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Development of Bacterial Contamination during Production of Yeast Extracts

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Baker's yeast suspensions having bacterial populations of 10^6 and 10^8 CFU/ml were subjected to autolysis processes designed to obtain yeast extracts (YE). The bacterial contaminants added to the yeast cell suspensions were produced with spent broths obtained from a commercial yeast production plant and contained 59% cocci (*Leuconostoc*, *Aerococcus*, *Lactococcus*) as well as 41% bacilli (*Bacillus*). Autolyses were conducted at four different pH levels (4.0, 5.5, 7.0, and 8.5) and with two autolysis-promoting agents (ethyl acetate and chitosan). Processing parameters were more important than the initial bacterial population in the development of contaminating bacteria during manufacture of YE. Drops in the viable bacterial population after a 24-h autolysis were observed when pH was adjusted to 4.0 or when ethyl acetate was added. A significant interaction was found between the effects of pH and autolysis promoters on the bacterial population in YE, indicating that the activity of ethyl acetate, as opposed to that of chitosan, was not influenced by pH.

Yeast extracts (YE) are used as ingredients in foods as well as in microbiological growth media. Various methods can be used to produce YE (11). Although it is possible to produce them without the use of autolysis promoters (7), increased production yields are achieved with enzymatic (11, 12), mechanical (2, 13), or chemical (4, 10, 11) treatments.

A number of studies have reported on yeast autolysis and YE production (1, 9, 18), but none has examined the effects of processing parameters on the development of bacterial contaminants in the yeast suspension during the YE manufacturing process. Industrial production of baker's yeast biomass is carried out in large fermentation units (>300 m^3), which makes it almost impossible to avoid some form of microbial contamination, and the presence of lactic acid bacteria has been reported (8, 16, 17). There is thus a need to evaluate the effects of YE processing conditions on the development of contaminating bacteria in order to ensure the microbiological quality of YE.

The aim of this study was to inoculate bacterial contaminants into industrial baker's yeast cell suspensions and to evaluate the effects of autolysis promoters, pH, and bacterial density on their development during the production of YE.

Yeast. For commercial baker's yeast, lots (40 liters each) were obtained from a yeast factory. Each lot, containing approximately 18% dry substance, was divided into 1-liter portions and frozen at −20°C. Sixteen hours prior to autolysis, the 1-liter units of yeast suspension were thawed at 4°C. Preliminary studies showed that freezing the yeast suspension for 1 month at −18°C generated initial lysis but did not have a significant effect on the 24-h autolysis yield (Fig. 1).

Bacterial contaminants. (i) Production of high-density bacterial suspensions of contaminating bacteria. Bacterial contamination of yeast suspensions was 10^6 CFU/ml. The experimental plan was also designed to study the impact of a very high level of bacterial contaminants, 10^8 CFU/ml of yeast suspension. To achieve such a level of bacterial contamination, a bacterial biomass production step was necessary. The biomass was produced in a solution of 111 ml of cane molasses (45°Brix), 10 g of YE (Oxoid), 10 ml of cycloheximide (Acti-Dione; Omega Inc., Lévis, Québec, Canada) (0.1% stock solution), and 879 ml of spent broth placed in a 2-liter fermentor (Biostat M; Braun, Melsungen, Germany). The molasses-YE solution was previously sterilized at 121°C for 15 min. Cycloheximide was included to prevent the development of yeast present in the fermentation medium. Fermentation was carried out at 30°C for 24 h, with agitation at 100 rpm and aeration at 0.1 volume of air per volume of growth medium per min. The pH was controlled at 5.5 with 5 N H_2SO_4 and 5 N NaOH. These conditions were designed to simulate conditions of baker's yeast production (medium, aeration, pH). After this 24-h fermentation, cells were harvested by centrifugation at 5,600 × *g* (Beckman JA-10 rotor) for 25 min at 4°C. The cell pellet was resuspended in a 10% solution of sterile glycerol (Aldrich Chemical Company Inc., Milwaukee, Wis.) at 1/10 of the original volume. This high-density bacterial suspension was divided into 15-ml fractions and frozen at −40°C without delay.

(ii) Isolation. Samples from bacterial suspensions were diluted in sterile peptone (0.1%)-water; plated on PCA, M17, and MRS agars (Difco, Detroit, Mich.); and incubated at 30°C for 48 h. Colonies from each lot were chosen randomly and subcultured twice prior to a microscopic purity check.

(iii) Strain storage. All isolates were grown on MRS agar slants (duplicate) covered with sterile mineral oil (previously heated at 150°C for 6 h). The samples were stored at 4°C.

(iv) Characterization and identification. Gram staining, spore staining, and oxidase testing were carried out as described previously (14). Production of lactic and acetic acids, as well as of ethanol, was determined by high-pressure liquid chromatography (6) following growth in a medium composed of 2% glucose, 0.5% peptone, 0.5% YE, and 0.3% potassium phosphate. Identification of bacterial isolates was attempted with the API 50 CH, API 20 STREP, API 20E (BioMérieux Vitek, Inc., Hazelwood, Mo.), and Biolog (GP Microplate and MicroStation System, version 3.50; Biolog Inc., Hayward, Calif.) commercial kits. The tests were performed according to the instructions of the manufacturers.

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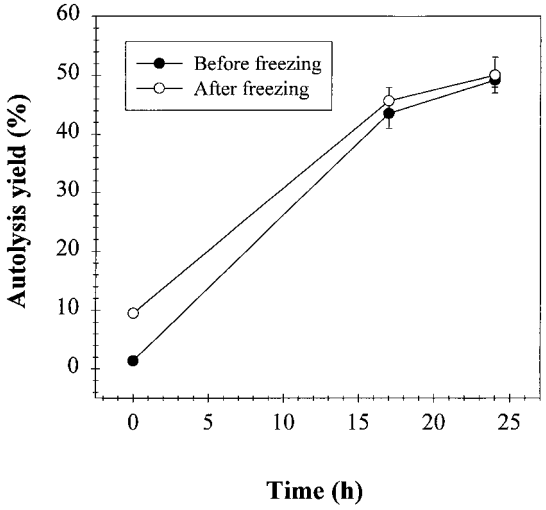

FIG. 1. Effect of freezing of baker's yeast suspension on the appearance of soluble solids during subsequent autolysis. Yield is expressed as percentages of total solids that became soluble.

Bacterial enumerations. Total bacterial population was estimated by plating peptone (0.1%)-water dilutions on a PCA medium (Difco) supplemented with cycloheximide (0.001%). Plates were incubated at 30°C for 48 h.

Autolysis. Three process parameters were modified for the production of YE: autolysis promoters (ethyl acetate [1.5%], chitosan [0.2%], and negative control), pH (4.0, 5.5, 7.0, and 8.5), and the presence of contaminating subcultured bacteria from spent yeast broths (10^8 CFU/ml). A random experimental plan was designed and applied with four identical Biostat M (Braun) fermentors; four independent assays were conducted, each treatment being applied once in each fermentation unit to avoid a fermentor effect.

(i) General procedure. Glass jars were sterilized (autoclaved at 121°C for 35 min). Under aseptic conditions, 1 liter of thawed (24°C) yeast suspension was added to the jar, along with the autolysis promoter and contaminants if required. Constant agitation (100 rpm), temperature (48°C), and pH were maintained during autolysis. Following 24 h of incubation under these conditions, a sample of autolysate (10 ml) was taken for bacterial enumeration.

(ii) Autolysis with ethyl acetate. Ethyl acetate (15 ml) was added to the yeast suspension, and the mixture was incubated as for the general (control) procedure.

(iii) Autolysis with chitosan. The chitosan autolysis method was adapted from the patent of Origane and Sato (10). A stock solution of 4% shrimp chitosan (Alpha-Biotech 2000, Québec, Canada) was prepared with glacial acetic acid (99.7%). To 1 liter of yeast suspension, 50 ml of the 4% solution of chitosan was added. Five milliliters of sterile (autoclaved for 15 min at 121°C) antifoam (Dow Corning Corporation, Midland, Mich.) was added immediately after the chitosan to prevent foaming. Chitosan combined with yeast suspension induced high viscosity. Therefore, autolysis with chitosan required agitation of 450 rpm during the first 3 h, after which the agitation rate was lowered to 200 rpm.

(iv) Autolysis with a high level of bacterial contaminants. At the beginning of autolysis, 10 ml of the frozen high-density bacterial suspension was thawed at 24°C and added to the yeast suspension.

Statistical analyses. Analysis of variance (F test) was performed on bacterial cell viabilities after 24 h of autolysis (with $\log_{10}$ values). Analysis of variance was calculated with the Genstat 5 statistical program. All interactions between parameters were verified. Multiple comparisons were done to detect significant differences ($P < 0.001$) between treatments, and the least significant differences test was used to form regrouping. Variance homogeneity was verified by graphic analysis of residues and by the Bartlett test.

Production of high-density bacterial suspensions. The spent broth obtained after removal of yeast cells by continuous centrifugation from the industrial fed-batch fermented culture medium was deemed representative of bacterial contaminants of the yeast suspension. The initial bacterial counts of the four tested lots varied from 1.1×10^5 to 5.0×10^5 CFU/ml, with an average of 1.7×10^5 CFU/ml.

After the lab fermentation step, the bacterial population was 4.7×10^9 CFU/ml, and it was further concentrated to 4.5×10^{10} CFU/ml by batch centrifugation. Freezing of the concentrate produced some mortality, and the thawed culture had a viable population of 1×10^{10} CFU/ml. In order to examine the development of these bacteria during the YE production process, the bacterial concentrate was inoculated into the yeast suspension to obtain an initial population of 1×10^8 CFU/ml. This bacterial contamination level is much higher than the average contamination level of yeast suspensions (10^5 to 10^6 CFU/ml) but is representative of the extremes that can be reached.

Growth of yeast was inhibited by cycloheximide, and the final population (after 24 h) was limited to 6×10^6 CFU/ml, which was approximately 800 times lower than the inoculated bacterial count.

Identification of bacterial contaminants. Thirty-four colonies of bacteria were selected randomly from the higher dilution (10^9) of the high-density suspensions. In all, 41% of the isolated bacteria were aerobic sporulating rods, characteristic of the genus *Bacillus*. Cocci represented 59% of the isolates and were all oxidase negative, as well as catalase negative. The carbon balance, determined by glucose assimilation and acid production, permitted identification of homofermentative lactic cultures. Among the cocci, 70% were homofermentative and 15% produced some ethanol. All isolates were gram positive.

Of the bacilli, 11 isolates were found to be *Bacillus* sp., of which 6 were identified as *B. megaterium* and 2 were identified as *B. circulans*. No definite identification was obtained with the cocci, but growth patterns on Biolog and API plates, catalase tests, and high-pressure liquid chromatography analyses suggested that *Leuconostoc*, *Aerococcus*, and *Lactococcus* were the most probable genera.

A concern should be raised as to the exact representation of the high-density bacterial suspension with respect to the contamination flora of baker's yeast suspensions. This study suggests that *Bacillus* sp. would constitute a significant fraction of fresh baker's yeast, but Viljoen and Lues (17) reported that lactobacilli were the main contaminating bacteria of compressed yeast blocks.

Effects of initial bacterial population levels. Two initial bacterial populations were examined: the native uninoculated yeast suspensions at 10^6 CFU/ml and the yeast suspensions inoculated at 10^8 CFU/ml with the high-density bacterial concentrate. Inoculation of the high-density bacterial suspension did not significantly influence populations obtained after 24 h of incubation ($P = 0.265$). In instances in which conditions were favorable for growth, bacterial populations reached up to 6.3×10^8 CFU/ml irrespective of the initial contamination level (Fig. 2). Under detrimental conditions, counts dropped to as low as 15 CFU/ml irrespective of the initial contamination

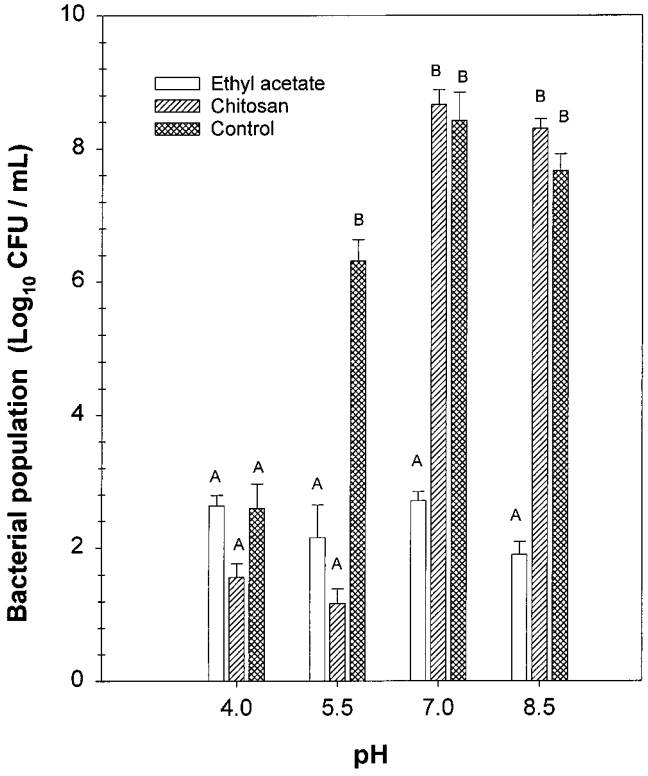

FIG. 2. Effects of pH adjustment to 4, 5.5, 7.0, and 8.5 on bacterial cell counts after 24 h of baker's yeast autolysis. Error bars represent standard errors of the means. Different letters (A and B) above columns indicate statistically significant differences ($P < 0.05$).

level. No interaction between bacterial inoculation and pH ($P = 0.592$) or between inoculation and the use of autolysis promoters was found ($P = 0.889$).

Effects of autolysis parameters. Significant effects of pH and autolysis promoters on bacterial populations after 24 h of autolysis were observed (Fig. 2). A pH of 4.0 or the addition of ethyl acetate generated drops in the viable bacterial population. A significant interaction was found between pH and the autolysis promoters. This reflects the fact that the effectiveness of chitosan is influenced by pH (15, 19), chitosan being inhibitory at pH 5.5 but not at pH 7 or 8.5, in contrast to the effectiveness of ethyl acetate. These results are consistent with previous reports that organic solvents have the ability to inhibit bacterial growth (5, 11).

In conclusion, this study shows that development of bacterial contaminants in baker's yeast suspensions can be suppressed during the autolysis process. Use of a pH of 4.0 will achieve this goal, but yields are lower at this level (3). Since YE are traditionally produced at a pH of about 5.5, and growth of contaminating bacteria occurs at this pH level, the use of an inhibitory compound such as ethyl acetate or chitosan is suggested.

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REFERENCES

1. **Arnold, W. N., ed.** 1981. Yeast cell envelopes: biochemistry, biophysics, and ultrastructure, p. 129–137. CRC Press Reprints, Blaker Wood.
2. **Baldwin, C., and C. W. Robinson.** 1990. Disruption of *Saccharomyces cerevisiae* using enzymatic lysis combined with high-pressure homogenization. Biotechnol. Tech. **4:**329–334.
3. **Barrette, J.** 1997. Production d'extraits de levures biostimulants destinés à la prolifération de lactocoques. M.S. thesis. Université Laval, Québec, Québec, Canada.
4. **Breddam, K., and T. Beenfeldt.** 1991. Acceleration of yeast autolysis by chemical methods for production of intracellular enzymes. Appl. Microbiol. Biotechnol. **35:**323–329.
5. **Chao, K. C., E. F. McCarthy, and G. A. McConaghy.** 1980. Yeast autolysis process. U.S. patent 4,218,481.
6. **Doyon, G., G. Gaudreau, D. St.-Gelais, Y. Beaulieu, and C. J. Randail.** 1991. Simultaneous HPLC determination of organic acids, sugars and alcohols. Can. Inst. Food Sci. Technol. J. **24:**87–94.
7. **Hough, J. S., and I. S. Maddox.** 1970. Yeast autolysis. Process Biochem. **5:**50–52.
8. **Jenson, I.** 1998. Bread and baker's yeast, p. 172–198. *In* B. J. B. Wood (ed.), Microbiology of fermented foods, 2nd ed., vol. 1. Blakie Academic & Professional, London, England.
9. **Lurton, L., J.-P. Segain, and M. Feuillat.** 1989. Étude de la protéolyse au cours de l'autolyse de levures en milieu acide. Sci. Aliments **9:**111–124.
10. **Origane, A., and T. Sato.** 1993. Production of yeast extract. U.S. patent 5,188,852.
11. **Peppler, H. J.** 1982. Yeast extracts. Econ. Microbiol. **7:**293–312.
12. **Reed, G., and T. W. Nagodawithana.** 1991. Yeast-derived products, p. 369–412. *In* V. N. Reinhold (ed.), Yeast technology, 2nd ed. AVI, New York, N.Y.
13. **Slaughter, J. C., and T. Nomura.** 1992. Autocatalytic degradation of proteins in extracts of a brewing strain of Saccharomyces cerevisiae. The role of endopeptidases and exopeptidases. Appl. Microbiol. Biotechnol. **37:**638–642.
14. **Speck, M. L.** 1984. Compendium of methods for the microbiological examination of foods, 2nd ed. American Public Health Association, Washington D.C.
15. **Sudarshan, N. R., D. G. Hoover, and D. Knorr.** 1992. Antibacterial action of chitosan. Food Biotechnol. **6:**257–272.
16. **Sugimoto, H.** 1974. Synergistic effect of ethanol and sodium chloride on autolysis of baker's yeast for preparing food-grade yeast extracts. J. Food Sci. **39:**939–942.
17. **Viljoen, B. C., and J. F. R. Lues.** 1993. The microbial populations associated with post-fermented dough and compressed baker's yeast. Food Microbiol. **10:**379–386.
18. **Vosti, D. C., and M. A. Joslyn.** 1954. Autolysis of baker's yeast. Appl. Microbiol. **2:**70–78.
19. **Wang, G.-H.** 1992. Inhibition and inactivation of five species of foodborne pathogens by chitosan. J. Food Prot. **55:**916–919.