Influence of Macromolecules and Treatments on the Behavior of Aroma Compounds in a Model Wine

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This study deals with the influence of macromolecules and clarification stabilization treatments on the retention of aroma compounds in a model wine. After the characterization of substrates, two techniques applied in oenology have been used: fining and tangential ultrafiltration. The assumed effects of the treatments on the fixation of volatile compounds were verified. Under our conditions, the results showed that these treatments play a significant role in the behavior of the volatile compounds. We notice strong binding of the two esters (isoamyl acetate, ethyl hexanoate) with sodium caseinate and an inclusion phenomenon of the esters with the mannans. For 1-hexanol and β-ionone, hydrogen bonds are observed with sodium caseinate. The cellular envelopes and the mannans create weak bonds with β-ionone. These hypotheses need to be confirmed.

Treatments of stabilize wines by fining or tangential microfiltration sometimes result in apparent loss of aroma (Feuillat, 1988).

For this reason an assumption has been made: Macromolecules strongly bind small molecules such as aroma compounds. The influence of yeast walls on the fixation of ethyl esters in reconstituted model wine has been shown by Geneix (1984).

At the outset, the concentrations of volatile compounds were 0.46 mg/L for ethyl hexanoate, 0.36 mg/L for ethyl octanoate, and 0.12 mg/L for ethyl decanoate. After a contact of 24 h, ethyl esters were not detected; treatment with a concentration of 1 g/L cell walls eliminated 78% of ethyl hexanoate and almost all ethyl octanoate and ethyl decanoate (Geneix, 1984). The fixation of aroma substances during stabilization of Australian Riesling wine by fining and ultrafiltration was studied on a polysulfone membrane with a nominal molecular weight cutoff of 10 000 (Miller et al., 1985). A decrease of 5% ethyl hexanoate during the fining and a slight increase of volatile compound concentrations during ultrafiltration were observed.

More fundamental work was done on interactions between aroma compounds and proteins. For example, Franzen and Kinsella (1974) observed that the addition of protein (casein, egg albumin) in aqueous solution with acetone and ethanol significantly increased the concentration of volatile compounds in the headspace phase. During these treatments, the loss of aroma was seldom studied and the origin of this phenomenon was never explained. The methods, fining and ultrafiltration, were not common in such research. However, they permitted us to verify the hypothesis proposed as to the bonds between aroma compounds and macromolecules, to study the effects of clarification-stabilization treatments on aroma substances, and to compare the influence of macromolecules with the effects of the other treatments. We used a model wine similar to that of Geneix (1984). This approach was more practical than theoretical.

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MATERIALS AND METHODS

The medium was simplified in order to show the interactions between macromolecules and aroma compounds (Table I).

Macromolecules from wines of different origins were added to this model medium. Red grapes were the source for the glucans, yeast was the source for the cell walls, and fining agent or grapes were the sources for the tannins. Large molecules, based on selected technologies, were sodium caseinate prepared at ENS.BANA, mannans extracted from walls of *Saccharomyces cerevisiae* by the method of Kocourek and Ballou (1969), and yeast walls from Fould Springer type *S. cerevisiae* prepared for use in wine (Table II). Solutions with a concentration of 0.1% (w/w) were prepared with the previous substances. Alcohols and esters are responsible for the fruity character and the aroma of wine, so isoamyl acetate (Merck), ethyl hexanoate (Merck), 1-hexanol (Merck) (100 μL/L), and β-ionone (Merck) (50 μL/L) were chosen; β-ionone was specific substance of Burgundy wine.

The fining consisted of eliminating wine macromolecules by means of floculation by food-grade fining agent.

For the organic fining agents, the mechanism was

$$\text{gelatin}^{+} \xrightarrow{\text{tannins}} \text{gelatin}^{-}$$

Floculation and then sedimentation were followed by a frontal filtration on Whatman filter GF/C made of fiberglass with a porosity of 1.2 μm. Two fining agents were tested at a concentration of 10 g/L: bentonite and lacta B (bentonite and casein). Tangential ultrafiltration was performed on a laboratory apparatus (Millipore-Minitan system); 0.2-2 L of solution was filtered on a polysulfone membrane (10 000 Da) with an area of 2.4×10^{-2} m^2.

The following conditions were established after trials: inlet pressure, $(2 \pm 0.2) \times 10^5$ Pa; inlet rate to prevent the membrane from plugging, 12 m/s; rate of retention, 11 m/s; rate of filtration, 1 m/s. The solution was recycled over 45 min (batch system); 63% of the filtrate is recovered at the end.

To determine the concentration of aroma substances before clarification-stabilization treatments, liquid injections of the model wines into a gas-liquid chromatograph were made. A Packard Series 427 chromatograph was used: FID, 190 °C; column with Chromosorb W AW (100-120 mesh) with Carbowax 20 M (10%); nitrogen flow, 25 mL/min; hydrogen flow, 20 mL/min; air flow, 200 mL/min; temperatures of isothermal column chromatography, 80 °C for isoamyl acetate and ethyl hexanoate, 140 °C for 1-hexanol, and 180 °C for β-ionone.

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Table I. Model Wine Composition

compd[a]	concn (w/w), %
ethanol	10
tartaric acid	0.4
malic acid	0.3
acetic acid	0.01
potassium sulfate	0.01
magnesium sulfate	0.0025
mineral water (Evian), pH 7.2	qsp

[a] Adjusted to pH 3 with NaOH (1 mol/L).

Table II. Characteristics of Macromolecules

macromolecule	characteristics
sodium caseinate	prepared in laboratory of food biochemistry and toxicology, 23 000 Da
mannans	prepared in laboratory of oenology, extracted from *S. cerevisiae*
	sugar fraction (85%) 23 500 Da, proteic fractions (1.58–40) × 10^5 Da
yeast walls (wall and membrane)	Fould Springer type *S. cervevisiae* for oenology uses

RESULTS AND DISCUSSION

All of the experiments were repeated three times.

Results expressed the specific fixation percentage of aroma on macromolecules and clarification–stabilization treatments: the losses were taken into account because the calculations were corrected with regard to volatile compound quantity lost in a system without macromolecules.

Influence of Macromolecules on Aroma Substances. *Fining.* The effects of macromolecules on the four aroma substances varied with the chemical nature of volatile compounds and macromolecules. For each fining agent (Figures 1 and 2) the binding increased when sodium caseinate was added. This effect was greater with β-ionone (about 30%) and lower so for the three other compounds (from 8% for 1-hexanol with lacta B to 18% for isoamyl acetate with bentonite). There were interactions between aldehydes and amino acid groups in an aqueous medium (Maier, 1970). Cellular walls and mannans had similar effects on each volatile substances, except for β-ionone, which was more easily fixed on the cellular walls than on mannans. These macromolecules only retained ketones. These results confirmed those of Geneix (1984) who observed a retention of ethyl esters by yeast walls, which increased with macromolecule concentration. The same phenomenon was noted between 1-hexanol and maltodextrin (Lebert and Richon, 1984).

Tests with 1% nonvolatile compounds (Figure 3) showed an increasing retention of every volatile compound during the fining with the bentonite (25% for 1-hexanol with sodium caseinate, 8.1% for β-ionone with cellular walls). This fact was confirmed by activity coefficient, γ^{∞}, measurements (Sadafian, 1986; Voilley et al., 1988).

Tangential Ultrafiltration. As shown previously, the binding of volatile compounds depended on the nature of macromolecules (Figure 4). The same effect as with the fining was observed, except in some cases. Accordingly, we could develop a hypothesis about the different strengths of the bonds: Fining is a "static method" because it is a floculation followed by a slow sedimentation due to gravity. The strength of the linkage set in action during the sedimentation was less than that of the bonds between macromolecules and aroma substances, and consequently these bonds were not broken. Tangential ultrafiltration and equilibrium dialysis were compared

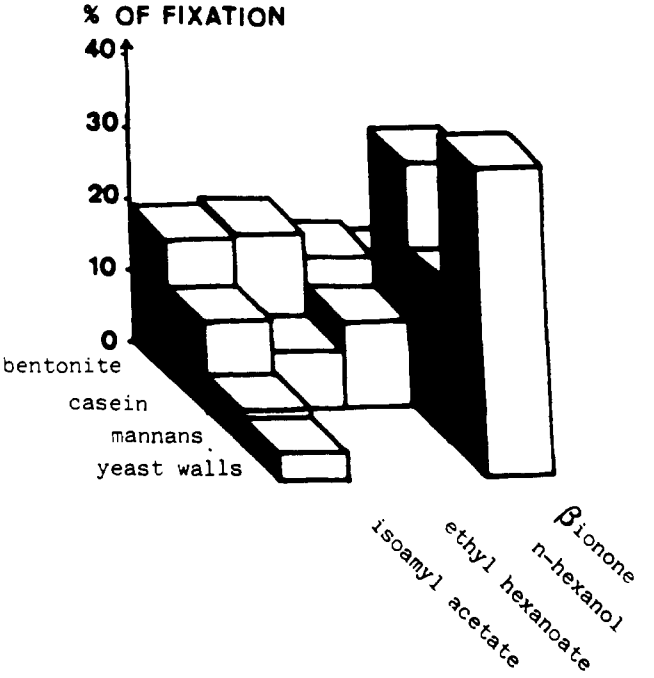

Figure 1. Percentage of aroma substance fixation during the fining with the bentonite.

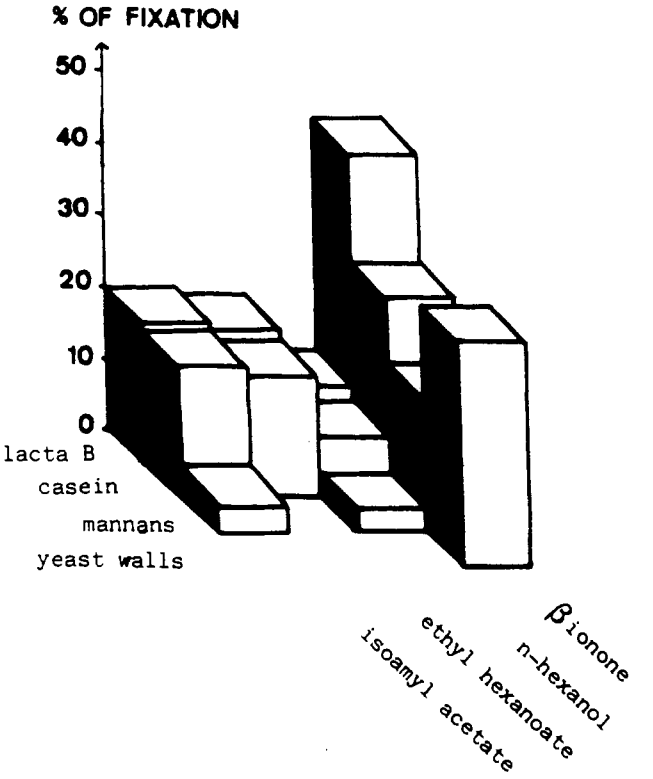

Figure 2. Percentage of aroma compound fixation during the fining with Lacta B.

in theory (Sophianopoulos and Sophianopoulos, 1985). This dynamic method cut weak bindings such as hydrogen bonds. An equilibrium dialysis coupled with headspace method was used in order to show the interactions.

Isoamyl acetate and ethyl hexanoate could be fixed on the macromolecules by weak bonds.

β-Ionone could be fixed more strongly in particular with proteic substrates.

In this experiment, fining and tangential ultrafiltration revealed interactions with the esters; it proved that

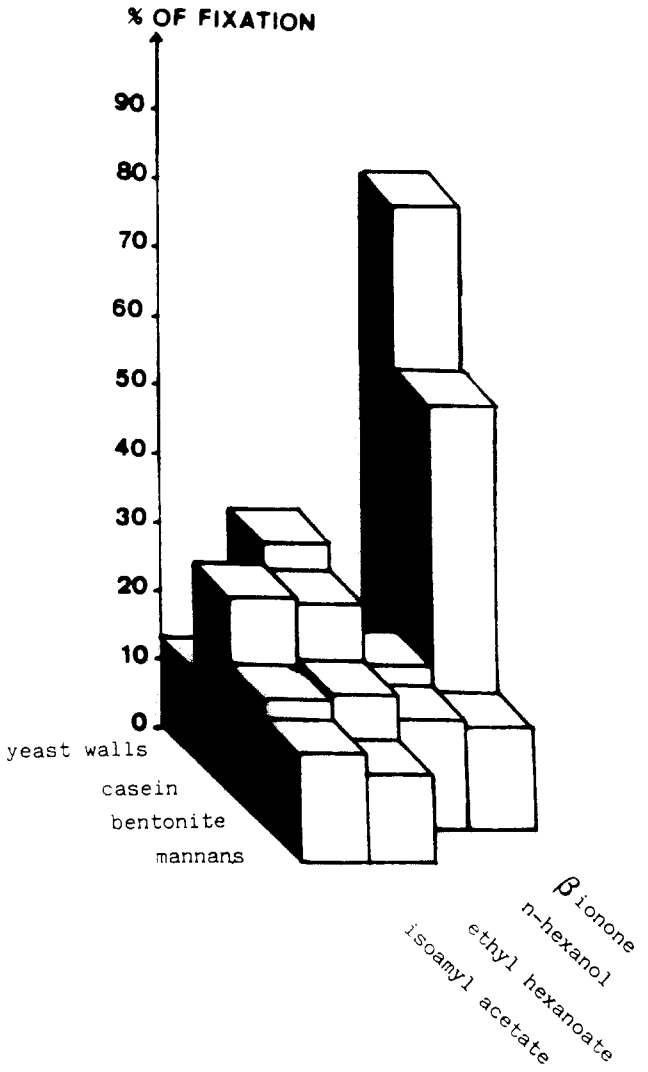

Figure 3. Percentage of aroma compound fixation during the fining with bentonite and 1% macromolecules.

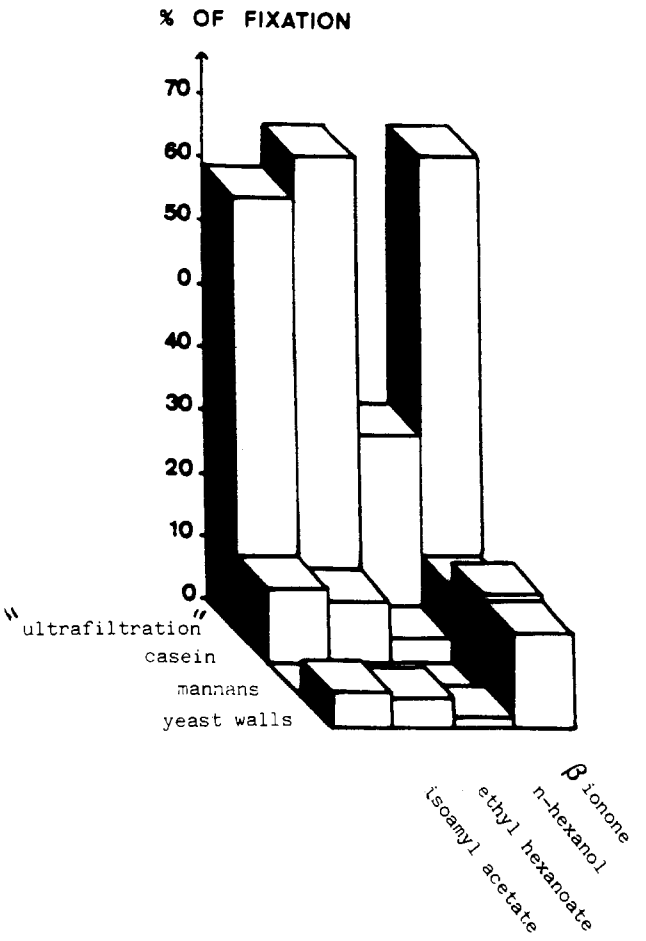

Figure 4. Percentage of volatile substance fixation during ultrafiltration.

the bonds were strong. On the contrary, interaction with 1-hexanol and β-ionone was weak. The hypothesis of Fares (1987), except with respect to esters, was confirmed. An inclusion phenomenon seemed to be more probable than binding between esters and mannans (Solms, 1986). Bonds between β-ionone and mannans were weak, as it was proposed, between four ketons (acetone, diacetyl, ethyl methyl ketone, diethyl ketone) and glucides (Maier, 1970). Aroma substances were poorly fixed by cellular walls.

Effect of Clarification–Stabilization Treatment on Volatile Compounds. The fixation by bentonite and lacta B was similar for the two esters (18.5%). But, bentonite fixed more 1-hexanol (12.1% against 7.7%) and lacta B more β-ionone (15.0% against 34.3%). They were two macromolecules in suspension much in the same way as those previously studied.

During the tangential ultrafiltration, important losses were noted (from 30 to 80%). They were due to the fixation of volatile compounds on the membrane as well as on the entire apparatus. The losses of aroma compounds during the proteic stabilization by bentonite and tangential ultrafiltration on a polysulfone membrane (10 000 Da) were studied (Miller et al., 1985). A decrease of ethyl hexanoate (5%) and *n*-ethyl octanoate (65%) was noted during the fining. Studies on mineral membranes M1 (5000 Da), M4 (20 000 Da), and M6 (2×10^6 Da) showed a decrease of 1-hexanol, of isoamyl acetate with M4 and M6, and of 3-hexanol with M1, M4, and M6 and an increase of ethyl acetate with M1 and M6 (Berger and Gaillard, 1985).

In our work the fixation was greater during the tangential ultrafiltration than during the fining. Few differences were observed between the two technologies (Miller et al., 1985). Our results were in accordance with studies comparing traditional treatment and filtration on M1, M4, and M6 (Berger and Gaillard, 1985).

According to Figures 1–3, the volatile substance fixation due to clarification–stabilization treatment was greater than that due to the macromolecules.

CONCLUSION

With these studies we are able to verify the proposed hypothesis on the macromolecules role in aroma loss and to predict the strength of possible bonds between the two types of compounds.

The clarification–stabilization treatments bound more aroma substances, in our system, than the macromolecules of the model wine. The hypothesis of the fixation of volatile compounds by the macromolecules alone did not explain the aroma defaults noted in treated wine. The treatment effect played a nonnegligible part in losses of aroma compounds.

This study should be developed with experiments on wine fractions that are similar to compounds naturally found in wine than those sometimes used (tannins) and with better characterization in order to determine more precisely the interactions between macromolecules and the aroma compounds.

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Effect of Methanol–Ammonia–Water Treatment on the Fate of Glucosinolates

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Canola seed, Altex variety, was treated with 10% ammonia in a 95% methanol–hexane extraction system under forcing conditions. The four specific aliphatic glucosinolates, namely gluconapin, progoitrin, glucobrassicanapin, and gluconapoleiferin, for which canola seed is generally analyzed were extracted, mainly in the intact form, into the polar phase. Nearly 10% of the total aliphatic glucosinolates was retained in the meal. Almost 19% of the original glucosinolates was degraded in this process, and these were predominantly extracted into the polar phase. Two isolated glucosinolates, namely progoitrin and gluconapin, were also subjected to this process in model experiments. The breakdown products of aliphatic glucosinolates were nitriles, hydroxy nitriles, epithio nitriles, and isothiocyanates. No oxazolidinethione was produced in this process.

Rapeseed could play an important role in the world's protein supplies due to its climatic adaptability and the excellent nutritional value of its protein. However, the usefulness of rapeseed as a source of food protein is limited by the presence of undesirable components such as glucosinolates, phytates, phenols, and fiber (hull). Glucosinolates, the principal antinutrients found in rapeseed, give rise to hydrolysates possessing goitrogenic and toxic properties (Fenwick et al., 1983). Canadian rapeseed varieties, canola, low in both glucosinolates (<30 μmol/g of meal) and erucic acid (<2% in oil) content, have been available for over a decade. Nonetheless, even these glucosinolate levels are still too high for inclusion of canola meal into food products. Extensive work on the detoxification of rapeseed meal with respect to glucosinolates and/or their breakdown products has been reported (Rutkowski, 1970; Afzalpurkar et al., 1974; Maheshwari et al., 1981). However, commercial application of these methods is not feasible due to high processing costs, high loss of proteins, poor functional properties of the resultant products, and incomplete removal of the glucosinolate degradation products. Thermal inactivation of endogeneous myrosinase, which is responsible for glucosinolate hydrolysis, is the current commercial practice (Eapen et al., 1968). The intact glucosinolates left in the meal are, however, still capable of inducing undesirable effects by forming toxic aglycons in the gastrointestinal tract. Oginsky et al. (1965) have shown that some bacteria, particularly *Para colobatrum*, common to the digestive system of man, have myrosinase activity. Recently, a new process for the removal of glucosinolates from canola and from Midas rapeseed and mustard seed was developed (Rubin et al., 1984; Naczk et al., 1986, 1988; Shahidi et al., 1988). Crushed seeds were treated with a two-phase solvent extraction system. The first phase was 95% methanol containing 10% ammonia, and hexane, which

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