Optimized Extraction Procedure for Quantifying Norisoprenoids in Honey and Honey Food Products

Christine Guyot-Declerck, Fabienne Chevance, Guillaume Lermusieau, and Sonia Collin*

Université Catholique de Louvain, Unité de Brasserie et des Industries Alimentaires, Place Croix du Sud 2 / Bte 7, B-1348 Louvain-la-Neuve, Belgium

Norisoprenoids appear as promising compounds for authenticating unifloral honeys. So far, however, no method has been optimized for their isolation from a matrix so rich in sugars. In this framework, an original extraction procedure based on the use of Amberlite XAD-16 was developed. Recovery factors were determined and compared with those obtained with another resin (XAD-2). This was done for different model media and various norisoprenoids. In aqueous or alcoholic solutions, the efficiency of both resins proved very high. As expected, addition of honey decreased the adsorption of nonpolar compounds. This effect was much more pronounced with the lower-porosity XAD-2 support. Sugar addition markedly improved the recovery factors obtained with the XAD-16 resin in the case of more polar norisoprenoids.

Keywords: *Honey; norisoprenoid; extraction; XAD-16; XAD-2*

INTRODUCTION

Norisoprenoids constitute one of the most important classes of flavor compounds identified in nature. Derived from carotenoids, they occur in many higher plants, especially tobacco (Enzell, 1985). Many have also been isolated from honey, where they constitute markers of floral origin. Australian blue gum honeys were authenticated by D'Arcy et al. (1997) on the basis of their 8,9-dehydrotheaspirone and 3-oxo-α-ionone contents, whereas 3,5,6-trihydroxymegastigm-7-en-9-one enabled Tan et al. (1989a; 1990) to identify New Zealand thyme honeys. High norisoprenoid contents have also been found in heather (Häusler and Montag, 1989, 1991; Tan et al., 1989b; Guyot et al., 1999) and strawberry tree honeys (Dalla Serra et al., 1999). Although these both come from plants belonging to the *Ericaceae* family, they appeared distinguishable on the basis of a few specific compounds, i.e. megastigma-4,6,7-trien-3,9-dione (III, Figure 1) in the former and C_{10} norisoprenoids and 5,6-epoxy-9-hydroxymegastigma-3,7-dione in the latter. As regards heather honeys, Guyot et al. (1999) recently demonstrated that *Calluna vulgaris* samples can be distinguished from *Erica arborea* honeys on the basis of their megastigm-4-en-7-yne-3,9-dione (I) and dehydrovomifoliol (VI) contents.

Although they appear so promising for authenticating unifloral honeys, norisoprenoids remain insufficiently studied because of difficulties in extracting them from such a sugar-rich matrix. So far, nonselective liquid/liquid extractions with solvents such as dichloromethane, diethyl ether, or ethyl acetate have been used for honeys. In the case of foods with lower sugar contents, such as fruits or fruit juices, other isolation procedures have been developed to isolate free norisoprenoids and glycosides. Based on the use of nonionic resins, such techniques offer the advantage of eliminating, by simple washing with water, numerous interfering substances such as sugars and acids, without significant loss of glycosides or aroma compounds (Gunata et al., 1985). In all cases, Amberlite XAD-2 has been chosen as the solid phase (Versini et al. (1988), grape; Winterhalter and Schreier (1988), quince; Sakho et al. (1997), mango; Knapp et al. (1997), white-fleshed nectarine; Chassagne et al. (1998), passion fruit). In the case of sugar-rich matrixes, data are scant. Regarding the best resin for extracting honey flavonoids, the chemical nature and physical characteristics of Amberlite XAD resins seem to have low effect on the extraction yield (in most cases higher than 80%, Tomas-Barberan et al., 1992). The use of XAD-2, however, has been recommended to minimize the amount of methanol needed for desorption. All optimizations have been performed, unfortunately, in sugarless aqueous solutions. XAD-2 was recently chosen by Dalla Serra et al. (1999) to isolate aroma compounds from strawberry tree honeys, but without preliminary tests. To our knowledge, no research concerning Amberlite resins has focused on optimizing norisoprenoid recoveries in sugar matrixes. The aim of the present work was, therefore, to develop an original extraction method allowing accurate quantification of norisoprenoids in unifloral honeys. Several parameters such as the nature of the Amberlite resin, the type of solvent, the elution volume, the extraction time, and the honey/resin ratio were optimized.

As heather honey is commonly used in the fabrication of some Belgian special beers, the chosen parameters were then applied to different model media including beer, to determine the influence of the matrix, and mainly its sugar content.

MATERIALS AND METHODS

Materials. A unifloral heather honey (*Calluna vulgaris*, France), stored at 4 °C until used, was chosen because of its well-known high norisoprenoids content (Häusler and Montag, 1989, 1991; Tan et al., 1989b; Guyot et al., 1999). Screening for floral purity was based on pollen analyses (Louveaux et al., 1978), sensory tests, electrical conductivity, pH, titratable

* To whom correspondence should be addressed. Phone: (32) 10 47 29 13. Fax: (32) 10 47 21 78. E-mail: collin@inbr.ucl.ac.be.

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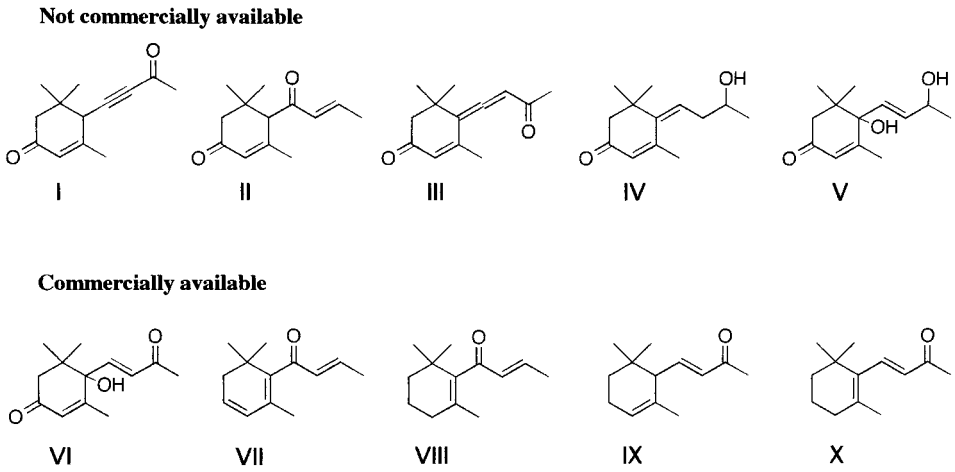

Figure 1. Structures of the norisoprenoids chosen for this study.

acidity (Journal Officiel de la République Française, 1977), and sugar composition (Pourtallier and Rognone, 1977).

A Pils-type beer was purchased from a local supermarket and used as a model medium.

Amberlite XAD-2 (pore size, 9 nm; specific surface, 330 $m^2 \cdot g^{-1}$) and Amberlite XAD-16 (pore size, 10 nm; specific surface, 650 $m^2 \cdot g^{-1}$) resins were supplied by Supelco (Bellefonte, PA). They were successively washed with dichloromethane and methanol (each for 4 h in a Soxhlet apparatus) and stored at 4 °C in methanol until used.

β-Damascenone (VII) (megastigma-3,5,8-trien-7-one) (>95%) and β-damascone (VIII) (megastigma-5,8-dien-7-one) (>95%) were gifts from Haarmann & Reimer GmbH (Saint-Ouen l'Aumône, France); α-ionone (IX) (megastigma-4,7-dien-9-one) (>90%) and β-ionone (X) (megastigma-5,7-dien-9-one) (>95%) were obtained from Aldrich (Bornem, Belgium); dehydrovomifoliol (VI) (6-hydroxymegastigma-4,7-dien-3,9-dione) was obtained from Sigma (Bornem, Belgium); decane (99%) was obtained from Fluka (Bornem, Belgium); and 1-chloroheptane (97%) and anhydrous sodium sulfate (99%) were purchased from Janssen (Geel, Belgium). The solvents (dichloromethane, pentane, methanol, and ethanol) were of pure analytical grade (purity > 99.8%) and were purchased from Romil (Gent, Belgium) and pentane was supplied by Aldrich (Bornem, Belgium). All the mentioned purities are stated purities and correspond to those on the labels of the concerned compounds. Dichloromethane was redistilled twice in glass prior to use. The water used was ultrapure water (Milli-Q water purification system, Millipore, Bedford, MA).

Norisoprenoid Extraction. Optimization parameters for each type of resin (XAD-2 or XAD-16) were the nature of the elution solvent (pentane or dichloromethane), the time of contact between the resin and the honey (1, 2, or 17 h), the resin/honey (w/w) ratio (1:2, 2:2, or 4:2), and the volume of solvent necessary to desorb most of the norisoprenoids from the resin (10, 20, or 40 mL).

For this optimization experiment, duplicate analyses were performed for each tested set of conditions. Extraction yields were obtained by standard addition of dehydrovomifoliol (VI) (50, 100, 150, and 200 μL of a ca. 8000 $\mu g \cdot g^{-1}$ solution in dichloromethane), initially present in the heather honey, and β-damascenone (VII), β-damascone (VIII), α-ionone (IX), and β-ionone (X) (100 μL of a ca. 200 $\mu g \cdot g^{-1}$ of each = stock solution A), absent in the initial sample, by comparing the slopes with usual calibration curves.

The Optimized Procedure. Resin (2 g) was thoroughly rinsed with ultrapure water (100 mL) and poured into a 100-mL Schott flask (Vel, Leuven, Belgium) containing 2 g of honey and 20 mL of ultrapure water (equivalent to medium C in the next experiment). This mixture was placed on a platform shaker at 200 rpm for 1 h at 20 °C. The content of the flask was then transferred to a glass column specially designed for norisoprenoid extraction. This column (60 × 1 cm i.d.) was ended with a PTFE tap. The lower part was fitted with glass balls (3 g with a diameter of 3.5−4.5 mm and 1 g with a diameter of 0.8−1.2 mm) in order to retain the resin. The resin was first rinsed with 100 mL of ultrapure water in order to eliminate sugars and other water-soluble substances. Norisoprenoids were then eluted with 40 mL of dichloromethane at a flow rate of 0.75 $mL \cdot min^{-1}$. The dichloromethane extract was dried with anhydrous sodium sulfate. It was then concentrated to 1 mL in a Snyder-Kuderna column at 45 °C ± 1 °C with 100 μL of a 250 $\mu g \cdot g^{-1}$ solution of 1-chloroheptane in dichloromethane, added as external standard (expected final concentration, 25 $\mu g \cdot g^{-1}$). The extract was analyzed by GC and GC−MS.

Application of the Optimized Extraction Procedures (With Both Resins) to Different Model Media. The model media investigated were (A), water; (B), water + honey (2.5% w/w); (C), water + honey (9% w/w); (D), water + ethanol (5% v/v); (E), water + ethanol (5% v/v) + honey (2.5% w/w); (F), beer; and (G), beer + honey (2.5% w/w). For each medium, 100 μL of stock solution A and 100 μL of dehydrovomifoliol (VI) solution in dichloromethane (ca. 2000 $\mu g \cdot g^{-1}$) were added to 20 mL of medium prior to the extraction. In beer, the external standard 1-chloroheptane was replaced with decane (100 μL of a 15 $\mu g \cdot g^{-1}$ solution in dichloromethane) because of interferences with other compounds. Analyses were performed in duplicate for each model medium.

Gas Chromatography−FID Analytical Conditions. Analyses were conducted on a Hewlett-Packard model 5890 gas chromatograph, equipped with a Hewlett-Packard model 7673 automatic sampler, a cold on-column injector, a flame ionization detector, and a Shimadzu CR4A integrator. The separation of volatile compounds was performed on a 50 m × 0.32 mm, wall-coated, open tubular (WCOT) CP−SIL5 CB (Chrompack, Antwerp, Belgium) capillary column (film thickness, 1.2 μm), which was preceded by a 1 m × 0.53 mm capillary column, coated with a thin film of methyl silicon phase (Hewlett-Packard, Brussels, Belgium). The oven temperature was programmed to rise from 36 to 85 °C at 20 °C min^{-1}, then to 145 °C at 1 °C min^{-1}, and to 250 °C at 3 °C min^{-1}. The carrier gas was helium at a flow rate of 1.5 mL min^{-1}. The injector temperature was maintained at 3 °C above the oven temperature. The detector temperature was maintained at 260 °C. The minimum peak area for data acquisition was set at 500 $\mu V \cdot sec$. The minimum detectable limits depend on the structure of the compound and ranged from 25 $ng \cdot g^{-1}$ (α-ionone) to 30 $ng \cdot g^{-1}$ (dehydrovomifoliol). Retention indices were determined by interpolation of the retention times of a *n*-alkanes ($C_6 - C_{19}$) mixture analyzed under identical conditions.

Gas Chromatography−Mass Spectrometry Conditions. Chromatographic conditions were the same as those used for FID detection. The column was directly connected to an HP 5988 quadrupole mass spectrometer. Electron impact mass spectra were recorded at 70 eV (filament current, 300 mA; electron multiplier voltage, 2500; scan rate, 4 s^{-1}; *m/z*

Table 1. Influence of the Resin/Honey Contact Time on Norisoprenoid Concentrations ($\mu g \cdot g^{-1}$ Dehydrovomifoliol Equivalent) Calculated with a 100% Recovery Factor[a]

noriso-prenoid	retention index	1 hour mean (standard deviation)		2 hours mean (standard deviation)		17 hours mean (standard deviation)	
		XAD-2	XAD-16	XAD-2	XAD-16	XAD-2	XAD-16
I	1475	$1.9^{b,a}$ (0.1)	$2.6^{a,a}$ (0.1)	$2.1^{a,a}$ (0.1)	$2.5^{a,a}$ (0.1)	$1.9^{a,a}$ (0.0)	$2.0^{a,b}$ (0.0)
II	1609	$0.7^{b,a}$ (0.0)	$1.0^{a,a}$ (0.0)	$0.8^{a,a}$ (0.1)	$0.9^{a,a}$ (0.1)	$0.6^{a,a}$ (0.1)	$1.0^{a,a}$ (0.2)
III	1625	$17^{a,a}$ (1)	$20^{a,a}$ (1)	$17^{a,a}$ (2)	$17^{a,a}$ (1)	$15^{a,a}$ (1)	$15^{a,a}$ (2)
IV	1696	$1.2^{a,a}$ (0.1)	$1.9^{a,a}$ (0.2)	$1.4^{a,a}$ (0.2)	$1.5^{a,a}$ (0.1)	$1.2^{a,a}$ (0.2)	$1.2^{a,a}$ (0.0)
V	1768	$5.8^{a,a}$ (0.9)	$7.8^{a,a}$ (0.7)	$7.0^{a,a}$ (0.2)	$8.8^{a,a}$ (0.2)	$6.2^{a,a}$ (0.3)	$6.0^{a,a}$ (0.6)
VI	1760	$145^{b,a}$ (10)	$212^{a,a}$ (6)	$177^{a,a}$ (14)	$210^{a,a}$ (9)	$172^{a,a}$ (9)	$179^{a,a}$ (8)

[a] Operating conditions: resin, 2 g; honey, 2 g; elution solvent, CH_2Cl_2; elution volume, 20 mL. For each norisoprenoid, the first and second superscript letters correspond respectively to the resin effect for a given contact time and to the time effect for a given resin; in each case, according to the results of the Student-Newman-Keuls test, values that do not share a common superscript are significantly different ($p < 0.05$).

Table 2. Influence of the Resin/Honey (w/w) Ratio on Norisoprenoid Concentrations ($\mu g \cdot g^{-1}$ Dehydrovomifoliol Equivalent) Calculated with a 100% Recovery Factor[a]

noriso-prenoid	retention index	resin/honey (w/w) 1 g/2 g mean (standard deviation)		resin/honey (w/w) 2 g/2 g mean (standard deviation)		resin/honey (w/w) 4 g/2 g mean (standard deviation)	
		XAD-2	XAD-16	XAD-2	XAD-16	XAD-2	XAD-16
I	1475	$0.9^{b,b}$ (0.0)	$2.0^{a,a}$ (0.0)	$1.9^{b,a}$ (0.1)	$2.6^{a,a}$(0.1)	$2.2^{a,a}$(0.0)	$2.5^{a,a}$(0.2)
II	1609	$0.3^{b,b}$ (0.0)	$0.6^{a,a}$ (0.0)	$0.7^{b,a}$(0.0)	$1.0^{a,a}$(0.0)	$0.8^{a,a}$(0.0)	$0.9^{a,a}$(0.1)
III	1625	$9.0^{b,b}$ (0.2)	$14^{a,b}$ (0)	$17^{a,a}$(1)	$20^{a,a}$(1)	$18^{a,a}$(1)	$22^{a,a}$(2)
IV	1696	$0.6^{b,b}$ (0.0)	$1.4^{a,a}$ (0.1)	$1.2^{a,a}$(0.1)	$1.9^{a,a}$(0.2)	$1.4^{a,a}$(0.0)	$1.5^{a,a}$(0.1)
V	1768	$3.4^{b,b}$ (0.1)	$7.5^{a,a}$ (0.1)	$5.8^{a,ab}$(0.9)	$7.8^{a,a}$(0.7)	$7.8^{a,b}$(0.6)	$7.4^{a,a}$(0.5)
VI	1760	$74^{b,c}$ (1)	$178^{a,a}$ (1)	$145^{b,b}$(10)	$212^{a,a}$(6)	$181^{a,a}$(2)	$210^{a,a}$(17)

[a] Operating conditions: honey, 2 g; contact time, 1 h; elution solvent, CH_2Cl_2; elution volume, 20 mL. For each norisoprenoid, the first and second superscript letters correspond respectively to the resin effect for a given resin/honey ratio and to the resin/honey ratio effect for a given resin; in each case, according to the results of the Student-Newman-Keuls test, values that do not share a common superscript are significantly different ($p < 0.05$).

range, 40 to 250). The minimum detectable limits depend on the structure of the compound and ranged from 250 $ng \cdot g^{-1}$ (α-ionone) to 300 $ng \cdot g^{-1}$ (dehydrovomifoliol). Spectral recording throughout elution was automatic using HP59970C software. Identification was achieved on the basis of peak enhancement by co-injection with authentic standard compounds and by comparison of mass spectra found in the literature.

Statistical Analyses. All the statistical analyses were performed with the Statistical Analysis System (SAS Institute, Inc., Cary, NC). Analysis of variance (ANOVA) was used in order to determine significant differences among samples. A Student-Newman-Keuls test was used to perform a multiple comparison of means.

RESULTS AND DISCUSSION

Optimization of Norisoprenoid Extraction. Using a heather honey where norisoprenoids naturally occur (Guyot et al., 1999), we first tried to assess quickly the impact of the nature of the solvent used for Amberlite XAD-2 or XAD-16 desorption. In particular, dehydrovomifoliol (VI) and five norisoprenoids identified only by mass spectrometry by comparison with published spectra (I and III, Guyot et al., 1999; II, Ohloff et al., 1973; IV, Winterhalter, 1990; V, Winterhalter and Schreier, 1988) were monitored in this study. For preliminary tests, the procedure described by Dalla Serra et al. (1999) was used. Quantitatively, both dichloromethane extracts proved much more rich in norisoprenoids (only compound (III) was found in the pentane extracts). Only dichloromethane was therefore tested in the following experiments.

The same honey was then used to compare the two resins for different extraction times (experimental conditions described under Materials and Methods, apart from the elution volume of dichloromethane, 20 mL). As shown in Table 1, the calculated concentrations were always higher with the XAD-16 resin, whatever the norisoprenoid or the extraction time (except for compound (V) when the extraction time was 17 h). Statistical differences ($p < 0.05$) were evident between the two resins in the case of compounds (I), (II), and (VI) with a 1-h extraction. For more prolonged extractions, differences were no longer significant.

With XAD-16, surprisingly, the shorter the extraction time, the higher the recovery. With the XAD-2 resin, a 2-h extraction time proved optimal, allowing recovery almost as high as with XAD-16. Although the adsorption rate of XAD-2 was a limiting factor, a contact time of 1 h was kept for all the following experiments, whatever the resin.

Table 2 depicts optimization of the resin/honey ratio. In most cases, maximal recoveries were obtained with the 2 g/2 g resin/honey ratio. The extraction efficiency was not, however, significantly influenced by the amount of XAD-16 resin (except in the case of compound (III), for which at least 2 g resin is necessary to reach maximal recovery). It is noteworthy that with our 1-h extraction time, 4 g Amberlite XAD-2 and 2 g XAD-16 gave similar results. This could be due to the specific surface of XAD-16, which is two times greater than that of XAD-2.

In the case of the 4 g/2 g resin/honey ratio, we checked that the elution volume was not limiting. Norisoprenoid recoveries with XAD-16 proved to be similar with 20 or 40 mL of dichloromethane (results not shown). With XAD-2 on the other hand, higher recoveries were found when 40 mL was used, indicating that the desorption rate of this resin is also, in all likelihood, below that of XAD-16.

Complete data concerning different dichloromethane volumes for the 2 g/2 g ratio are depicted in Table 3. In

Table 3. Influence of the Elution Volume on norisoprenoid Concentrations ($\mu g.g^{-1}$ Dehydrovomifoliol Equivalent) Calculated with a 100% Recovery Factor[a]

noriso-prenoid	retention index	10 mL mean (standard deviation)		20 mL mean (standard deviation)		40 mL mean (standard deviation)	
		XAD-2	XAD-16	XAD-2	XAD-16	XAD-2	XAD-16
I	1475	$1.5^{a,a}$ (0.2)	$2.0^{a,a}$ (0.1)	$1.9^{b,a}$ (0.1)	$2.6^{a,a}$ (0.1)	$1.6^{b,a}$ (0.1)	$2.5^{a,a}$ (0.1)
II	1609	$0.5^{a,b}$ (0.0)	$0.7^{a,b}$ (0.0)	$0.7^{b,b}$ (0.0)	$1.0^{a,a}$ (0.0)	$1.0^{a,a}$ (0.1)	$1.0^{a,a}$ (0.0)
III	1625	$11^{b,b}$ (0.5)	$16^{a,b}$ (0)	$17^{a,a}$ (1)	$20^{a,a}$ (1)	$17^{a,a}$ (1)	$21^{a,a}$ (1)
IV	1696	$1.0^{a,a}$ (0.1)	$1.3^{a,a}$ (0.1)	$1.2^{a,a}$ (0.1)	$1.9^{a,a}$ (0.2)	$1.2^{a,a}$ (0.2)	$1.6^{a,a}$ (0.1)
V	1768	$4.8^{b,a}$ (0.5)	$8.0^{a,a}$ (0.3)	$5.8^{a,a}$ (0.9)	$7.8^{a,a}$ (0.7)	$5.3^{b,a}$ (0.5)	$8.7^{a,a}$ (0.3)
VI	1760	$118^{a,a}$ (16)	$175^{a,b}$ (4)	$145^{b,a}$ (10)	$212^{a,a}$ (6)	$131^{b,a}$ (10)	$224^{a,a}$ (7)

[a] Operating conditions: resin, 2 g; honey, 2 g; contact time, 1 h; elution solvent, CH_2Cl_2. For each norisoprenoid, the first and second superscript letters correspond respectively to the resin effect for a given elution volume and to the elution volume effect for a given resin; in each case, according to the results of the Student-Newman-Keuls test, values that do not share a common superscript are significantly different ($p < 0.05$).

Table 4. Average Concentrations ($\mu g \cdot g^{-1}$ Dehydrovomifoliol Equivalent) Calculated with a 100% Recovery Factor, Standard Deviations, and Variation Coefficients (%) Obtained for Ten Analyses of the Same Honey with XAD-16

noriso-prenoid	retention index	average concentration	standard deviation	variation coefficient (%)
I	1475	2.5	0.1	4.9
II	1609	1.0	0.1	5.0
III	1625	20	1.0	5.2
IV	1696	1.8	0.1	6.0
V	1768	8.5	0.3	3.7
VI	1760	214	8.6	4.0

most cases, 20 mL and 40 mL proved better than 10 mL, whatever the resin. A volume of 40 mL dichloromethane was selected for further experiments.

The reproducibility of the final optimized method was determined in 10 consecutive extractions (Table 4). Depending on norisoprenoid structure, the variation coefficients were between 3.7 and 6.0%, with the most polar compounds (i.e., vomifoliol (V) and dehydrovomifoliol (VI)) showing the lowest variability. The extraction yield for commercially available dehydrovomifoliol (VI) was as high as 97% ± 4 (standard addition method), leading to a real concentration of 212 $\mu g \cdot g^{-1}$ in the sample.

Application of the Optimized Norisoprenoid Extraction Procedure to Different Model Media. Five commercial norisoprenoids were selected for this study: dehydrovomifoliol (VI), β-damascenone (VII), β-damascone (VIII), α-ionone (IX), and β-ionone (X). Three model media without sugar were first selected: water, (A); water + ethanol (5% v/v), (D); and beer, (F). The effect of adding honey to 2.5% was then measured in media B, E, and G. For comparison, results of the optimization procedure obtained with 9% honey medium (medium C) are also reported in Figure 2. Extraction yields of β-damascone (VIII) were not determined in beer because of coelution.

Hydrophobic Compounds. Whatever the resin and matrix considered, the extraction yields of β-damascenone (VII), β-damascone (VIII), α-ionone (IX), and β-ionone (X) proved similar, as is logical. In the aqueous (A) and alcoholic (D) reference media, whatever the resin, similar extraction yields (all close to 80%) were obtained (Figure 2). Thus, as previously described by Tomas-Barberan et al. (1992) for flavonoids, neither the physical characteristics of the resins, nor the alcohol content significantly influence norisoprenoid recovery.

In beer (F), probably owing to the presence of macromolecules, a significant decrease ($p < 0.05$) in XAD-16 efficiency was observed. Interactions between macromolecules or dextrins and β-ionone (X) have been evidenced by Langourieux and Crouzet (1995). Therefore, we suspect that such interactions could be more favored with XAD-16-adsorbed norisoprenoids because of the large pores in this Amberlite resin as compared to those in XAD-2.

The presence of sugar in the extraction medium radically modified the adsorption capacity of the resins, especially in the case of XAD-2 (Figure 2). Addition of 2.5% sugar to water (medium B) led to losses ranging from 28% for β-damascenone (VII) to 56% for α-ionone (IX). This effect was even more pronounced with 9% sugar (medium C) (losses close to 55% and 77% for compounds oxygenated at C_7 (VII and VIII) or C_9 (IX and X), respectively). In the case of XAD-16, limited sugar addition (medium B) had almost no effect. Slight improvement of the extraction was even observed in the case of β-damascenone (VII) and β-ionone (X). On the other hand, around 20% was still lost when the sugar content was higher (medium C). Similar results were obtained when sugar was added to the alcoholic solutions. A significant decrease (38−65%) was measured with Amberlite XAD-2 compared to medium D. As few interactions occur between volatiles and carbohydrates at such low concentrations (Land, 1979; Le Thanh et al., 1992), the extraction yield losses observed here were probably due to decreased resin adsorption rates. This effect was, expectedly, more pronounced in the case of XAD-2, used here under kinetically unfavorable conditions (see previous experiments).

Sugar addition to beer media led to similar conclusions, although the presence of other constituents seemed to attenuate the effect (recovery decrease of 20−37% with XAD-2) (Figure 2). Hence, for the first time, XAD-2 appeared best suited for these media despite the presence of sugar

Hydrophilic Compounds. Very low recoveries (around 20%) were obtained for dehydrovomifoliol (VI) in aqueous (A) and alcoholic (D) media. Dehydrovomifoliol (VI) exhibited a high affinity for water, making its extraction from the medium difficult whatever the resin used.

Surprisingly in this case, addition of honey (2.5%) significantly enhanced ($p < 0.05$) the recovery factors (increase of 250% for XAD-2 and 300% for XAD-16) (Figure 2). Sugars are known to reduce water activity (Davis, 1995), favoring increased polar compound activity coefficients (as shown for acetone by Le Thanh et al. (1992)). In our experiments with 2.5% honey, the "competition" for water was probably favorable to sugars, thus improving dehydrovomifoliol (VI) adsorption to the resin. Nevertheless, at a higher sugar level

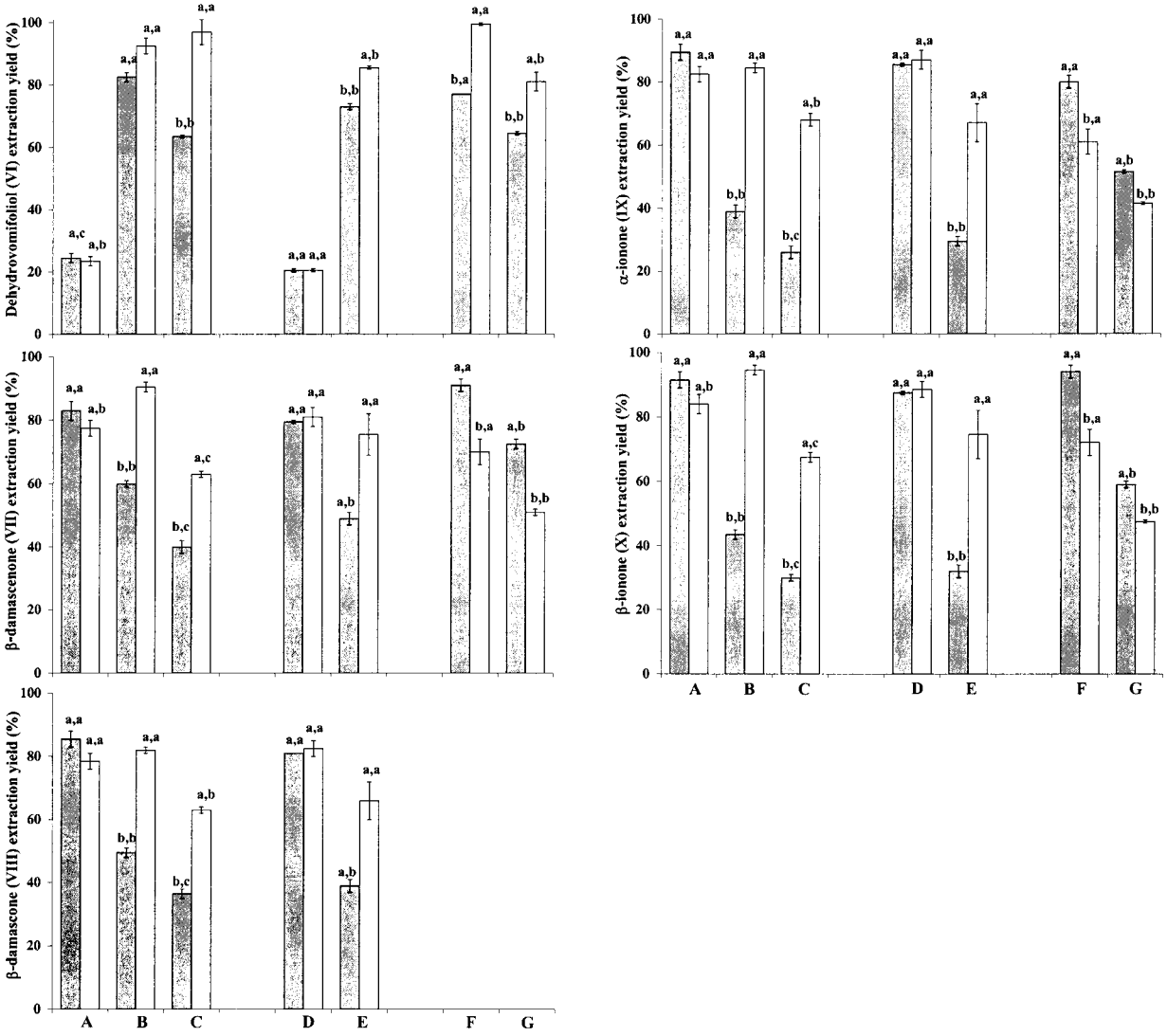

Figure 2. Extraction yields of dehydrovomifoliol (VI), β-damascenone (VII), β-damascone (VIII), α-ionone (IX), and β-ionone (X) from model solutions: water, (A); water + honey (2.5% w/w), (B); water + honey (9% w/w), (C); water + ethanol (5% v/v), (D); water + ethanol (5% v/v) + honey (2.5% w/w), (E); beer, (F); beer + honey (2.5% w/w), (G). Symbols: ■, with XAD-2; □, with XAD-16. For each norisoprenoid, in the case of the media A, B, and C, or D and E, or F and G, the first and second superscript letters correspond respectively to the resin effect for a given medium and to the medium effect for a given resin; in each case, according to the results of the Student-Newman-Keuls test, values that do not share a common superscript are significantly different ($p < 0.05$).

(medium C), this effect was significantly counterbalanced by kinetic factors, as in the case of hydrophobic compounds, especially with the XAD-2 resin.

Extraction yields of 99% and 77% were again obtained with XAD-16 and XAD-2 in beer, even when no sugar was added (medium F). The small carbohydrates naturally present in beer must logically induce the "sugar effect" described above. Additional sugars (medium G) led, therefore, to a 20% decrease in efficiency (Figure 2). Contrary to the results obtained with hydrophobic compounds, XAD-16 turned out to be the best resin for dehydrovomifoliol (VI) extraction in all sugar-containing media, even beer.

CONCLUSION

The choice of an XAD resin depends strongly on medium composition and compound polarity. The performances of XAD-2 and XAD-16 resin are equivalent as regards extraction of norisoprenoids from aqueous and alcoholic solutions (close to 80 and 20% for hydrophobic and hydrophilic compounds, respectively). In the case of hydrophobic compounds, sugar addition considerably decreases the efficiency of XAD-2, characterized by smaller pores, while increasing the recovery of more polar norisoprenoids by its influence on water activity. This latter effect is especially pronounced with XAD-16. In complex media such as beer, interactions with macromolecules must also be considered, mainly when pores of larger diameter are involved.

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