Conversion of *meso*-2,3-Butanediol into 2-Butanol by Lactobacilli. Stereochemical and Enzymatic Aspects

Giovanna Speranza,* Sandra Corti, Gabriele Fontana, and Paolo Manitto

Dipartimento di Chimica Organica e Industriale, Università di Milano, e Centro di Studio per le Sostanze Organiche Naturali, via Venezian 21, I-20133 Milano, Italy

Antonietta Galli and Mauro Scarpellini

Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università di Milano, via Celoria 2, I-20133 Milano, Italy

Franco Chialva

Centro Studi Maria Branca, via Edoardo Porro 1, I-20158 Milano, Italy

A number of strains of *Lactobacillus* spp. from foods were screened for their ability to convert *meso*-2,3-butanediol into 2-butanol. Only three strains of *L. brevis* transformed the *meso*-diol into the secondary alcohol. These strains as well as the others unable to metabolize *meso*-2,3-butanediol exhibited the capacity to hydrogenate 2-butanone to 2-butanol. In both types of lactobacilli, an inverse relationship was observed between the diol or ketone concentration and the abundance of the *R* form of 2-butanol. This fact has been interpreted in terms of a co-occurrence of two dehydrogenases, both acting on the ketone with different kinetic parameters and opposite enantioselectivities. These results represent a further support to the assumption that 2-butanol present in distillates originates from the enzymatic activity of lactobacilli growing on mashes and give the most likely explanation of the enantiomeric excess of (*R*)-2-butanol generally found in distillates.

Keywords: *2-Butanol; meso-2,3-butanediol; lactobacilli; diacetyl; acetoin; dehydrogenases*

INTRODUCTION

2-Butanol is a typical constituent of certain spirits such as Italian grappa, where its concentration ranges from 20 to 300 mg/100 mL of pure ethanol, French marc (1−100 mg), and German tresterbrandwein (30−50 mg) (Postel and Adam, 1989). All of these distillates are obtained from the fermented residues of the grape-pressing procedure in the wine-making process. By contrast, 2-butanol normally occurs in much smaller amounts in wine (<6 mg/L) (Hieke, 1972; Versini et al., 1983) and in wine brandies (0−60 mg/L) (Nykänen and Suomalainen, 1983; Tandoi et al., 1984).

It is generally assumed that this secondary alcohol, which is present in fermented beverages and particularly abundant also in fruit brandies (Postel and Adam, 1989), originates from *meso*-2,3-butanediol (**1**) by the action of lactic acid bacteria on the mashes (Usseglio-Tomasset, 1971; Hieke, 1972; Hieke and Vollbrecht, 1974a; Manitto et al., 1994). Thus, high concentrations of 2-butanol in fermented grape beverages could be indicative of bacterial spoilage in the wines or in the lees and pomaces used for distillation (Postel and Adam, 1985, 1989) . It must be pointed out, however, that an inverse correlation between the level of 2-butanol and the quality of a commercial product is still considered questionable (Bertrand and Suzuta, 1976; Postel, 1982).

The fact that lactic acid bacteria are responsible for the appearance of 2-butanol in wines and pomaces is supported by the following observations: (i) its production increases during storage of the grape marc after fermentation and is pronounced especially near the bottom of the tanks, where living conditions are more satisfactory for lactobacilli (Usseglio Tomasset, 1971; Postel, 1984); (ii) certain strains of *Lactobacillus brevis* originally isolated from spoiled wine were found to be able to transform *meso*-2,3-butanediol (**1**) into 2-butanol (**3**) (Hieke and Vollbrecht, 1974a,b, 1980; Bertrand and Suzuta, 1976; Radler and Zorg, 1986); (iii) an enzyme catalyzing the dehydration of *meso*-2,3-butanediol (**1**) to 2-butanone (**2**) was obtained and characterized from cell-free extracts of one of the above strains (Radler and Zorg, 1986); (iv) some cryptostereochemical features of the conversion of stereospecifically ^{2}H-labeled *meso*-2,3-butanediol into 2-butanol by *L. brevis* were well explained in terms of a B_{12}-dependent diol dehydratase reaction followed by hydrogenation of the resulting 2-butanone (**2**) (Speranza et al., 1996). Thus, the two-step process summarized in Scheme 1 appears to be the most likely origin of 2-butanol occurring in wines and distillates.

Regarding the source of *meso*-2,3-butanediol, it can be pointed out that the pathway pyruvate−α-acetyllactate (or diacetyl)−acetoin, which is common among *Saccharomyces* spp. (Tittel and Radler, 1979; Hagenauer-Hener et al., 1990; Heidlas and Tressl, 1990), has also been demonstrated to occur in heterofermentative lactic acid bacteria (Axelsson, 1993; Kandler, 1983).

Recently we found that the *R* form of 2-butanol (**3a**) is largely predominant in distillates of different origin, while the *S* stereoisomer (**3b**) is the product of hydrogenation of **2** by *Saccharomyces cerevisiae* and *Saccha-*

* Author to whom correspondence should be addressed (fax +39-2-236 43 69; e-mail giospe@icil64.cilea.it).

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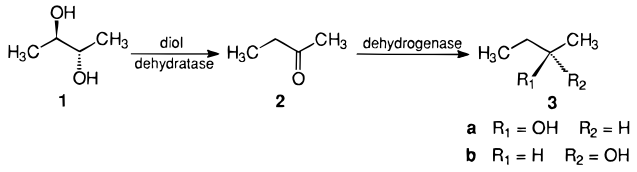
Scheme 1



romyces bayanus both in water and in freshly prepared must (Manitto et al., 1994). These results could represent conclusive evidence that 2-butanol is produced mostly, if not completely, by enzymatic activities of lactobacilli, provided the absolute configuration and the enantiomeric excess (ee) of the alcohol, which is recovered from these bacteria fed with *meso*-2,3-butanediol (**1**) or 2-butanone (**2**), are in agreement with the corresponding properties exhibited by 2-butanol in distillates. This consideration prompted us to investigate the stereoselectivity in lactobacilli-mediated transformations of *meso*-2,3-butanediol and 2-butanone into 2-butanol. In addition, we examined the possibility that *meso*-2,3-butanediol comes from diacetyl and/or acetoin in these microorganisms.

MATERIALS AND METHODS

Chemicals. *d*-, *l*-, and *meso*-2,3-butanediol, *d*,*l*-acetoin, and diacetyl were purchased from Aldrich Chimica (Milano, Italy); L-lactic acid dehydrogenase from *Lactobacillus leichmannii*, L-lactic acid dehydrogenase from *Bacillus stearothermophilus*, and NADH were obtained from Sigma-Aldrich (Milano, Italy). All chemicals and solvents were of analytical grade.

Microorganisms, Culture Conditions, and Fermentations. The screening was performed with strains of *Lactobacillus fermentum*, *Lactobacillus buchneri*, and *Lactobacillus brevis*. About half of these strains were freshly isolated from foods, while the others were from the Centre National de Recherches Zootechniques (Jouy En Josas, France). All strains were identified according to *Bergey's Manual* (Sneath et al., 1986) and mantained in the collection of the Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche (University of Milan) as freeze-dried cells. Each inoculum was grown in MRS (Difco, Detroit, MI) liquid medium tubes for 24 h at 30 °C.

Fermentation experiments (1% inoculum) were carried out at 30 °C for 7 days, unless otherwise stated, in a modified synthetic medium SM2 (Radler and Zorg, 1986) containing *meso*-2,3-butanediol, 2-butanone, diacetyl, or *d*,*l*-acetoin in the range 0.05−4 g/L. Anaerobic conditions were obtained in jars (BBL gas pack system, Italian BBL, Milano, Italy).

Ethanol (95−96%, 30 mL) was added to the liquid cultures (200 mL) and the resulting mixture distilled. Fractions in the range 78−85 °C were collected and analyzed by gas chromatography (GC) for the 2-butanol content. The remaining solution was extracted continuously with ethyl ether (100 mL) for 15 h to recover the isomeric 2,3-butanediols. The ether extract was dried with Na_2SO_4 and analyzed by GC.

Chromatographic and Spectroscopic Methods. The GC analyses of 2-butanone (R_t = 4.2 min), diacetyl (R_t = 5.5 min), 2-butanol (R_t = 6.8 min), *d*,*l*-acetoin (R_t = 12.5 min), *meso*-2,3-butanediol (R_t = 18.5 min), and *d*,*l*-2,3-butanediol (R_t = 17.6 min) were carried out by injecting samples prepared as above on a DANI 3800 gas chromatograph equipped with a FID using a homemade glass column (2 m × 2 mm i.d.) packed with 20% Carbowax 20M on Chromosorb W (60−80 mesh). The GC parameters were as follows: carrier gas, N_2 at 30 mL/min; injector temperature, 210 °C; detector temperature, 220 °C; the oven temperature was held at 60 °C for 4 min, then increased to 150 °C at 10 °C/min, and held at 150 °C for 8 min. For quantitative analyses *n*-amyl alcohol (R_t = 10.5 min) was used as internal standard. The ee of 2-butanol was determined by bidimensional GC as previously reported (Manitto et al., 1994). The ratio between (*R*,*R*)-*l*- and (*S*,*S*)-*d*-2,3-butanediol was measured by chromatographic separation performed on a Mega dimethylpentyl-β-cyclodextrin (30%)−OV 1701 (70%) capillary column (25 m × 0.25 mm i.d., 0.25 μm film thickness) (Mega, Legnano, Italy). The oven temperature was programmed from 40 to 90 °C with a gradient of 2 °C/min; injector and FID temperatures were 250 °C; R_t values of *d*-, *l*-, and *meso*-2,3-butanediol were 16.2, 17.5, and 18.5 min, respectively.

Table 1. Strains of Obligatory Heterofermentative Lactobacilli Tested for Conversion of *meso*-2,3-Butanediol into 2-Butanol

strain		source
L. fermentum	LF-1	crackers sponge
	LF-2	crackers sponge
	LF-3	white flour
	LF-88	white flour
L. buchneri	LH-1	CNRZ 56[a]
	LH-6	CNRZ 214[a]
L. brevis	LB-1	panettone sour dough
	LB-2	bread sour dough
	LB-5[b]	crackers sponge
	LB-6	sauerkraut
	LB-8	sauerkraut
	LB-9	minced meat
	LB-10	bread sour dough
	LB-14	CNRZ 741[a]
	LB-15	CNRZ 215[a]
	LB-16	CNRZ 234[a]
	LB-17	CNRZ 423[a]
	LB-18[b]	CNRZ 734[a]
	LB-19[b]	CNRZ 735[a]
	T_1	white flour
	T_2	white flour

[a] Centre National de Recherches Zootechniques, Jouy en Josas (France). Strains isolated from different French cheeses. [b] 2-Butanol-producing strains.

Table 2. Conversion of *meso*-2,3-Butanediol (1) (4 g/L) into 2-Butanol (3) by Strains of *L. brevis*

expt no.	*L. brevis* strain	2-butanol formed (g/L)	conversion yields[a] (%)	predominant enantioform *R* (**3a**) or *S* (**3b**)	*R*/*S* ratio
1	LB-5	0.26	97	*R*	3.11
2	LB-18	0.29	97	*S*	0.73
3	LB-19	0.41	97	*R*	1.54

[a] Chromatographic yields based on the consumed *meso*-2,3-butanediol.

Tests for 2-Butanone Reduction by Lactic Acid Dehydrogenase. Enzymatic assays were carried out according to literature procedures (Bergmeyer et al., 1983) using a Hewlett-Packard 8452A diode array spectrophotometer. Phosphate buffer (pH 7.2, 50 mM) solutions of NADH (0.2 mM, 0.8 mL), of the substrate (0.01 M, 0.16 mL), and of the enzyme (165 units/L, 0.03 mL) were combined in a 2-mL cuvette at 30 °C. A decrease of absorbance at 339 nm was found with both D- and L-lactic acid dehydrogenase with pyruvate as the substrate. On the contrary, no change in absorbance was observed when 2-butanone was used in parallel experiments.

RESULTS

Conversion of *meso*-2,3-Butanediol (1) into 2-Butanol (3) by Lactobacilli. Among 15 strains of *L. brevis*, 4 strains of *L. fermentum*, and 2 strains of *L. buchneri*, only 3 strains of *L. brevis* were found to be able to transform *meso*-2,3-butanediol (**1**) into 2-butanol (**3**) (Table 1). The conversion yield, the predominant enantiomeric form of **3**, and its *R*/*S* ratio were determined gas chromatographically for each experiment performed with a strain capable of metabolizing **1** (Table 2). In parallel experiments no biotransformation of either (*S*,*S*)-**4** or (*R*,*R*)-2,3-butanediol (**5**) was observed, as previously reported (Radler and Zorg, 1986).

Table 3. Enantiomeric Excess of (*R*)-2-Butanol (3a) with Respect to the *meso*-2,3-Butanediol (1) Concentration in Experiments Carried out with *L. brevis* (LB-5) Strain

meso-2,3-butanediol concentration (g/L)	2-butanol formed (g/L)	ee (%) of (*R*)-2-butanol
0.05	0.01	84.3
0.5	0.05	80.2
1	0.08	81.5
2	0.12	69.1
4[a]	0.26	51.4

[a] Entry 1 of Table 2.

Table 4. Reduction of 2-Butanone (2) to 2-Butanol (3) by *L. brevis* Strains[a]

expt no.	*L. brevis* strain	yield[b] (%)	predominant enantioform *R* (**3a**) or *S* (**3b**)	*R*/*S* ratio
	Able To Metabolize *meso*-2,3-Butanediol			
1	LB-5	100	*R*	2.49
2	LB-18	100	*S*	0.59
3	LB-19	75	*S*	0.87
	Unable To Metabolize *meso*-2,3-Butanediol			
4	LB-15	92	*S*	0.03
5	LB-16	53	*R*	1.12
6	LB-17	96	*R*	5.60

[a] Substrate concentration, 0.8 g/L. [b] Determined by GC.

Table 5. Effect of 2-Butanone (2) Concentration on Enantiomeric Excess of (*R*)-2-Butanol (3a)

L. brevis strain	2-butanone concentration (g/L) (A_0)	ee[a] (%) of (*R*)-2-butanol
LB-5[b]	0.05	93.7
	0.1	89.2
	0.2	86.1
	0.4	65.9
	0.8	42.8
LB-17[c]	0.1	89.6
	0.2	82.3
	0.4	80.4
	0.8	69.7
	1.2	61.4

[a] Measured at complete conversion of the substrate. [b] Able to metabolize *meso*-2,3-butanediol. [c] Unable to metabolize *meso*-2,3-butanediol.

The dependence of the ee of (*R*)-2-butanol on the concentration of the substrate, when tested for the LB-5 strain of *L. brevis*, is shown in Table 3.

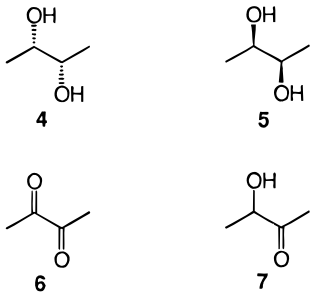
Reduction of 2-Butanone (2) to 2-Butanol (3) by Lactobacilli. Under the same experimental conditions as for the conversion of *meso*-2,3-butanediol (**1**) into 2-butanol, 2-butanone (**2**) was subjected to reduction by the action of a number of strains of *L. brevis*. Experiments were carried out using strains both able and unable to metabolize *meso*-2,3-butanediol. All of them exhibited a great capability to hydrogenate the carbonyl group of the ketone, even if a large variation in enantioselectivity was apparent (Table 4). Considering that two or more competing dehydrogenases with opposite stereochemical preferences could concur in the reduction of 2-butanone, two sets of experiments were then performed to reveal a possible connection of the product ee's with the substrate concentration (Chen et al., 1984). In fact, a coherent relationship between the two parameters was observed with both a *meso*-2,3-butanediol-metabolizing strain (LB-5) and a strain (LB-17) unable to transform the diol (Table 5).

Reduction of Diacetyl (6) and *d,l*-Acetoin (7) by Lactobacilli. *L. brevis* (LB-19) was shown to be able to reduce both *d,l*-acetoin (**7**) and diacetyl (**6**) completely (in 10 days) to a mixture of the three isomeric 2,3-butanediols (**1**, **4**, and **5**) and 2-butanol (**3**) (Table 6).

Table 6. Transformation of *d,l*-Acetoin (7) and Diacetyl (6) by *L. brevis* (LB-19) Strain

	product,[a] mg			
substrate[b] (mg)	(*S*,*S*)-BD (**4**)	(*R*,*R*)-BD (**5**)	*meso*-BD (**1**)	2-butanol (**3**)
d,l-acetoin (150)	27.1	8.5	9.3	22.3
diacetyl (150)	21.2	6.4	13.1	10.7

[a] After 10-day fermentation; BD, 2,3-butanediol. [b] Substrate concentration, 1g/L.



DISCUSSION

Table 4 shows that the capability of reducing 2-butanone (**2**) into the corresponding alcohol (**3**) is presumably widespread among *L. brevis* strains. Its occurrence in all strains examined that are able to convert *meso*-2,3-butanediol (**1**) into 2-butanol (**3**) (cf. Table 1) is consistent with the reaction sequence of Scheme 1. The large variability in enantioselectivity exhibited by the strains listed in Table 4 appears to be in agreement with some of the results obtained when the reduction of noncyclic ketones by heterofermentative lactic acid bacteria was investigated (Maconi and Aragozzini, 1989; Aragozzini et al., 1992).

Inspection of Table 5 clearly reveals a correlation between the ee values of the *R* form of 2-butanol (**3a**) and the initial concentration of 2-butanone in the growing medium of two *L. brevis* strains. It can be noted that the *R* ee increases in both cases as the initial concentration (A_0) of the ketone decreases. The dependence of the ee on the initial substrate concentration was treated theoretically and experimentally by Chen et al. (1984), and a quantitative expression was developed for systems such as yeast reductions in which two competing enzymes act on one substrate to yield two enantiomeric products. Each set of data reported in Table 5 (A_0 and % ee) is strongly indicative of the involvement of two dehydrogenases having opposite stereoselectivities and different kinetic parameters, i.e. Michaelis constants, K_R and K_S, and maximal velocities, V_R and V_S, for enzyme *R* and *S* forms, respectively. The shapes of the curves obtained by plotting % ee vs A_0, when compared with the theoretical ones reported by Chen et al. (1984), suggest $K_R \ll K_S$ and $V_R < V_S$ or $V_R \approx V_S$ for experiments with LB-5 or LB-17 strain, respectively. An analogous trend, i.e., a higher enantiomeric excess of (*R*)-2-butanol at lower substrate concentration, was observed in the transformation of *meso*-2,3-butanediol by LB-5 strain (Table 3).

The involvement of both D- and L-lactate dehydrogenases (Dennis and Kaplan, 1960; Axelsson, 1993) seems to be unlikely since no reduction of 2-butanone was

observed by us when the ketone was tested with the above enzymes isolated from lactic acid bacteria [see also Maconi and Aragozzini (1989)]. On the other hand, butanediol dehydrogenases could be responsible for the 2-butanone hydrogenation. Two types of these enzymes are known, i.e. one specific to *R,R* and *meso*- forms and the other specific to (*S,S*)- and *meso*-2,3-butanediol (Taylor and Juni, 1960). This fact could indicate that each type is characterized by one enantiospecificity toward the $CH_3CH(OH)-$ grouping, independent of the configuration of the connected carbon atom. Such enantiospecifically different dehydrogenases have been reported to co-occur in microorganisms (Ui et al., 1983) including lactobacilli (Crow, 1990). Actually, we found that both exogenous diacetyl and *d,l*-acetoin were converted by *L. brevis* (LB-19 strain, under proper conditions for metabolizing *meso*-2,3-butanediol) into mixtures of (*S,S*)-, (*R,R*)-, *meso*-2,3-butanediol and 2-butanol, the three isomeric diols appearing in both cases in similar molar ratios, i.e. ca. 3:1:4, when the secondary alcohol is reckoned as the *meso* form (Table 6). These results can be interpreted in terms of the presence in that strain of both (*S*)- and (*R*)-acetoin reductases (the activity of the former appearing to be prevalent in agreement with entry 3 of Table 4) beside a nonstereospecific diacetyl reductase (or two specific enzymes and an acetoin racemase) (Taylor and Juni, 1960).

Now, if one compares the *R*/*S* ratios of 2-butanol resulting from reduction of 2-butanone by LB-5, LB-18, and LB-19 strains (Table 4) with the corresponding ones for the alcohol produced by the same strains in the presence of *meso*-2,3-butanediol (Table 2), a significative increase in the formation of the *R* isomer results on going from the ketone to the diol as substrate, even though the initial concentration of the former (0.8 g/L) is remarkably lower than that of the latter (4 g/L). From this observation it can be inferred that of the two consecutive reactions of Scheme 1, the dehydration of *meso*-2,3-butanediol (**1**) is much slower than the second one (catalyzed by a pair of enantioselectively opposite oxidoreductases), thus setting up a nearly stationary state characterized by a (very) low concentration of the intermediate 2-butanone (**2**). Such a situation, together with the inverse relationship between the concentration of 2-butanone and the preference for the *R* form of its reduction product exhibited by *L. brevis* (LB-5) (Table 5), would explain the high % ee's in (*R*)-2-butanol listed in Table 3.

In conclusion, our previous finding that the enantiomeric form of 2-butanol in all distillates analyzed is *R* with an ee ranging from 50 to 80% (Manitto et al., 1994) is understandable provided the assumption is made that the enzymatic reactions involved in the transformation of *meso*-2,3-butanediol by lactic acid bacteria growing on mashes are kinetically and stereoselectively similar to those performed by the lactobacilli we examined.

LITERATURE CITED

Aragozzini, F.; Valenti, M.; Santaniello, E.; Ferraboschi, P.; Grisenti, P. Biocatalytic, enantioselective preparations of (*R*)- and (*S*)-ethyl 4-chloro-3-hydroxybutanoate, a useful chiral synthon. *Biocatalysis* **1992**, *5*, 325−332.

Axelsson, L. T. Lactic acid bacteria. Classification and physiology. In *Lactic Acid Bacteria*; Salminen, S., von Wright, A., Eds.; Dekker: New York, 1993.

Bergmeyer, H. U. *Methods of Enzymatic Analysis*, 3rd ed.; Verlag Chemie: Weinheim, Germany, 1983; Vol. III, pp 118−126.

Bertrand, A.; Suzuta, K. Formation of 2-butanol by lactic acid bacteria isolated from wine. *Connaiss. Vigne Vin* **1976**, *10*, 409−426; *Chem. Abstr.* **1977**, *86*, 137900p.

Chen, C.-S.; Zhou, B.-N.; Giraukas, G.; Shieh, W.-R.; Van-Middlesworth, F.; Gopalan, A. S.; Sih, C. J. Stereochemical control of yeast reductions. 2. Quantitative treatment of the kinetics of competing enzyme systems for a single substrate. *Bioorg. Chem.* **1984**, *12*, 98−117.

Crow, V. L. Properties of 2,3-butanediol dehydrogenases from *Lactococcus lactis* subsp. *lactis* in relation to citrate fermentation. *Appl. Environ. Microbiol.* **1990**, *56*, 1656−1665.

Dennis, D.; Kaplan, N. O. d- and l-Lactic acid dehydrogenases in *Lactobacillus plantarum*. *J. Biol. Chem.* **1960**, *235*, 810−818.

Hagenauer-Hener, U.; Henn, D.; Dettmar, F.; Mosandl, A.; Schmitt, A. Direct determination of 2,3-butanediol stereoisomers in wine. *Dtsch. Lebensm.-Rundsch.* **1990**, *86*, 273−276.

Heidlas, J.; Tressl, R. Purification and characterization of a (*R*)-2,3-butanediol dehydrogenase from *Saccharomyces cerevisiae*. *Arch. Microbiol.* **1990**, *154*, 267−273.

Hieke, E. Analysis of volatile compounds (flavors) present in wine and in other alcoholic beverages. *Allg. Dtsch. Weinfachztg.* **1972**, *108* (1/2), 24.

Hieke, E.; Vollbrecht, D. On the volatile ingredients in wine and other alcoholic beverages. IV. The influence of yeasts and lactobacilli on the content of volatile ingredients in wine from exerted wine yeast (yeast-wine). *Chem. Mikrobiol. Technol. Lebensm.* **1974a**, *3*, 65−68.

Hieke, E.; Vollbrecht, D. On the formation of butanol-2 by lactic acid bacteria and yeast. *Arch. Microbiol.* **1974b**, *99*, 345−351.

Hieke, E.; Vollbrecht, D. Metabolic activities of *Lactobacillus brevis*. Effect of pH, glucose and butane-2,3-diol. *Z. Lebensm. Unters. Forsch.* **1980**, *171*, 38−40.

Kandler, O. Carbohydrate metabolism in lactic acid bacteria. *Antonie van Leeuwenhoek* **1983**, *49*, 209−224.

Maconi, E.; Aragozzini, F. Stereoselective reduction of noncyclic ketones with lactic acid bacteria. *Appl. Microbiol. Biotechnol.* **1989**, *31*, 29−31.

Manitto, P.; Chialva, F.; Speranza, G.; Rinaldo, C. Absolute stereochemistry and enantiomeric excess of 2-butanol in distilled spirits of different origin. *J. Agric. Food Chem.* **1994**, *42*, 886−889.

Nykänen, L., Suomalainen, H., Eds. *Aroma of Beer, Wine and Distilled Alcoholic Beverages*; Reidel Publishing: Dodrecht, Holland, 1983; pp 22−41.

Postel, W. Butanol-(2) as an evaluation criterion in wine, wines for distillation, and wine distillates. *Dtsch. Lebensm. Rdsch.* **1982**, *78*, 211−215.

Postel, W. Volatile components of fruit brandies. In *Proceedings of the Alko Symposium on Flavour Research of Alcoholic Beverages*; Nykänen, L., Lehtonen, P., Eds.; Foundation for Biotechnical and Industrial Fermentation Research: Helsinki, 1984; pp 175−187.

Postel, W.; Adam, L. Quantitative determination of volatiles in distilled alcoholic beverages. In *Topics in Flavour Research*; Berger, R. G., Nitz, S., Schreier, P., Eds.; Eichorn: Marzling-Hangenham, Germany, 1985; pp 79−108.

Postel, W.; Adam, L. Fruit distillate flavours. In *Distilled Beverage Flavour*; Piggott, J. R., Paterson, A., Eds.; Horwood: Chichester, U.K., 1989; pp 133−147.

Radler, F.; Zorg, J. Characterization of the enzyme involved in formation of 2-butanol from *meso*-2,3-butanediol by lactic acid bacteria. *Am. J. Enol. Vitic.* **1986**, *37*, 206−210.

Sneath, P. H. A.; Mair, N. S.; Sharpe, M. E.; Holt, J. G. *Bergey's Manual of Systematic Bacteriology*; William and Wilkins: Los Angeles, 1986; Vol. 2, pp 1209−1234.

Speranza, G.; Manitto, P.; Fontana, G.; Monti, D.; Galli, A. Evidence for enantiomorphic-enantiotopic group discrimination in diol dehydratase-catalyzed dehydration of *meso*-2,3-butanediol. *Tetrahedron Lett.* **1996**, *37*, 4247−4250.

Tandoi, P.; Guidotti, M.; Stacchini, P. Composition of brandies. *Riv. Ital. Sci. Aliment.* **1984**, *13*, 69−76; *Chem. Abstr.* **1985**, *102*, 44333q.

Taylor, M. B.; Juni, E. Stereoisomeric specificities of 2,3-butanediol dehydrogenases. *Biochim. Biophys. Acta* **1960**, *39*, 448−457.

Tittel, D.; Radler, F. Formation of 2,3-butanediol by acetoin reductase in *Saccharomyces cerevisiae*. *Monatsschr. Brau.* **1979**, *32*, 260, 262−267; *Chem. Abstr.* **1979**, *91*, 138796z.

Ui, S.; Masuda, H.; Muraki, H. Stereospecific and electrophoretic natures of bacterial 2,3-butanediol dehydrogenases. *J. Ferment. Technol.* **1983**, *61*, 467−471.

Usseglio-Tomasset, L. Characteristics of brandy derived from volatile constituents which develop during ensilage and from modifications due to distillation. *Vini Ital.* **1971**, *13*, 453−461; *Chem. Abstr.* **1972**, *76*, 111661e.

Versini, G.; Inama, S.; Pellegrini, R. Observations on a possible correlation between the amount of 2-butanol and propionic acid in wines. *Vigne Vini* **1983**, *10* (11), 23−27; *Chem. Abstr.* **1984**, *100*, 101559c.

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