Differentiation between Yeast Species, and Strains within a Species, by Cellular Fatty Acid Analysis. 2. *Saccharomyces cerevisiae**

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Fatty acid extracts of 50 *Saccharomyces cerevisiae* strains, grown under rigidly standardised conditions, were subjected to capillary gas chromatographic analysis on a polar column. Strains contained saturated, mono-unsaturated and trace amounts of dienoic fatty acids. The mean relative percentages of 10 fatty acids were used to differentiate between the strains studied. Forty-six strains could be differentiated from all others in the group, based on the criterion that when comparing two strains the CFAP's were considered unique if the MRP's of at least one fatty acid differed at the 1% level. Holman's Index of Relationship proved to be a useful tool for indicating degree of similarity between fatty acid profiles.

Efforts to distinguish between the species *Sacch. cerevisiae* and *Sacch. bayanus* were not successful. More analyses on authentic strains (determined by DNA homology) are necessary to confirm whether such a separation is possible or not.

Oenologically important changes that occurred in a commercial dried yeast during production were reflected in the fatty acid profiles of the dried products. Index of Relationship between the fatty acid profiles of five other dried yeast products, and the mother cultures from which they were produced, was very high indicating no change during the commercial production phase. Changes, or lack of change, were confirmed by fermentation studies.

South African wine-makers rely almost exclusively on selected strains of *Saccharomyces cerevisiae,* in the active dried form, to convert must to wine. Over the last decade the local industry has experienced recurrent stuck/lagging fermentation problems resulting in serious cellar capacity problems during the harvest as well as financial losses due to reduction in wine quality. A number of factors contributing to this problem have been identified to date. These include a too low level of assimilable nitrogen (Vos, Zeeman & Heymann, 1978), insufficient levels of grape solids (Groat & Ough, 1978; Houtman & Du Plessis, 1981), a too low fermentation temperature (Tromp, 1984) and the action of killer yeasts (Tredoux, Tracey & Tromp, 1986; Van Vuuren & Wingfield, 1986).

The inability of classical taxonomic techniques to characterise yeast strains hampers the resolution of the stuck fermentation problem as it is never clear whether the yeast strain inoculated in a particular must is the same as the dominant yeast present upon completion of fermentation. Against this background the development of a yeast strain characterisation technique was identified as a research priority by leaders of the local wine industry.

Augustyn & Kock (1989) indicated that it was possible to differentiate between 13 strains of *Sacch. cerevisiae* by means of cellular fatty acid analysis (CFAA). This paper explores the utility of the technique when a large number of strains have to be differentiated. The technique was also applied to fatty acid extracts from six dried yeast preparations, and the respective mother cultures from which they were prepared, in an effort to detect possible changes induced by the commercial production and drying processes.

MATERIALS AND METHODS

Organisms studied

The fifty *Sacch. cerevisiae* strains used in this study are listed in Table 1. Stock cultures were maintained on YM agar slants. In addition the mother cultures of six commercial strains, viz. N6 (WE 14), N66 (WE 372), N76 (228), N93 (WE 500), N95 and N96 (maintained under liquid N_2 at the VORI) were used to compare their fatty acid profiles with the profiles of the corresponding dried yeast products.

Cultivation of organisms

Organisms were cultivated according to the method described by Augustyn & Kock (1989). Culture medium consisted of 6,7 g/l yeast nitrogen base (Difco) and 80 g/l glucose. Organisms were cultivated on a rotary shaker at 30°C in conical flasks equipped with a side-arm to facilitate direct reading in a Klett apparatus equipped with a 640 nm filter. Cultivation proceeded in two stages. The preculture consisted of 40 ml culture medium (250 ml flask) and organisms were cultivated for 16 h (Klett 190 – 200 minimum). Slow-growing organisms were maintained in the preculture until the required Klett reading was reached. Some organisms failed to reach the required Klett reading and these were harvested in the stationary phase. For the final culture, 10 ml of the preculture was added to 300 ml culture medium in a 1 litre flask. Cultivation then proceeded for two days or until the organism reached stationary phase in the case of very slow growers. Cells were harvested by centrifugation at 8000 g for 15 min at 4°C and the sediment washed once with cold saline so-

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TABLE 1. *Saccharomyces cerevisiae* strains studied

VORI Collection no.	ADDITIONAL INFORMATION
N1	—
N2	Elsenburg N
N3	Elsenburg Q
N4	K 35
N5	D.48(a) 1
N7	K.W.V. 19(b)
N8	K.W.V. white
N9	Champ. Hautvilliers
N10	N-27a
N11	N-33
N12	N-58a
N13	K-13
N14	K-14
N16	K.W.V. 14
N17	K.W.V. 28
N18	K-19a
N19	D 48 cl
N23	N-3
N29	K.W.V. 60
N71	Epernay; *Sacch. uvarum**
N72	C.B.S 435; ex Sake-moto
N73	CBS 1598; *Sacch. cerevisiae*
N77	D.G.I. 243; Danish red wine – tannin resistant. *Sacch. cerevisiae**
N78	D.G.I. 259; desert wine 73% sugar tolerant
N81	STV 142; INRA, Dijon, France
N83	S 47c
N85	S 46c
N86	STV 142; INRA, Dijon, France, May 1982
N87	Eg 16; INRA, Colmar, France. May 1982
N88	Eg 8; INRA, Colmar, France. May 1982
N89	OC # 2 ex Goto; Yamanishi Univ. Kikkoman
N90	W - 3 ex Goto; Yamanishi Univ, Kikkoman
N92	Red Star Champagne
N97	Wädenswil 27
N98	71B; INRA, Narbonne, France
N100	SAF OENOS
N101	SB - 1 (Lalvin 1119); *Sacch. bayanus,* INRA, Montpellier, start stuck fermentation.
N102	CBS 1171; *Sacch. cerevisiae** (Type)
N103	CBS 380; *Sacch. bayanus* (Type)
N104	NCYC # 738
N105	NCYC # 1006
N110	CSIR - Y2
N111	CSIR - Y31
N117	CBS 380; *Sacch. bayanus* (Type)
N118	CBS 400; *Sacch. chevalieri*
N131	CBS 4054; *Sacch. aceti**
N161	CSIR - Y106
N180	SIHA 1, *Sacch. cerevisiae**
N234	CBS 5155; *Sacch. prostoserdovii*
N237	CBS 6413; Sake yeast

*Confirmed to be *Sacch. cerevisiae* by J.P. van der Walt (CSIR, Division of Food Science and Technology, Pretoria, Republic of South Africa).

lution. After centrifugation the recovered cells were lyophilised and stored in glass bottles in a dessicator at -8°C to -10°C.

In a modification of the cultivation method described above, fresh slants were prepared from the stock cultures four days before strains were inoculated into the preculture. These fresh slants were maintained at 30°C. The effect of this modification on the growth and fatty acid mean relative percentages was monitored for a number of strains. Duplicate cultivations of four strains were made 30 days after initial cultivation to determine repeatability of the method over time.

Commercial dried yeasts were rehydrated in water at 37°C and then streaked out onto malt extract agar. After incubation at 30°C, single uniform colonies were streaked out on YM agar slants. These slants were used as above to prepare material for fatty acid analysis. Rehydrated dried yeasts, and yeasts from the respective mother cultures of N93 and N96, were also inoculated into Chenin blanc and Morio Muscat musts. Fermentation rate was monitored by measuring CO_2 loss.

Sporulation of some commercial yeast strains

Strains N6, N93, N95 and N96 (from the respective mother cultures) were inoculated into a pre-sporulation medium consisting of 50% must/water. After 48 h at 25°C a light inoculum was streaked out onto McLeary's Acetate medium and the slants incubated at 20 – 25°C. Some strains needed up to 30 days to reach a high percentage sporulated cells. The whole sporulated mass was transferred to the standard preculture medium to simulate the fate of sporulated material that possibly appeared during the commercial preparation of dried yeast and cultivation proceeded as above. A second preculture was prepared from the first in order to give the yeasts more time to stabilise their fatty acid profiles and cultivation proceeded as described.

Fatty acid extraction and preparation of methyl esters

Esters were prepared by the method of Augustyn & Kock (1989). Briefly; dried cells (0,120 g) were saponified with 2,5% KOH in 50% methanol/water. Non-saponifiable material was extracted with 1:4 chloroform/hexane and the aqueous layer acidified to pH 2. Free acids were extracted with 1:4 chloroform/hexane, the solvent evaporated under N_2 and the acids esterified with 20% boron trifluoride-methanol. The methyl esters were extracted with 1:4 chloroform/hexane and the extracts dried over anhydrous $MgSO_4$ prior to concentration and analysis.

Possible losses of fatty acid material during the first extraction step (removal of non-saponifiable material) were examined by excluding this step in the preparation of esters from a number of organisms. Fatty acid profiles from these extracts were then compared with profiles generated from extracts prepared by the original technique.

Gas chromatography and mass spectrometry

Fatty acids extracts were analysed by gas chromatography on a J & W DB wax capillary column (30 m x 0,32 mm I.D., coating 0,15 micron) and identified by analysis of methyl and picolinyl esters (Augustyn & Kock, 1989).

Throughout this paper fatty acids were named according to the delta system in which the carboxyl carbon atom is designated number one. Abbreviated fatty acid notation, eg. C 16:1(9), designates; number of carbon atoms, number of double bonds (position of double bond[s]).

Differentiation between organisms by comparison of fatty acid relative percentages

The relative percentages of 10 fatty acids, viz. C14:0, C14:1(9), C15:0, C15:1(9), C16:0, C16:1(9), C16:1(11), C18:0, C18:1(9) and C18:1(11), were used to differentiate between the strains studied. Raw data (quadruplicates) were subjected to a two-way analysis of variance and mean relative percentages differing at

the 1% level used to differentiate between strains.

The relatedness of any two strains was computed by applying the formula developed by Holman (1978):

$$Rx,y = \left(\frac{Cx}{Cy}\right)_1\left(\frac{Cx + Cy}{200}\right)_1 + \cdots\cdots + \left(\frac{Cx}{Cy}\right)_n\left(\frac{Cx + Cy}{200}\right)_n$$

In this formula R represents the Index of Relationship, x and y are the two strains being compared, C is the concentration expressed as a relative percentage and 1 through n are the fatty acids used to differentiate between the strains (n = 10 in this instance). It is important to note that $\frac{Cx}{Cy}$ represents the *minor* ration of the particular fatty acid in the two strains.

RESULTS AND DISCUSSION

Fatty acids of *Saccharomyces cerevisiae*

For the purposes of this general discussion the epithet *Sacch. cerevisiae* was applied as in Yarrow (1984). Fatty acids containing more than 18 carbon atoms were not considered in this study. The fourteen fatty acids identified in this study, viz. C12:0, C14:0, C14:1(9), C15:0, C15:1(9), C16:0, C16:1(9), C16:1(11), C17:0, C17:1(9), C17:1(11), C18:0, C18:1(9) and C18:1(11), corresponded to those identified by Augustyn & Kock (1989). Some strains also contained C17:1(8) tentatively identified by these authors.

Fragmentary mass spectral data indicated the presence of trace amounts of dienoic fatty acids (C16:2 and/or C18:2) in some of the strains. No trace of polyunsaturated fatty acids was detected in any of the strains. Three of the four cultures of strain N131 contained a larger amount of C18:2 (retention time, methyl ester mass spectrum: M^+ 294, base peak m/z 67). The absence of C18:2 in the fourth sample coupled to the large variation in the relative percentage C18:2 recorded in the other three cultures (0,199%, 0,315% and 0,542%), cast doubt on the origin of this C18:2. Strain N131 was examined by J.P. van der Walt (CSIR, Division of Food Science and Technology, Pretoria, Republic of South Africa) and subjected to standard taxonomic tests. It was determined as a strain of *Sacch. aceti* Santa Maria (= *Sacch. cerevisiae* sensu Yarrow). This strain will be re-examined and other authentic strains of *Sacch. aceti* acquired to clarify the position regarding the presence or absence of significant amounts of C18:2 in these strains. Baraud, Maurice & Napias (1970) reported the presence of, amongst others, C12:2, C16:3 and C18:3 in the cellular fatty acid profile (CFAP) of *Sacch. cerevisiae* strain S_2 (Springer), while Bulder & Reinink (1974) reported C18:2 and C18:3 in a number of *Sacch. cerevisiae* strains including strain CBS 380. Malfeito – Ferreira, Fonseca & Loureiro (1987) distinguished between *Sacch. cerevisiae* (strains IGC 4072 & IGC 4074) and *Torulaspora delbrueckii* (strain IGC 4269) by means of the lower relative percentage C16:1 and higher relative percentage C18:2 in the latter organism. On the other hand Kock, Cottrell & Lategan (1986), Tredoux, Kock & Lategan (1987) and Augustyn & Kock (1989) found no trace of dienoic fatty acids in various strains of *Sacch. cerevisiae*. Reasons for these discrepant results are not apparent.

Fatty acid relative percentages

The mean relative percentages (MRP's) for the 10 fatty acids used for strain differentiation are listed in Table 2. Maximum and minimum values for each fatty acid are printed in bold type. It is clear that these relative percentages vary widely. These variations were of the same order as those reported by Ribes *et al.* (1988) for 6 fatty acids in 18 *Sacch. cerevisiae* strains. *Saccharomyces cerevisiae* as defined by Yarrow (1984) is a heterogeneous species and it is extremely unlikely that analysis of the fatty acid profiles of a limited number of strains would allow the analyst to gauge accurately the variation in fatty acid MRP's occurring within the species. Kock *et al.* (1986) determined the fatty acids in four strains of *Sacch. cerevisiae* and used the MRP's to differentiate that species from others within the genus *Saccharomyces*. Applying the parameters set down in that study to the data presented in Table 2, would result in many strains not being recognised as belonging to *Sacch. cerevisiae*.

Tredoux *et al.* (1987) studied the CFAP's of 41 strains of *Sacch. cerevisiae*, many of which were also included in this study. Their results, generated on packed columns, also showed a large variation in fatty acid MRP's for the various strains, but were limited to data for six fatty acids because of the poor resolution obtained on the packed columns. A comparison of the data generated by Tredoux *et al.* (1987), and those obtained in this study, is made in Table 3. Data for C16:1(9)/C16:1(11) and C18:1(9)/C18:1(11) generated here were combined and expressed as C16:1 and C18:1 to conform with data from Tredoux *et al.* (1987). The sharper peaks generated by capillary gas chromatographic analysis resulted in more accurate integration of minor fatty acid peaks and could account for the variations noted for the MRP's of C14:0 and C14:1. Perusal of the data for individual and total C18 acids revealed differences that were not easily explained. As the same culture medium and general cultivation conditions were used in both studies, almost identical values for the MRP's of the major fatty acids should have been obtained. Augustyn & Koch (1989) pointed out that the esterification procedure used by Tredoux *et al.* (1987) did not affect complete esterification. If this incomplete esterification distorted the natural ratio of C16 and C18 acids found in the various strains and left relatively more free C16:1 acid in the extract, it could well be the cause of the elevated C18:1 MRP's reported by Tredoux *et al.* (1987).

Differentiation between strains based on fatty acid mean relative percentages

The strains studied here represent only 48 different organisms as N81/N86 and N103/N117 respectively represent two isolates of the same organisms (Table 1). Statistical analysis revealed that 46 of the strains had unique fatty acid profiles that distinguished each strain from the others (Augustyn & Kock, 1989). When comparing two strains the CFAP's were considered unique if the MRP's of at least one fatty acid differed at the 1% level. Comparisons can be made by utilising the MRP's and D-values presented in Table 2.

This strain differentiation criterion must, however, be applied with discression. If two strains were distinguished by the MRP's of one or two fatty acids that exceed the limits for similarity, as determined by the D value, by a very small margin, the differentiation must be considered doubtful. The situation could very easily

TABLE 2. Mean Relative Percentage (MRP) of 10 fatty acids in 50 strains of *Saccharomyces cerevisiae*.

Yeast Strain	Fatty acid (MRP)									
	C14:0	C14:1(9)	C15:0	C15:1(9)	C16:0	C16:1(9)	C16:1(11)	C18:0	C18:1(9)	C18:1(11)
N 1	1,264	0,618	0,180	0,183	8,315	48,091	0,407	4,959	34,322	1,160
N 2	0,929	0,367	0,182	0,188	7,880	44,306	0,338	4,951	38,656	1,442
N 3	1,201	0,611	0,228	0,220	8,272	46,865	0,425	5,545	34,343	1,776
N 4	1,241	0,813	0,121	0,199	6,378	47,733	0,444	4,568	36,486	1,519
N 5	1,944	0,515	0,309	0,117	16,440	41,825	0,195	3,041	34,074	1,223
N 7	0,687	0,256	0,173	0,209	6,038	48,766	0,464	5,249	34,564	**2,818**
N 8	1,376	1,077	0,134	0,237	6,594	47,470	0,536	5,103	36,092	0,949
N 9	0,707	0,269	0,136	0,144	8,213	46,809	0,410	4,970	35,939	1,835
N 10	1,060	0,193	0,615	0,171	16,399	37,079	0,193	2,862	39,256	1,609
N 11	1,934	0,880	0,172	0,178	11,067	49,612	0,439	3,842	30,252	1,245
N 12	1,400	0,606	0,323	0,330	10,367	50,813	0,491	4,262	29,189	1,648
N 13	1,226	1,084	0,256	0,385	5,902	53,530	**0,763**	4,550	30,147	1,685
N 14	1,031	0,488	0,180	0,209	7,191	47,580	0,458	5,552	34,929	1,796
N 16	1,842	1,031	0,158	0,222	8,552	50,382	0,441	3,858	31,390	1,528
N 17	2,153	0,746	0,245	0,205	15,691	46,491	0,174	6,441	26,336	1,046
N 18	1,192	0,370	0,151	0,122	12,380	45,525	0,389	5,016	32,884	1,442
N 19	0,799	0,581	0,211	0,328	5,833	47,352	0,614	5,102	35,942	2,208
N 23	1,111	0,536	0,181	0,195	8,961	47,220	0,375	6,531	32,812	1,614
N 29	1,657	0,757	0,187	0,204	11,332	51,615	0,518	4,001	27,820	1,367
N 71	**3,367**	0,756	0,144	0,070	**24,329**	43,636	**0,047**	3,489	**23,191**	0,612
N 72	0,413	0,413	0,234	0,321	8,298	45,404	0,404	**7,979**	32,713	2,805
N 73	0,585	0,723	0,100	0,315	**5,719**	**55,916**	0,537	3,144	29,507	2,696
N 77	0,750	0,309	0,182	0,189	6,970	36,629	0,169	6,364	**46,880**	0,770
N 78	0,643	0,257	0,264	0,238	9,056	44,265	0,394	6,194	36,282	1,557
N 81	1,305	0,187	0,377	0,124	17,750	36,845	0,142	3,052	38,209	1,619
N 83	2,181	0,550	0,343	0,158	16,317	36,388	0,151	5,200	37,374	0,934
N 85	1,056	0,352	0,173	0,162	11,198	47,243	0,332	4,319	33,054	1,703
N 86	1,118	**0,158**	0,378	0,101	18,338	36,431	0,116	3,571	38,018	1,510
N 87	1,647	0,294	0,299	0,078	20,290	40,142	0,119	3,392	32,051	1,480
N 88	1,594	0,333	0,478	0,132	17,969	37,481	0,159	3,943	36,027	1,606
N 89	0,854	0,361	0,236	0,210	8,315	42,069	0,357	6,538	38,859	1,482
N 90	1,230	0,686	0,169	0,226	7,810	43,959	0,309	5,991	37,907	1,150
N 92	1,324	0,278	0,410	0,114	18,870	37,784	0,136	4,031	35,356	1,315
N 97	1,985	1,320	0,321	0,326	9,296	44,985	0,361	5,152	34,331	1,227
N 98	1,025	0,561	0,148	0,192	7,606	45,297	0,430	5,484	36,869	1,789
N100	**0,397**	0,169	0,389	0,363	6,064	45,371	0,285	7,474	36,308	1,997
N101	0,527	0,226	0,289	0,273	7,729	42,360	0,253	5,791	39,886	1,865
N102	1,304	0,633	**0,057**	0,062	12,198	38,306	0,225	7,709	38,480	**0,578**
N103	1,608	0,351	0,476	0,186	20,496	47,163	0,070	3,394	25,119	0,879
N104	0,912	0,253	0,265	0,151	14,452	39,456	0,206	6,816	35,025	1,707
N105	0,606	0,218	0,075	0,095	9,042	37,944	0,235	5,680	44,685	1,008
N110	0,608	0,446	0,344	0,418	7,692	47,253	0,343	5,503	34,305	2,118
N111	0,661	0,395	0,415	0,488	8,666	45,699	0,276	6,447	33,837	1,936
N117	1,405	0,242	0,628	0,227	20,131	47,234	0,057	3,274	25,368	0,880
N118	1,325	0,629	0,185	0,230	9,100	48,066	0,354	4,413	33,964	1,257
N131	1,272	0,218	**0,745**	0,218	20,935	**34,517**	0,141	5,562	34,155	0,983
N161	2,925	**1,693**	0,603	**0,939**	14,473	49,080	0,592	3,066	23,863	0,948
N180	0,732	0,387	0,168	0,206	5,939	43,783	0,442	6,448	39,246	1,760
N234	1,422	0,335	0,309	0,169	17,360	42,887	0,205	**2,497**	33,348	1,139
N237	0,507	0,265	0,167	0,192	7,793	41,519	0,237	6,840	39,971	1,865
D(p≤0,01)	0,192	0,112	0,043	0,040	1,008	2,319	0,035	0,931	2,195	0,194

Values printed in bold represent the minimum and maximum values for each fatty acid

TABLE 3. Comparison between the fatty acid relative percentages generated in this study and that of Tredoux *et al.* (1987).

Yeast Strain	FATTY ACID (relative %)							
	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	Tot. C16	Tot. C18
N5 T	1,0	0,2	10,2	41,6	1,4	45,7	51,8	47,1
P	1,9	0,5	16,4	42,0	3,0	35,3	58,0	38,3
N22 T	0,4	0,07	8,1	35,8	1,7	53,8	43,9	55,5
P*	1,3	0,05	16,0	32,6	4,6	43,3	48,6	47,9
N29 T	1,2	0,6	7,7	49,4	2,5	38,6	57,1	41,1
P	1,7	0,8	11,3	52,1	4,0	29,2	63,4	33,2

T = data generated by Tredoux *et al.* (1987).
P = data generated in present study, C16 : 1(9) + C16 : 1 (11) and C18 : 1 (9) + C18 : 1 (11) combined as C16 : 1 and C18 : 1 respectively.
P* strain not included in original study (Table 1), specially cultivated for comparative purposes.

TABLE 4. Effect of the mean relative percentages (MRP's) of the major fatty acids on the Index of Relationship (R).

Yeast Strain	FATTY ACID (MRP)										$R_{x,y}$
	C14:0	C14:1(9)	C15:0	C15:1(9)	C16:0	C16:1(9)	C16:1(11)	C18:0	C18:1(9)	C18:1(11)	
N103 (x)	1,61	0,35	0,48	0,19	20,50	47,16	0,07	3,39	25,12	0,88	—
N117 (y)	1,41	0,24	0,63	0,23	20,13	47,23	0,06	3,27	25,37	0,88	0,983
N 81 (x)	1,31	0,19	0,38	0,12	17,50	36,85	0,14	3,05	38,21	1,62	—
N 86 (y)	1,12	0,16	0,38	0,10	18,34	36,43	0,12	3,57	38,02	1,51	0,977
N 1 (x)	1,26	0,62	0,18	0,18	8,32	48,09	0,41	4,96	34,32	1,16	—
N118 (y)	1,33	0,63	0,19	0,23	9,10	48,01	0,35	4,41	33,96	1,26	0,976
N 14 (x)	1,03	0,49	0,18	0,21	7,19	47,58	0,46	5,55	34,93	1,80	—
N 98 (y)	1,03	0,56	0,15	0,19	7,61	45,30	0,43	5,48	36,87	1,79	0,947
N 2 (x)	0,93	0,37	0,18	0,19	7,88	44,31	0,34	4,95	38,66	1,44	—
N 3 (y)	1,20	0,61	0,23	0,22	8,27	46,87	0,43	5,55	34,34	1,78	0,910
N 8 (y)	1,38	1,08	0,13	0,24	6,59	47,47	0,54	5,10	36,09	0,95	0,910
D($p \geq 0,01$)	0,192	0,112	0,043	0,040	1,008	2,319	0,035	0,931	2,195	0,194	

be reversed after repeated cultivation and analysis of a new set of isolates. Strains N14/N98 and N81/N86 could not be differentiated whilst strains N103/N117, representing two isolates of the type strain of *Sacch. bayanus*, were differentiated by the MRP's of two fatty acids. The variations were small and the same argument as above may be valid in this case.

Large differences between the MRP's of all fatty acids were stable (Augustyn & Kock, 1989) and could be used with confidence when differentiating between strains, even when a single fatty acid was involved.

The Index of Relationship

The Index of Relationship (R) for all possible combinations of the strains studied is presented in Addendum 1. Values for R ranged from a minimum of 0,591 [$R_{N71, N77}$] to a maximim of 0,983 [$R_{N103, N117}$]. For $R_{x,y} = 1$ strains x and y will have exactly the same MRP's for all the fatty acids used to determine the relationship.

The major fatty acids, C16:1(9) and C18:1(9), made by far the largest contribution to the value of R. A high R (>0,960) is dependent on a small difference between the respective MRP's of these acids in the strains being compared, as is illustrated by the data in Table 4. Note the $R_{N1, N118}$(0,976) is greater than $R_{N14,N98}$(0,947) although the latter strains could not be differentiated by comparing the MRP's of the 10 fatty acids used for differentiating between strains.

Some strains had the same R with a number of other strains. This did not mean that differentiation between such strains was impossible as is illustrated by the data for N2, N3 and N8 (Table 4). The minor fatty acids which contributed a minute proportion of R afforded easy differentiation between these strains (D-value – Table 4). The R values, therefore, offered an easy method for grouping yeast strains according to similarity of the respective fatty acid profiles, while final strain differentiation was afforded by considering the D-values and MRP's of all the fatty acids used to determine R.

Differentiation between *Saccharomyces cerevisiae* and *Saccharomyces bayanus* by means of cellular fatty acid analysis

Only one strain [N104] had a R greater than 0,900 with the type of *Sacch. cerevisae* [N102], while 33 strains had a R greater than 0,800 (Addendum I). Two strains [N17, N117] had a R greater than 0,900 and 15 strains a R of greater than 0,800 with the type strain of *Sacch. bayanus*. Values expressing the similarity of each strain to the group as a whole ($\frac{\varepsilon R}{n}$) are presented in Table 5. Strain N3 exhibited the highest similarity to the group as a whole and a R greater than 0,900 with 21 of the 50 strains studied (Addendum I). This fact again illustrates the necessity of analysing a large number of strains when attempting species differentiation by means of CFAA. It also indicates that the type strain does not necessarily have a fatty acid profile closely related to the majority of strains of a particular species.

Yarrow (1984) considered *Sacch. bayanus* a synonym of *Sacch. cerevisiae*. Vaughan Martini & Martini (1987), however, indicated that *Sacch. cerevisiae* should in fact be subdivided into three species, viz. *Sacch. cerevisiae, Sacch. bayanus* and *Sacch. pastoria-*

TABLE 5. Similarity of each strain to all other strains in the group

Strain	Similarity to group ($\frac{\varepsilon R}{n}$)	Strain	Similarity to group ($\frac{\varepsilon R}{n}$)
N 3	0,869	N234	0,835
N 9	0,867	N104	0,832
N 1	0,865	N 16	0,832
N118	0,864	N237	0,829
N 98	0,864	N 11	0,829
N 14	0,864	N 12	0,818
N 97	0,863	N 88	0,815
N 78	0,863	N 92	0,814
N 85	0,862	N102	0,813
N110	0,861	N 87	0,809
N 23	0,861	N 83	0,806
N 18	0,861	N 17	0,806
N111	0,860	N 81	0,800
N 8	0,858	N 29	0,800
N 90	0,857	N 86	0,798
N 4	0,857	N 10	0,797
N 2	0,856	N 13	0,794
N 19	0,852	N105	0,782
N 7	0,845	N117	0,780
N100	0,844	N103	0,779
N 89	0,844	N131	0,773
N 72	0,842	N 73	0,760
N180	0,838	N161	0,758
N 5	0,838	N 77	0,749
N101	0,837	N 71	0,730

nus. These authors also indicated that DNA homology was the only method that could distinguish between these species. No authenic strains of *Sacch. pastorianus* were included in this study and a definite statement on the fatty acid relative percentage ranges typical of *Sacch. cerevisiae* and *Sacch. bayanus* can, therefore, not be made.

Strain N234, an authentic strain of *Sacch. prostoserdovii* (CBS 5155), had a R of 0,841 with the type strain of *Sacch. bayanus* [N103] and a R of 0,816 with the type of *Sacch. cerevisiae* [N102] (Addendum I). *Saccharomyces prostoserdovii,* a synonym of *Sacch. cerevisiae* (Yarrow, 1984), exhibited a 96% DNA reassociation with the type strain of *Sacch. cerevisiae* (Vaughan Martini & Kurtzman, 1985). The R between N234 and N3 was 0,846, slightly higher than its R with *Sacch. bayanus*. Although *Sacch. bayanus* and *Sacch. cerevisiae* are genetically unrelated (Vaughan Martini & Martini, 1987), Bousfield *et al.* (1983) pointed out that there was no fundamental reason why taxonomically very different organisms should not have similar fatty acid compositions. These results, therefore, indicate either that *Sacch. cerevisiae* and *Sacch. bayanus* cannot be differentiated by CFAA or that the R manipulates the fatty acid data in such a way that the differences that do exist are not exploited. An attempt at resolving these questions will be made in future work by utilising all the strains studied in the DNA homology work of Vaughan Martini & Kurtzman (1985) and Vaughan Martini & Martini (1987) and a variety of data manipulation techniques.

Effect of technique modification and time elapsed between successive cultivations of the same strain, on the cellular fatty acid profile of selected strains of *Saccharomyces cerevisiae*

Streaking out fresh isolates from the stock cultures four days before inoculation into the preculture resulted in faster growth of many cultures. Although some slight variations were apparent amongst the minor fatty acid relative percentages, the high R-values (Table 6) indicated that the adapted cultivation method had a very small effect on the CFAP's of the yeasts examined. Reasons for the low R for N5 (old/new) were not apparent but it should be mentioned that the first cultivation (old method) was made at the onset of this study when methods were not as accurate as at the present time. All strains were subsequently cultured by the adapted method.

The potential loss of CFA's from the sample during removal of the non-saponifiable material was examined by omitting this step during esterification of fatty acids from five strains. The R between these respective profiles and the profiles of duplicate esterifications which included the step removing the non-saponifiable material, indicated that a very small amount of CFA material, if any, was lost during removal of the non-saponifiable material (Table 6). The step was, therefore, retained to obtain cleaner samples.

Effect of the commercial drying process on the fatty acid profile of six commercial yeast strains

Local commercial dried yeast products were prepared from mother cultures maintained under liquid N_2 at the VORI. For five of the six commercial products there was a high R between the mother cultures and the appropriate dried yeasts (Table 7). The low R recorded for the two batches of N93 (WE 500) resulted from pronounced changes in the MRP's of three major fatty acids, eg:

	C16:0	C16:1(9)	C18:1(9)
N93 (mother culture)	17,12%	38,12%	36,13%
N93 (dried, batch 6)	7,01%	40,11%	43,54%

Dramatic changes also occurred in the MRP's of some of the minor fatty acids but these changes would not have had a significant effect on the value of R. The reason for these changes was not apparent. It seems highly unlikely that the manufacturer would experience almost identical contamination problems during the preparation of only these two batches of dried material.

The yeast strains used by the local wine industry are all genetically unstable and sporulate very easily (I.S. Pretorius – lecture presented to the S.A.S.E.V. congress – November 1987). The effect of sporulation on the fatty acid profile of four commercial yeasts was studied by comparing the fatty acid profiles of sporulated

TABLE 6. Effect of cultivation method, time between duplicate cultivations and esterification technique on the Index of Relationship (R) for selected strains of *Saccharomyces cerevisiae*

Yeast Strain	$R_{x,y}$		
	Cultivation Method (old/new)	Time between cultivations (new method/30 days later)	Esterification non-saponifiable (in/out)
N1	0,965	0,973	0,989
N2	0,957	0,969	0,988
N5	0,822	0,931	—
N7	0,944	0,964	—
N11	—	—	0,977
N72	0,946	—	0,958
N77	0,908	—	0,983

TABLE 7. Index of Relationship (R) between mother culture and the active dried yeast produced from that culture

Saccharomyces cerevisiae strain no.	R
N6 (WE 14)*	0,954
N66 (WE 372)	0,968
N76 (228)	0,987
N93 (WE 500) Batch 6/1988	0,804
N93 (WE 500) Batch 7/1988	0,820
N95	0,972
N96	0,947

* Common names used by industry

material to those of the respective mother cultures. It is clear that for all strains the fatty acid profiles of the whole sporulated mass differed very little from the pro-

TABLE 8. Index of Relationship (R) between sporulated and non-sporulated (mother cultures) material of *Saccharomyces cerevisiae* for two cultivation procedures

Yeast Strain	Degree of Sporulation %	Cultivation Procedure: Normal $R_{Mother/sporulated}$	Cultivation Procedure: Additional Preculture $R_{Mother/sporulated}$
N6	90	0,917	0,919
N93	10	0,941	0,957
	>90	0,954	0,940
N95	5	0,952	0,919
	>90	0,956	0,942
N96	90	0,965	0,962

file of the respective mother cultures (Table 8). Sporulation was, therefore, an unlikely cause of the changes noted in the fatty acid profiles of the dried batches of N93 (Table 7).

To determine if the changes recorded in the CFAP of the dried N93 had any oenological significance, fermentation studies were conducted using mother cultures and respective dried yeasts of two strains. While the fermentation curves for mother culture and dried N96 were indistinguishable, the dried batches of N93 clearly had inferior fermentation characteristics as the fermentation showed serious lagging (data not shown).

CONCLUSIONS

The variations in fatty acid relative percentages occurring in different strains of *Sacch. cerevisiae* could be reproduced reliably. These variations made reliable differentiation between strains possible. Differentiation was based on the MRP's of 10 fatty acids. As the number of strains to be characterised rises, problems may arise concerning the differentiation between closely related strains. Incorporation of additional fatty acids into the data base should help to alleviate this problem.

Additional authentic strains of *Sacch. bayanus* and *Sacch. cerevisae* must be analysed to determine if differentiation by CFAA is possible. Strains of *Sacch. pastorianus* must be included in such a study.

While it is unlikely that all oenologically important changes to a yeast strain will be reflected in its fatty acid profile, CFAA nevertheless adds a useful additional technique to the series of tests used to monitor the quality of the dried product.

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ADDENDUM 1

Index of Relationship (R) for all possible combinations of the strains studied.

	N1	N2	N3	N4	N5	N7	N8	N9	N10	N11
N 1	1,000	0,904	0,970	0,943	0,848	0,942	0,946	0,952	0,760	0,899
N 2	—	1,000	0,910	0,914	0,835	0,877	0,910	0,932	0,833	0,818
N 3	—	—	1,000	0,934	0,850	0,934	0,939	0,964	0,769	0,878
N 4	—	—	—	1,000	0,820	0,936	0,972	0,948	0,778	0,867
N 5	—	—	—	—	1,000	0,811	0,822	0,835	0,885	0,830
N 7	—	—	—	—	—	1,000	0,935	0,929	0,739	0,870
N 8	—	—	—	—	—	—	1,000	0,952	0,767	0,864
N 9	—	—	—	—	—	—	—	1,000	0,787	0,863
N10	—	—	—	—	—	—	—	—	1,000	0,734
N11	—	—	—	—	—	—	—	—	—	1,000

ADDENDUM 1

Index of Relationship (R) for all possible combinations of the strains studied.

	N12	**N13**	**N14**	**N16**	**N17**	**N18**	**N19**	**N23**	**N29**	**N71**
N 1	0,887	0,866	0,958	0,920	0,828	0,915	0,931	0,945	0,859	0,717
N 2	0,810	0,798	0,907	0,840	0,780	0,887	0,903	0,882	0,784	0,716
N 3	0,873	0,855	0,968	0,903	0,839	0,921	0,937	0,957	0,840	0,719
N 4	0,856	0,869	0,953	0,888	0,799	0,883	0,958	0,911	0,830	0,700
N 5	0,795	0,751	0,831	0,813	0,844	0,885	0,813	0,836	0,789	0,797
N 7	0,861	0,876	0,951	0,890	0,794	0,879	0,949	0,910	0,832	0,685
N 8	0,850	0,861	0,957	0,884	0,811	0,887	0,963	0,915	0,825	0,704
N 9	0,856	0,839	0,955	0,887	0,816	0,909	0,955	0,931	0,825	0,709
N10	0,715	0,675	0,764	0,723	0,750	0,798	0,763	0,754	0,703	0,705
N11	0,949	0,895	0,870	0,948	0,861	0,896	0,846	0,894	0,942	0,758
N12	1,000	0,912	0,864	0,937	0,848	0,875	0,842	0,887	0,953	0,741
N13	—	1,000	0,861	0,911	0,790	0,830	0,861	0,857	0,894	0,689
N14	—	—	1,000	0,893	0,819	0,901	0,954	0,942	0,833	0,703
N16	—	—	—	1,000	0,823	0,879	0,866	0,913	0,916	0,727
N17	—	—	—	—	1,000	0,868	0,798	0,859	0,864	0,829
N18	—	—	—	—	—	1,000	0,881	0,930	0,862	0,765
N19	—	—	—	—	—	—	1,000	0,911	0,811	0,691
N23	—	—	—	—	—	—	—	1,000	0,855	0,724
N29	—	—	—	—	—	—	—	—	1,000	0,757
N71	—	—	—	—	—	—	—	—	—	1,000
	N72	**N73**	**N77**	**N78**	**N81**	**N83**	**N85**	**N86**	**N87**	**N88**
N 1	0,905	0,820	0,748	0,907	0,764	0,787	0,934	0,762	0,792	0,792
N 2	0,880	0,756	0,817	0,940	0,825	0,827	0,875	0,823	0,786	0,814
N 3	0,925	0,809	0,758	0,926	0,771	0,791	0,937	0,769	0,797	0,799
N 4	0,869	0,820	0,766	0,910	0,783	0,794	0,909	0,782	0,772	0,804
N 5	0,825	0,729	0,728	0,852	0,886	0,889	0,865	0,874	0,915	0,904
N 7	0,893	0,851	0,744	0,886	0,740	0,760	0,902	0,739	0,763	0,768
N 8	0,876	0,811	0,773	0,912	0,772	0,801	0,904	0,770	0,769	0,801
N 9	0,909	0,801	0,771	0,941	0,788	0,803	0,925	0,787	0,786	0,817
N10	0,740	0,654	0,813	0,808	0,963	0,932	0,785	0,948	0,851	0,929
N11	0,884	0,857	0,673	0,834	0,736	0,750	0,925	0,735	0,806	0,766
N12	0,842	0,878	0,664	0,828	0,718	0,725	0,906	0,715	0,780	0,746
N13	0,823	0,931	0,667	0,797	0,678	0,686	0,854	0,677	0,741	0,704
N14	0,903	0,819	0,769	0,916	0,764	0,784	0,926	0,764	0,780	0,793
N16	0,874	0,870	0,690	0,851	0,726	0,733	0,907	0,725	0,798	0,757
N17	0,826	0,759	0,666	0,812	0,748	0,790	0,853	0,743	0,808	0,775
N18	0,913	0,784	0,728	0,901	0,799	0,818	0,954	0,798	0,852	0,826
N19	0,884	0,827	0,761	0,909	0,765	0,786	0,904	0,764	0,763	0,794
N23	0,937	0,813	0,748	0,920	0,754	0,771	0,951	0,752	0,807	0,780
N29	0,809	0,867	0,642	0,797	0,705	0,715	0,888	0,703	0,771	0,736
N71	0,718	0,677	0,596	0,724	0,725	0,717	0,746	0,731	0,825	0,748
N72	1,000	0,803	0,745	0,908	0,740	0,756	0,902	0,738	0,798	0,766
N73	–	1,000	0,635	0,760	0,656	0,646	0,811	0,649	0,714	0,669
N77	–	–	1,000	0,798	0,800	0,814	0,719	0,800	0,710	0,779
N78	–	–	–	1,000	0,808	0,825	0,891	0,807	0,801	0,832
N81	–	–	–	–	1,000	0,937	0,784	0,977	0,875	0,954
N83	–	–	–	–	–	1,000	0,789	0,941	0,849	0,932
N85	–	–	–	–	–	–	1,000	0,783	0,832	0,813
N86	–	–	–	–	–	–	–	1,000	0,880	0,953
N87	–	–	–	–	–	..	–	–	1,000	0,903
N88	–	–	–	–	–	–	–	–	–	1,000

ADDENDUM 1

Index of Relationship (R) for all possible combinations of the strains studied.

	N89	**N90**	**N92**	**N97**	**N98**	**N100**	**M101**	**M102**	**N103**	**N104**
N 1	0,871	0,904	0,800	0,938	0,922	0,886	0,858	0,805	0,797	0,831
N 2	0,949	0,964	0,807	0,912	0,951	0,907	0,941	0,864	0,741	0,839
N 3	0,888	0,916	0,803	0,944	0,944	0,908	0,881	0,814	0,794	0,850
N 4	0,875	0,913	0,796	0,900	0,940	0,922	0,872	0,810	0,776	0,815
N 5	0,847	0,842	0,905	0,880	0,836	0,814	0,832	0,838	0,819	0,902
N 7	0,844	0,873	0,773	0,897	0,907	0,907	0,850	0,772	0,764	0,816
N 8	0,872	0,915	0,795	0,916	0,938	0,920	0,871	0,814	0,781	0,819
N 9	0,898	0,920	0,810	0,925	0,953	0,925	0,895	0,819	0,782	0,846
N 10	0,847	0,818	0,910	0,780	0,803	0,773	0,841	0,889	0,735	0,878
N 11	0,788	0,818	0,767	0,877	0,836	0,806	0,776	0,767	0,835	0,787
N 12	0,781	0,806	0,747	0,858	0,830	0,801	0,773	0,746	0,824	0,772
N 13	0,762	0,796	0,707	0,826	0,822	0,817	0,764	0,700	0,765	0,734
N 14	0,878	0,910	0,796	0,919	0,947	0,916	0,880	0,804	0,783	0,845
N 16	0,812	0,837	0,755	0,886	0,859	0,827	0,800	0,747	0,803	0,775
N 17	0,772	0,798	0,775	0,835	0,805	0,794	0,755	0,762	0,900	0,819
N 18	0,853	0,879	0,831	0,929	0,904	0,874	0,841	0,841	0,820	0,873
N 19	0,868	0,899	0,787	0,900	0,934	0,932	0,871	0,797	0,768	0,822
N 23	0,877	0,892	0,782	0,924	0,912	0,893	0,853	0,811	0,806	0,847
N 29	0,754	0,781	0,736	0,832	0,800	0,769	0,743	0,737	0,839	0,756
N 71	0,695	0,723	0,762	0,746	0,714	0,690	0,690	0,684	0,887	0,723
N 72	0,874	0,886	0,769	0,911	0,908	0,919	0,859	0,813	0,765	0,837
N 73	0,724	0,753	0,671	0,777	0,781	0,784	0,730	0,664	0,749	0,695
N 77	0,845	0,822	0,770	0,755	0,797	0,783	0,849	0,837	0,610	0,791
N 78	0,931	0,948	0,823	0,934	0,948	0,932	0,917	0,858	0,751	0,878
N 81	0,834	0,822	0,936	0,784	0,803	0,775	0,822	0,886	0,756	0,875
N 83	0,836	0,837	0,915	0,819	0,824	0,787	0,823	0,895	0,737	0,892
N 85	0,843	0,865	0,815	0,915	0,895	0,866	0,834	0,814	0,831	0,850
N 86	0,831	0,820	0,943	0,782	0,802	0,773	0,817	0,878	0,761	0,871
N 87	0,796	0,786	0,917	0,818	0,784	0,765	0,783	0,810	0,860	0,875
N 88	0,822	0,814	0,971	0,816	0,816	0,796	0,809	0,872	0,782	0,902
N 89	1,000	0,946	0,814	0,882	0,919	0,893	0,958	0,896	0,710	0,874
N 90	–	1,000	0,810	0,914	0,954	0,915	0,940	0,877	0,739	0,851
N 92	–	–	1,000	0,820	0,807	0,787	0,801	0,867	0,795	0,904
N 97	–	–	–	1,000	0,927	0,897	0,872	0,827	0,782	0,856
N 98	–	–	–	–	1,000	0,941	0,921	0,844	0,756	0,849
N100	–	–	–	–	–	1,000	0,892	0,835	0,737	0,846
N101	–	–	–	–	–	–	1,000	0,868	0,703	0,851
N102	–	–	–	–	–	–	–	1,000	0,697	0,905
N103	–	–	–	–	–	–	–	–	1,000	0,747
N104	–	–	–	–	–	–	–	–	–	1,000

ADDENDUM 1

Index of Relationship (R) for all possible combinations of the strains studied.

	N105	**N110**	**N111**	**N117**	**N118**	**N131**	**N161**	**N180**	**N234**	**N237**
N 1	0,788	0,956	0,929	0,799	0,976	0,769	0,796	0,860	0,845	0,844
N 2	0,851	0,903	0,899	0,741	0,889	0,752	0,721	0,944	0,834	0,927
N 3	0,798	0,970	0,951	0,795	0,954	0,779	0,776	0,882	0,846	0,865
N 4	0,785	0,929	0,894	0,778	0,936	0,737	0,768	0,898	0,817	0,857
N 5	0,773	0,837	0,849	0,819	0,858	0,851	0,789	0,812	0,958	0,828
N 7	0,762	0,944	0,906	0,767	0,928	0,742	0,773	0,878	0,809	0,834
N 8	0,791	0,936	0,899	0,785	0,932	0,749	0,773	0,892	0,821	0,855
N 9	0,811	0,953	0,935	0,784	0,936	0,755	0,763	0,896	0,832	0,881
N 10	0,845	0,756	0,766	0,741	0,766	0,848	0,705	0,823	0,863	0,843
N 11	0,720	0,867	0,856	0,834	0,913	0,719	0,864	0,782	0,830	0,764
N 12	0,711	0,863	0,852	0,827	0,903	0,702	0,846	0,776	0,804	0,758
N 13	0,683	0,853	0,828	0,768	0,867	0,672	0,786	0,794	0,760	0,748
N 14	0,789	0,967	0,930	0,785	0,943	0,762	0,771	0,891	0,828	0,864
N 16	0,732	0,891	0,880	0,802	0,925	0,709	0,827	0,803	0,815	0,787
N 17	0,696	0,822	0,839	0,900	0,831	0,749	0,879	0,771	0,844	0,750
N 18	0,777	0,904	0,922	0,821	0,919	0,797	0,808	0,849	0,896	0,827
N 19	0,779	0,945	0,906	0,770	0,918	0,737	0,754	0,902	0,810	0,854
N 23	0,787	0,944	0,951	0,808	0,950	0,760	0,789	0,868	0,847	0,852
N 29	0,687	0,828	0,817	0,838	0,874	0,687	0,862	0,748	0,792	0,730
N 71	0,632	0,706	0,720	0,876	0,727	0,744	0,824	0,697	0,811	0,677
N 72	0,775	0,919	0,944	0,766	0,898	0,744	0,748	0,867	0,837	0,860
N 73	0,652	0,819	0,793	0,754	0,821	0,631	0,770	0,758	0,735	0,717
N 77	0,929	0,757	0,766	0,611	0,734	0,748	0,591	0,835	0,711	0,859
N 78	0,845	0,914	0,937	0,753	0,907	0,776	0,735	0,924	0,851	0,906
N 81	0,826	0,757	0,765	0,761	0,770	0,873	0,701	0,804	0,878	0,823
N 83	0,836	0,778	0,782	0,736	0,783	0,885	0,710	0,804	0,857	0,822
N 85	0,767	0,929	0,919	0,833	0,949	0,771	0,816	0,840	0,877	0,821
N 86	0,822	0,756	0,764	0,763	0,768	0,886	0,690	0,799	0,866	0,817
N 87	0,753	0,785	0,801	0,857	0,804	0,884	0,752	0,764	0,916	0,785
N 88	0,816	0,786	0,794	0,780	0,799	0,892	0,715	0,792	0,895	0,810
N 89	0,875	0,873	0,894	0,711	0,858	0,766	0,692	0,945	0,831	0,960
N 90	0,851	0,903	0,906	0,740	0,889	0,767	0,721	0,944	0,841	0,927
N 92	0,813	0,789	0,796	0,797	0,807	0,909	0,713	0,782	0,897	0,802
N 97	0,809	0,928	0,937	0,780	0,939	0,788	0,778	0,873	0,869	0,855
N 98	0,826	0,936	0,928	0,756	0,907	0,761	0,738	0,926	0,833	0,905
N100	0,794	0,913	0,924	0,739	0,875	0,740	0,717	0,924	0,813	0,891
N101	0,883	0,882	0,881	0,705	0,845	0,756	0,685	0,942	0,822	0,971
N102	0,877	0,796	0,818	0,699	0,804	0,815	0,689	0,859	0,816	0,886
N103	0,647	0,787	0,776	0,983	0,807	0,775	0,877	0,711	0,841	0,690
N104	0,828	0,838	0,861	0,749	0,829	0,855	0,742	0,841	0,879	0,869
N105	1,000	0,789	0,801	0,648	0,786	0,769	0,633	0,844	0,754	0,884
N110	–	1,000	0,947	0,789	0,942	0,765	0,771	0,878	0,833	0,864
N111	–	–	1,000	0,777	0,929	0,773	0,759	0,890	0,852	0,875
N117	–	–	–	1,000	0,810	0,774	0,877	0,711	0,844	0,691
N118	–	–	–	–	1,000	0,767	0,807	0,848	0,856	0,831
N131	–	–	–	–	–	1,000	0,670	0,737	0,846	0,754
N161	–	–	–	–	–	–	1,000	0,691	0,788	0,671
N180	–	–	–	–	–	–	–	1,000	0,812	0,937
N234	–	–	–	–	–	–	–	–	1,000	0,808
N237	–	–	–	–	–	–	–	–	–	1,000