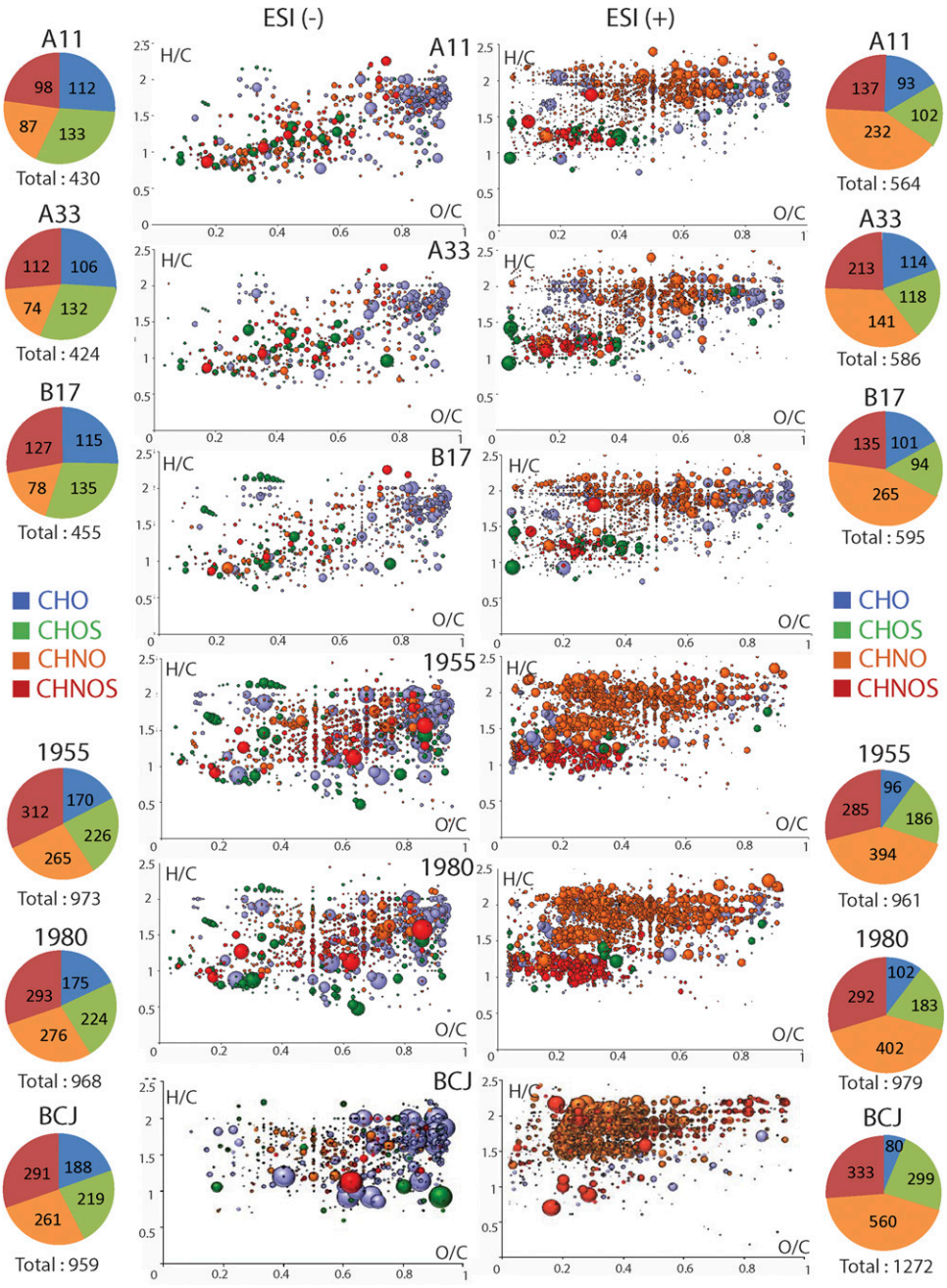

Fig. S5. Van Krevelen diagrams of the samples in negative and positive ionization modes showing the differences in relative amounts of CHO, CHOS, CHNO, and CHNOS molecules.

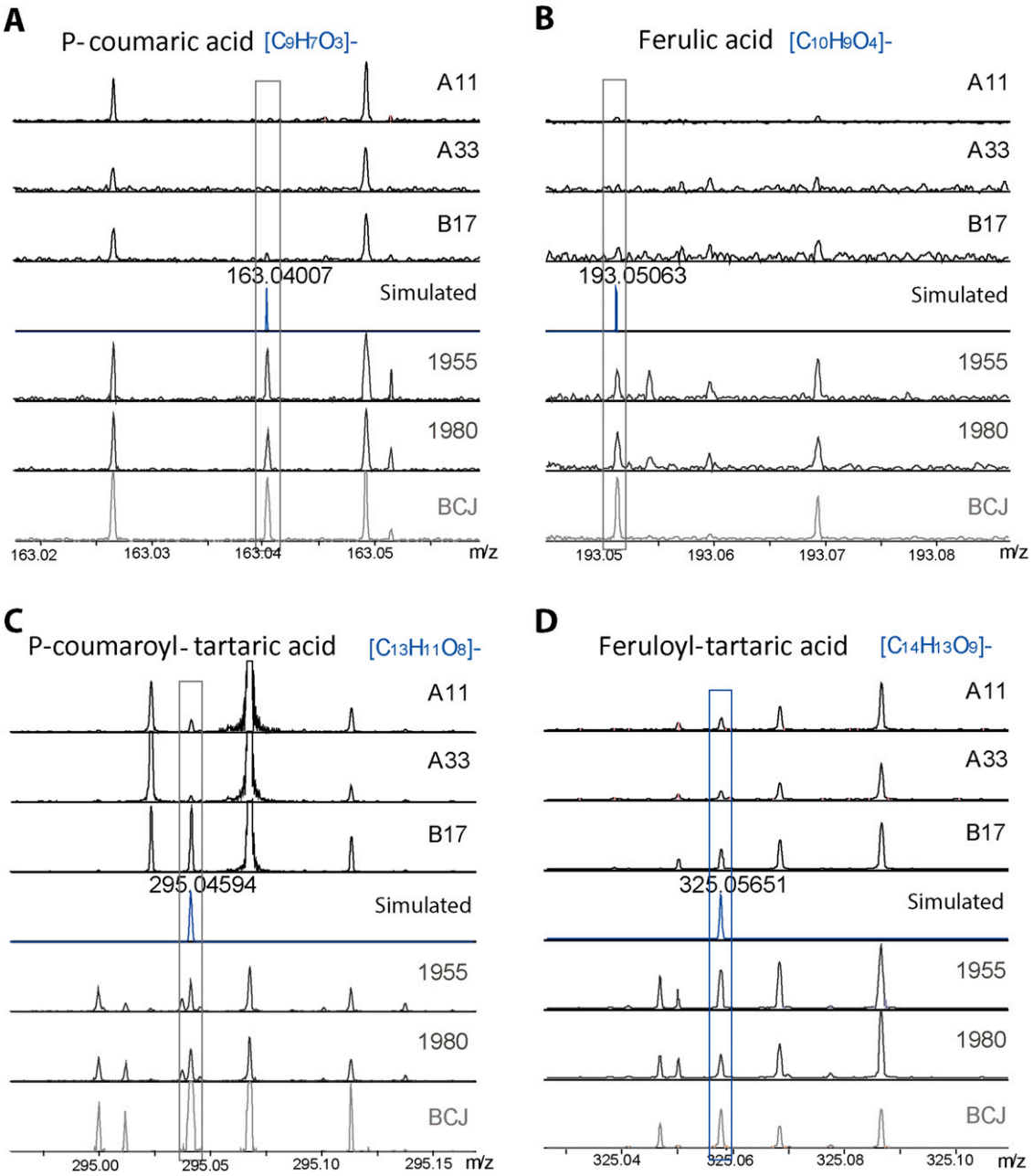

Fig. S6. FTICR/MS characterization of volatile phenol precursors. (*A*) Details of the mass spectra *m/z* 163 showing the presence of the *m/z* 163.04007 signal only in modern champagnes, assigned to $[C_9H_7O_3]^-$; (*B*) *m/z* 193 showing the presence of the *m/z* 193.05063 signal only in modern champagnes, assigned to $[C_{10}H_9O_4]^-$; (*C*) *m/z* 295 showing the presence of the *m/z* 295.04594 signal in all champagnes, assigned to $[C_{13}H_{11}O_6]^-$; and (*D*) *m/z* 325 showing the presence of the *m/z* 325.05651 signal in all champagnes, assigned to $[C_{14}H_{13}O_9]^-$.

Table S1. Current analyses of modern champagnes vs. champagnes from the Baltic Sea

Measured parameter	Modern champagnes	Champagnes from the Baltic Sea		
		A11	B17	A33
Alcohol, % V/V	12.33	9.34	9.84	9.40
Density at 20 °C	0.990	1.032	1.022	1.032
pH	3.03	3.16	3.16	3.14
Total acidity, g H_2SO_4/L	4.7	4.2	4.1	4.2
Acetic acid, g/L	0.23	0.41	0.54	0.38
Malic acid, g/L	0.1	1.6	1.3	1.6
Tartaric acid, g/L	3.5	1.8	1.3	1.8
Sugars, g/L	1.6	144	118	144
Gluconic acid, mg/L	60	156	110	155

Table S2. Elemental analyses of modern champagnes vs. champagnes from the Baltic Sea

	Modern champagnes			Champagnes from the Baltic Sea		
Element	BCJ	1980	1950	A11	B17	A33*
Al	726	1,220	1,120	2,870	2,550	3,710
B	2,630	2,820	3,310	2,250	2,530	3,380
Ba	36	45	65	71	170	149
Br	nd	nd	nd	4,020	2,080	2,390
Ca	83,000	58,200	57,200	58,400	63,700	85,700
Cl	6,030	7,200	12,500	966,000	925,000	1,540,000
Cu	27	40	78	100	303	1,430
Fe	1,100	3,630	4,630	118,000	84,200	13,300
K	297,000	390,000	505,000	359,000	280,000	489,000
Mg	64,600	70,300	58,400	79,900	84,300	129,000
Mn	743	1,070	847	922	1,100	1,370
Na	8,020	18,700	9,480	510,000	443,000	1,050,000
NO_3^-	1,550	2,160	10,400	7,200	10,600	513,000
P	118,000	110,000	73,000	92,200	82,600	113,000
PO_4^{3-}	263,000	237,000	168,000	150,000	142,000	210,000
S	104,000	120,000	168,000	88,800	77,600	165,000
SO_4^{2-}	130,000	166,000	315,000	131,000	228,000	189,000
Sr	217	292	261	537	545	845
Ti	25	101	24	16	271	256
V	19	54	19	22	48	94
Zn	574	989	721	422	1,020	944
Elements closed to their limits of detection						
As	82	83	82	207	416	415
Cd	5	8	8	10	26	39
Co	26	13	13	51	127	199
Cr	17	20	17	20	50	87
Hg	8	9	8	22	45	44
Li	6	8	4	11	29	41
Mo	24	16	12	47	116	225
Ni	16	21	17	21	53	81
Pb	27	67	575	554	357	508
Sn	38	38	38	96	193	193

Concentrations are given in micrograms per liter; nd, not detected. The striking differences in Cl and Na concentrations between Baltic champagnes and modern ones straightforwardly suggest some seawater contamination of the former ones. However, several arguments can be made against such a hypothesis, even for the A33 sample, which was considered to be contaminated given the salty flavor detected during tasting. Assuming that A33 was contaminated, the relative proportion of sea-derived compounds in this wine is an indication of expected concentrations resulting from contamination from the Baltic Sea, the composition of which is known to be consistent throughout open seas and oceans. If all of the samples were contaminated, the relative proportion of Cl and Br that was found in A33 (Cl/Br = 645) should be found in champagnes A11 and B17. However, the Cl/Br ratios for these two champagnes are 444 and 240, respectively, which are significantly different from that of A33. The same conclusion can be drawn from Cl/SO_4^{2-} ratios (around 8, 4, and 7 for A33, B17, and A11, respectively). Furthermore, a possible seawater contamination raises the question of diffusion, either through the cork or along defects at the cork/bottle interface, on the basis of the osmosis from high to low concentrated media. In the particular case of K, it is expected that the wine concentration is initially higher than the Baltic Sea one. Consequently, the K concentration of A33 should reflect an equilibrated concentration between the wine and the sea. In that case, the lower K concentrations for champagnes A11 and B17 are not consistent with seawater contamination, unless these three samples were stopped with corks having different permeability and selectivity. No literature data can be found to evaluate this hypothesis. Finally, the extremely high NO_3^- concentration of A33 is certainly the most difficult to explain without considering, indeed, some contamination of this particular bottle.

*Seawater-contaminated sample.

Table S3. Relative determinations of metabolites in champagnes by ^{1}H NMR spectroscopy

	Champagnes from the Baltic Sea			Modern champagnes			Average		
Metabolite*	A11	B17	A33	BCJ	1980	1955	Baltic champagnes	Modern champagnes	*t* test
Glucose	279.5	195.2	430.3	16.1	12.2	6.7	301.6	11.7	0.015
Fructose†	120.3	120.1	116.9	18.6	35.6	19.1	119.1	24.4	<0.001
Tartaric acid	7.6	5.5	9.6	13.3	12.9	15.3	7.6	13.8	0.011
Malic acid	10.8	7.8	13.8	1.3	2.2	2.3	10.8	2.0	0.007
Lactic acid	13.3	17.0	17.9	32.8	30.1	30.7	16.1	31.2	0.032
Acetic acid	3.30	3.96	3.66	1.96	4.26	4.44	3.6	3.6	0.919
Formic acid	0.6	0.3	0.7	0.1	0.2	0.1	0.5	0.1	0.022
Succinic acid	0.1	0.1	0.1	0.1	0.0	0.1	0.1	<0.1	0.823
Gallic acid	0.0	0.0	0.0	0.1	0.2	0.1	0.0	0.1	0.032
Ethanol	416.4	792.1	768.5	923.1	1137	804.7	659.0	955.1	0.13
2,3-Butanediol	3.6	2.7	3.0	4.0	5.7	6.2	3.1	5.3	0.038
Hydroxymethylfurfural	0.4	0.4	0.5	0.0	0.0	0.0	0.4	0.0	<0.001
Ratio malic acid/lactic acid	0.81	0.46	0.77	0.04	0.07	0.07	0.68	0.06	0.005
Glucose + fructose	399.8	315.3	547.2	34.7	47.8	25.8	420.8	36.1	0.005

*Values are based on area integration of ^{1}H NMR peaks, normalized to the added standard TSP and stoichiometrically corrected for the number of protons.
†The fructose signal at δ4.12 showed overlap with lactate (HO-CH-CH_3), and values might therefore be biased.

Table S4. Identified volatile compounds of Baltic champagnes showing concentrations higher than their detection threshold

Compounds	Mean, μg/L rel Kow	Error, %	Threshold, μg/L	Descriptors	Chemical family
Propylphenol	177,692	5	0.29*	animal note cowshed	volatile phenols
Isoamyl alcohol	176,192	3	30	alcoholic, malty, fusel	higher alcohols
2-Phenylethanol	21,309	3	60	rose, floral, honey	higher alcohols
Ethyl pentanoate	390	3	1.5	fruit, apple, floral	ethyl esters
5-Methylfurfural	1,771	4	16	caramel, spicy	Maillard product
Diethyl succinate	97,795	4	1,200	fruit, wine, wet	ester
Eugenol	277.25	4	5	clove, spicy, sweet	volatile phenols
Ethyl hexanoate	152.63	5	3	fruit, green apple, sweet	ethyl esters
Ethyl lactate	491,607	1	14,000	fruit, raspberry, perfume	ethyl esters
Butyric acid	5,244	8	173	rancid, cheesy, sweaty	medium-chain fatty acid
1-Hexanol	2,605	4	110	green, floral, resinous	alcohols
Ethyl dihydrocinnamate	32.85	2	1.60	floral, fruit, sweet	ester
1-Heptanol	58.80	5	3	green, fruit	alcohols
trans-Oak lactone	13,346	7	790	spicy, oak	lactone
Ethyl-2-hydroxy-2-methyl butanoate	11,326	8	1,000	floral, pineapple,	ethyl esters
Isoamyl lactate	1,701	6	200	unknown	esters
Isoamyl acetate	251	6	30	banana, fruit, sweet	esters
1-Octanol	782	2	110	green, floral, rose	alcohols
Hexanoic acid	2,219	44	420	cheesy, rancid, sweaty	medium-chain fatty acid
Ethyl octanoate	8.78	12	2	fruit, sweet, floral	ethyl esters
cis-Oak lactone	154.76	18	46	spicy, maple	lactone
Octanoic acid	1,587	12	500	fatty, rancid	medium chain fatty acid
Methyl eugenol	25.08	5	10	strawberry	volatile phenols
cis-3-Hexenol	134.59	10	70	green, grassy, fresh	alcohols
Eucalyptol	3.52	12	3.20	minty, sweet, spicy	terpenes
Isobutanol	15,566	48	1,000	wine, solvent	higher alcohol

All compounds were identified by Mass Spectrometry and Linear Retention Index (KOVATS) (see Table S5). rel Kow, related to the Kowats index.
*Thresholds are calculated in wine, except for water.

Table S5. KOVATS indexes and techniques used for aroma identification

Compounds	Experimental KOVATS indexes	KOVATS indexes range In the literature	Identification*
Propylphenol	2,240	2,251	MS, LRI
Isoamyl alcool	1,184	1,168–1,342	MS, LRI, std
2-Phenylethanol	2,176	1,915–2,262	MS, LRI, std
Ethylpentanoate	1,136	1,116–1,170	MS, LRI, std
5-Methylfurfural	1,375	1,366–1,411	MS, LRI
Diethylsuccinate	1,669	1,666–1,750	MS, LRI, std
Eugenol	2,151	1,994–2,237	MS, LRI, std
Ethyl lactate	1,317	1,311–1,374	MS, LRI, std
Butyric acid	1,816	1,853	MS, LRI, std
1-Hexanol	1,328	1,319–1,398	MS, LRI, std
Ethyldihydrocinnamate	1,880	1,353–1,908	MS, LRI, std
1-Heptanol	1,447	1,425–1,481	MS, LRI, std
trans-Oak lactone	1,887	1,862–1,987	MS, LRI
Ethyl-2-hydroxy-2-methyl butanoate	1,419	1,397–1,431	MS, LRI
Isoamyl lactate	1,563	1,555–1,619	MS, LRI, std
Isoamyl acetate	1,128	1,160–1,363	MS, LRI, std
1-Octanol	1,654	1,619–1,830	MS, LRI, std
Hexanoic acid	1,859	1,820–1,897	MS, LRI, std
Ethyl octanoate	1,432	1,392–1,497	MS, LRI, std
cis-Oak lactone	1,959	1,886–2,135	MS, LRI
Octanoic acid	2,060	2,001–2,120	MS, LRI, std
Methyl eugenol	2,002	1,923–2,099	MS, LRI
cis-3-Hexenol	1,365	1,320–1,415	MS, LRI
Eucalyptol	1,209	1,115–1,232	MS, LRI, std
Isobutanol	1,103	1,058–1,252	MS, LRI, std

*MS, LRI, identified on the basis of both mass spectral data and Linear Retention Index data (in-house database and refs. 7–9) and/or by comparison with an authentic standard (std).