daily amount of vitamin E originates from the group butter, margarine, oils, particularly from vegetable oils.

Although seasonality with respect to vitamin concentrations does not seem so obvious, Figures 6 and 7 show that, for most vitamins analyzed, highest daily amounts could be observed in November/December, while the daily intakes were lowest in May. But even when the lowest daily amounts of all vitamins are used to calculate total vitamin A or vitamin E intake, the recommendations for male adolescents are met.

In conclusion, the amounts of both vitamin A (or retinol equivalents) and vitamin E analyzed in total diets of Dutch male adolescents can be regarded as more than sufficient. For vitamin A, the group meat and meat products is the most important one for daily supply, for carotenoids the groups leafy vegetables and root vegetables are the most important sources, and the group butter, margarine, oils is the most important source for the daily vitamin E intake. A group of male adolescents formed the basis for our total diet study. For other age categories, consuming less food, or for groups or individuals with a more extreme dietary pattern, conclusions may diverge from ours. At present, a third total diet study is carried out, in which individual food products are analyzed rather than food groups. This approach will enable us to evaluate the quality of dietary food intake of any population group if their food consumption pattern (or market basket) is known.

Registry No. Vitamin A, 68-26-8; β-carotene, 7235-40-7; α-tocopherol, 59-02-9; β-tocopherol, 148-03-8; γ-tocopherol, 7616-22-0; δ-tocopherol, 119-13-1.

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Extraction and Analysis of Volatile Compounds in White Wines Using Amberlite XAD-2 Resin and Capillary Gas Chromatography

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A rapid and simplified technique for the analysis of major volatile compounds in wines has been developed by modification of a previously published procedure for beer analysis. Modifications include adjustment of the alcohol content of standards and samples to 10% (v/v) ethanol, use of larger sample size, use of two internal standards, and employment of a newly developed capillary column with a modified poly(ethylene glycol) stationary phase. Relative recoveries of higher alcohols, esters, and medium-chain fatty acids extracted from a white wine ranged from 90 to 114%. Precision, as measured by coefficients of variation, were less than 5% with the exceptions of isobutyl alcohol (22%) and decanoic acid (9%). Analysis of white wines fermented with and without insoluble grape solids and/or yeast ghosts revealed differences in the concentration of higher alcohols, esters, and medium-chain fatty acids in the bottled wines.

Various techniques for the extraction and analysis of volatile compounds in wines have included using extraction solvents such as carbon disulfide or Freon (Snyman, 1977; Nelson and Acree, 1978; Marais and Houtman, 1979) or have used other solvents or procedures (Cobb et al., 1978; Usseglio-Tomasset and Di Stefano, 1981; Simpson and Miller, 1984; Shinohara, 1985; Baumes et al., 1986). Although widely used for analysis of volatiles, these methods often require several hours for extraction and analysis of one wine sample, a major disadvantage to their use. Recently, a technique for analysis of volatile

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compounds in beer was developed by Hawthorne et al. (1987). This method utilizes Amberlite XAD-2 resin to adsorb volatile compounds from beer prior to elution of the major volatiles using diethyl ether. With use of this method, more samples could be extracted and analyzed compared to traditional analytical techniques including solvent extraction. In fact, the most time-consuming step was the gas–liquid chromatography. In the present work, this method was adapted for analysis of major volatile compounds in a French hybrid white wine.

The presence (or absence) of insoluble materials during the alcoholic fermentation has long been known to alter the volatile composition of the resultant wines (Crowell and Guymon, 1963; Groat and Ough, 1978; Houtman et al., 1980a,b; Klingshirn et al., 1987; Liu et al., 1987). Most reports concerning production of higher alcohols (fusel oils) are similar to that of Guymon et al. (1961) who found that the presence of insoluble grape solids increased the levels produced during alcoholic fermentation. Houtman et al. (1980a) was able to show that synthesis of reported medium-chain fatty acids was dependent on the presence of insoluble grape solids and the initial sugar level of Chenin blanc juice. The influence of insoluble solids on esters is less clear in that Groat and Ough (1978) reported ester production to be enhanced in one must but retarded in another due to the presence of insoluble grape solids. Thus, the second objective of this study was to determine the influence of two different insoluble materials, insoluble grape solids and yeast ghosts (hulls or rinds), on the levels of major volatile compounds in wines extracted and analyzed by the XAD-2 method.

MATERIALS AND METHODS

Prior to use, Amberlite XAD-2 resin (Aldrich Chemical Co., Milwaukee, WI) was sequentially washed with distilled water, methanol, diethyl ether, methanol, and distilled water to remove impurities as outlined by Hawthorne et al. (1987). The resin was stored wet at 7 °C until use.

Higher alcohols, esters, and medium-chain fatty acids (≥99% purity, Aldrich) were dissolved in 40% (v/v) ethanol at concentrations similar to those present in wine and used for gas–liquid chromatography calibration standards. Dodecanol (Aldrich) and nonanoic acid (Sigma Chemical Co., St. Louis, MO) at concentrations of 157 and 97 mg/L, respectively, served as the internal standards. Dodecanol was used for quantitation of higher alcohols (isobutyl alcohol, isoamyl alcohol, phenylethyl alcohol) and esters (ethyl acetate, isoamyl acetate, phenylethyl acetate, ethyl hexanoate, ethyl octanoate) while nonanoic acid was used for quantitation of medium-chain fatty acids (hexanoic acid, octanoic acid, decanoic acid). All prepared standards were stored at 7 °C and were allowed to equilibrate to room temperature (25 °C) prior to use.

A 50-mL sample of wine or calibration standard was pipetted into 100-mL wide-mouth glass jars. Prior to extraction, the alcohol contents of calibration standards and wine samples were standardized to 10% (v/v) ethanol. Hawthorne et al. (1987) observed that the alcohol content of a sample influences the relative recovery of certain higher alcohols, especially isobutyl alcohol. Consequently, the alcoholic content of the wines was determined with use of an ebulliometer, and both samples and standards were adjusted to 10% (v/v) with 10-mL mixtures of ethanol and/or distilled water. Corrections to the ebulliometer readings due to the presence of soluble solids were made with the tables published by Love (1939).

Washed XAD-2 resin (2 g, wet weight), granular sodium chloride (15 g), internal standard solution (2 mL), and 3.5% (w/v) HCl (2 mL for wine samples or 1 mL for calibration standards) were added to the jars. The jars were sealed with Teflon-lined lids and placed on a platform shaker at 200 rpm for 90 min. The contents of the jars were then poured into 11 × 150 mm stopcocked glass columns plugged with glass wool. Complete transfers of the resin were achieved with cold (7 °C) saturated salt solution (3 × 10 mL).

Once the resin was transferred to the columns, the salt solutions were drained away and diethyl ether (2 mL) was added. After a 5-min period, the ether was collected into vials and additional ether (1 mL) added. This elution was repreated until a total of 10 mL of ether was used. The diethyl ether extracts were dried with anhydrous $MgSO_4$, transferred to Teflon-lined screw-capped vials, and stored at 7 °C prior to gas–liquid chromatography.

Table I. Conditions for Gas–Liquid Chromatography of Diethyl Ether Extracts of Wine Samples

carrier gas flow rate (He)	1.5 mL/min
H_2 flow rate	30 mL/min
air flow rate	350 mL/min
makeup gas flow rate	30 mL/min
column[a]	Nukol (0.25 μm × 30 m)
split ratio	30:1
injection size	4 μL
injector temperature	220 °C
detector temperature	220 °C
oven temperature program	60 °C for 3 min, increase at 6 °C/min to 190 °C, hold at 190 °C for 25 min

[a] Supelco.

Wines were prepared from ultrafiltered juice from French hybrid grapes (cv. Aurore). The juice (pH 3.5, 20° Brix) was ultrafiltered at the Taylor Wine Co. (Hammondsport, NY) using a Romicon ultrafiltration unit (Woburn, MA) with 50 000 molecular weight cutoff membranes. Insoluble grape solids, collected from the ultrafiltration unit, were steamed at 100 °C and transported along with the juice to The Pennsylvania State University for fermentation.

The juice was transferred to eight 5-gal glass carboys, and insoluble grape solids (13 g/L, dry weight) and/or yeast ghosts (1 g/L; Universal Foods Corp., Milwaukee, WI) were added so that there were two carboys per treatment. The juice was then inoculated with *Saccharomyces cervisiae* Montrachet No. 522 (Universal Foods) and *Leuconostoc oenos* (PSU-1) at initial viable populations of 1×10^6 and 6×10^5 CFU/mL, respectively. Fermentation was carried out at 25 °C. The wines were racked twice, once 18 days after inoculation and again on day 43. At the time of the second racking, 50 ppm sulfur dioxide was added. Wines were then clarified with bentonite and bottled in preparation for analysis.

Two bottles of wine from each treatment (one from each fermentation carboy) were analyzed in triplicate by the XAD-2 extraction technique. Gas–liquid chromatography was accomplished on a Hewlett-Packard gas chromatograph (Model 5890A) with split-mode capillary injection port and flame ionization detector using a Nukol acidic bonded-phase capillary column (Supelco Inc., Bellefonte, PA) under the conditions listed in Table I. Major volatile compounds in the wines were identified by retention times and by a Finnigan 3200 gas chromatograph–mass spectrometer (GC–MS) with chemical ionization (R. Minard, Department of Chemistry, The Pennsylvania State University, University Park, PA) and a Finnigan INCOS 50 mass spectrometer with electron impact ionization (R. Sciree and L. M. Sidisky, Supelco). Mean concentrations of the major volatiles in the wines were statistically analyzed with Fisher's protected LSD at $p = 0.05$ (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Relative recoveries of the volatile compounds extracted from a French hybrid (cv. Aurore) wine previously made by traditional wine-making techniques (Amerine et al., 1980) are shown in Table II. Most recoveries were found to be quite acceptable, in the range 90–114%, with the majority being greater than 95%. The recoveries of isoamyl acetate and ethyl hexanoate were slightly lower, being 90 and 94%, respectively.

Table II. Relative Recoveries of Volatile Compounds from Aurore Wine

compound	rel rec,[a] %
ethyl acetate	95
isobutyl alcohol	110
isoamyl acetate	90
isoamyl alcohol	114
ethyl hexanoate	94
ethyl octanoate	107
phenylethyl acetate	101
hexanoic acid	99
phenylethyl alcohol	111
octanoic acid	100
decanoic acid	102

[a] % relative recovery = $([C]_{sw} - [C]_w)/[C]_s \times 100$, where $[C]_{sw}$ = concentration in spiked wine, $[C]_w$ = concentration in wine, and $[C]_s$ = concentration added to spiked wine. Means of four replications.

Table III. Means and Coefficients of Variation (CV) of Concentrations of Volatile Compounds Present in Aurore Wine

peak identifcn	compound	ret time, min	mean,[a] mg/L	CV, %
A	ethyl acetate	2.51	46.9	4.92
B	ethyl alcohol	3.02		
C	isobutyl alcohol	5.55	131	21.5
D	isoamyl acetate	6.13	9.60	2.55
E	isoamyl alcohol	8.31	346	3.79
F	ethyl hexanoate	8.77	0.850	1.47
G	ethyl octanoate	13.64	1.09	1.93
H	phenylethyl acetate	22.16	1.12	1.79
I	hexanoic acid	22.75	2.83	3.46
J	phenylethyl alcohol	24.14	41.6	2.48
K	dodecanol (int std)	24.65		
L	octanoic acid	26.70	3.76	1.77
M	nonanoic acid (int std)	29.26		
N	decanoic acid	32.62	0.866	9.68

[a] Means of eight replications.

The precision of the XAD-2 extraction technique as measured by the coefficients of variation (CV) of the different major volatiles are illustrated in Table III. Generally, the CV for any given volatile was less than 5%, the major exception being isobutyl alcohol which approached 22%. Of the eight replicates, one replicate had a very high value for isobutyl alcohol (199 mg/L), and if this replicate was not included in calculations, the mean and CV were lowered to 121 mg/L and 5%, a value close to the CV of 3% previously reported for the analysis of isobutyl alcohol in beer (Hawthorne et al., 1987). Since CV significantly decreases without the value for this replicate, this value may not be valid. The high value for this replicate may in fact be related to errors in alteration of the alcohol content of the wine. Hawthorne et al. (1987) noted that the relative recovery of isobutyl alcohol from beer decreased when the alcohol content increased from 2 to 4%. On the basis of this observation, a lower alcohol level than the standardized level of 10% would increase the relative recovery and, thus, the measured value, resulting in a high coefficient of variation.

One concern in using XAD-2 resin is that the resin can fracture, resulting in the release of impurities. James et al. (1981) and Wigilius et al. (1987) observed that impurities from improperly cleaned XAD-2 resin may interfere with subsequent GC–MS analysis. James et al. (1981) recommended sequential washing of the resin using methanol–diethyl ether–water, which minimizes the presence of impurities. Satisfactory levels of impurities were observed by Wigilius et al. (1987) in blanks either using the method of James et al. (1981) or substituting acetone for diethyl ether. A more complex washing technique involving water–methanol–diethyl ether–methanol–water was used by Hawthorne et al. (1987). Although Hawthorne et al. (1987) did not ascertain the level of impurities, a satisfactory blank was achieved with this washing procedure (Figure 1). The identities of the contaminants shown in the chromatogram were not confirmed by GC–MS, but peak 1 was probably methanol, a contaminant from the washing steps of the resin. Peaks 2–4 were impurities from both the resin and the diethyl ether (i.e., butylated hydroxytoluene).

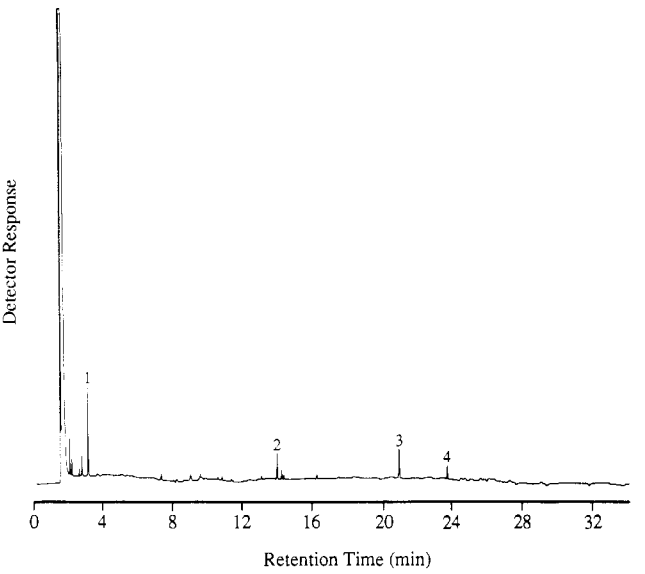

Figure 1. Diethyl ether extract of Amberlite XAD-2 resin. See text for peak identifications.

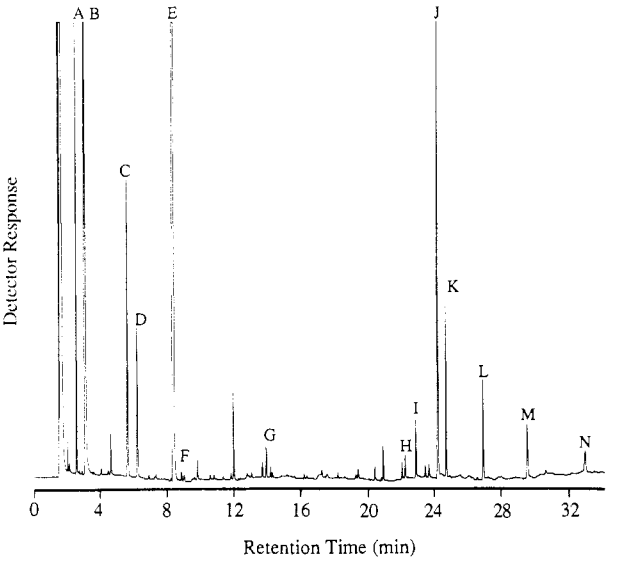

Figure 2. Chromatogram of a diethyl ether extract of an Aurore wine. Peak identifications are listed in Table III.

A typical chromatogram of a diethyl ether extract of Aurore wine is shown in Figure 2 (peak identifications are listed in Table III). Many commercial capillary columns are unsuitable for analysis of wine volatiles due to excessive tailing of medium-chain fatty acids and sometimes higher alcohols. In this study, analysis of wine extracts was carried out using a newly developed acidic bonded-phase capillary column. Although this column (Nukol) was originally developed for analysis of volatile free fatty acids and other acidic compounds, good resolution as well as peak symmetry was achieved for fatty acids and higher alcohols as well as esters.

Table IV. Concentration of Volatile Compounds (mg/L) in Aurore Wine Fermented with and without Insoluble Material[a]

compound	without insol matl	insol grape solids	yeast ghosts	insol grape solids and yeast ghosts
ethyl acetate	24.9[a]	33.6[b,c]	38.9[c]	29.1[a,b]
isobutyl alcohol	11.0[a]	108[b]	37.5[c]	129[d]
isoamyl acetate	3.79[a]	7.83[b]	6.50[c]	8.21[b]
isoamyl alcohol	110[a]	304[b]	211[c]	363[d]
ethyl hexanoate	1.40[a]	1.19[b]	1.19[b]	1.16[b]
ethyl octanoate	2.34[a]	1.75[b]	1.86[b]	1.62[c]
phenylethyl acetate	1.14[a]	1.60[b]	1.56[b]	1.75[b]
hexanoic acid	5.21[a]	2.26[b]	2.41[b]	1.57[c]
phenylethyl alcohol	8.90[a]	39.1[b]	28.5[c]	51.0[d]
octanoic acid	8.41[a]	3.08[b]	4.23[c]	2.06[d]
decanoic acid	2.48[a]	0.53[b]	1.35[c]	0.33[d]

[a] Treatment means within a row followed by different letters are significantly different at $p = 0.05$.

While most compounds present in extracts of Aurore wine were identified and confirmed by GC–MS, the identity of a few peaks remains unknown. Cobb et al. (1978) identified ethyl butanoate, ethyl L-lactate, diethyl succinate, and a few others in Aurore wine not identified and/or confirmed by the present study. However, Cobb et al. (1978) did not report the presence of decanoic acid, an acid identified and confirmed as being present in Aurore wine. Unfortunately, Cobb et al. (1978) did not quantitate the volatiles found in Aurore wine so comparison with the concentrations listed in Table III is not possible.

From the analysis of Aurore wines, the presence of insoluble material during fermentation altered their volatile compositions (Table IV). Wines fermented with insoluble grape solids and/or yeast ghosts had elevated levels of higher alcohols (isobutyl alcohol, isoamyl alcohol, phenylethyl alcohol) in comparison to wines made without insoluble material. This is in agreement with the findings of Guymon et al. (1961), Crowell and Guymon (1963), Groat and Ough (1978), Houtman et al. (1980a,b), Klingshirn et al. (1987), and Liu et al. (1987). Formation of medium-chain fatty acids followed an opposite trend than the higher alcohols; higher concentrations were present in wines fermented without insoluble materials. Previous research similarly showed that wines made from sterile filtered juices had higher concentrations of these acids than wines made from settled juices (Houtman et al., 1980a). As for ester formation, wines fermented with insoluble material had higher levels of isoamyl acetate and phenylethyl acetate but lower levels of ethyl hexanoate and ethyl octanoate. Differences were also observed between the two insoluble materials in that wines made with yeast ghosts (alone) had lower concentrations of higher alcohols and higher concentrations of medium-chain fatty acids than with insoluble grape solids or a combination of the two.

Since addition of insoluble materials to a fermenting must alters the volatile composition of the resultant wines, these materials must somehow influence the metabolism of the active yeast. Interaction between insoluble solids and yeast is not well understood but has been thought to be related to oxygen addition. In considering higher alcohol formation, Guymon et al. (1961) proposed that insoluble materials may aerate a must by incorporating entrapped air. Later work by Crowell and Guymon (1963) showed that higher alcohol production decreased during fermentation in the presence of insoluble solids that had been deaerated. The formation of medium-chain fatty acids also appears to be dependent on the presence of oxygen on the basis of the finding of Houtman et al. (1980a) that wines made from deaerated sterile filtered juice had higher levels than wines made from aerated juice.

However, air entrapment as the mechanism by which insoluble materials influence yeast metabolism cannot be currently fully accepted as the only explanation. In data presented by Crowell and Guymon (1963) concerning higher alcohol formation, wines fermented without insoluble solids (control) had lower levels than wines made from juice and insoluble solids deaerated by vacuum. Although firm conclusions cannot be made on the basis of these data because statistical analysis was not applied and it is not clear whether the control wine was also deaerated, other mechanism(s) including increased enzymatic activity (Klingshirn et al., 1987) have been proposed. Certainly, more research is needed in this area.

CONCLUSIONS

The XAD-2 extraction method is a rapid and simple technique for analysis of volatile compounds in wines. With the method, relative recoveries were excellent while the coefficients of variation remained low. The principal advantage of the method over other commonly used methods is decreased time of extraction. Excellent precision and recovery as well as speed give this technique great potential for both research and quality control applications.

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Effect of Nitrogen Fertilizers on Celery Volatiles

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The volatiles of two varieties of celery (*Apium graveolens* L. var. dulce and rapaceum) were isolated both by Likens-Nickerson extraction and by Soxhlet extraction. The extracts were analyzed and quantified by two-dimensional capillary gas chromatography. Celery grown with different levels of organic and/or anorganic fertilizers was investigated. The addition of high levels of mineral and/or organic nitrogen fertilizers significantly decreased the amounts of the character-impact flavor compounds. Yields and nitrate and dry matter contents of the two vegetables were also determined.

Celery (*Apium graveolens* L.) (Umbelliferae = Apiaceae) ranks with the more important vegetables cultivated in Belgium. From the wild celery two familiar garden varieties were developed, *Apium graveolens* L. var. dulce, the common blanching celery and *Apium graveolens* L. var. rapaceum, the root vegetable celery or celeriac. Celery is used for its unique texture and appetizing flavor. Gold and Wilson (1961) were the first to investigate the volatile flavor substances of the vegetable celery. Among the components described by these authors were several phthalides. The characteristic odor of celery is due to a series of phthalide derivatives (Wilson, 1970; Fehr, 1979; Gijbels et al., 1985). The separation of phthalides in the complex matrix of the flavor compounds of celery is very difficult. Two-dimensional capillary gas chromatography gives a solution to this problem and may contribute to the objective measurement of the quality of the celery flavor (Van Wassenhove et al., 1988).

The application of fertilizers on arable crops may have adverse effects on crop production and crop quality such as lodging of cerials, decreases in starch content of potatoes and sugar content of sugar beets (Smilde, 1980), and increase of the nitrate concentration of vegetables (Vulsteke and Biston, 1978).

The aim of this study was to determine the effect of different rates of pig and cattle manure and/or mineral nitrogen application on the flavor components of celery. The results of 2 years of work are presented in this paper.

EXPERIMENTAL SECTION

Field Experiments. The field plots were situated in the middle of a field with blanching celery or celeriac. The experimental fields were laid down at random with four replications, except for celeriac in 1987 with three parallels.

Specimens of *A. graveolens* L. var. dulce cultivar Golden Spartan and *A. graveolens* L. var. rapaceum cultivar Monarch were used in this fertilization study.

Details of the different types of fertilizers used and the rates of application are given in Table I.

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