JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
REVIEW

J. Agric. Food Chem. 2010, 58, 3243–3250 3243
DOI:10.1021/jf9041112

Acrolein in Wine: Importance of 3-Hydroxypropionaldehyde and Derivatives in Production and Detection

Rolene Bauer,*,† Donald A. Cowan,† and Andrew Crouch§

†Institute for Microbial Biotechnology and Metagenomics, Department of Biotechnology, University of the Western Cape, Private Bag X17, Bellville, 7535 Cape Town, South Africa, and §Institute of Molecular Sciences, Faculty of Science, University of the Witwatersrand, Private Bag 3, Wits, 2050 Johannesburg, South Africa

Certain lactic acid bacteria strains belonging to the genus *Lactobacillus* have been implicated in the accumulation of 3-hydroxypropionaldehyde (3-HPA) during anaerobic glycerol fermentation. In aqueous solution 3-HPA undergoes reversible dimerization and hydration, resulting in an equilibrium state between different derivatives. Wine quality may be compromised by the presence of 3-HPA due to the potential for spontaneous conversion into acrolein under winemaking conditions. Acrolein is highly toxic and has been implicated in the development of bitterness in wine. Interconversion between 3-HPA derivatives and acrolein is a complex and highly dynamic process driven by hydration and dehydration reactions. Acrolein is furthermore highly reactive and its steady-state concentration in complex systems very low. As a result, analytical detection and quantification in solution is problematic. This paper reviews the biochemical and environmental conditions leading to accumulation of its precursor, 3-HPA. Recent advances in analytical detection are summarized, and the roles played by natural chemical derivatives are highlighted.

KEYWORDS: Acrolein; acrolein dimer; 3-hydroxypropionaldehyde; wine; analytical detection

INTRODUCTION

Acrolein is an α,β-unsaturated carbonyl compound and is also known as 2-propenal or acrylaldehyde. Among the compounds in its class, acrolein is by far the strongest electrophile, shows the highest reactivity with nucleophiles, and is therefore a dangerous substance for the living cell (*1*). The compound is a pulmonary toxicant and an irritant of mucous membranes (*2*) and is considered by regulatory agencies to be one of the greatest noncancer health risks of all organic air pollutants (*3*). Thresholds for acute effects of acrolein in humans, according to the International Program on Chemical Safety (IPCS), are summarized in **Table 1**. Acrolein has furthermore been implicated in the development of bitterness in wine, where it is nonenzymatically produced by a secession of H_2O from 3-hydroxypropionaldehyde (3-HPA), a product of bacterial glycerol fermentation (**Figure 1**). In vivo, a coenzyme B12-dependent glycerol/diol dehydratase (EC 4.2.1.30 and EC 4.2.1.29, respectively) converts glycerol into 3-HPA (*4*, *5*). In the presence of glucose, 3-HPA may be reduced to 1,3-propanediol (1,3-PD) by a NADH-linked dehydrogenase (1,3-PD oxidoreductase; EC 1.1.1.202). Several microorganisms are known to transform glycerol into 3-HPA during anaerobic glycerol fermentation and include the genera *Bacillus*, *Klebsiella*, *Citrobacter*, *Enterobacter*, *Clostridium*, and *Lactobacillus* (*6*). 3-HPA is mostly reduced as an intracellular intermediate and does not accumulate. To date, lactic acid bacteria (LAB) strains belonging to the genus *Lactobacillus* are the only isolates shown to accumulate 3-HPA in the extracellular media (*7*−*11*).

3-HPA plays a central role not only in the synthesis but also in the analytical detection of acrolein in aqueous solutions and fermented products such as wine (pH 3−4), where glycerol is one of the most important byproducts of alcoholic hexose fermentation by yeasts. Acrolein is spontaneously formed by thermal intramolecular dehydration of 3-HPA, and this transformation is enhanced by low pH and/or heat. Analytical detection in an alcoholic water solution is complicated due to nearly complete interaction with ethanol and water to form 3-ethoxypropionaldehyde (3-EPA) and 3-HPA, respectively. As acrolein is highly reactive, its steady-state concentration in complex systems is not expected to be high. For these reasons, few studies have evaluated its content in beverages.

Available quantitative data for acrolein in alcoholic products are limited. Distillation has been employed to separate acrolein prior to its determination (*12*), and free 3-HPA is likely to convert to acrolein during this process. Levels of up to 2.8 mg/L in wine have been reported in isolated cases in the 1980s (*13*, *14*), well above the thresholds for acute effects in humans (**Table 1**). The mean concentration of acrolein in samples (n = 3) of aged lager was 5 μg/L (*15*), and fermented distillery mashes contained up to 420 mg/L (*16*). The presence of acrolein was also reported in brandies (*17*, *18*), rums and whiskies (*19*), apple eau-devie (*20*), and ciders (*21*).

This review highlights the importance of 3-HPA and derivates in the detection of acrolein in wine. The biochemical and environmental conditions leading to 3-HPA production are also reviewed.

*Corresponding author (telephone +27-21-959-2817; fax +27-21-959-3505; e-mail rbauer@uwc.ac.za).

Published on Web 03/01/2010

PHYSICAL AND CHEMICAL PROPERTIES OF ACROLEIN

At room temperature acrolein is a highly flammable, clear, and colorless liquid with an intense acrid odor reminiscent of tomato fruit (R. Bauer, personal observation). The compound is highly volatile with a conversion factor in air at 25 °C and 101.3 kPa of 1 mg/m^3 air = 0.44 ppm (*22*). It is very polar and highly soluble in water and many polar organic solvents including ethanol (*23*). Acrolein is the most reactive of the α,β-unsaturated aldehydes due to the conjugation of a carbonyl group with a vinyl group within its structure, conferring two reactive centers: one at the carbon−carbon double bond and the other at the aldehydic group. Typical reactions involving acrolein include Diels−Alder condensations, carbonyl and carbon−carbon double-bond additions, oxidation, reduction, dimerization, and polymerization. Commercial acrolein is at least 95.5% pure, containing water (up to 3.0% by weight) and other carbonyls (up to 1.5% by weight), mainly propanal and acetone. The pH of commercial acrolein is set with acetic acid between 5 and 6, providing stability by preventing aldol condensation. Hydroquinone is added as an inhibitor of vinyl polymerization (0.1−0.25% by weight). In the absence of an inhibitor, acrolein is subject to highly exothermic polymerization, which is catalyzed by light and air at room temperature to an insoluble, cross-linked solid. In the presence of strong bases or acids, polymerization occurs even with the inclusion of the inhibitor. Relevant physical and chemical data on acrolein are presented in **Table 2** (*24*).

Table 1. Thresholds for the Acute Effects of Acrolein in Humans[a]

effect	concentration[b] mg/m^3	concentration[b] ppm	time (min)
odor perception	0.07	0.03	
eye irritation	0.10	0.04	5
nasal irritation	0.30	0.13	10
increased eye blinking	0.30	0.13	30
decreased respiratory rate	0.70	0.31	40
lacrimation	1.00	0.44	5
extreme irritation of mucosal membranes	2.00	0.88	0.3

[a] Adapted from IPCS Health and Safety Guide No. 67. [b] Conversion factor: 1 mg/m^3 air = 0.44 ppm at 25 °C and 101.3 kPa (760 mmHg).

DYNAMICS IN AQUEOUS SOLUTION

Acrolein. Acrolein does not contain hydrolyzable groups, but reacts with water in a reversible hydration reaction to form 3-HPA. The hydration of acrolein is an equilibrium reaction that approximates first-order kinetics with respect to acrolein (*25*−*27*).

Table 2. Physical and Chemical Properties of Acrolein[a]

property	data
chemical name	2-propenal
alternate names	acrolein, acrylaldehyde
CAS Registry No.	107-02-8
structural formula	CH_2=CHCHO
molecular weight	56.06
boiling point (°C at 101.3 kPa)	52.1−53.5
melting point (°C)	−86.95
vapor pressure (kPa at 20 °C)	29.3−36.5
water solubility (g/L at 20 °C)	206−270
organic solvent solubility	miscible
Henry's law constant (dimensionless at 25 °C)	7.8−180
log K_{ow}	−1.1 to 1.02
log K_{oc}	−0.210 to 2.43
relative density (20 °C)	0.8427−0.8442
relative vapor density	1.94
vapor pressure (kPa at 20 °C)	29.3
log *n*-octanol−water partition coefficient	0.9
odor perception threshold (mg/m^3)	0.07
odor recognition threshold (mg/m^3)	0.48
explosive limits of vapor and air (%)	2.8−31

[a] Adapted from IPCS Health and Safety Guide No. 67.

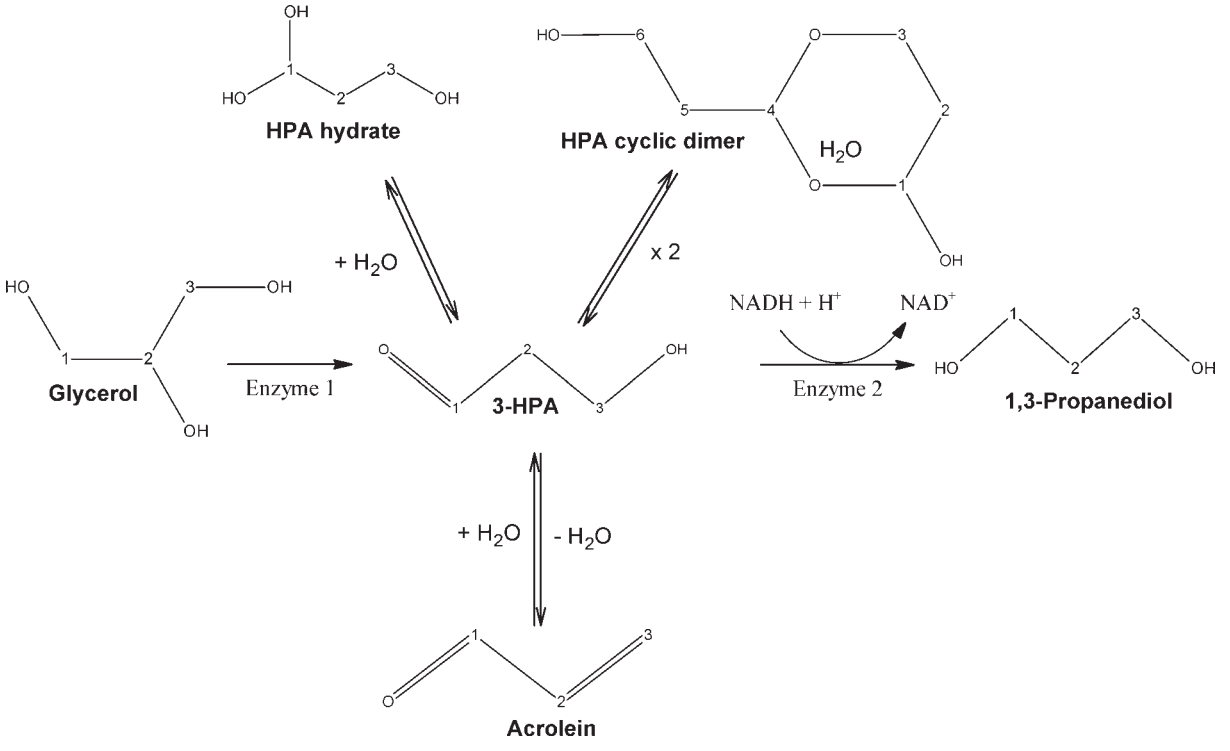

Figure 1. Glycerol catabolism and acrolein production: (enzyme 1) coenzyme B_{12}-dependent glycerol/diol dehydratase; (enzyme 2) 1,3-propanediol oxidoreductase; (+ H_2O) hydration reaction; (− H_2O) dehydration reaction.

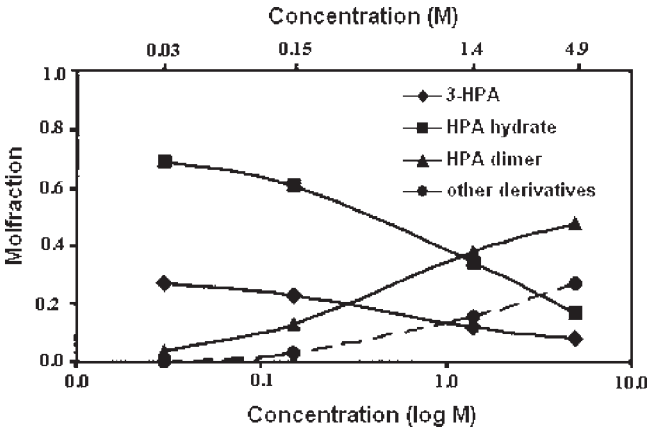

Figure 2. Concentration-dependent distribution of the main forms of the HPA system in D_2O at 20 °C measured by quantitative ^{13}C NMR (adapted from ref *30*).

In dilute solution, whether in distilled water or when buffered between pH 5 and 9, the equilibrium constant is pH independent and approaches 12 at 20 °C, indicating that approximately 92% of acrolein is hydrated in equilibrium. This constant increases with a decrease in temperature but also with a rise in initial acrolein concentration (*27*). Dimerization of 3-HPA probably displaces the equilibrium in favor of hydration.

The rate constant, on the other hand, is pH dependent, and acrolein appears to be most stable between pH 5 and 6. The constant has been reported to increase with increasing acid concentrations (*25*) and also when the pH was raised from 5 to 9 (*26*). In dilute buffered solution, the rate constant (0.015/h at 21 °C) equates to a half-life of 46 h at pH 7 compared to 38 h at pH 8.6. Data for the dependence of rate constant on temperature are not available in the literature, but when acidified solutions of acrolein were heated to 100 °C, equilibrium with its hydrated form was reached in approximately 5 min (*25*). The authors have also shown that the rate constant is independent of the initial acrolein concentration (*25*).

In contrast to laboratory conditions, loss of acrolein (< 3 mg/L initial concentration) in field experiments was faster and decay continued to completion (*26*). In complex aquatic systems, processes other than hydration may contribute to acrolein dissipation, for example, volatilization, adsorption, and absorption or uptake by organisms and sediments. Acrolein moderately absorbs light within the solar spectrum at 315 nm (molar extinction coefficient of 26 L mol^{-1} cm^{-1}), so the compound may even be photoreactive, resulting in photolysis (*28*). The contribution of photolysis to acrolein dissipation is, however, not well established (*29*).

HPA System. In aqueous solution 3-HPA undergoes a reversible dimerization and hydration, resulting in equilibrium among three main forms, also referred to as the HPA system (*30*). As shown in **Figure 1**, the forms include monomeric HPA (3-HPA), hydrated monomeric HPA (1,1,3-trihydroxypropane), and cyclic dimeric HPA (2-(2-hydroxyethyl)-4-hydroxy-1,3-dioxane). Axelsson et al. (*31*) first reported a broad-spectrum antimicrobial action displayed by 3-HPA produced by a probiotic strain of *Lactobacillus reuteri*. The dimeric form of the compound was patented in 1988 under the name reuterin and was postulated to be largely responsible for the antimicrobial effect (*32*). Today, *L. reuteri* is widely used as a starter culture and is commercially available as a probiotic agent (*6*). A probiotic is defined as a live microbial feed supplement that confers a health benefit on the host when administered in adequate amounts (*33*). In aqueous solution reuterin displays activity against Gram-positive and Gram-negative bacteria, yeasts, molds, and protozoa (*34*), but due to the complexity of the HPA system chemistry, the mode of action of reuterin has not been elucidated (*35*).

Few analytical results have been obtained in experimental conditions that were relevant to biological systems (*30*). ^{13}C NMR studies revealed that the HPA system is strongly influenced by concentration. Hydration in aqueous solution increased with dilution to the extent that dimeric HPA and other undetermined derivates (probably polymeric forms), predominant at concentrations above 1.2 M, were largely replaced by HPA hydrate when diluted to 0.03 M (**Figure 2**). The equilibrium state between the different HPA forms was reached within 15 min after dilution. HPA hydrate is likely to remain the dominant form at concentrations below 0.03 M, more relevant for biological systems, as seen by extrapolating the curve.

Figure 3 illustrates various oligomers of 3-HPA that may be present due to the addition of a hydroxyl to an aldehyde group (*36*). As shown in this figure, the HPA system and oligomers appear to be pH dependent. 3-HPA was, however, shown to be relatively stable in acidic solution at room temperature (*25*). ^{13}C NMR studies revealed little influence of moderate changes in pH (from 4.1 to 7.0) on the distribution of the three main forms in organic solution (*30*). Unspecified HPA derivatives formed under strong acidic conditions (12% DCl) or at pH 8.9. Clearly, not only is the HPA system highly dynamic, but complex and chemical characterization has been proven to be difficult.

The effect of temperature on the composition of the HPA system has not been extensively studied. The system appears to be relatively stable at 4 °C (*29*), whereas the production of acrolein was favored over time at 20 °C. Acrolein arises from the spontaneous intramolecular dehydration of 3-HPA, and the process seems to be accelerated under acidic conditions and heat (*37*).

ACROLEIN IN WINE

Glycerol Metabolism and Bitterness. Flavonoid phenols have been described as the primary cause of bitterness in wine (*38*). The polyphenolic content and antioxidant capacity in red wines are directly influenced by the choice of vinification techniques (*39*). A reduction of bitterness and astringency is generally anticipated as a wine ages due to oxidative polymerization and precipitation of the flavonoid phenols. The development of bitterness over time, on the other hand, remains one of the least understood wine defects. Pasteur (*40*) first connected the development of bitterness in red wines with bacterial growth and a concomitant loss of glycerol, whereas Voisenet (*12*) first correlated bitterness with the presence of acrolein. Acrolein is not a bitter compound, but may contribute to bitterness upon interaction with, as yet undetermined, phenolic compounds in wine (*41*), explaining why high-phenolic red wines rather than white wines are associated with this problem. Acrolein concentrations as low as 10 ppm were shown to cause a bitter taint (*42*).

Wines that have undergone malolactic fermentation (MLF) have been reported to contain acrolein at levels of up to 2.8 mg/L and were characterized by reduced glycerol content (*13*, *14*). Limited information is available on the acrolein concentration range to be expected in wine or the role played by bacteria in its production (*10*, *11*).

Glycerol Metabolism and 3-HPA Yield. During alcoholic fermentation by yeast, glycerol is the major end product after ethanol and carbon dioxide (*43*) and could serve as a carbon source for bacteria. Microorganisms either produce glycerol from glucose, like yeast, or metabolize glycerol. LAB play an important

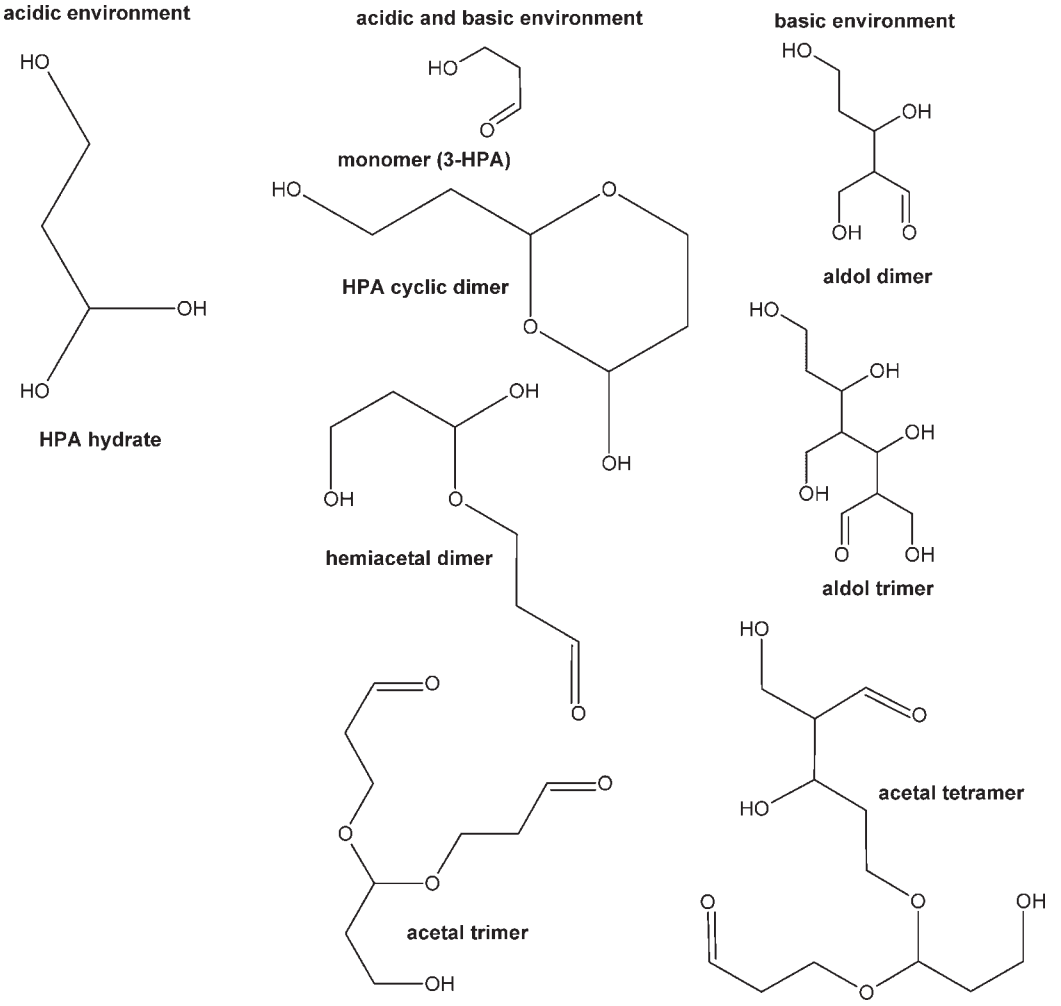

Figure 3. Chemical structures of the HPA system and oligomers that may be present in acidic and basic environments (adapted from ref *36*).

role in MLF of wine (*44*) and may depend on glycerol to maintain viability when fermentable sugars have been exhausted (*10*, *11*).

Anaerobic glycerol catabolism is not widespread among bacteria and may occur via an oxidative and/or reductive pathway (**Figure 4**). The reductive branch requires the presence of a functional coenzyme B_{12}-dependent glycerol/diol dehydratase that catalyzes dehydration of glycerol into 3-HPA (*44*). Microorganisms that use glycerol as the sole carbon and energy source, more common among members of the genera *Citrobacter*, *Klebsiella*, and *Enterobacter*, also involve a parallel oxidative biochemical pathway. Glycerol is oxidized by glycerol dehydrogenase to dihydroxyacetone (DHA), which is phosphorylated by DHA kinase before entering glycolysis. Enzymatic reactions up to pyruvate are universal, whereas microorganisms differ with regard to pyruvate utilization (**Figure 4**). The oxidative pathway is associated with carbon incorporation into cell mass and provides not only energy for anaerobic growth but also reducing equivalents in the form of reduced nicotinamide adenine dinucleotide ($NADH + H^+$). Regeneration of the oxidized form (NAD^+) is achieved through the production of 1,3-PD, the end product of the reductive pathway, which serves as an electron sink. Yield of 1,3-PD per glycerol molecule is determined by the availability of $NADH + H^+$, which in turn is affected by the product distribution of the oxidative pathway. When simultaneously metabolized by both pathways, the yield of the reductive way accounts for about 50−65% of the glycerol consumed (*45*). The oxidative pathway does not appear to feature in heterofermentative LAB (*45*), due to the supply of reducing equivalents and energy for growth by the fermentation of an additional substrate such as glucose or fructose (*46*).

Aerobic glycerol catabolism mainly involves the glycerol kinase pathway and does not concede to 3-HPA production. A homofermentative *Pediococcus pentosaceus* wine isolate has been shown to switch to this pathway when exposed to microaerophilic conditions (*47*). Low levels of glycerol dehydratase activity were, however, still detected.

Anaerobic utilization of glycerol does not guarantee supply of the acrolein precursor. 3-HPA is normally an intracellular intermediate that does not accumulate but is reduced to 1,3-PD, which is excreted into the extracellular media (*45*). Until recently members of *L. reuteri*, *Lactobacillus coryniformis* (*7*), and *Lactobacillus collinoides* (*8*, *9*) were the only strains shown to accumulate 3-HPA in the fermentation medium. As these species do not usually occur in wine, the source of acrolein in wine is disputed. Acrolein is, after all, also a product of lipid peroxidation reactions that could be ubiquitously generated in biological systems (*1*). Strains of *Lactobacillus pentosus*, *Lactobacillus brevis* (*10*), and *Lactobacillus hilgardii* (*11*) isolated from wine were recently implicated to have the ability to accumulate the acrolein precursor, 3-HPA. New questions may now be asked, for example, how common is extracellular 3-HPA production in the microbial world and to what extent do 1,3-PD producers leak 3-HPA into the fermentation media?

Environmental Conditions Affecting Microbial 3-HPA Yield. Product yield from glycerol and the fate of 3-HPA are dependent not only on the microorganisms and specialized enzymatic

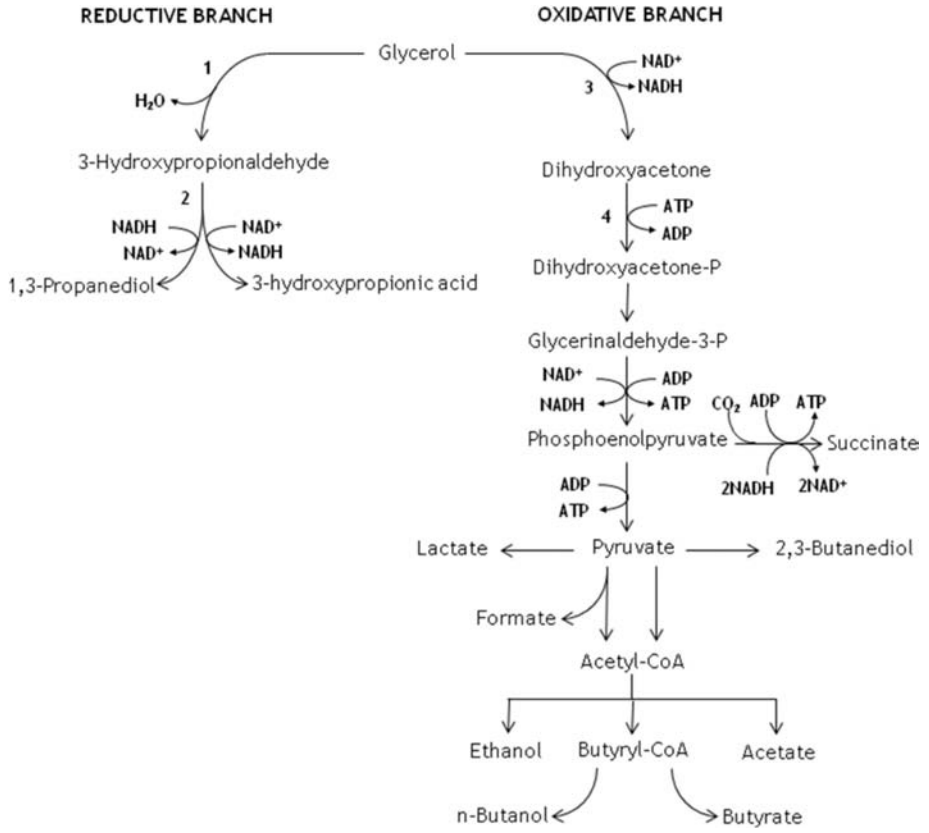

Figure 4. Proposed summary for anaerobic glycerol catabolism (adapted from ref *37*): (1) glycerol/diol dehydratase; (2) 1,3-propanediol oxidoreductase; (3) glycerol dehydrogenase; (4) dihydroxyacetone kinase.

pathways involved but also on the process conditions prevailing in wine, such as control of MLF, acidification of grape must, and fermentation temperature (*44*). Inconclusive results with regard to the impact of several critical environmental parameters such as cell concentrations, pH, and temperature may stem from the toxic effect displayed by 3-HPA on the producer strains (*48*) because cell viability is critical for glycerol biotransformation (*6*).

Cell Concentration. Bauer et al. (*10*) evaluated the effect of individual environmental parameters on bacterial production by *L. reueri* DSMZ 20016 under conditions that restrict 3-HPA yield to levels below the threshold affecting cell viability. 3-HPA production was shown to increase with an increase in cell concentration up to a defined value, followed by a sudden and severe drop in 3-HPA content at higher cell concentrations (*10*). The study suggests that the toxic effect of 3-HPA may be circumvented through regulation of its accumulation. Sudden disappearance of 3-HPA from the external cell environment may conceivably be ascribed to a consequent enzymatic reduction of 3-HPA into 1,3-PDO and/or the dismutation of 3-HPA and 1,3-PDO into 3-hydroxypropionic acid by the same enzyme (enzyme 2, **Figure 4**) (*8*).

Temperature and pH. Anaerobically cultured *L. reuteri* was reported to produce 3-HPA under physiological conditions of temperature and pH (*32*). Subsequently it was shown that there is no significant difference in 3-HPA production for temperatures between 15 and 37 °C (*48*), whereas production was strongly favored at pH 6 (*10*). Although not a wine lactobacilli strain, production was drastically reduced at pH values applicable to the winemaking process (pH 3−4). A recent study shows that at least a portion of the diol-dehydratase of *L. reuteri* is metabolosome-associated and may be in part responsible for 3-HPA production from glycerol (*49*). A very large and complex operon (*pdu*) encodes for the diol dehydratase and metabolosome structural genes in *L. reuteri* and appears to be regulated by external pH (*50*). Physiological constraints posed by a fermentative background on the electrochemically properties of the metabolosome and its involvement in 3-HPA production require further investigation.

Substrate Availability. The presence of glycerol and fructose, the most common residual sugars in wine, is favorable for glycerol metabolism and accumulation of 3-HPA by heterofermentative LAB strains (*9*). 3-HPA production by *L. reuteri* was shown to increase with an increase in glycerol concentrations up to 300 mM (*10*), and glycerol dehydratase activity appeared to be inhibited by higher concentrations. When fructose was used as an electron acceptor to reoxidize NADH, the NAD^+/NADH ratio was increased (*51*). This ratio, rather than the concentration of the nucleotides, is positively correlated to accumulation of 3-HPA (*8*,*51*,*52*). Together with fructose, glucose is the main sugar in fermenting grape juice. For resting *L. reuteri* cells, 1,3-PD was shown to be the major product of glycerol conversion when the molar ratio of glucose to glycerol is >1.6 (*51*). A ratio of <0.33 favored accumulation of 3-HPA, whereas no 1,3-PD was formed in the absence of glucose. The ratio between glucose and glycerol levels therefore appears to be the determining factor regarding the product distribution of the reductive pathway. On the other hand, the presence of glucose represses reduction of glycerol, probably through the disturbance of the redox balance in resting cells that affects the NADH-linked reduction of 3-HPA to 1,3-PD (*51*). An adequate supply of glycerol, however, appears to outweigh glucose repression.

Process Conditions Affecting 3-HPA Conversion into Acrolein. The HPA system is stable at 4 °C, hence studies on the application of reuterin in food stored at low temperatures such as dairy products (*6*). Spontaneous intramolecular dehydration of 3-HPA results in the formation of acrolein, and the process appears to be

Table 3. Methods for the Determination of Acrolein[a]

sample matrix	sample preparation	assay	LOD	ref
exhaust gas	derivitize with *O*-benzylhydroxylamine; brominate, reduce, and extract with diethyl ether	GC-ECD	not reported	*55*
exhaust gas	derivatize with DNPH impregnated filters; toluene extraction	GC-FID	0.05 mg/m^3	*56*
air	draw air through sodium bisulfite containing cartridge; react with 4-hexylresorcinol in an alcoholic TCA solvent with $HgCl_2$ as catalyst to form a colored complex	colorimetry	22.9 μg/m^3	*57*
air	adsorb on sorbent coated with 2-(hydroxymethyl)piperidine; desorp with toluene	GC-NSD	6.1 μg/m^3	*58*
air	collect in sodium bisulfite mist chamber; liberate carbonyls from bisulfite addicts; derivitize with PFBHA; solvent extraction	GC-MS	0.012 μg/m^3	*3*
moist air	collect in DNPH-impregnated adsorbent tubes in the presence of CaCl; acetonitrile extraction	HPLC-UV	0.01 mg/ m^3	*59*
biological samples	derivitize with DNPH; extract with chloroform HCl solvent; dry with nitrogen; dissolve in methanol	HPLC-UV	1 ng	*60*
liquid and solid wastes	purge with inert gas; trap with suitable adsorbent; desorp as vapor onto GC column	GC-FID	matrix dependent	*61*
water	derivitize with *O*-methoxylamine; brominate, reduce, and extract with diethyl ether	GC-ECD	0.4 μg/L	*62*
water	derivitize with PFBHA	GC-MS	not reported	*63*
aqueous solution	derivitize with PFBHA	MIMS-EIMS	10 μg/L	*64*
cider and Calvados	derivatize with MBTH	GC-NPD	0.6−60 μg/L	*65*
urine	SPME	GC-MS	60 μg/L	*66*
wine	SPME/SEP; detection of acrolein dimer, a natural derivative	GC-MS	0.2−20 mg/L	*67*

[a] LOD, limit of detection; DNPH, 2,4-dinitrophenylhydrazine; TCA, trichloroacetic acid; PFBHA, *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine; MBTH, 3-methylbenzothiazolone hydrazine; ECD, electron capture detection; FID, flame ionization detection; GC, gas chromatography; HPLC, high-performance liquid chromatography; UV, ultraviolet; MIMS, membrane introduction mass spectrometry; EIMS, electron impact mass spectrometry; MS, mass spectrometry; NSD, nitrogen selective detection; SPME, solid-phase microextraction; SEP, sample enrichment probe.

accelerated under acidic conditions and heat. The formation of acrolein in food or upon indigestion may be an unappreciated risk that deserves further investigation. Temperatures as low as 20 °C favor production of acrolein over time (*30*), emphasizing the importance of temperature control during the production and storage of high-risk products such as wine that contain glycerol. Although recent progress has been made in determining the conditions required for bacterial production of 3-HPA (see the previous section), further work is required to fully elucidate the effect of process conditions relevant to wine on the conversion of 3-HPA into acrolein. On the basis of existing information, Bauer et al. (*10*) made recommendations with regard to minimizing acrolein and its precursor in wine.

ANALYTICAL DETECTION

Methods available for the determination of acrolein have been reviewed (*53*, *54*); examples are summarized in **Table 3** (*55*−*67*). Spectrophotometric determination with 4-hexylresorcinol and a fluorometric method with *m*-aminophenol are common procedures. More recent practices involve gas chromatography (GC) and high-performance liquid chromatography (HPLC). Compounds such as acrolein that display poor chromatographic performance, high reactivity, high volatility, or thermal instability often need derivatization during the sample preparation procedure. Sampling methods for acrolein and other airborne aldehydes in emissions, for example, EPA method TO-11A (*68*), are generally based on carbonyl derivatizing agents such as 2,4-dinitrophenylhydrazine (DNPH), which produce hydrazones. Environmental samples and drinking water are typically derivatized with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBHA). These derivatives are solvent desorbed and separated by HPLC followed by UV detection or, more recently, identified with GC-MS. Detection limits reported for methods involving GC with electron capture detection and GC-MS with ion-selective monitoring were 3.5 and 16.4 μg/L, respectively (*69*).

Such methods are effective for the quantification of certain aldehydes and ketones, but have not proved to be reliable for acrolein and other unsaturated carbonyls (*70*). Problems with derivative analysis include instability, long sample collection times, coelution of similar compounds, and ozone interferences. Although recent advances have been made in establishing derivatization methodology for measuring acrolein in ambient air (*3*), analysis from liquid samples remains problematic.

General trends in the development of modern extraction techniques center on the use of adsorbents or absorbents for selective analyte extraction as an alternative to solvent extraction, and various systems have been developed for this purpose (*69*). Solid-phase microextraction (SPME) is generally preferred, because it is simple to use and the nonautomated version requires neither the adaptation of the GC nor the need for additional expensive instrumentation (*71*). Whereas traditional sorptive phase extraction (SPE) methods tend to be based on phases similar to that of liquid chromatography (LC), SPME phases tend to be similar to GC stationary phases. In this regard SPME is more versatile, because it can be employed for extracting solutes from both liquid and gaseous samples. The major disadvantage of SPME is the amount of available phase, which limits the mass of analyte extracted. Techniques such as stir bar sorptive extraction (SBSE) have been developed to deliver more sorptive stationary phase mass and surface area and result in correspondingly higher sensitivity (*72*). In SBME the analytes are enriched in a sorptive rubber sleeve on a magnetic stir bar allowing sampling from gas as well as liquid. Two other promising sample enrichment methods are the high-capacity sorption probe (HCSP) and solid-phase aroma-concentrate extraction (SPACE), developed by Pettersson et al. (*73*) and Ishikawa et al. (*74*), respectively. Because a very small volume of sorptive phase is used in SPME, thermal desorption of the enriched material takes place almost instantaneously. On the other hand, the large volumes of sorptive material employed with SBME, HCSP, and SPACE require cryofocusing of volatiles on the GC column after desorption from the fiber. The recent introduction of the high-capacity sample enrichment probe (SEP) overcomes this problem and allows analysis of volatiles from solid, aqueous, and gaseous samples (*75*, *76*). As with SPME, desorption and GC separation of the volatiles run almost concurrently; therefore, no auxiliary thermal desorption and cryotrapping equipment are required. Another advantage of SEP analysis is the absence of ice formation in the column, a problem that could be encountered if small quantities of moisture are adsorbed during sampling and are subsequently cryotrapped on the column, resulting in the interruption of the carrier gas flow.

Few studies have reported the use of modern extraction techniques as alternatives to solvent extraction for direct analysis of acrolein (*66*, *67*, *77*). Bauer et al. (*67*) recently reported on the suitability of SPME and SEP, in combination with headspace GC-MS, to measure a natural derivative, acrolein dimer, as a marker for the detection of acrolein in complex matrices such as wine.

LITERATURE CITED

(1) Uchida, K.; Kanematsu, M.; Morimitsu, Y.; Osawa, T.; Noguchi, N.; Niki, E. Acrolein is a product of lipid peroxidation reaction. *J. Biol. Chem.* **1998**, *273*, 16058–16066.

(2) Esterbauer, H.; Schaur, R. J.; Zollner, H. Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde and related aldehydes. *Free Radical Biol. Med.* **1991**, *11*, 81–128.

(3) Seaman, V.; Charles, M.; Cahill, T. A sensitive method for the quantification of acrolein and other volatile carbonyls in ambient air. *Anal. Chem.* **2006**, *78*, 2405–2412.

(4) Smiley, K. L.; Sobolov, M. A cobamide-requiring glycerol dehydrase from an acrolein-forming *Lactobacillus*. *Arch. Biochem. Biophys.* **1962**, *97*, 538–43.

(5) Toraya, T.; Shirakashi, T.; Kosuga, T.; Fukui, S. Substrate specificity of coenzyme B12-dependent diol dehydrase: glycerol as both a good substrate and a potent inactivator. *Biochem. Biophys. Res. Commun.* **1976**, *69*, 475–480.

(6) Vollenweider, S.; Lacroix, C. 3-Hydroxypropionaldehyde: applications and perspectives of biotechnological production. *Appl. Microbiol. Biotechnol.* **2004**, *64*, 16–27.

(7) Martin, R.; Olivares, M.; Marin, M. L.; Xaus, J.; Fernandez, L.; Rodríguez, J. M. Characterization of a reuterin-producing *Lactobacillus coryniformis* strain isolated from a goat's milk cheese. *Int. J. Food Microbiol.* **2005**, *104*, 267–277.

(8) Sauvageot, N.; Gouffi, K.; Laplace, J. M.; Auffray, Y. Glycerol metabolism in *Lactobacillus collinoides*: production of 3-hydroxypropionaldehyde, a precursor of acrolein. *Int. J. Food Microbiol.* **2000**, *55*, 167–170.

(9) Garai-Ibabe, G.; Ibarburu, I.; Berregi, I.; Claisse, O.; Lonvaud-Funel, A.; Irastorza, A.; Dueñas, M. T. Glycerol metabolism and bitterness producing lactic acid bacteria in cidermaking. *Int. J. Food Microbiol.* **2008**, *121*, 253–261.

(10) Bauer, R.; du Toit, M.; Kossmann, J. Influence of environmental parameters on production of the acrolein precursor 3-hydroxpropionaldehyde by *Lactobacillus reuteri* DSMZ 20016 and its accumulation by wine lactobacilli. *Int. J. Food Microbiol.* **2010**, *137*, 28–31.

(11) Pasteris, S. E.; Strasser de Saad, A. M. Sugar−glycerol cofermentations by *Lactobacillus hilgardii* isolated from wine. *J. Agric. Food Chem.* **2009**, *57*, 3853–3858.

(12) Voisenet, E. Formation d'acroleine dans la maladie de l'amertume des vins. *C. R. Acad. Sci.* **1910**, *150*, 1614–1616.

(13) Dittrich, H. H.; Sponholz, W. R.; Wünsch, B.; Wipfler, M. Zur veränderung des weines durch bakteriellen säureabbau. *Wein-Wiss.* **1980**, *35*, 421–429.

(14) Sponholz, W. R. Analyse und vorkommen von aldehyden in weinen. *Eur. Food Res. Technol.* **1982**, *174*, 458–462.

(15) Greenhoff, K.; Wheeler, R. E. Analysis of beer carbonyls at the part per billion level by combined liquid chromatography and high pressure liquid chromatography. *J. Inst. Brew.* **1981**, *87*, 35–41.

(16) Mills, D. E.; Baugh, W. D.; Conner, H. A. Studies on the formation of acrolein in distillery mashes. *Appl. Microbiol.* **1954**, *2*, 9–13.

(17) Rosenthaler, L.; Vegezzi, G. Acrolein in spirituosen. *Eur. Food Res. Technol.* **1955**, *102*, 117–123.

(18) Postel, W.; Adam, L. Gas chromatographic characterization of raspberry brandy. *Dtsch. Lebensm. Rundsch.* **1983**, *79*, 117–122.

(19) Lencrerot, P.; Parfait, A.; Jouret, C. Rôle des corynebactéries dans la production d'acroléine (2-propenal) dans les rhums. *Ind. Aliment. Agric.* **1984**, *101*, 763–765.

(20) Subden, R.; Krizus, A.; Akhtar, M. Mutagen content of Canadian apple eau-de-vie. *Can. Inst. Food Sci. Technol. J.* **1986**, *19*, 134–136.

(21) Claisse, O.; Lonvaud-Funel, A. Primers and a specific DNA probe for detecting lactic acid bacteria producing 3-hydroxypropionaldehyde from glycerol in spoiled ciders. *J. Food Prot.* **2001**, *64*, 833–837.

(22) Albin, T. B. Handling and toxicology. In *Acrolein*; Smith, C. W., Ed.; Wiley: New York, 1962; pp 234−239.

(23) Beauchamp, R. O.; Andjelkovich, D. A.; Kligerman, A. D.; Morgan, K. T.; d'A Heck, H.; Feron, V. J. A critical review of the literature on acrolein toxicity. *CRC Crit. Rev. Toxicol.* **1985**, *14*, 309–380.

(24) Anderson, E. A.; Hood, G. C. Physical properties. In *Acrolein*; Smith, C. W., Ed.; Wiley: New York, 1962; pp 7−50.

(25) Pressman, D.; Lucas, H. J. Hydration of unsaturated compounds. XI. Acrolein and acrylic acid. *J. Am. Chem. Soc.* **1942**, *64*, 1953–1957.

(26) Bowmer, K. H.; Higgins, M. L. Some aspects of the persistence and fate of acrolein herbicide in water. *Arch. Environ. Contam. Toxicol.* **1976**, *5*, 87–96.

(27) Hall, R. H.; Stern, E. S. Acid-catalysed hydration of acraldehyde. Kinetics of the reaction and isolation of β-hydroxypropaldehyde. *J. Chem. Soc.* **1950**, 490–498.

(28) Lyman, W. J.; Reehl, W. F.; Rosenblatt, D. H. In *Handbook of Chemical Property Estimation Methods*; McGraw-Hill Book: New York, 1982.

(29) Magneron, I.; Thévenet, R.; Mellouki, A.; Le Bras, G. A study of the photolysis and OH-initiated oxidation of acrolein and *trans*-crotonaldehyde. *J. Phys. Chem. A* **2002**, *106*, 2526–2537.

(30) Vollenweider, S.; Grassi, G.; König, I.; Puhan, Z. Purification and structural characterization of 3-hydroxypropionaldehyde and its derivatives. *J. Agric. Food Chem.* **2003**, *51*, 3287–3293.

(31) Axelsson, L.; Chung, T. C.; Dobrogosz, W. J.; Lindgren, L. E. Discovery of a new antimicrobial substance produced by *Lactobacillus reuteri*. *FEMS Microbiol. Rev.* **1987**, *46*, 65–71.

(32) Talarico, T. L.; Casas, I. A.; Cung, T. C.; Dobrogosz, W. J. Production and isolation of reuterin, a growth inhibitor produced by *Lactobacillus reuteri*. *Antimicrob. Agents Chemother.* **1988**, *32*, 1854–1858.

(33) WHO. *Guidelines for the Evaluation of Probiotics in Food*; Geneva, Switzerland, 2002.

(34) El-Ziney, M. G.; Debevere, J. M.; Jakobsen, M. Reuterin. In *Natural Food Antimicrobial Systems*; Naidu, A. S., Ed.; CRC Press: Boca Raton, FL, 2000; pp 567−588.

(35) Cleusix, V.; Lacroix, C.; Vollenweider, S.; Duboux, M.; Le Blay, G. Inhibitory activity spectrum of reuterin produced by *Lactobacillus reuteri* against intestinal bacteria. *BMC Microbiol.* **2007**, *7*, 101–110.

(36) Sung, H.; Chen, C.; Liang, H.; Hong, M. A natural compound (reuterin) produced by *Lactobacillus reuteri* for biological-tissue fixation. *Biomaterials* **2003**, *24*, 1335–1347.

(37) Sobolov, M.; Smiley, K. L. Metabolism of glycerol by an acrolein-forming *Lactobacillus*. *J. Bacteriol.* **1959**, *79*, 261–266.

(38) Noble, A. C. Bitterness in wine. *Physiol. Behav.* **1994**, *56*, 1251–1255.

(39) Netzel, M.; Strass, G.; Bitsch, I.; Könitz, R.; Christmann, M.; Bitsch, R. Effect of grape processing on selected antioxidant phenolics in red wine. *J. Food Eng.* **2003**, *56*, 223–228.

(40) Pasteur, L. In *Études sur le Vin*, 2nd ed.; Savy: Paris, France, 1873.

(41) Rentschler, H.; Tanner, H. Das bitterwerden der Rotweine. *Mitt. Lebensm Unters. Hyg.* **1951**, *42*, 463–475.

(42) Margalith, P. Z. In *Flavour Microbiology*; Thomas, C. G., Ed.; Springfield, IL, 1983; pp 173−224.

(43) Nieuwoudt, H. H.; Prior, B. A.; Pretorius, I. S.; Bauer, F. F. Glycerol in South African table wines: an assessment of its relationship to wine quality. *S. Afr. J. Enol. Vitic.* **2002**, *23*, 22–30.

(44) Bauer, R.; Dicks, L. M. T. Control of malolactic fermentation in wine. A review. *S. Afr. J. Enol. Vitic.* **2004**, *25*, 74–88.

(45) Biebl, H.; Menzel, K.; Zeng, A.-P.; Deckwer, W.-D. Microbial production of 1,3-propanediol. *Appl. Microbiol. Biotechnol.* **1999**, *52*, 289–297.

(46) Veiga da Cunha, M.; Foster, M. A. Sugar−glycerol cofermentation in lactobacilli: the fate of lactate. *J. Bacteriol.* **1992**, *174*, 1013–1019.

(47) Pasteris, S. E.; Strasser de Saad, A. M. Aerobic glycerol catabolism by *Pediococcus pentosaceus* isolated from wine. *Food Microbiol.* **2005**, *22*, 399–407.

(48) Doleyres, Y.; Beck, P.; Vollenweider, S.; Lacroix, C. Production of 3-hydroxypropionaldehyde using a two-step process with *Lactobacillus reuteri*. *Appl. Microbiol. Biotechnol.* **2005**, *68*, 467–474.

(49) Sriramulu, D. D.; Liang, M.; Hernandez-Romero, D.; Raux-Deery, E. *Lactobacillus reuteri* DSM 20016 produces cobalamin-dependent diol dehydratase in metabolosomes and metabolizes 1,2-propanediol by disproportionation. *J. Bacteriol.* **2008**, *13*, 4559–4567.
(50) Wall, T.; Bath, K.; Britton, R. A.; Jonsson, H.; Versalovic, J.; S. Roos, S. The early response to acid shock in *Lactobacillus reuteri* involves the ClpL chaperone and a putative cell wall-altering esterase. *Appl. Environ. Microbiol.* **2007**, *73*, 3924–3935.
(51) Lüthi-Peng, Q.; Dileme, E. B.; Puhan, Z. Effect of glucose on glycerol bioconversion by *Lactobacillus reuteri*. *Appl. Microbiol. Biotechnol.* **2002**, *59*, 289–296.
(52) Barbirato, F.; Grivet, J. P.; Soucaille, P.; Bories, A. 3-Hydroxypropionaldehyde, an inhibitory metabolite of glycerol fermentation to 1,3-propanediol by Enterobacterial species. *Appl. Environ. Microbiol.* **1996**, *62*, 1448–1451.
(53) Agency for Toxic Substances and Disease Registry (ATSDR). *Toxicological Profile for Acrolein*; U.S. Department of Health and Human Services, Public Health Service: Atlanta, GA, **2007**.
(54) Peters, E. D. Analytical methods. In *Acrolein*; Smith, C. W., Ed.; Wiley: New York, 1962; pp 247−249.
(55) Nishikawa, H.; Hayakawa, T.; Sakai, T. Determination of acrolein and crotonaldehyde in automobile exhaust gas by gas chromatography with electron-capture detection. *Analyst* **1987**, *112*, 859–862.
(56) Otson, R.; Fellin, P.; Tran, Q.; Stoyanoff, R. Examination of sampling methods for assessment of personal exposures to airborne aldehydes. *Analyst* **1993**, *118*, 1253–1259.
(57) Feldstein, M.; Bryan, R. J.; Hyde, D. L.; Levaggi, D. A.; Locke, D. C.; Rasmussen, R. A.; Warner, P. O. Determination of acrolein in air. In *Methods of Air Sampling and Analysis*, 3rd ed.; Lodge, J. P., Ed.; Lewis Publishers: Chelsea, MI, 1989; pp 646−648.
(58) Eller, P. M. Methods 2501 and 2539. In *NIOSH Manual of Analytical Methods*, 4th ed., U.S. Government Printing Office: Washington, DC, 1994; Vol. 1, pp 94−113.
(59) Vainiotalo, S.; Matveinen, K. Determination of acrolein in air with 2,4 dinitrophenylhydrazine impregnated adsorbent tubes in the presence of water. In *Clean Air at Work. New Trends in Assessment and Measurement for the 1990s*; Brown, R. H., Curtis, M., Saunders, K. J., Vandendriessche, S., Eds.; European Commission: Luxembourg, 1992; pp 204−206.
(60) Boor, P. J.; Ansari, G. A. S. High-performance liquid chromatographic method for quantification of acrolein in biological samples. *J. Chromatogr.* **1986**, *375*, 159–164.
(61) U.S. Environmental Protection Agency. Method 8030. Acrolein, acrylonitrile, acetonitrile. In *Test Methods for Evaluating Solid Waste: Physical/Chemical Methods*, 3rd ed.; Office of Solid Waste and Emergency Response: Washington, DC, 1986; Vol. 1A, pp 1−11.
(62) Nishikawa, H.; Hayakawa, T.; Sakai, T. Gas chromatographic determination of acrolein in rain water using bromination of *O*-methyloxime. *Analyst* **1987**, *112*, 45–48.
(63) Le Lacheur, R. M.; Sonnenberg, L. B.; Singer, P. C.; Christman, R. F.; Charles, M. J. Identification of carbonyl compounds in environmental samples. *Environ. Sci. Technol.* **1993**, *27*, 2745–2753.
(64) Choudhury, T. K.; Kotiaho, T.; Cooks, R. G. Analysis of acrolein and acrylonitrile in aqueous solution by membrane introduction mass spectrometry. *Talanta* **1992**, *39*, 1113–1120.
(65) Ledauphin, J.; Barillier, D.; Beljean-Leymarie, M. Gas chromatographic quantification of aliphatic aldehydes in freshly distilled Calvados and Cognac using 3-methylbenzothiazolin-2-one hydrazone as derivative agent. *J. Chromatogr., A* **2006**, *1115*, 225–232.
(66) Takamoto, S.; Sakuraa, N.; Yashikib, M.; Kojimab, T. Determination of acrolein by headspace solid-phase microextraction gas chromatography and mass spectrometry. *J. Chromatogr., B* **2001**, *758*, 123–128.
(67) Bauer, R.; Crouch, A.; Kossmann, J.; Hiten, F.; Burger, B. Acrolein dimer as marker for direct detection of acrolein in wine. Unpublished results.
(68) U.S. Environmental Protection Agency. Method TO-11A, EPA-625/R-96/010b. *Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air*; U.S. EPA: Research Triangle Park, NC, 1999.
(69) Raynie, D. E. Modern extraction techniques. *Anal. Chem.* **2006**, *78*, 3997–4003.
(70) Goelen, E.; Lambrechts, M.; Geyskens, F. Sampling intercomparisons for aldehydes in simulated workplace air. *Analyst* **1997**, *122*, 411–419.
(71) Arthur, C. L.; Pawliszyn, J. Solid phase microextraction with thermal desorption using fused silica optical fibers. *Anal. Chem.* **1990**, *62*, 2145–2148.
(72) Baltussen, E.; Sandra, P.; David, F.; Cramers, C. Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: theory and principles. *J. Microcolumn Sep.* **1999**, *11*, 737–747.
(73) Pettersson, J.; Kloskowski, A.; Zaniol, C.; Roeraade, J. Automated high-capacity sorption probe for extraction of organic compounds in aqueous samples followed by gas chromatographic analysis. *J. Chromatogr., A* **2004**, *1033*, 339–347.
(74) Ishikawa, M.; Ito, O.; Ishizaki, S.; Kurobayashi, Y.; Fujita, A. Solid-phase aroma concentrate extraction (SPACE): a new headspace technique for more sensitive analysis of volatiles. *Flavour Fragrance J.* **2004**, *19*, 183–187.
(75) Burger, B. V.; le Roux, M.; Burger, W. J. G. Headspace analysis: a novel method for the production of capillary traps with ultra-thick stationary phase layers. *J. High Resolut. Chromatogr.* **1990**, *13*, 777.
(76) Burger, B. V.; Marx, B.; le Roux, M.; Burger, W. J. G. Simplified analysis of organic compounds in headspace and aqueous samples by high-capacity sample enrichment probe. *J. Chromatogr., A* **2006**, *1121*, 259–267.
(77) Takamoto, S.; Sakura, N.; Namerab, A.; Yashiki, M. Monitoring of urinary acrolein concentration in patients receiving cyclophosphamide and ifosphamide. *J. Chromatogr., B* **2004**, *806*, 59–63.

Received for review September 11, 2009. Revised manuscript received February 10, 2010. Accepted February 15, 2010.