DISTRIBUTION OF ESTERS PRODUCED DURING SUGAR FERMENTATION BETWEEN THE YEAST CELL AND THE MEDIUM

By Lalli Nykänen, Irma Nykänen and Heikki Suomalainen
(*Research Laboratories of the State Alcohol Monopoly (Alko), Box 350, SF-00101 Helsinki 10, Finland*)

The distribution of the esters formed during sugar fermentations between the yeast cells and the medium was investigated in fermentations by 5 strains of *Saccharomyces cerevisiae* and 3 strains of *S. uvarum* (*carlsbergensis*). The esters studied included the acetates of isoamyl alcohol and phenethyl alcohol and the ethyl esters of the C_6–C_{12} fatty acids. All of both acetates appeared in the medium. The proportion of the fatty acid ethyl esters transferred to the medium decreased with increasing chain length: all in the medium for ethyl caproate, 54–68% for ethyl caprylate, 8–17% for ethyl caprate, and all remaining in the yeast cell for ethyl laurate.

A higher proportion of the esters formed appeared to remain in the cells of the *S. uvarum* strains than in cells of *S. cerevisiae*.

Key words: *distribution, ester, fermentation, Saccharomyces, yeast.*

Introduction

A great variety of volatile metabolic by-products is formed in yeast cells during fermentations; the numerically largest group consists of esters.[7,8,10] A part of the esters permeates through the cell membrane to the medium and part remains in the yeast cell. Wine lees, which contains the yeast grown during the wine fermentation, gives on steam distillation an oily product called lees oil that has the characteristic aroma of cognac. The lees oil has been shown to contain ethyl caprate, ethyl laurate and ethyl caprylate as major components and small amounts of the ethyl esters of higher fatty acids.[1,4,6]

While investigating the formation of aroma compounds in sugar fermentations, Suomalainen & Nykänen[9] noted that the amount of esters in a distillate of the medium increased if the distillation was performed without removing the yeast. The same observation was later reported by Parfait *et al.*[5] Nordström,[2] while investigating the esterification of fatty acids added to the fermentation medium and the distribution of the esters formed between the yeast cell and the liquid phase, found that the longer the chain length of the ester the more the cell phase was preferred.

We have previously examined[3] the production of esters in sugar fermentations by *Saccharomyces cerevisiae* and *S. uvarum*, but we then determined the ester contents only in the fermentation media. In the present work we were especially concerned with determining the distribution of the esters formed during sugar fermentations by *S. cerevisiae* and *S. uvarum* between the yeast cells and the medium.

Materials and Methods

Yeasts.—Wine yeasts, *S. cerevisiae* (*ellipsoideus*), were from the collection of Research Laboratories of the Finnish State Alcohol Monopoly (Alko). Three of these strains (Champagne Epernay, Champagne Hautvilliers, and Sauternes) were originally from the Institut für Mikrobiologie und Biochemie, Geisenheim am Rhein, one (Mosel) from Dr Kielhöfer, Trier, and one was isolated from a German dried yeast. Brewer's yeasts, *S. uvarum* (*carlsbergensis*) were from the Biotechnical Laboratory of the Technical Research Center of Finland (2 strains) and from Ab Pripps Bryggerier, Stockholm (1 strain). Two fermentations were carried out with each yeast.

Fermentation medium.—The fermentation media contained 80 g/litre sucrose and 6·7 g/litre Difco yeast nitrogen base, and the fermentations were performed in the same semi-aerobic conditions as have been described before.[3]

Sample preparation.—When the fermentation was complete the yeast and medium were mixed thoroughly. The suspension obtained was divided into two parts. One part was steam distilled in presence of the yeast and the other was steam distilled after the yeast had been removed by centrifugation at 5000 rpm for 10 min at 0°C. The distillates were extracted with re-distilled isopentane (4 × 50 ml) and 2-octanol was added to the extracts as internal standard. The combined extracts were dried over anhydrous sodium sulphate and concentrated by distillation.[3]

Sample analysis.—The esters in the concentrated extracts, isoamyl acetate, phenethyl acetate, ethyl caproate, ethyl caprylate, ethyl caprate and ethyl laurate, were determined gas chromatographically using 3 m × ⅛ in stainless-steel columns packed with 10% FFAP on Chromosorb W AW, 60–80 mesh. The F & M 810 chromatograph equipped with FID and the chromatographic conditions were the same as have been described previously.[3] An Infotronics CRS-11HSB/42 integrator connected to the chromatograph was used in determining the peak areas of the individual esters.

Results and Discussion

In all, 16 sugar fermentations were performed with 5 *S. cerevisiae* and 3 *S. uvarum* strains. Fig. 1 shows gas chromato-

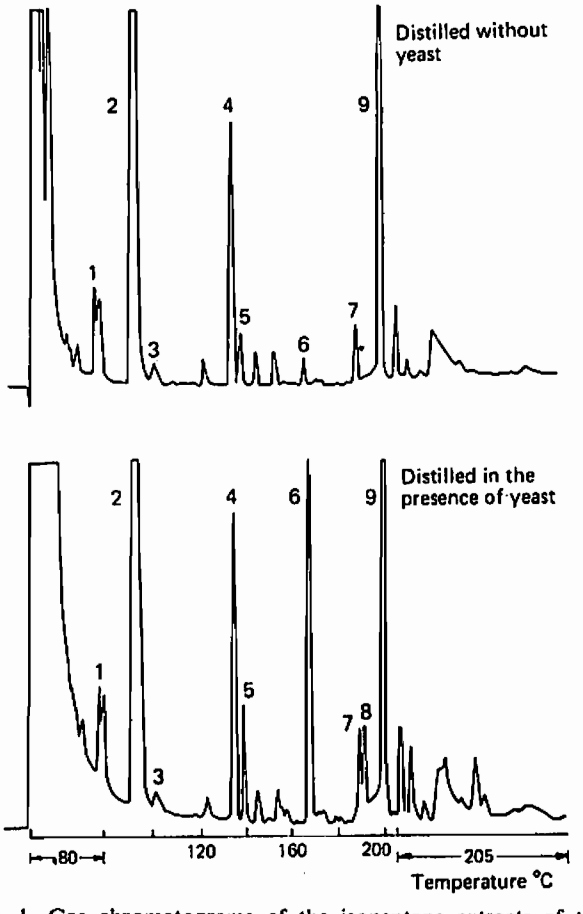

Fig. 1. Gas chromatograms of the isopentane extracts of the volatile aroma fraction formed during sugar fermentations by brewer's yeast. A 3 m × ⅛ in FFAP column was used. 1 Isoamyl acetate, 2 isoamyl alcohol, 3 ethyl caproate, 4 2-octanol (internal standard), 5 ethyl caprylate, 6 ethyl caprate, 7 phenethyl acetate, 8 ethyl laurate, 9 phenethyl alcohol.

grams of the isopentane extracts of media fermented by brewer's yeast and distilled with and without yeast. The identification of the compounds has been described before.[3]

The average ester contents found after steam distillation with and without yeast are presented in Tables I and II for fermentations by, respectively, the *S. cerevisiae* strains and *S. uvarum* strains. Isoamyl acetate was the major ester component in samples distilled without yeast. Its mean concentration was 0·26 ppm in the media fermented by the *S. cerevisiae* strains and 0·10 ppm when the *S. uvarum* strains were used. The finding that wine yeasts form more isoamyl acetate during sugar fermentation than do brewer's yeasts is in accordance with the results reported in our previous work,[3] in which we compared the mean production of isoamyl acetate by 56 *S. cerevisiae* yeasts with that by 3 *S. uvarum* yeasts. The difference between the means is statistically significant ($P < 0.05$). For both species the isoamyl acetate content was independent of whether yeast was present or absent during the steam distillation. The average content of phenethyl acetate, 0·04 ppm, was independent of both the presence of yeast during the steam distillation and the yeast species used.

A similar concentration of ethyl caproate was obtained whether the steam distillation was performed in the presence of yeast or not. The wine yeasts produced a mean concentration of ethyl caproate of 0·11 ppm and the brewer's yeasts 0·003 ppm, the difference being statistically significant ($P < 0.01$). The concentration of ethyl caprylate was greatly increased by retaining the yeast during steam distillation. In samples distilled without yeast an average concentration of 0·12 ppm was found for the *S. cerevisiae* strains and 0·07 ppm for the *S. uvarum*; in the presence of yeast the corresponding values were 0·17 ppm and 0·12 ppm. The differences in the amounts of ethyl caprylate produced by the two species were statistically significant. Assuming that all of the esters within the cells are released and that no marked hydrolysis of esters occurs during the steam distillation, these values show that an average of 68% of the total ethyl caprylate is in the medium after fermentation with the *S. cerevisiae* strains and 54% after the fermentation with *S. uvarum*. Nordström[2] found that the medium contained 67% of the total ethyl caprylate formed in fermentations by brewer's top yeast (*S. cerevisiae*) when caprylic acid had been added to the fermentation medium at the start of fermentation. The presence of yeast during the distillation stage had an even greater effect on the measured content of ethyl caprate. The average concentration of ethyl caprate in the medium was 0·06 ppm for the *S. cerevisiae* strains and 0·02 ppm for the *S. uvarum* yeasts when the ester contents were measured after removing the yeast cells. The values for samples distilled with yeast present rose to 0·37 ppm and 0·28 ppm, respectively, so that ethyl caprate became the major ester component in the samples distilled with yeast. There was no significant difference between the average contents of the total ethyl caprate in samples fermented by the wine and brewer's yeasts, although the media alone contained more ethyl caprate when the fermentations had been performed by the wine yeasts than by the brewer's yeasts. Of the ethyl caprate produced by the strains of *S. cerevisiae* and *S. uvarum*, an average of 17% and 8%, respectively, appeared in the medium. Nordström[2] has estimated that the medium contained about 5% of the total ethyl caprate formed during fermentations by brewer's top yeast (*S. cerevisiae*) when capric acid had been added to the fermentation medium.

Ethyl laurate, which could not be detected in the samples prepared from the fermentation media alone, assumed average concentrations of 0·18 ppm and 0·05 ppm in, respectively, fermentations by *S. cerevisiae* and *S. uvarum* when the steam distillations were performed in the presence of the yeast cells. This considerable difference in the amounts produced by the two species was, however, not statistically significant because

TABLE I. Means (ppm) with 95% Confidence Limits and Maximum and Minimum Values for Some of the Esters Formed during Sugar Fermentations by *Saccharomyces cerevisiae* Yeasts and Steam Distilled from the Mixture without (A) and with (B) the Yeast Cells

Ester	A Distilled without yeast n = 10			B Distilled with yeast n = 10			Significance of the difference between A and B[a]
	$\bar{X}$	X_{max}	X_{min}	$\bar{X}$	X_{max}	X_{min}	
Isoamyl acetate	0·26 ± 0·12	0·52	0·10	0·32 ± 0·14	0·65	0·08	—
Ethyl caproate	0·11 ± 0·03	0·22	0·06	0·12 ± 0·05	0·29	0·03	—
Ethyl caprylate	0·12 ± 0·02	0·16	0·08	0·17 ± 0·03	0·26	0·11	+++
Ethyl caprate	0·06 ± 0·02	0·13	0·02	0·37 ± 0·15	0·80	0·13	+++
Phenethyl acetate	0·04 ± 0·01	0·08	0·02	0·04 ± 0·01	0·07	0·02	—
Ethyl laurate	none detected			0·18 ± 0·12	0·51	0·01	++

[a] Calculated from original data by matched pair *t*-test.
+++ Very significant, $P < 0.001$; ++ significant, $P < 0.01$; — not significant, $P > 0.05$.

TABLE II. Means (ppm) with 95% Confidence Limits and Maximum and Minimum Values for Some of the Esters Formed during Sugar Fermentations by *Saccharomyces uvarum* Yeasts and Steam Distilled from the Mixture without (A) and with (B) the Yeast Cells

Ester	A Distilled without yeast n = 6			B Distilled with yeast n = 6			Significance of the difference between A and B[a]
	$\bar{X}$	X_{max}	X_{min}	$\bar{X}$	X_{max}	X_{min}	
Isoamyl acetate	0·10 ± 0·02	0·12	0·08	0·09 ± 0·02	0·10	0·06	—
Ethyl caproate	0·03 ± 0·01	0·04	0·02	0·03 ± 0·01	0·04	0·01	—
Ethyl caprylate	0·07 ± 0·01	0·08	0·05	0·12 ± 0·02	0·15	0·09	+++
Ethyl caprate	0·02 ± 0·01	0·03	0·02	0·28 ± 0·05	0·38	0·23	+++
Phenethyl acetate	0·04 ± 0·01	0·04	0·03	0·04 ± 0·02	0·06	0·02	—
Ethyl laurate	none detected			0·05 ± 0·02	0·08	0·03	+++

[a] Calculated from original data by matched pair *t*-test.
+++ Very significant, $P < 0.001$; — not significant, $P > 0.05$.

of the wide dispersion in the concentrations produced by strains of *S. cerevisiae*.

The results presented above clearly show that the distribution of the fatty acid esters produced during fermentation between the yeast cells and the fermentation medium is dependent on the chain length of the acid part of the esters. Both of the acetates, isoamyl acetate and phenethyl acetate, and the ethyl caproate appear solely in the medium. Ethyl caprylate and ethyl caprate are present in both the medium and the cells, whereas the ethyl laurate is present only in the cells. It also seems that the distribution of esters between medium and cells is to some extent dependent on the yeast species used. The *S. uvarum* strains appear to retain in the cells relatively more of the total amount of ethyl caprylate and ethyl caprate formed during fermentation than do the *S. cerevisiae* strains.

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