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Analysis of Beers from an 1840s' Shipwreck

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ABSTRACT: Two bottles of beer from an about 170-year-old shipwreck (M1 Fö 403.3) near the Åland Islands in the Baltic Sea were analyzed. Hop components and their degradation compounds showed that the bottles contained two different beers, one more strongly hopped than the other. The hops used contained higher levels of β-acids than modern varieties and were added before the worts were boiled, converting α-acids to iso-α-acids and β-acids to hulupones. High levels of organic acids, carbonyl compounds, and glucose indicated extensive bacterial and enzyme activity during aging. However, concentrations of yeast-derived flavor compounds were similar to those of modern beers, except that 3-methylbutyl acetate was unusually low in both beers and 2-phenylethanol and possibly 2-phenylethyl acetate were unusually high in one beer. Concentrations of phenolic compounds were similar to those in modern lagers and ales.

KEYWORDS: *beer flavor, flavor stability, hop components, shipwreck beer, spoilage*

INTRODUCTION

In the summer of 2010 the wreck of a schooner (M1 Fö 403.3) was discovered in the Baltic Sea a short distance south of the Åland Islands, Finland, at a depth of about 50 m. Archeological evidence suggests the shipwreck occurred during the 1840s, but the schooner's name, its destination, and its last port-of-call have not yet been identified. The cargo consisted of luxury items, including more than 150 bottles of champagne. Five bottles that look like typical early 19th century beer bottles were also brought to the surface. One of these cracked in the divers' boat. The liquid that foamed from the cracked bottle looked and, according to the divers, tasted like beer.

Although at least one older (1825) beer sample has been reported,[1] we are not aware of previous chemical analyses of any beer this old. Here we compare the physicochemical characteristics and flavor compound profiles of beer from two of these about 170-year-old bottles with those of modern beers. In contrast to the 100-year-old Scotch whiskey excavated from the ice under Shackleton's 1907 base camp in the Antarctic and then thoroughly analyzed,[2] these beers have not been stored under ideal conditions, as evidenced by some deterioration in quality. However, although both spontaneous and microbiologically driven chemical changes have occurred, the results give some indication of the original nature of the beers and the techniques used to manufacture them.

MATERIALS AND METHODS

Opening the Shipwreck Beer Bottles. Bottles A56 and C49 were raised to the sea surface, and their corks and necks were protected with plastic wrappings. The bottles were stored in water at 2−4 °C and brought from Åland Islands to VTT's laboratories in Espoo, Finland. The bottles were opened (on separate occasions) under sterile conditions because samples were also taken for microbiological examination (R. Juvonen, M. Raulio, A. Wilhelmson, and E. Storgårds, manuscript in preparation). The part of the cork protruding from the bottle was cut off. A slightly slanting hole was drilled through the rest of the cork using a sterilized drill. A surgical needle fitted with an air filter was inserted into the cork to allow sterile air to enter the bottle to replace the beer withdrawn. (During this procedure, the cork of bottle A56 broke horizontally into two pieces. The upper two-thirds of the cork was removed from the bottle by hand. The lower third remained tightly in the neck of the bottle, but later fell into the beer during an attempt to remove it.) A sterile steel pipe was inserted to the bottom of the bottle. Samples of beer were then slowly removed by syringe through this pipe. Samples for physicochemical analyses were centrifuged twice (10 min at 1000*g*, then 10 min at 9000*g*). The supernatants were analyzed immediately or stored in portions at −25 °C. Samples (50 mL) for hop analyses were sent to the Technical University of Munich, Germany, packed in dry ice.

Reference Beers. Bottles of six reference beers, Leffe Brune, Koff Porter, Weihenstephan Hefe Weissbier, Paulaner Hefe Weissbier, Aldaris Porteris Alus, and Olvi Sandels (lager beer), were purchased from a retailer in Helsinki, Finland.

Beer Analyses. Water was deionized and filtered through active carbon (Milli-Q Water Sytem; Millipore Corp., Bedford, MA, USA). Color (EBC method 9.6), bitterness (EBC method 9.8), SO_2 (EBC method 9.25.3), and free amino nitrogen (FAN; EBC method 9.10) were determined as described by European Brewery Convention.[3] Specific gravities were determined with an Anton-Paar DMA58 density meter and used to calculate apparent extracts according to EBC method 9.4.[3] Real extracts and original gravities were estimated from the apparent extracts and ethanol contents by using EBC method 9.4.[3] Samples for metal analyses were dry-ashed and then dissolved in dilute nitric acid. Sodium and potassium contents were determined by flame atomic absorption spectrometry using a PerkinElmer AAnalyst 800.

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Ethanol, Glycerol, Acetic Acid, and Lactic Acid. These compounds were quantified by using a HPLC-RI (PerkinElmer, Flexar) system equipped with Aminex column (HPX-87H, 300 mm × 7.8 mm; Bio-Rad) under isocratic conditions (40 °C) at a flow of 0.5 mL min^{-1} of 2.5 mM H_2SO_4.

Fermentable Sugars. Fermentable sugars were analyzed by high-performance anion exchange chromatography (HPAEC) (Dionex ICS-3000) with pulse amperometric detection (PAD) using a CarboPac PA-1 (4 mm × 250 mm) analytical column and a CarboPac PA-1 (4 mm × 50 mm) guard column at 30 °C (Dionex Corp, Sunnyvale, CA, USA). The system was equilibrated with 100 mM NaOH. After injection of a 100 μL filtered (0.45 μm), diluted sample, 100 mM NaOH was run through the column (5 min). Separation was achieved with a gradient (1 mL min^{-1}) of 100−300 mM NaOH in 3 min and then 300−250 mM NaOH + 75 mM sodium acetate in 15 min, and a final washing step with 100 mM NaOH + 300 mM sodium acetate and 300 mM NaOH. The flow rate was 1 mL min^{-1}. The results were confirmed by MSQ detection (HPAEC-MS) using a CarboPac PA200 (3 mm × 250 mm) with a CarboPac PA200 guard (3 mm × 50 mm) column (Dionex) with a configuration as described by Bruggink et al.[4] and a gradient as described by Mikkelson et al.[5]

Hop-Derived Bitter Compounds. For quantitation of 96 hop-derived bitter compounds, beer samples were degassed by ultrasonification and filtered (0.45 μm), and an aliquot (5 μL) was analyzed by HPLC-MS/MS_{MRM} as reported recently.[6,7]

Yeast-Derived Flavor Compounds. These compounds in bottle A56 and reference beers were determined by GC analysis as described[8] and those in bottle C49 by headspace-GC-MS. 1-Butanol was used as internal standard. Samples (4 mL) of C49 were filtered (0.45 μm) and incubated at 60 °C for 30 min, and then 1 mL was injected (splitless; 260 °C; flow = 14.9 mL min^{-1}) into a gas chromatograph (Agilent 6890 series; Palo Alto, CA, USA) combined with an MS detector (Agilent 5973 Network MSD, USA) and an SPME autosampler (Combipal, Varian Inc., USA). Analytes were separated on a BPX5 capillary column (60 m × 0.25 mm with phase thickness = 1.0 μm; SGE Analytical Science Pty Ltd., Australia). The carrier gas was helium (1.7 mL min^{-1}). The temperature program was 50 °C for 3 min, 10 °C min^{-1} to 100 °C, 5 °C min^{-1} to 140 °C, 15 °C min^{-1} to 260 °C, and then isothermal elution for 1 min. MSD was operated in electron impact mode at 70 eV, in the full scan mode (m/z 40−550). The ion source temperature was 230 °C, and the interface was 280 °C. Compounds were identified by comparison with authentic standards and the mass spectra in Palisade Complete 600 K Mass Spectral Library (Palisade Mass Spectrometry, USA) and were quantified using standard curves.

Total Vicinal Diketones (VDKs). VDKs were measured according to Analytica-EBC method 9.10.[3]

Carbonyl Compounds. Carbonyl compounds were analyzed as oximes by using GC-ECD as described by Ojala et al.[9] To 5 mL beer samples were added 500 μL of 0.4% (w/v) PFBOA (*O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride) solution and 100 μL of internal standard (benzaldehyde; 0.1% v/v). Samples were then incubated at room temperature for 2 h. Reaction was stopped by adding 100 μL of 9 M H_2SO_4, and oximes were extracted into 2 mL of hexane. Samples (2 μL, split injection 12.5:1) were analyzed using GC-ECD (Agilent 6890 Series). Analytes were separated in constant flow mode with helium as carrier gas (1.2 mL min^{-1}) on a DP-5 capillary column (30 m × 0.32 mm) with a phase thickness of 0.50 μm (Agilent). The injector was kept at 260 °C, and the ECD was kept at 300 °C. The temperature program was 100 °C for 2 min, 8 °C min^{-1} to 250 °C, and a 5 min hold. Compounds were identified with authentic standards and quantified with standard calibration curves.

Short-Chain Fatty Acids. Short-chain fatty acids were extracted with diethyl ether from 4 mL of acidified beer samples as described by Schooley et al.[10] Internal standard was heptanoic acid (C7). Diethyl ether extracts (2 μL, splitless injection) were analyzed by GC-FID (Agilent 6890 series). Analytes were separated on a DP-FFAP capillary column (30 m × 0.32 mm; phase thickness = 0.25 μm; Agilent). The carrier gas was helium (2.7 mL min^{-1}). The injector and FID were at 250 °C. The temperature program was 50 °C for 3 min, 25 °C min^{-1} to 100 °C, 10 °C min^{-1} to 240 °C, and a 10 min hold. Compounds were quantified using authentic standards.

Bound and Free Phenolic Acids (PAs). Beer samples (250 μL) were mixed with 2 M NaOH (1.1 mL, for hydrolysis of bound PAs) or water (1.1 mL, for free PAs). After 16 h in the dark, samples were acidified with 5 M HCl and extracted with ethyl acetate (3 × 2 mL). The extracts were dried under nitrogen and residues dissolved in 0.5 mL of methanol, filtered, and used to quantify free and total PAs by ultraperformance liquid chromatography (UPLC). Portions (200 μL) of the methanol samples were dried and dissolved in 30 μL of dichloromethane (DCM) and derivatized with MSTFA (*N*-methyl-*N*-trimethylsilyltrifluoroacetamide, Pierce, Rockford, IL, USA; 25 μL, 80 °C for 20 min) for GC-MS analysis of minor PAs.

Volatile Phenols. Beer samples (0.5 mL) were vortexed, spiked with heptadecanoic acid (10 μg), and extracted with DCM (2 × 4 mL). The separated DCM extracts were dried under nitrogen and redissolved in 100 μL of DCM. Half (50 μL) of each extract was trimethylsilylated with MSTFA (25 μL, 80 °C for 20 min). Underivatized and derivatized samples were both analyzed by GC-MS.

Phenolic compounds were analyzed using UPLC and GC-MS. A Waters Acquity UPLC was equipped with a Waters Acquity BEH C18 column (1.7 μm, 2.1 × 100 mm) combined with a VanGuard pre-column (2.1 × 5 mm 1.7 μm). The solvent was 5% formic acid and acetonitrile. Flow was 0.43 mL min^{-1}. The proportion of acetonitrile was increased linearly from 5 to 90% in 9 min and returned to 5% in 2 min. The diode array detector was used at 190−600 nm. Ferulic, sinapic, and *p*-coumaric acids were quantified at 320 nm by using standard curves of authentic compounds (0.1−100 μg mL^{-1}). For GC-MS analyses, an Agilent 7890A GC was equipped with a 5975C mass selective detector and Rtx-5MS silica capillary column (15 m, 0.25 mm i.d., phase thickness of 0.25 μm (Restek, Bellefonte, PA, USA)). The oven temperature was increased from 70 °C (1 min) at 10 °C min^{-1} to 270 °C (4 min). The split ratio was 25:1, and the samples were injected by a Gerstel Maestro MPS 2 sampling system (Gerstel GmbH & Co.KG, Mühlheim an der Ruhr, Germany). Data were collected in the full scan mode (m/z 40−600). Compounds were identified by retention times and library comparison (NIST '08, Scientific Instrument Services, Inc., Ringoes, NJ, USA).

Protein Content. Proteins were precipitated from beers (400−500 μL) with acetone (1600 μL) at −20 °C. Precipitates from centrifugation (720*g*, 5 min) were dissolved in 25 μL of rehydration buffer (2 M thiourea, 7 M urea, 0.5% Triton-X-100, 0.5% Pharmalyte 3−10) and assayed by 2-D Quant Kit (GE Healthcare UK Limited, Buckinghamshire, UK) with bovine serum albumin as standard.

Amino Acids. Total and free amino acids were analyzed with Mass TRAK Amino Acid Analysis Application Solution (Waters, Milford, MA, USA). Samples for total amino acids were hydrolyzed in 6 N HCl at 110 °C for 20 h and then dried under nitrogen, dissolved in 200 μL of water, redried, and dissolved in 200 μL of 0.1 N HCl. Hydrolyzed and untreated samples (10 μL) were mixed with internal standard (10 μL of 250 μM norvaline) and derivatized (AccQ·Fluor reagent kit; Waters). AccQ·Fluor reagent (20 μL) and boric acid buffer (70 μL) were added, and the mixture was vortexed for 60 s. UPLC-MS analysis involved an Acquity UPLC system (Waters) with a diode array detector. Chromatography involved an Acquity Mass TRAK (2.1 × 150 mm, 1.7 μm) column (Waters) at 43 °C. The injection volume was 2.0 μL. Separation was achieved by gradient elution with 10% (v/v) Amino Acid Analysis Concentrate A in water and Amino Acid Analysis Eluent B at 0.4 mL min^{-1}, monitoring the effluent at 260 nm.

RESULTS

Bottles. Bottles A56 (Figure 1) and C49 were similar brown glass bottles, 245 mm high including the 80 mm neck. The diameters increased from 85 mm at the base to 90 mm at the shoulder. The necks were about 37 mm in diameter. The bases had conical depressions about 40 mm deep (as in traditional wine bottles). Bottle A56 showed no signs of sand abrasion or glass corrosion (Risto Aalto, Riikka Alvik, Markku Annila, Ulla Klemelä, and Kaisa Koivisto, personal communication). It

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Figure 1. Bottle A56.

appeared to have been mouth blown while revolving in a cylindrical mold, which shaped the wall (marks from the mold were visible on the wall). The bottle's mouth was apparently formed by wrapping strips of hot glass round the cut neck. No labels remained on either bottle. Both bottles were closed with rather coarse-grained corks that protruded several millimeters out of the necks.

Bubbles of gas, presumably CO_2, formed during sampling, producing a light foam. Both beers were bright golden yellow, with little haze. Both beers smelt of autolyzed yeast, dimethyl sulfide, Bakelite, burnt rubber, over-ripe cheese, and goat, with phenolic and sulfury notes. As the samples warmed to room temperature, the smell of hydrogen sulfide disappeared and that of butyric acid (particularly strong in C49) strengthened.

Physicochemical Analyses. Compared to modern beers, the shipwreck beers contained similar levels of potassium but 15−60-fold more sodium (Table 1), presumably derived from seawater. This may have diluted the beers up to 30%. Ethanol contents were low (2.8−3.2% ABV) compared to typical modern lagers and ales. The mass ratios of glycerol/ethanol were 4.5% for both shipwreck beers, which is typical for a yeast fermentation product. Both beers were acidic, with pH about 1 unit below modern values. The color strengths were in the range of modern ales and lagers (and much lower than those of porters or stouts). Bitterness was lower in A56 (9.9 IBU, corresponding to a modern light lager) and higher in C49 (16 IBU). Sulfur dioxide was not detected in A56 (not tested in C49): original sulfur dioxide would probably be oxidized over the 170 years under water. FAN was low (61 mg L^{-1}) for A56 but normal (114 mg L^{-1}) for C49. Protein levels (5−7 mg L^{-1}) were very low in both beers.

Table 1. Physicochemical Properties of Shipwreck Beers

property	unit	beer A56	beer C49	modern beers[a]
ethanol	ABV[b] (% v/v)	2.8	3.2	3−6
glycerol	g L^{-1}	1.0	1.1	1−2
pH		3.18	3.39	3.9−4.5
color	EBC[c] units	8.9	10.5	2−27
bitterness	IBU[d]	9.9	16	6−30
apparent extract	°Plato	1.32	3.10	1−5
real extract	°Plato	2.24	3.98	3−6
SO_2 (sulfite)	mg L^{-1}	<0.4	NA[f]	0−10
FAN[e]	mg L^{-1}	61	114	40−250
protein	mg L^{-1}	5−7	5−7	200−500
glucose	g L^{-1}	1.35	9.8	0
fructose	g L^{-1}	<0.04	<0.04	0
sucrose	g L^{-1}	<0.04	<0.04	0
maltose	g L^{-1}	0.14	1.2	0.0−2.5
maltotriose	g L^{-1}	0.073	0.11	0.0−3.0
Na^+ (sodium)	mg L^{-1}	1200	890	20−60
K^+ (potassium)	mg L^{-1}	440	510	500

[a]Results for shipwreck beers A56 and C49 are compared with typical values for modern ales and lagers. Concentrations are for the beer "as is", i.e., values have not been normalized to a standard ethanol concentration. [b]ABV, alcohol by volume. [c]EBC, European Brewing Convention. [d]IBU, International Bitterness Unit. [e]FAN, free amino nitrogen. [f]NA, not analyzed.

Fermentable Sugars. A small amount of maltose was present in A56 and much more in C49, which contained more maltose than is typical in modern beers (Table 1). Both shipwreck beers contained less maltotriose than maltose, whereas modern beers contain more maltotriose than maltose.[11] Both beers, especially C49, contained unexpectedly large amounts of glucose (glucose is usually consumed completely early in fermentation). Fructose and sucrose were not detected in either shipwreck beer. Like glucose, these sugars are consumed in early fermentation (their presence would suggest deliberate sweetening).

Apparent extracts (1.32 °Plato for A56 and 3.10 °Plato for C49) were at the low end of the range for modern ales and lagers (Table 1). Real extracts, calculated from the apparent extracts and ethanol concentrations, were 2.24 °Plato (A56) and 3.98 °Plato (C49). Total carbohydrate levels were not measured, but the difference between real extracts and residual sugars and salts suggests that starch was still present at about 17 g L^{-1} (A56) and 25 (C49) g L^{-1}.

Hop Components. The shipwreck beers contained small amounts of some iso-α-acids, which are considered the key bitter compounds in beer (Table 2). Among these iso-α-acids, *cis*-isohumulone and *cis*-isocohumulone were found in both beers, whereas additional isomers were detectable in C49 only. Besides the iso-α-acids, some of their degradation products such as tri- and tetracyclic degradation products, allo-iso-α-acids, scorpiohumols, and the tricyclohumolactols were detected (Table 2). These compounds are formed upon beer aging by an acid-catalyzed degradation of iso-α-acids.[12−14] In addition, hulupone and hulupinic acid were found as degradation products of hop β-acids. Only small amounts of xanthohumol, isoxanthohumol, and 6- and 8-prenylnaringenin were detected, although their presence was unequivocally confirmed. Hydroperoxides of allo-iso-α-acids, tricyclic degradation products of β-acids, and hydroperoxides and hydroxides of tricyclolupones are known hop derivatives,[12−14] but were not detected in either shipwreck beer. Their absence is, however, not unexpected as they are susceptible to chemical degradation from autoxidation and acid-catalyzed reactions. A series of oxidation products of α-, iso-α-, and β-acids such as humulinons, allohumulonhydroxides, and humulinic acids was detected in C49 but not in A56. Most other hop-derived compounds present in A56 were detected in C49 at higher concentrations. The higher concentration of most hop components in C49 than in A56 agrees with the more pronounced bitterness of C49 (Table 1).

Yeast-Derived Flavor Compounds. Yeasts produce compounds that contribute to the flavor of fermented beverages. Some of these compounds were analyzed in the

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Table 2. Hop-Derived Bitter Taste Compounds

compound	A56 (μmol L^{-1})	C49 (μmol L^{-1})	substance group
phloroisobutyrophenone	0.03	0.01	precursors of desoxyhumulones
phloroisovalerophenone	nd[a]	nd	
o-prenylphloroisobutyrophenone	nd	nd	
o-prenylphloroisovalerophenone	nd	nd	
desoxycohumulone	0.12	nd	precursors of α- and β-acids
desoxyhumulone	0.03	nd	
desoxyadhumulone	nd	nd	
humulone	nd	0.05	α-acids
adhumulone	nd	nd	
cohumulone	nd	nd	
prähumulone	nd	nd	
adprähumulone	nd	nd	
posthumulone	nd	nd	
humulinone	nd	0.65	oxidation products of α-acids
adhumulinone	nd	0.20	
cohumulinone	nd	0.52	
cis-isohumulone	0.29	0.72	iso-α-acids
cis-isoadhumulone	nd	0.44	
cis-isocohumulone	0.27	0.69	
cis-isoprähumulone	nd	nd	
cis-isoposthumulone	nd	0.29	
trans-isohumulone	nd	1.02	
trans-isoadhumulone	nd	0.39	
trans-isocohumulone	nd	nd	
trans-isoprähumulone	nd	nd	
trans-isoposthumulone	nd	nd	
cis-alloisohumulone	0.24	0.57	allo-iso-α-acids
cis-alloisoadhumulone	nd	nd	
cis-alloisocohumulone	0.20	0.51	
trans-alloisohumulone	nd	nd	
trans-alloisoadhumulone	nd	nd	
trans-alloisocohumulone	nd	nd	
cis-alloisohumulonhydroperoxide	nd	nd	hydroperoxides of allo-iso-α-acids
cis-alloisoadhumulonhydroperoxide	nd	nd	
cis-alloisocohumulonhydroperoxide	nd	nd	
trans-alloisohumulonhydroperoxide	nd	nd	
trans-alloisoadhumulonhydroperoxide	nd	nd	
trans-alloisocohumulonhydroperoxide	nd	nd	
cis-alloisohumulonhydroxide	nd	8.37	hydroxides of allo-iso-α-acids
cis-alloisoadhumulonhydroxide	nd	21.29	
cis-alloisocohumulonhydroxide	nd	10.27	
trans-alloisohumulonhydroxide	nd	6.89	
trans-alloisoadhumulonhydroxide	nd	8.20	
trans-alloisocohumulonhydroxide	nd	2.56	
tricyclocohumol	2.31	7.76	tricyclic degradation products of iso-α-acids
tricyclohumol	2.59	8.65	
tricycloadhumol	0.82	2.88	
tricyclocohumene	nd	0.36	
tricyclohumene	nd	0.46	
tricycloadhumene	nd	nd	
isotricyclocohumene	nd	4.91	
isotricyclohumene	nd	4.56	
isotricycloadhumene	nd	1.48	

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Table 2. continued

compound	A56 (μmol L^{-1})	C49 (μmol L^{-1})	substance group
tetracyclocohumol	1.60	4.24	tetracyclic degradation products of iso-α-acids
tetracyclohumol	1.98	1.24	
tetracycloadhumol	0.59	4.86	
tricyclocohumolactol	0.35	0.90	tricyclic degradation products of iso-α-acids
tricyclohumolactol	0.33	1.25	
tricycloadhumolactol	0.40	0.60	
scorpiohumol	0.17	nd	tricyclical degradation products of iso-α-acids
sorpioadhumol	nd	nd	
sorpiocohumol	0.26	0.23	
cis-cohumulinic acid	nd	0.12	oxidation products of iso-α-acids
cis-humulinic acid	nd	0.24	
cis-adhumulinic acid	nd	0.11	
trans-cohumulinic acid	nd	0.21	
trans-humulinic acid	nd	0.36	
trans-hdhumulinic acid	nd	0.19	
lupulone	nd	nd	β-acids
adlupulone	nd	nd	
colupulone	nd	nd	
postlupulone	nd	nd	
prälupulone	nd	nd	
adprälupulone	nd	nd	
cohulupone	24.26	68.43	oxidation products of β-acids
hulupone	8.07	17.84	
adhulupone	3.35	9.38	
hulupinic acid	15.55	59.01	oxidation product of hulupones
nortricyclocolupone	nd	nd	tricyclic degradation products of β-acids
nortricyclolupone	nd	nd	
dehydrotricyclocolupone	nd	nd	
dehydrotricyclolupone	nd	nd	
dehydrotricycloadlupone	nd	nd	
tricyclocolupone	nd	nd	
tricyclolupone	nd	nd	
tricycloadlupone	nd	nd	
peroxytricyclocolupone	nd	nd	hydroperoxides of tricyclolupones
peroxytricyclolupone	nd	nd	
peroxytricycloadlupone	nd	nd	
hydroxytricyclocolupone	nd	nd	hydroxides of tricyclolupones
hydroxytricyclolupone	nd	nd	
hydroxytricycloadlupone	nd	nd	
xanthohumol	0.002	0.005	chalconaringenine
isoxanthohumol	0.004	0.167	flavonoids
6-prenylnaringenin	<0.001	0.004	
8-prenylnaringenin	<0.001	0.002	

[a]nd, not detected.

shipwreck beers and six reference beers. Results were normalized to a standard ethanol concentration of 35 g L^{-1}. The (normalized) concentrations of most compounds in the shipwreck beers were within or close to the range found in the reference beers (Figure 2). However, there were exceptions. Acetaldehyde, with a Tb (taste threshold in beer) of 25 mg L^{-1} (unless stated otherwise, taste thresholds in beer and flavor descriptions are from Meilgaard[15]) and a green leaf flavor, was

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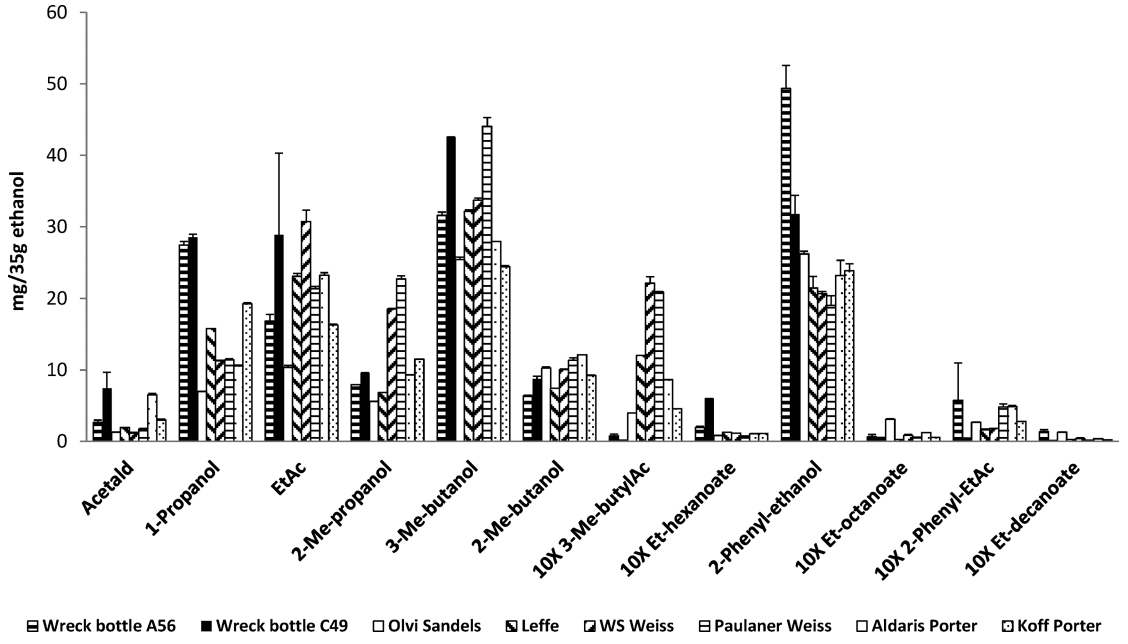

Figure 2. Yeast-derived flavor compounds in shipwreck and six modern reference beers. Concentrations are normalized to an ethanol concentration of 35 g L^{-1}. Actual ethanol concentrations (g L^{-1}) were as follows: A56, 22.2; C49, 25.6; Olvi Sandels, 37.4; Leffe Brune, 49.7; Weihenstephan Hefe Weissbier, 45.0; Paulaner Hefe Weissbier, 43.8; Aldaris Porter, 54.3; and Koff Porter, 59.2. Abbreviations are acetald, acetaldehyde; EtAc, ethyl acetate; 2-Me-propanol, 2-methylpropanol; 3-Me-butanol, 3-methylbutanol; 2-Me-butanol, 2-methylbutanol; 3-Me-butylAc, 3-methylbutyl acetate; Et-hexanoate, ethyl hexanoate; Et-octanoate, ethyl octanoate; 2-Phenyl-EtAc, 2-phenylethyl acetate; Et-decanoate, ethyl decanoate. Where indicated (10 X), the concentrations of some compounds have been multiplied by 10. Results are averages of duplicate or triplicate analyses, and error bars show the SDs.

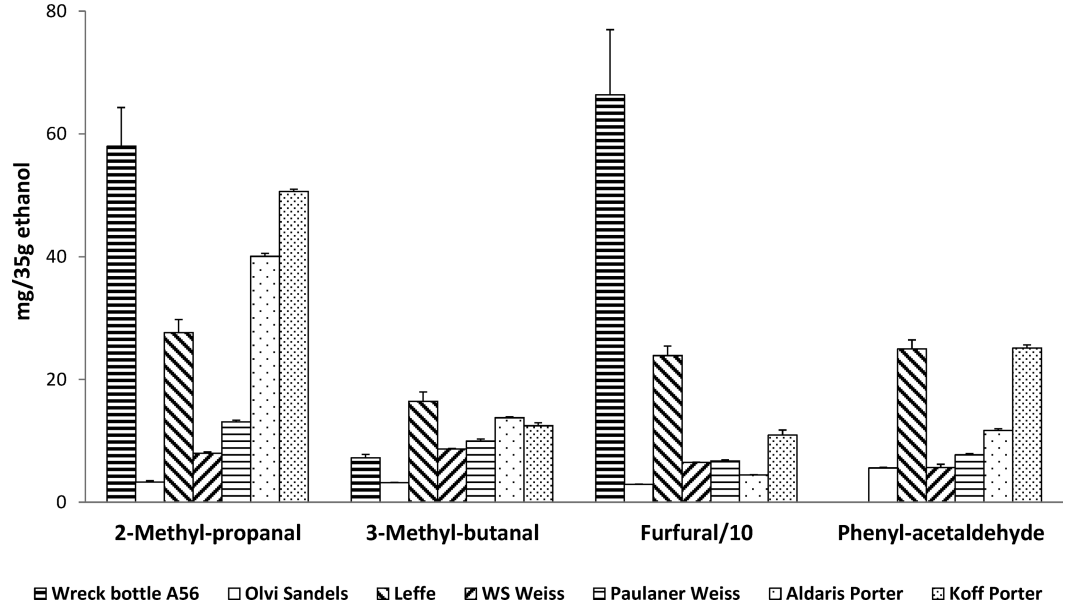

Figure 3. Carbonyl concentrations in shipwreck beer A56 and six reference beers. Concentrations are normalized to an ethanol concentration of 35 g L^{-1} (see Figure 2). Furfural/10 indicates the furfural concentration divided by 10. Results are averages of two or (wreck bottle A56) three determinations, and error bars show the SDs.

relatively high in C49 as was 1-propanol (Tb = 800 mg L^{-1}; alcohol) in both shipwreck beers. 3-Methylbutyl acetate (Tb = 1.6 mg L^{-1}; banana) was markedly low in both shipwreck beers. Ethyl hexanoate (Tb = 0.23 mg L^{-1}; apple) was high in both shipwreck beers, especially C49. Ethyl decanoate (Tb = 0.9 mg L^{-1}; sweet apple) was high in A56 (and also in the beer Olvi Sandels). 2-Phenylethanol (Tb = 125 mg L^{-1}; rose) was high in A56 and slightly high in C49. 2-Phenylethyl acetate (Tb = 3.8 mg L^{-1}; rose) was possibly high in A56, but low in C49.

Carbonyl Compounds. Two vicinal diketones, which are also yeast-derived and can cause a buttery or butterscotch-like flavor considered unacceptable in lagers but appreciated in some ales, were measured in A56 and the reference beers. Normalized concentrations of diacetyl (Tb = 150 μg L^{-1}) were 36 μg L^{-1} for A56 compared to 15 μg L^{-1} for Olvi Sandels and

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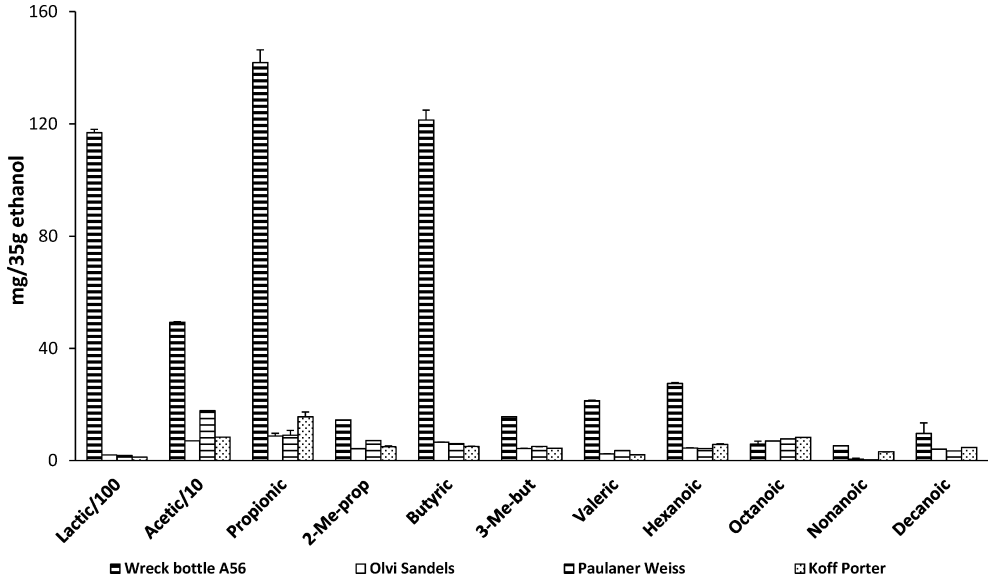

Figure 4. Organic acid concentrations in shipwreck beer A56 and three reference beers. Concentrations are normalized to an ethanol concentration of 35 g L^{-1} (see Figure 2). Abbreviations are Lactic/100, lactic acid divided by 100; Acetic/10, acetic acid divided by 10; 2-Me-prop, 2-methylpropionic acid; 3-Me-but, 3-methylbutyric acid. Results are averages of duplicate determinations. Error bars show half the range.

Table 3. Phenolic Compounds[a]

	A56	C49	Olvi Sandels	Paulaner Weiss	Koff Porter
hydroxycinnamic acids					
total ferulic acid	7.5	9.4	9.4	10.3	12.3
free ferulic acid	1.8 (24%)	1.4 (15%)	2.5 (27%)	<0.004 (0%)	0.88 (7%)
total sinapic acid	0.92	1.2	2.1	4.1	2.3
free sinapic acid	0.14 (16%)	0.11 (9%)	0.46 (22%)	0.21 (5%)	0.31 (14%)
total *p*-coumaric acid	1.3	1.5	1.5	0.44	1.5
free *p*-coumaric acid	0.87 (67%)	1.0 (67%)	1.2 (78%)	0.06 (15%)	0.93 (61%)
total caffeic acid	1.3	1.2	0.40	0.21	0.93
hydroxybenzoic acids					
total syringic acid	0.77	0.75	0.55	0.82	0.37
total vanillic acid	2.1	3.4	1.0	1.1	1.3
other aromatic					
total 4-hydroxyphenylacetic acid	2.0	2.1	0.93	0.67	0.95
ferulic acid-derived					
total 4-vinlyguaiacol	0.16	0.04	0.07	0.87	0.04
total 4-ethylguaiacol	<0.008	<0.014	<0.005	<0.004	<0.006
sinapic acid-derived					
total 4-vinylsyringol	0.08	0.014	0.03	0.17	0.05
other phenolic					
total 4-hydroxyphenyl ethanol	6.7	10.2	7.9	13.7	6.8

[a]Concentrations (mg L^{-1}) are averages of two analyses and normalized to an ethanol concentration of 35 g L^{-1}. Values in parentheses denote the percentage of free, unbound compound with respect to the total concentration for that particular compound.

34−64 μg L^{-1} for the other reference beers. Pentanedione (Tb = 900 μg L^{-1}) was low in A56 (7 μg L^{-1}) and was below the average concentration (14 μg L^{-1}) found in the reference beers. The levels of four other carbonyl compounds in shipwreck beer A56 and the reference beers are compared in Figure 3 (not measured in C49). All of the beers contained between 3.2 μg L^{-1} (Olvi Sandels) and 16.5 (Leffe Brune) μg L^{-1} 3-methylbutanal (Tb = 600 μg L^{-1}; unripe banana). There was greater variation for the other carbonyls. For 2-methylpropanal (Tb = 1000 μg L^{-1}; banana, melon) reference beers contained between 3.3 μg L^{-1} (Olvi Sandels) and 51 (Koff Porter) μg L^{-1}, whereas the shipwreck beer contained 58 μg L^{-1}. Phenylacetaldehyde (Tb = 1600 μg L^{-1}; hyacinth) could not be detected in the shipwreck beer and was between 5.7 μg L^{-1} (Weihenstephan Weissbier) and 25 (Koff Porter) μg L^{-1} in the reference beers. Furfural (Tb = 150 mg L^{-1}) ranged between 29 μg L^{-1} (Olvi Sandels) and 240 (Koff Porter) μg L^{-1} in the references and was much higher (664 μg L^{-1}), but still below threshold, in the shipwreck beer.

Organic Acids. Several fatty acids were measured in shipwreck beer A56 and three reference beers (Figure 4). The (normalized) concentration of lactic acid (Tb = 400 mg L^{-1}; acid) was extremely high (11.7 g L^{-1}) in A56 (and 10.5 g L^{-1} in C49; not shown in Figure 4) compared to the reference beers (100−200 mg L^{-1}). Acetic acid (Tb = 175 mg L^{-1}; vinegar) levels were also relatively high: 70−180 mg L^{-1} in the

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reference beers compared with 493 mg L^{-1} in A56 and 542 mg L^{-1} in C49 (not shown in Figure 4). The other measured organic acids were present at much lower concentrations in the reference beers (0.2−16 mg L^{-1}). A56 contained higher levels of most of these acids, including about 20-fold more butyric acid (Tb = 2.2 mg L^{-1}; buttery, sweaty) and 15-fold more propionic acid (Tb = 150 mg L^{-1}; vinegar).

Phenolics. Several phenolic compounds were measured in the shipwreck beers and three reference beers (Table 3). Among these five beers, the wheat beer, Paulaner Weiss, was exceptional. It contained at least 5-fold more 4-vinylguaiacol and 2-fold more 4-vinylsyringol than any other beer and exhibited the highest concentrations of sinapic acid and 4-hydroxyphenylethanol and the lowest concentrations of *p*-coumaric, caffeic, and 4-hydroxyphenylacetic acids. The proportions of free compared to total hydroxycinnamic acids were much smaller (0% ferulic, 5% sinapic, and 15% *p*-coumaric) for Paulaner Weiss than for all other beers (7−17% ferulic, 9−22% sinapic, and 61−78% *p*-coumaric). The shipwreck beers more closely resembled the other references, Olvi Sandels (a lager) and Koff Porter. Total concentrations of phenolic acids in the shipwreck beers were within ±60% of those in the Olvi Sandels and/or Koff Porter references, except that vanillic and 4-hydroxyphenylacetic acids were 2−3-fold higher in the shipwreck beers. A56 contained more 4-vinylguaiacol than the Olvi Sandels and Koff Porter references, but much less than Paulaner Weiss. 4-Ethylguaiacol was below the limit of quantification in all beers.

Amino Acids. After acid hydrolysis, the main (total) amino acids in shipwreck beers were glutamate/glutamic acid, proline, and glycine (Table 4). The amino acid composition of the shipwreck beers was similar to that of modern commercial beers,[16] except that shipwreck beers had relatively more proline and alanine and less asparagine/aspartic acid. Proline was the main free amino acid in both shipwreck beers. The amino acid profiles of the two beers were similar, but C49 had a higher proportion of free glycine and a smaller proportion of free threonine and proline than A56. Differences of the same magnitude are found between different modern beers.[17]

Table 4. Amino Acid Profiles with (Total Amino Acids) and without (Free Amino Acids) Acid Hydrolysis

	total amino acids (%)		free amino acids (%)	
	A56	C49	A56	C49
alanine	9.6	9.6	12.2	12.9
arginine	1.9	1.8	3.2	3.0
aspartic acid + asparagine	5.1	4.9	5.8	5.0
cysteine	0.4	0.3	0.0	0.0
glutamic acid + glutamine	22.0	23.1	7.4	6.7
glycine	11.6	13.0	9.6	14.8
histidine	1.3	1.7	1.1	1.8
isoleucine	2.0	2.0	1.3	1.8
leucine	3.3	3.4	3.7	4.0
lysine	3.1	3.0	2.1	2.2
methionine	0.7	0.5	1.2	1.4
phenylalanine	2.4	2.5	3.1	3.2
proline	22.7	21.1	35.5	29.5
serine	4.6	4.8	4.0	5.5
threonine	3.4	2.2	2.5	0.0
tyrosine	1.5	1.2	1.7	2.0
valine	4.5	4.9	5.7	6.2
total	100.0	100.0	100.0	100.0

DISCUSSION

The overall shape and detailed features of bottles A56 and C49 indicate a high-quality technology that was not yet used in Finland in 1840, but had been used to manufacture beer bottles for two or three decades in central and northern Europe (personal communication; Risto Aalto, Riikka Alvik, Markku Annila, Ulla Klemelä, and Kaisa Koivisto). The presence of hop components (extensively degraded), maltose, and maltotriose identifies the bottles' contents as beers. The higher concentrations of hop components in beer C49 than in A56 cannot be explained by different degrees of chemical degradation or dilution by seawater and indicates that the bottles contained two different beers. Both shipwreck beers contained too little protein (Table 1) to permit protein identification by 2D gel electrophoresis. Most of the original protein was probably hydrolyzed (e.g., by proteolytic activity of lactic acid bacteria) and partially consumed by microorganisms during aging (both beers contained large numbers of dead bacteria and yeast). Peptides that may have been liberated by hydrolysis and still present in the beer would not have been detected in the protein assay employed as the acetone precipitation step is much less efficient for peptides than for proteins. The amino acid profiles of both beers were broadly similar to those of modern commercial beers (Table 4) and clearly different from, for example, that of apple cider.[18] Features such as the relatively high free proline content are consistent with the raw material being cereal grain but do not distinguish between barley and wheat, which have very similar amino acid profiles.[16] Furthermore, the amino acid profiles of the shipwreck beers have been disturbed by the activity of microbial contaminants.

The presence of hop-derived bitter compounds confirms the use of hops for bittering the beers. Kettle-boiling induces the transformation of α-acids to iso-α-acids and that of β-acids to hulupones.[6] The lack of α- and β-acids and the presence of iso-α-acids and hulupones therefore indicate that hops were added to the worts before kettle-boiling. The amounts of *cis*-iso-α-acids were higher than those of the corresponding *trans*-iso-α-acids, which is in line with the higher stability of *cis*-iso-α-acids and literature findings that *trans*-iso-α-acids are readily transformed into tri- and tetracyclocohumols, scorpiohumols, and tricyclolactohumols by proton catalysis during aging of beer.[7] Compared to modern beers, rather high amounts of these four compounds were detected; for example, 7.76 and 4.24 μmol L^{-1} of tri- and tetracyclocohumol were found in C49 compared to 1.00 and 0.46 μmol L^{-1} in a fresh Pilsner-type beer.[7] The high levels of these aging products can be explained by the low pH and long "reaction time" in the shipwreck. Interestingly, the unexpectedly large amounts of β-acid degradation products hulupones and hulupinic acid are consistent with old hop varieties containing higher levels of β-acids than modern varieties, which have been bred to maximize the α-acid content.

The lack of hydroxides and hydroperoxides of iso-α- and β-acids in beer A56 indicates that the degradation of the hop components was primarily driven by proton-catalyzed reactions rather than by autoxidation involving oxygen. In contrast, beer C49 contained oxidation products of α-, iso-α-, and β-acids such as hydroxides of allo-iso-α-acids, hulupones, and humulinic acids, clearly indicating autoxidation of hop

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components during aging. These differences may be due partly to ingress of air after bottling of C49 but also suggest that the two beers originated from different batches of hops.

Both shipwreck beers contained much more sodium than is usual in beers. Presumably either Na^+ ions diffused into the beers through the cork or seawater entered the bottles. Assuming average concentrations during the last 170 years of 3000 mg Na L^{-1} and 100 mg K L^{-1} in the sea around the wreck at 50 m depth (Baltic salinity varies with time, location, and depth[19]), the beers may have been diluted with seawater by up to about 30%. Concentrations of other analytes may be too low by up to 30% from this cause alone. This may account for the low levels of ethanol in the beers. Concentrations in Figures 2−4 and Table 3 are normalized to 35 g ethanol L^{-1}, which corrects for simple dilution and provides a more meaningful comparison with the reference beers.

The predominant organoleptic properties of both shipwreck beers were unpleasant (acidic, salty, burnt, sulfury, etc.). These negative qualities masked any fruitiness, maltiness, or hopiness. The levels of several organic acids were unusually high in the shipwreck beers (Figure 4), which presumably caused their low pH (Table 1) and vinegary, goaty, and soured milk flavors. Optical and electron microscopy revealed numerous bacteria in both beers and four species of (non-spore-forming) lactic acid bacteria were recovered by cultivation (R, Juvonen, M. Raulio, A. Wilhelmson, and E. Storgårds, E; manuscript in preparation). The organic acids found in the beers can be produced by bacterial activity (e.g., lactic acid bacteria can produce several volatile fatty acids as well as larger amounts of lactic and acetic acids[20−22]). In the 1840s, "microbiological hygiene" was a matter of trial and error with empirical experience but no scientific basis. Flavors now known to be caused by bacteria may have been intended (as for Belgian Geuze beers) or production faults. In either case, the bacterial activity continued after the shipwreck and eventually produced unacceptably large amounts of organic acids. The presence of live, non-spore-forming bacteria means that vegetative cells have been metabolizing continuously, albeit slowly, for about 170 years.

Compared to modern beers,[11] beer A56 contained less maltose and both beers contained much less maltotriose and relatively high concentrations of glucose (Table 1). A plausible explanation is that after the initial (yeast-driven) fermentation, contaminating microbes excreted enzymes (e.g., amyloglucosidase) able to degrade residual carbohydrates to glucose. This glucose supply probably supported the growth and fermentative activity of lactic acid bacteria and other microbes. As conditions deteriorated (e.g., acidity increasing), the production of glucose exceeded the fermentative capacity of the remaining viable microbes, and glucose began to accumulate. This hypothesis would explain the high glucose and low maltotriose in the shipwreck beers, but does not immediately explain the relatively high maltose in beer C49.

Despite the unpleasant organoleptic features probably resulting from bacterial spoilage, chemical analyses revealed profiles of yeast-derived flavor compounds broadly similar to those of modern beers (Figure 2). There were some notable peculiarities. Both beers contained very little 3-methylbutyl acetate, but rather high levels of 2-phenylethanol and 1-propanol; A56 contained a high level of 2-phenylethyl acetate, but C49 contained very little; A56 (but not C49) contained a high level of ethyl decanoate and C49 especially contained a high level of ethyl hexanoate. A problem is to determine how much these results reflect the original character of the two beers rather than chemical changes during 170 years at about 4 °C. To our knowledge, there are no studies of the chemical stability of beer over such a long time. Vanderhaegen et al.[23] studied the stability of top-fermented beer for 6 months at 0, 20, or 40 °C. Rates of change were very temperature-sensitive. Many compounds that changed markedly in 6 months at 20 or 40 °C were stable at 0 °C. The amounts of ethyl acetate and 3-methylbutyl acetate decreased by 25 and 60%, respectively, at 40 °C, but did not change at 0 °C. Thus, possibly both shipwreck beers originally contained only little 3-methylbutyl acetate, an important flavor component (banana) of modern beers. More probably, its concentration has decreased during the long aging. Lambic beers contain little 3-methylbutyl acetate, and this is thought to result from the activity of an esterase produced by *Dekkera* (*Brettanomyces*) yeasts during the lambic fermentation.[24] Considering the lack of ethylphenol compounds in the beers, it may be more likely that an esterase derived from lysed *Saccharomyces* cells contributed to the loss of 3-methylbutyl acetate.[25]

Vanderhaegen et al.[23] found that ethyl hexanoate, octanoate, and decanoate in beer decreased rapidly during storage at 20 or 40 °C, and ethyl octanoate and decanoate decreased by 10 and 25% during 6 months at 0 °C. Malfliet et al.[26] observed similar lowering of flavor compound concentrations in a range of modern beers stored at 30 °C for 60 days or at 22 °C for 9 months. Thus, the original levels of ethyl hexanoate in both shipwreck beers and of ethyl decanoate in beer A56 were probably at least as high as shown in Figure 2 and would have contributed fruity (apple) flavors to the fresh beers. Ethyl hexanoate was above its taste threshold[15] of 0.2 mg L^{-1} in both beers. However, if an appropriate esterase was present, these esters could, instead, have been formed in the bottles from ethanol and hexanoic and decanoic acids produced by spoilage micro-organisms, so that the levels in Figure 2 may overestimate those of the fresh beers.

The level of 1-propanol may have been increased by metabolic interactions of lactic acid bacteria. *Lactobacillus buchneri* can convert lactate into 1,2-propanediol, which *Lactobacillus diolivorans* can further convert into 1-propanol and propionic acid.[21,2728]

We did not find long-term stability studies for phenylethanol. Malfliet et al.[26] noted that the phenylethyl acetate concentration was lowered by 10−20% during aging, possibly explaining the relatively low level of this compound compared to the phenylethanol in beer C49. The phenylethanol concentration in A56 shipwreck beer (Figure 2) was nearly twice that of modern beers but still below the taste threshold[15] of 125 mg L^{-1}. Phenylethyl acetate in beer A56 (0.68 mg L^{-1}), although higher than in the reference beers (Figure 2), was below its taste threshold[15] (3.8 mg L^{-1}). It is known that compounds present in concentrations below their taste threshold concentrations can still contribute to the general flavor of beers because of synergistic effects,[15] so that in typical modern beers phenylethanol and phenylethyl acetate contribute to the general floral flavor, rather than imparting a specific rose flavor. Yet, the higher concentrations of these compounds in one or both shipwreck beers may have introduced clearer rose notes. At least sake strains of *S. cerevisiae* can evolve to produce high levels of phenylethanol.[29,30,46] Unusually high levels of phenylalanine in the wort can also result in higher phenylethanol levels.[31−33] Moreover, increased uptake of phenylalanine occurs at higher temperatures[34] and may contribute to higher concentrations of phenylethanol in wines

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fermented at higher temperatures.[35] Fermentation temperatures were poorly controlled in the 1840s and may have been relatively high during the summer. However, high phenylethanol concentrations might also have been caused by non-*Saccharomyces* yeasts in the brewing process. In the 1840s, pure yeast cultures were an unknown concept. Top fermentation was still the principal method. Fermentations were performed in open, wooden vessels that provided good attachment surfaces for microbes and were difficult to clean. Fermentations were open to airborne contamination and were pitched with the entire microbiota collected from a previous fermentation by skimming. This could have included several yeasts that today are classified as brewery contaminants, including *Dekkera* spp., *Kluyveromyces marxianus* and *Hanseniaspora* spp. that can produce high levels of phenylethanol.[33,36−41]

The level of total diacetyl (i.e., free diacetyl plus its α-acetolactic acid precursor) in beer A56 was within the typical range for beer, whereas the total pentanedione concentration was low (these two vicinal diketones were not analyzed in beer C49). In contrast to that, Vanderhaegen et al.[23] found large increases in diacetyl (3-fold at 0 °C; 50-fold at 40 °C) and pentanedione (30% at 0 °C and 8-fold at 40 °C) during storage of top-fermented beer for 6 months. Apparently this did not happen during aging of beer A56. Rather, this beer may have initially contained low levels of α-acetolactate and the 2,3-pentanedione precursor α-acetohydroxybutyrate, but a high fermentation temperature and low pH due to bacterial activity in the open fermentation vessels could have favored rapid oxidative decarboxylation of the α-acetohydroxy acids and prompted yeast-catalyzed reduction of the resulting vicinal diketones to the corresponding, flavor-neutral alcohols.

Shipwreck beer A56 contained slightly more 2-methylpropanal than the two reference porters but much more than the lager (Olvi Sandels) (Figure 3). 2-Methylpropanal increases during aging, and levels in the A56 beer are similar to those in pale lagers aged for 6 months at 22 °C[26] The level of 3-methylbutanal in A56 was about twice that in Olvi Sandels, but less than that found in modern wheat beers and porters. Levels of 3-methylbutanal can increase 6-fold (reaching 21−43 μg L^{-1}) during storage of top-fermented beers and lagers, especially in the presence of oxygen.[23,26] The relatively low value for 3-methylbutanal in beer A56 suggests that this bottle did not contain much oxygen or that the reactions leading to its formation were very slow at deep Baltic sea temperatures (approximately 4 °C). This assumption is clearly supported by the fact that, in contrast to beer C49, no hop-derived oxidation products of the α-, iso-α-, or β-acids were detected in beer A56.

Furfural is formed during aging of beers.[23] However, its level was less than that (2500 μg L^{-1}) reached during aging of a modern ale for 6 months at 40 °C[23] and much less than the reported taste threshold in beer[15] (150,000 μg L^{-1}). Yet, A56 contained much more furfural (660 μg L^{-1}) than any of the reference beers and very much more than a modern lager (29 μg L^{-1} for the Olvi Sandels). It might also have been formed thermally during mashing. Presumably the mash and wort were heated over an open fire, requiring very effective stirring to prevent burning of local patches of mash against the inner wall of the pot. Whether the resulting burnt flavor (not necessarily from furfural itself) was perceived as positive or negative is unclear.

We have no information about the stability of phenolic compounds in beer at about 4 °C. Still, the concentrations of phenolic compounds remaining in the shipwreck beers were similar to those found in a bottom-fermented lager (Olvi Sandels) and top-fermented porter (Koff Porter) but differed from those in a modern wheat beer (Paulaner Weiss). Paulaner Weiss contained 5−20-fold more 4-vinylguaiacol (clove flavor) than the shipwreck beers and much smaller proportions of the free forms of ferulic, sinapic, and *p*-coumaric acids (Table 3). 4-Vinylguaiacol is formed by decarboxylation of (free) ferulic acid catalyzed by phenylacrylic decarboxylase (Pad1). Pad1 is absent from bottom-fermenting (lager) strains of brewer's yeast[42] but present in some top-fermenting strains (including all tested strains used for production of wheat beers[42]) and in *Brettanomyces* (*Dekkera*) yeasts[44] and some lactic acid bacteria.[45] Table 3 results suggest that all of the free ferulic acid in Paulaner Weiss has been decarboxylated to 4-vinylguaiacol, but that, in contrast, most of the microorganisms responsible for the fermentation of the shipwreck beers did not produce active Pad1. The small amounts of 4-vinylguaiacol found in the shipwreck beers and the other two reference beers may derive from thermal degradation of ferulic acid during wort boiling.[43] The very small amounts of 4-ethylguaiacol in both shipwreck beers (Table 3) suggest that *Dekkera* spp. did not contribute significantly to their fermentation, because these yeasts produce large amounts of ethylphenols.[43] Levels of total and free ferulic acid are in agreement with those reported by Vanbeneden et al.[43] for 58 beers of many types (bound, 11.4 ± 2.6 mg L^{-1}; free, 1.3 ± 0.8 mg L^{-1}), except that the concentration of free ferulic acid in Paulaner Weiss was lower than in many wheat beers (0.81 ± 0.44; $n = 9$).

In summary, these two about 170-year-old bottles contained two different beers, one (C49) more strongly hopped than the other (A56) with the low α-acid yielding hop varieties common in the 19th century. Both beers exhibited typical profiles of yeast-derived flavor compounds and of phenolics. Present knowledge of the long-term chemical and microbiological stability of these compounds is not adequate to assess how closely the observed profiles indicate the original flavor of the beers. The flavors of these compounds were hidden by very high levels of organic acids, probably produced by bacterial spoilage. The composition of the microbial mixture used to produce these beers is unclear, but it probably did not include many strains producing the Pad1 enzyme responsible for the volatile phenols characteristic of wheat beers. Pad1 activity is common in wild yeast, and its absence suggests that the yeasts employed were domesticated rather than wild.[47]

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