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Principal-Component Analysis of the Characteristics Desirable in Baker's Yeasts

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Twenty-seven properties considered to be required for good bakery products were examined in 56 industrial and 2 laboratory yeast strains. The data obtained were applied to principal-component analysis, one of the multivariate statistical analyses. The first and second principal components together were extracted, and these accounted for 77.7% of the variance. The first principal component was interpreted as the glycolytic activity of yeast in dough, and the second one was interpreted as the balance of leavening abilities in sweet and flour doughs from the factor loadings. The scattergram on the two principal components was effective in grouping the 58 yeast strains used.

Saccharomyces strains are generally grouped into two types: laboratory strains, which are experimental microorganisms in fundamental biology and which have contributed to recent advances in molecular biology (3), and industrial strains, which are widely used in baking, brewing, distilling, and wine-making processes (10). These industrial yeasts contain both common and distinct characteristics; those for brewing and distilling are similar but different from those for wine making or baking (16). These characteristics consist of many complex properties. As for baker's yeasts, Burrow (5) stated that the following properties were necessary for a fast dough fermentation: (i) high potential glycolytic activity; (ii) ability to adapt rapidly to changing substrates; (iii) high invertase activity; (iv) high potential maltose fermentation; and (v) ability to grow and synthesize enzymes and coenzymes under anaerobic conditions. Osmotic stability in the presence of high sugar concentrations is also desirable in Japan, since sweet goods are favored much more than in North America and Europe. To construct a new, improved yeast for baking, we should first evaluate the properties mentioned above simultaneously and quantitatively.

Multivariate statistical analyses, which are widely used in pure and applied sciences, include techniques such as principal-component analysis, cluster analysis, discriminant analysis, and regression analysis (6). Cluster analysis is a common practice in the numerical taxonomy of bacteria, actinomycetes, and yeasts (7). The group of microorganisms under study is subjected to a wide variety of tests, and the similarity of characteristics between pairs of microorganisms is expressed numerically (15). Microorganisms with a high percentage of similarity are arranged together to form taxonomic groups or clusters in a tree-like diagram or dendrogram (15). Principal-component analysis, an ordination method, has a firm mathematical basis compared with that used in cluster analysis (4). This method can condense all of the variables, with a minimum mathematical loss of information, into two or three principal components which may be used as axes on which the data can be plotted and visualized structurally (13). Furthermore, this technique enables research of the underlying relationships among variables and interpretation of the classification achieved in terms of these variables (13).

From the above-mentioned reasons, principal-component analysis seems to be a powerful technique for investigating the characteristics of industrial yeasts. In the present paper we subjected the yeast properties considered to be required for good bakery products to principal-component analysis and observed the differences between baker's yeasts, laboratory yeasts, and other industrial yeasts.

MATERIALS AND METHODS

Abbreviations. The following abbreviations are used: PC, principal component; DF (0), dough fermentation, gas production rate from dough without the addition of sugar; DFg and DFs, gas production rates from doughs containing glucose and sucrose, respectively; LFm, liquid fermentation, gas production rate from aqueous medium containing maltose and a small amount of glucose; LFg and LFs, gas production rates from aqueous media containing glucose and sucrose, respectively. The numbers in brackets following DF and LF represent the amounts (percentages) of sugars contained in doughs and aqueous media.

Organism. The types and numbers of *Saccharomyces cerevisiae* strains used were as follows: baking (Japan), 8; baking (North America and Europe), 15; wine, 10; sáke, 5; brewing, 9; shochu, 3; whiskey, 3; alcohol, 3; and laboratory, 2. The organisms were maintained in yeast extract-malt extract agar slants (18).

Cultures. Yeast cells were grown in 30 ml of YPD medium, which contained 1% yeast extract, 2% peptone, and 2% glucose, in a 300-ml Erlenmeyer flask. The broth of the YPD medium was inoculated into 270 ml of medium containing 3% sugar (as cane molasses), 0.193% urea, and 0.046% KH_2PO_4 in a 2-liter baffled Erlenmeyer flask. Both of the cultures were incubated for 24 h at 28°C on a rotary shaker (180 rpm). Cultured cells were harvested, washed twice with distilled water, and placed on a porous plate (Nippon Kagaku Togyo Co., Osaka, Japan) for 5 min to adjust the moisture to about 67%. The cells were resuspended in distilled water (66 mg of dry cells per ml) for the following experiments.

Gas-producing activity of yeasts. The leavening and liquid fermentative abilities of the yeast cells were determined at 30°C for 2 h as the volume of evolved gas from doughs and aqueous solutions, respectively (Table 1). The ingredients of the dough (10 g of flour, 5.5 ml of sugar solution [Table 1], and 1.0 ml of yeast suspension) were kept at 30°C and mixed quickly by hand for 1 min after the addition of yeast suspension. Doughs containing from 5 to 30% sucrose (based

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TABLE 1. Amounts of sugar in doughs and liquid fermentation media

Dough		Liquid fermentation medium	
Designation	Sugar content (g/5.5 ml[a])	Designation	Sugar content (g/10 ml[b])
DF[0]	None	LFm[5]	0.69, Maltose, + 0.035, glucose
DFs[5]	0.5		
DFs[10]	1.0	LFs[5]	0.69
DFs[15]	1.5	LFs[10]	1.38
DFs[20]	2.0	LFs[20]	2.76
DFs[25]	2.5	LFs[30]	4.14
DFs[30]	3.0	LFs[40]	5.52
DFg[5]	0.5	LFg[5]	0.69
DFg[10]	1.0	LFg[10]	1.38
DFg[15]	1.5	LFg[20]	2.76
DFg[20]	2.0	LFg[30]	4.14
DFg[25]	2.5		

[a] Distilled water.
[b] Medium (see text).

on the weight of flour) were prepared to correspond to various white bread and sweet goods formulations. The leavening ability of dough containing glucose was assessed to estimate the fermenting activity irrespective of extracellular invertase. With a similar purpose in mind, liquid fermentative abilities were determined by the method of Hino et al. (9) with a mixture of 2.0 ml of yeast suspension and 10 ml of medium containing, per liter, 10 mM phosphate buffer (pH 5.6), 4.6 mg of thiamine hydrochloride, 4.6 mg of pyridoxine hydrochloride, 46 mg of nicotinic acid, 2.29 g of $MgSO_4 \cdot 7H_2O$, 2.86 g of $(NH_4)_2SO_4$, 5.71 g of urea, and a defined amount of sugar (Table 1). Liquid medium containing maltose corresponded to dough without the addition of sugar and in which endogenous maltose was the major sugar.

Enzyme assays. The reaction mixture for determining extracellular invertase contained 100 mM acetate buffer (pH 4.5), 5% (wt/vol) sucrose, and the appropriate amount of intact cells in a total volume of 0.5 ml. After incubation at 30°C for 3 min, the reaction was stopped by the addition of 3,5-dinitrosalicylic acid reagent, and the reducing sugars produced were determined (2).

α-Glucosidase of yeast cells was induced in the aqueous medium used for maltose fermentation, LFm[5] (Table 1). Samples (2 ml) were removed from the medium after 0, 1, 2, 3, and 4 h of incubation. Washed cells were permeabilized by freezing and thawing (11) and used for the assay of α-glucosidase. The activity was determined at 30°C as the hydrolysis of *p*-nitrophenyl-α-D-glucopyranoside by the method of Operti et al. (11). Specific activities of both enzymes were expressed as nanomoles per minute per milligram of cells.

PC analysis. The PC (Z_i) was computed from the following equation (1): $Z_i = a_{i1}x_1 + a_{i2}x_2 + \ldots + a_{ik}x_k + \ldots + a_{im}x_m$, where x_k refers to the k^{th} value of the datum obtained, and the variance in Z_i ($i = 1, 2, 3 \ldots p$) and a_{ik} ($i = 1, 2 \ldots p$, $k = 1, 2 \ldots m$) refers to the eigen value and the eigen vector, respectively. First, a_{1k} values are computed on the basis of a correlation matrix among the data obtained to maximize the variance in Z_1 among all PCs. Then, a_{2k} is calculated to maximize the variance in Z_2 except for Z_1. The computation can be continued until $(m - 1)^{th}$ PC is calculated, but this analysis is usually stopped before the eigen value becomes less than 1.0. Factor loading refers to the coefficient of correlation between x_i and Z_i. Calculation was conducted by the multivariate analysis program (IBC Co. Ltd., Miyazaki, Japan) with the NEC personal computer PC9801.

RESULTS AND DISCUSSION

Twenty-seven properties considered to be required for good bakery products, such as gas-producing activities, enzyme activities, and cell yield, were assayed in 58 yeast strains. Since a good practice in PC analysis is to ensure that the number of variables is less than 25% of the samples (6), the data measured were limited by the following procedure. Four groups of gas-producing activities (DFs, DFg, LFs, and LFg; Table 1) and α-glucosidase (at 0, 1, 2, 3, and 4 h) were subjected to PC analysis. The eigen values of the first PCs extracted from DFg, LFs, LFg, and α-glucosidase surpassed 1.0 and explained 79.4% of the variance in DFg, 75.2% of that in LFs, 88.7% of that in LFg, and 93.1% of that in α-glucosidase. The eigen values of the first and second PCs extracted from DFs surpassed 1.0 and explained 96.7% of the variance. Thus, DFs[15], DFs[30], DFg[15], LFs[20], LFg[20], and α-glucosidase (2 h) were selected as the DFs, DFg, LFs, LFg, and α-glucosidase variables with the highest factor loading for the respective PCs. The correlation coefficient matrix was calculated for these selected variables (Table 2). Significant correlations were observed among gas-producing activities, except for between DFs[30] and LFg[20] and between DFs[30] and LFs[20]. The negative correlation observed between invertase activity and DFs[30] supports the results of a previous report (14). A higher extracellular invertase activity might reduce the fermentative ability of yeast cells, since the hydrolysis of sucrose into glucose and fructose doubles the osmotic pressure around the cells (14). In dough without sugar yeast fermentation depends mainly on maltose, which is derived from the starch in the flour by the action of endogenous β-amylase (11). The leavening ability of the flour dough was expected to correlate closely with both LFm and α-glucosidase, but there have been controversial findings (8). In the present experiment DF[0], LFm[5], and α-glucosidase activity correlated significantly with each other.

The PCs of which the eigen values surpassed 1.0 were the first and second ones, and these PCs accounted for 77.7% of the variance of the data. The factor loadings for each of the 10 variables for the first and second PCs were plotted to investigate the significance of each PC (Fig. 1). The first PC, with which all of the yeast activities correlated except for DFs[30], could be interpreted as the glycolytic activity of

TABLE 2. Properties applied to PC analysis

Parameter	DFs[15] (ml/2 h)	DFs[30] (ml/2 h)	DFg[15] (ml/2 h)	DF[0] (ml/2 h)	LFs[20] (ml/2 h)	LFg[15] (ml/2 h)	LFm[5] (ml/ 2 h)	α-Glucosidase (nmol/min per mg of cells)	Invertase (nmol/min per mg of cells)	Cell yield (g [dry matter])
Avg	30.2	3.3	21.6	32.7	29.6	25.5	15.6	226	6,365	2.53
Minimum	8.0	0	3.5	6.0	6.5	5.0	0	0.8	254	1.23
Maximum	45.1	10.1	32.5	53.0	53.0	47.0	50.5	773	31,200	3.03

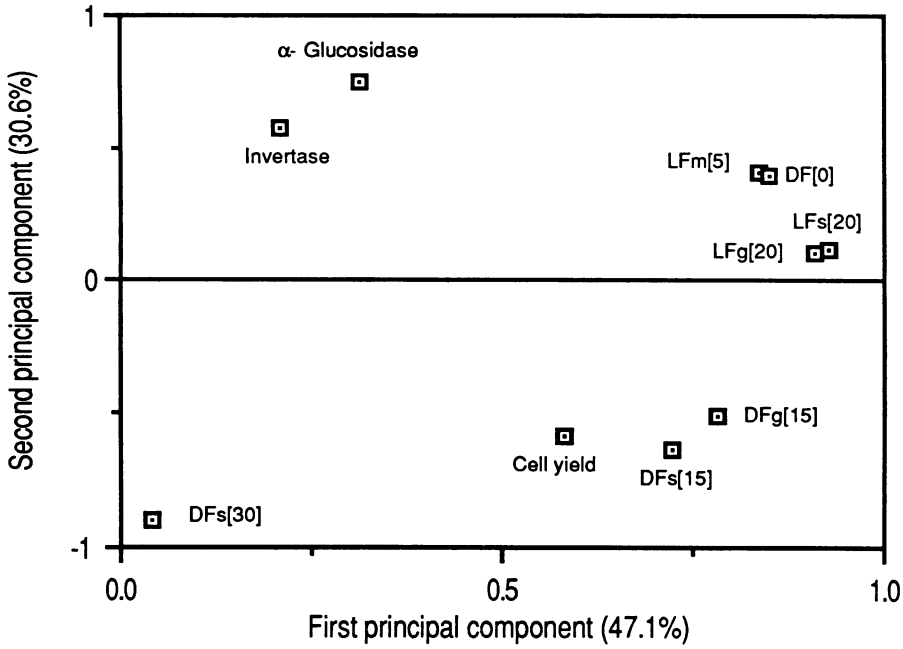

FIG. 1. Scattergram of factor loadings for the first and second PCs.

yeast in dough. DFs[30] and α-glucosidase correlated negatively and positively with the second PC, respectively, suggesting that this PC is related to the balance of leavening abilities in sweet and flour doughs.

The scattergram of 58 yeast strains for the first and second PCs is shown in Fig. 2. The first and second PCs accounted for 47.1 and 30.6% of the variance, respectively, and were effective in grouping the 58 yeast strains. When PC analysis was conducted on all the data except those for α-glucosidase, invertase, and cell yield, some groups overlapped and could not be distinguished from each other (data not shown). The leavening abilities of doughs containing sucrose and the α-glucosidase activities of four representative strains (FSC6001, a Japanese baking strain [no. 1 in Fig. 2]; FSC6014, a European baking strain [no. 12]; BK1111, a brewing strain [no. 43]; and X2180-1A, a laboratory strain [no. 57]) are shown in Fig. 3 and 4.

All of the baker's yeasts were found on the right half of the

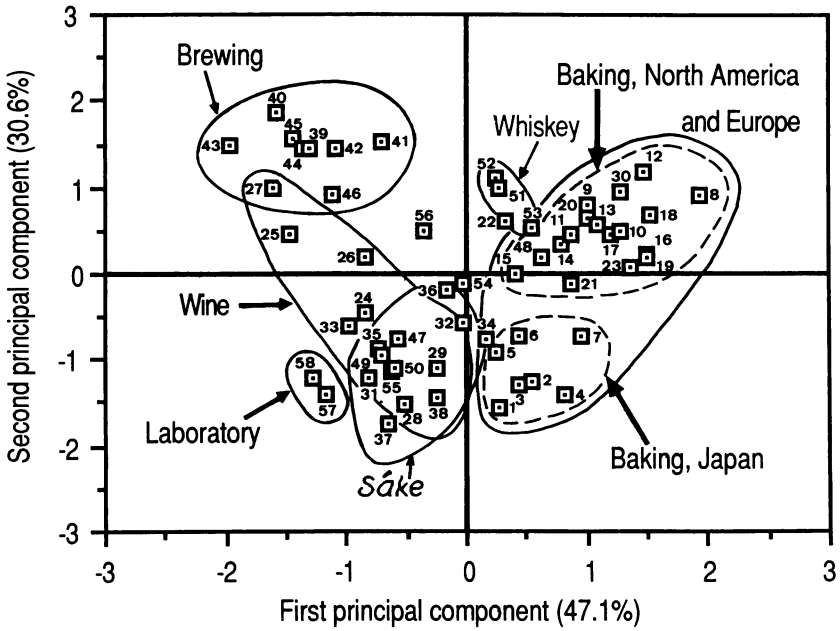

FIG. 2. Scattergram of 58 yeast strains for the first and second PCs. The types of *S. cerevisiae* strains were as follows: 1 to 8, Japanese baker's yeasts; 9 to 23, North American-European baker's yeasts; 24 to 33, wine yeasts; 34 to 38, sáke yeasts; 39 to 47, brewer's yeasts; 48 to 50, shochu yeasts; 51 to 53, whiskey yeasts; 54 to 56, alcohol yeasts; and 57 and 58, laboratory yeasts.

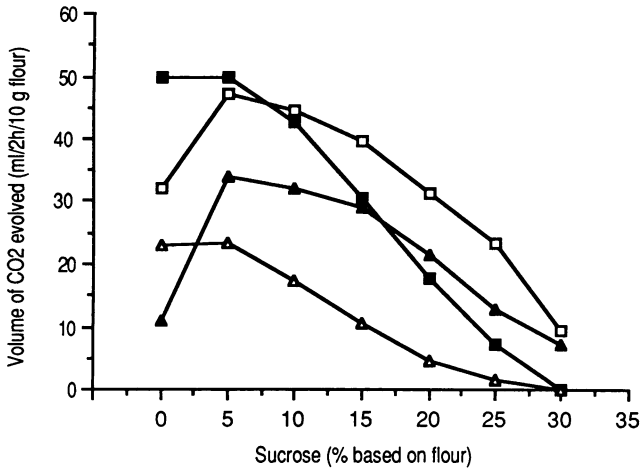

FIG. 3. Leavening abilities of doughs containing sucrose. Symbols: □, FSC6001, a Japanese baking strain; ■, FSC6014, a European baking strain; △, BK1111, a brewing strain; ▲, X2180-1A, a laboratory strain.

scattergram. These positions indicated that baker's yeasts indeed had higher glycolytic activities in dough than did the other industrial yeasts. The separation of Japanese and North American-European baker's yeasts into subgroups along the second axis reflected that accepted dough formulas are different in these countries (17). The exceptional position of FSC6224, a Japanese baking strain (no. 8 in Fig. 2) was reasonable, since the compressed form of this strain is exclusively used for dough containing lower amounts of sugar in Japan.

Brewer's yeasts were clearly differentiated from baker's yeasts. The former organisms have been highly adapted to maltose fermentation but possess poor leavening ability. Since the early records of the history of fermentation showed that foam from beer fermentation was used to raise dough, baker's and brewer's yeasts may have originated from the same ancestral strains (12). Unintentional selection in bread making and brewing over a long period could have caused the differences in these two yeasts. Although the classification of microorganisms by cluster analysis is now common practice, there have been few reports on industrial yeasts except for brewer's yeasts (4). A total of 235 strains of brewer's yeasts from the National Collection of Yeast Cultures (Norwich, United Kingdom) were divided into five groups, each of which consisted of two subgroups determined by principal-coordinate analysis as an alternative to PC analysis on the basis of the data on brewing properties (4).

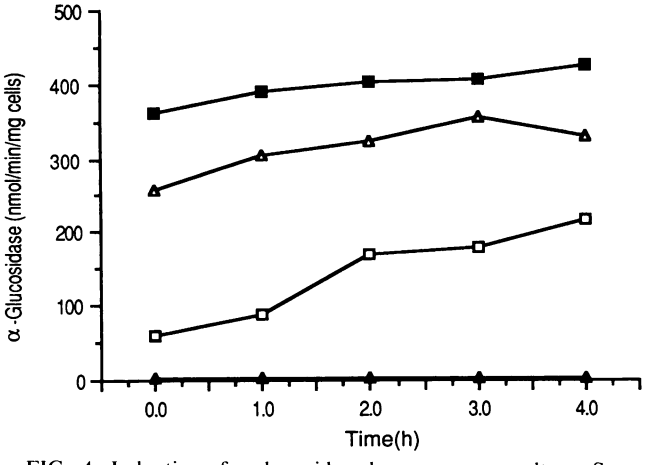

FIG. 4. Induction of α-glucosidase by exogenous maltose. Symbols are the same as in Fig. 3.

Two laboratory yeasts were mapped far from baker's yeasts, clearly indicating that these strains could not be used for baking. Whiskey yeasts were placed in the middle of brewer's and North American-European baker's yeasts. Wine, sáke, shochu, and alcohol yeasts overlapped in the map. Brewing and whiskey yeasts, which fermented in the mash containing maltose as a main sugar, seemed to be separated from sáke, shochu, and wine yeasts, which fermented in moromi mash or must containing glucose as a main sugar.

On the basis of PC analysis, we were able to visualize the differences between baker's yeasts, laboratory yeasts, and other industrial yeasts. We plan to search for and evaluate suitable isolates or constructed strains on the scattergram obtained from this analysis.

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