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Interaction between pH, autolysis promoters and bacterial contamination on the production of yeast extracts

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Abstract

Bakers' yeast (*Saccharomyces cerevisiae*) cell suspensions were submitted to autolysis at various pH values (4.0, 5.5, 7.0 and 8.5) and with chemical autolysis promoters (ethyl acetate, chitosan). In one series of assays, bacterial contaminants were added at 10^8 CFU/ml. Autolysis yields, total nitrogen (total N) and α-amino nitrogen (α-amino N) composition as well as turbidity of the yeast extracts (YE) were examined. The addition of bacterial contaminants at 10^8 CFU/ml to the yeast cell suspension did not significantly influence any of the response variables tested. There was a significant effect of pH on autolysis yields, total N and α-amino N of YE as well as on the turbidity of the YE. There was an interaction between pH and the autolysis promoter. The highest autolysis yields and total N contents of YE were obtained with a combination of pH 5.5 and the addition of ethyl acetate. The α-amino N represented between 50 and 60% of total N in the YE produced at pH 5.0 and 7.0, suggesting good peptidase activities at these pH values. The YE produced at pH 7.0 and 8.5 had much more turbidity than those obtained by incubating at pH levels of 4.0 and 5.5.

Keywords: *Saccharomyces*; Chitosan; Ethyl acetate; Hydrolysis; Total nitrogen; Turbidity; Yield

1. Introduction

Yeast extracts (YE) are commonly used as flavor enhancers or as flavoring ingredients in the food industry (Nagodawithana, 1992). They are obtained by a controlled autolysis of brewers' or bakers' yeast (Peppler, 1982). Many YE processing parameters have been examined. Reagents (Fenton, 1982; Shetty & Kinsella, 1978), temperature (Babayan, Bezrukov, Latov, Belikov, Belatseva & Titova, 1981), pH (Lurton, Segain & Feuillat, 1989; Slaughter & Nomura, 1992), high pressure homogenization (Baldwin & Robinson, 1990), enzyme addition (Ryan & Ward, 1985, 1988) as well as vitamin supplementation (Akin & Murphy, 1981), all influence the rate of autolysis, yields or composition of the resulting YE. In most cases, the effect of a parameter was studied independently, but there are a few studies that note interactions between autolysis parameters. Examples of such interactions were observed between papain and glucanases (Ryan & Ward, 1988), NaCl and incubation temperature (Behalova & Beran, 1979) as well as ethanol and NaCl (Sugimoto, 1974). Therefore, there are relationships between the autolysis parameters, and information of such interactions are useful in optimizing yields. Although the usefulness of reagents (solvents or chitosan) in promoting autolysis is well established, there is little information on the interaction between pH and reagents on yields and chemical composition of the YE. One of the aims of this study was to conduct such an evaluation.

Bacterial contamination of commercial compressed bakers' yeast commonly reaches 10^7 CFU/g (Viljoen & Lues, 1993), and surveys of commercial products report values attaining 10^{10} CFU/g (Jenson, 1998). There is no information, however, on the effect of bacterial contaminants on the autolysis process and YE composition.

The aim of this study was thus to evaluate the interactions between bacterial contamination, pH and autolysis promoters on the autolysis yields as well as the chemical composition and the turbidity of the YE obtained.

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2. Materials and methods

2.1. Yeast

Four fresh liquid bakers' yeast lots (40 l each), were obtained from a yeast production factory. Each lot, containing approximately 18% of dry matter, was divided into 1 l fractions and frozen at −20°C. Sixteen hours prior to autolysis, the 1 l units of yeast cell suspensions were thawed at 4°C.

2.2. Production of high-density suspensions of bacteria

The bacterial population of the yeast suspension was 10^6 CFU/ml, and this constituted a typical level of contamination for this particular plant. The experimental plan was also designed to study a very high level of bacterial contaminants, at 1×10^8 CFU/ml of yeast suspension. The bacterial contamination of the industrial spent broth (centrifugation residue) was insufficient to provide this biomass, and would have required the centrifugation of approximately 4000 l of spent broth for the assays that were conducted in this study. Therefore, a bacterial contaminant biomass production step was carried out, in conditions that would resemble a bakers' yeast manufacturing process. For this bacterial biomass production step, a 2 l bioreactor Biostat M (B. Braun, Germany) was used with a working volume of 1 l. Ten g of YE (Oxoid) were added to 111 ml of cane molasses (45° Brix) and this solution was sterilized at 121°C for 15 min. To that sterile medium, 879 ml of spent broth (broth obtained after the industrial centrifugation carried out to recuperate the yeast cells) obtained from the yeast factory, and 10 ml of actidion (0.1% stock solution) were added. Actidion (Omega Inc., Lévis, QC) was included so as to prevent the development of yeast present in the spent broth. The fermentation was carried out at 30°C for 24 h, with agitation at 100 rpm and with aeration at 0.1 volume of air per volume of growth medium per min (0.1 v/v/m). The pH was controlled at 5.5 with H_2SO_4 (5N) and NaOH (5N). After this 24 h fermentation, the bacterial cells were harvested by centrifugation at 5600×g (Beckman, Beckman rotor JA-10) for 25 min at 4°C. The cell pellet was resuspended in a 10% solution of sterile glycerol (Aldrich Chemical Company Inc., Milwaukee) at 1/10th of the original volume so as to obtain a 10× concentration factor. This high-density bacterial suspension was divided in 15 ml fractions, and frozen at −40°C without delay. Isolates from the bacterial suspensions were shown to be gram-positive lactic-acid-producing homofermentative cocci (40%), heterofermentative cocci (20%) and spore forming bacilli (40%) (Barrette, Champagne & Goulet, 1999). Although this data confirms the predominance of lactic acid-producing bacteria in commercial bakers' yeast, these data differ from those of Viljoen and Lues (1993), who had noted a greater content of *Lactobacillus*. This variation is not unusual, as contamination is largely plant-related. Therefore, the bacterial contaminant flora obtained was high in *Bacillus* sp., but is representative of the bacterial contamination flora of bakers' yeast.

2.3. Autolysis

Three process parameters were modified for the production of the YE: (1) autolysis promoters (ethyl acetate 1.5%, chitosan 0.2% and negative control), (2) pH (4.0, 5.5, 7.0 and 8.5) and (3) addition or not of contaminating bacteria from the spent broth (10^8 CFU/ml). Twenty-four different combinations of YE productions were possible, considering all parameters. A random experimental plan was designed and applied with four identical Biostat M (B. Braun) bioreactors; four independent assays were conducted, each treatment being applied once in each fermentation unit to avoid a bioreactor effect.

2.3.1. General procedure

Under aseptic conditions, 1 l of thawed yeast suspension was added into a sterilized (121°C, 35 min) bioreactor jar. The autolysis agent and the contaminants were added if required by the random combination. The pH was chosen randomly and held constant during all the autolysis (24 h). pH-meters probes were calibrated with standard solutions (buffers 4.0 and 7.0), dipped in ethanol (70%) and flamed before their use. Constant agitation (100 rpm) and temperature (48°C) were maintained during autolysis. Following 24 h of incubation under these conditions, the autolysates (in 500 ml portions) were submitted to a first centrifugation at 5000 g for 15 min (Beckman, rotor JA-10; DuPont Instruments). The supernatants were collected and the pellet resuspended in deionized water. A second centrifugation was conducted under the same conditions, and the supernatants collected. The YE were then heated at 80°C for 30 min, unless otherwise stated. After the heat treatment, the YE were freeze-dried in a Lyo-Tech unit (Lyo-San, Lachute, QC, Canada), for 48 h at 24°C under a vacuum of 100 μ or less.

2.3.2. Autolysis with ethyl acetate

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

2.3.3. Autolysis with chitosan

This method was adapted from the patent of Origane and Sato (1993). A stock solution of 4% shrimp chitosan (Alpha-Biotech 2000, Quebec) was prepared with glacial acetic acid (99.7%). No sterilization was needed because the viable count of bacteria in the chitosan before solubilization was less than 150 CFU/g. To 1 l of

yeast suspension, 50 ml of the 4% solution of chitosan was added. Five ml of sterile (autoclaved 15 min at 121°C) anti-foam (Dow Corning Corporation, Midland, USA) was added, immediately after the chitosan, to avoid foaming. Chitosan combined with yeast suspension induced high viscosity. Therefore the autolysis with chitosan required an agitation of 450 rpm during the first 3 h after which the agitation was lowered to 200 rpm.

2.3.4. 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.

2.4. Yeast extract recovery

The autolysate (300 ml) was harvested and centrifuged 15 min at 5000×g (Beckman, rotor Beckman JA-10). The supernatant was collected and approximately 300 ml of deionized water was mixed with the cell pellet. A second centrifugation was carried out, in the same conditions. The supernatant was combined to the first and pasteurized (80×C, 30 min). Samples were taken every 10 min during the heat treatment in order to determine the residual ethyl acetate level. The pasteurized YE were placed in freeze-drying pans in order to obtain 1 cm thick layers, and frozen at −40°C. They were then freezed-dried in a Lyo-Tech (LYO-SAN Inc., Lachute, Canada) unit at 24°C for 48 h, under a vacuum of at least 100 μ. The YE powders were stored in sealed bottles at −18°C to avoid the rehydration of hygroscopic YE.

2.5. Bacterial enumerations

Total bacterial population was estimated by plating appropriate peptone (0.1%) water dilutions on PCA medium (Difco) supplemented with actidion (0.001%). Plates were incubated at 30°C/48 h.

2.6. Yield and chemical analyses

For the determination of residual ethyl acetate in the heated YE, the samples were filtered (0.45 μm filters, Nylon HVLP, Millipore) and the filtrate compounds were separated on a HPX87H (Biorad) column maintained at 45°C, using a mobile phase of 0.008N H2SO4 at a flow rate of 0.6 ml/min. A Waters (model No. 410) refractive index monitor enabled the detection of the peaks.

In the determination of yields, 15 ml of autolysate was first centrifuged at 5000 g in a SS-34 Sorvall rotor. The supernatant was collected and its volume determined. An equivalent volume of deionized water was mixed with the cell pellet, and a second centrifugation was carried out. The supernatants, constituting the YE, were combined and their volume measured. The total solids in the yeast suspensions and in YE were determined by dry weights following drying at 100°C for 24 h. The yield was expressed as a % of the solids recuperated in the YE (taking into account dilution due to two centrifugations) with respect to the total solids present in the yeast suspensions.

Total nitrogen (total N) in the freeze-dried YE powders was determined by macro-Kjeldahl with the AOAC (1984) methodology. The α-amino nitrogen (α-amino N) content was determined by titration using the USP XXI (1985) procedure. The hydrolysis degree was estimated by determining the proportional content of α-amino N in the total N corresponding value.

2.7. Turbidity

Turbidimetry was determined by nephelometry, using an Orbeco Analytical Systems (Farmingdale, NY, USA) which provided direct readings in NTU. The validity of the unit's NTU calibration was verified with standard turbidity solutions (0–40 NTU; Orbeco). The analyses were made on YE solutions diluted to 5% (w/w) total solids.

2.8. Statistical analyses

Analysis of variance (F test) was performed on yield, total N, α-amino N, hydrolysis degree and turbidity after 24 h of autolysis. The ANOVA was calculated with the Genstat 5 statistical program. All the interactions between the parameters were verified. Multiple comparisons were done to detect the significant differences ($P < 0.05$) between the treatments and the LSD test (least significant difference) was used to form regrouping. The variance homogeneity was verified by the graphic analysis of the residues and by the Bartlett test.

3. Results and discussion

3.1. Effect of freezing the yeast suspension

Since each complete assay required 3 weeks to carry out, it was our concern that the fresh yeast suspensions would initiate autolysis during storage prior to the controlled autolysis experiments. It was thus examined if freezing the yeast suspensions and storing at −18°C for 4 weeks could be done to stabilize the raw material, and if this affected its subsequent autolysis. Freezing significantly influenced the initial soluble solids content in the yeast suspension, but as the incubation time progressed, the effect of freezing on yields becomes non-significant (Barrette et al., in 1999). Therefore, analyses

in this study were limited to the 24 h autolysis samples, which provide an accurate picture of phenomena that would occur with fresh yeast suspensions.

3.2. Effect of fermentation units

The laboratory was equipped with four bioreactors, which enabled us to conduct four simultaneous fermentations. Even though they were identical, a preliminary study had shown that there was a potential effect of the bioreactor unit. Every treatment was therefore performed on each unit, and the effect of bioreactor unit was examined. This resulted in an experimental plan having four independent replicates. Results showed that there was no significant effect of bioreactor on any of the variables tested (Tables 1–5), and data were thus averaged with confidence.

3.3. Effect of initial bacterial populations

Some *Bacillus* species produce glucanases, and such enzymes influence the production of YE (Ryan & Ward, 1985). Various *Bacillus* species produce proteinases (Champagne, Laing, Roy, Mafu & Griffiths, 1994), which also influence the production of YE (Knorr, Shetty, Hood & Kinsella, 1979). Therefore, bacterial contamination has potential for a significant effect on yeast autolysis. The bacterial suspension used in this study was complex (Barrette et al., 1999), but it can be

Table 1
Variance analysis of production yields (%)

Source of variation	d.f.	s.s.	m.s.	F	$Pr > F$
Repetition	3	56.5	18.8	5	
Autolysis promoter (AP)	2	194.4	97.2	25.6	<0.001
Bacterial inoculation (BI)	1	3.9	3.9	1.0	0.328
AP×BI	2	4.0	2.0	0.5	0.602
Residual	15	56.9	3.8	0.9	
pH	3	2207.1	735.7	173.7	<0.001
pH×AP	6	1774.9	295.8	69.8	<0.001
pH×BI	3	17.0	5.6	1.3	0.273
pH×AP×BI	6	29.2	4.9	1.2	0.347
Reactor	3	14.5	4.8	1.1	0.341
Residual	51	216.0	4.2		
Total	95	4574.4			

Table 2
Variance analysis of total N content of yeast extract powder

Source of variation	d.f.	s.s.	m.s.	F	$Pr > F$
Repetition	3	4.1	1.4	8.7	
Autolysis promoter (AP)	2	0.1	0.0	0.2	0.810
Bacterial inoculation (BI)	1	0.4	0.4	2.4	0.139
AP×BI	2	0.8	0.4	2.5	0.114
Residual	15	2.4	0.2	0.9	
pH	3	331.3	110.4	654.3	<0.001
pH×AP	6	18.2	3.0	18.0	<0.001
pH×BI	3	0.6	0.2	1.2	0.314
pH×AP×BI	6	0.6	0.1	0.6	0.731
Reactor	3	0.3	0.1	0.5	0.668
Residual	51	8.6	0.2		
Total	95	367.4			

Table 3
Variance analysis of α-amino N content of yeast extract powder

Source of variation	d.f.	s.s.	m.s.	F	$Pr > F$
Repetition	3	0.3	0.1	0.8	
Autolysis promoter (AP)	2	0.1	0.0	0.3	0.766
Bacterial inoculation (BI)	1	0.0	0.0	0.2	0.667
AP×BI	2	0.0	0.0	0.2	0.807
Residual	15	1.8	0.1	2.8	
pH	3	106.4	35.5	824.9	<0.001
pH×AP	6	2.3	0.4	9.0	<0.001
pH×BI	3	0.0	0.0	0.4	0.761
pH×AP×BI	6	0.1	0.0	0.4	0.841
Reactor	3	0.1	0.0	0.6	0.634
Residual	51	2.2	0.0		
Total	95	113.3			

Table 4
Variance analysis of protein hydrolysis degree in yeast extract powder (%)

Source of variation	d.f.	s.s.	m.s.	F	$Pr > F$
Repetition	3	224.8	74.9	8.7	
Autolysis promoter (AP)	2	31.0	15.5	1.8	0.200
Bacterial inoculation (BI)	1	4.2	4.2	0.5	0.498
AP×BI	2	13.5	6.7	0.8	0.476
Residual	15	129.8	8.6	1.3	
pH	3	4318.4	1439.5	215.8	<0.001
pH×AP	6	1045.0	174.2	26.1	<0.001
pH×BI	3	16.0	5.3	0.8	0.499
pH×AP×BI	6	10.2	1.7	0.3	0.954
Reactor	3	33.5	11.2	1.7	0.184
Residual	51	340.2	6.7		
Total	95	6166.6			

Table 5
Variance analysis of turbidity (NTU) values of 5% yeast extract solids solutions

Source of variation	d.f.	s.s.	m.s.	F	$Pr > F$
Repetition	3	2.8×10^6	9.2×10^5	43	
Autolysis promoter (AP)	2	8.4×10^6	4.2×10^6	2.0	0.176
Bacterial inoculation (BI)	1	2.6×10^4	2.6×10^4	0.0	0.913
AP×BI	2	8.1×10^5	4.0×10^5	0.2	0.830
Residual	15	3.2×10^7	2.1×10^6	1.0	
pH	3	2.2×10^8	7.2×10^7	33.9	<0.001
pH×AP	6	1.3×10^7	2.1×10^6	1.0	0.429
pH×BI	3	7.2×10^6	2.4×10^6	1.1	0.345
pH×AP×BI	6	4.8×10^6	8.0×10^5	0.4	0.890
Reactor	3	4.4×10^6	1.4×10^6	0.7	0.565
Residual	51	1.1×10^8	2.1×10^6		
Total	95	4.0×10^8			

estimated that 40% of the population consisted of the spore forming gram-positive bacilli which are of concern.

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. Although bacterial contamination levels of 10^{10} CFU/g of compressed yeast are reported (Jenson, 1998) typical values are in the 10^7 CFU/g range (Viljoen & Lues, 1993). Therefore, the levels used in this study represent typically low and moderately high contamination levels of commercial bakers' yeast. A preliminary study had shown that inoculation of the high-density bacterial suspension did not significantly influence populations obtained after 24 h of incubation (Barrette et al., 1999). This experiment did not generate any significant effect of bacterial inoculation on any of the response variables tested (Tables 1–5). These results show that low and moderately high levels of bacterial populations do not significantly affect YE production. It remains to be determined if higher contamination levels are problematic.

There was no interaction between bacterial inoculation at 10^8 CFU/ml and other factors (Tables 1–5). Since no effects of a 10^8 CFU/g bacterial contamination level on yield, turbidity or N contents of YE were noted and there was no interaction with pH or autolysis promoter, the two series of data (10^6 or 10^8 CFU/g) were pooled for the determination of the means used in the subsequent statistical analyses.

3.4. Effect of heating time on ethyl acetate content of YE

Ethyl acetate (EA) is added to promote the autolysis process. However, some food additives have regulated contents in EA (Health Canada, 1999), and they are partially removed in the preparation of YE, as part of good manufacturing processes. It was thus examined how a heat treatment could eliminate this compound. At the term of the 24 h incubation, there remained approximately a third of the EA originally added (1.5%). Heating at 80°C gradually decreased its content (Fig. 1). In this study, the centrifugation supernatants were heated during 30 min prior to drying and conducting the various chemical analyses.

3.5. Effect of autolysis parameters on yields

Yield is an important economical aspect in an autolysis process, and literature reports values between 50 and 70% (Kelly, 1973). In this study, yields varied between 26 and 54%, which means that some procedures would be commercially viable.

Saccharomyces cerevisiae has at least 30 proteolytic enzymes (Achstetter & Wolf, 1985), that demonstrate activities over a wide rage of pH values (Hough & Maddox, 1970). A pH of 8.5 was best to enable the extraction of Proteinase yscA from the cell in presence of solvents, but the enzyme only becomes active in acid conditions (Breddam & Beenfelt, 1991). Therefore, the extraction and the activation of enzymes may occur at different pH levels. Data on optimum pH values of *S. cerevisiae* vary as a function of strain (Hough & Maddox). The variance analysis showed that there was a significant effect of pH on autolysis (Table 1). The best yields were obtained at pH 5.5 and 4.5 (Fig. 2), which

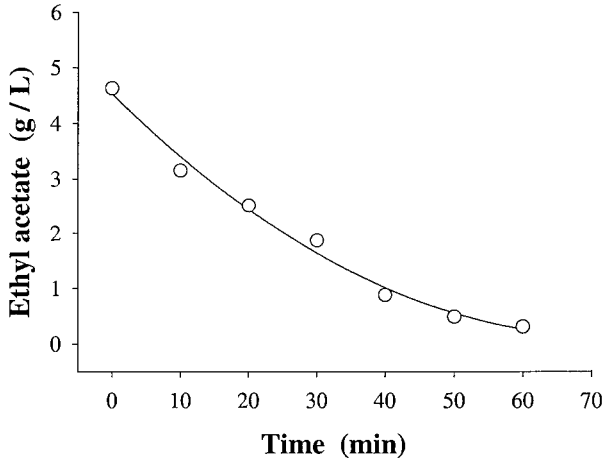

Fig. 1. Effect of heating time at 80°C on removal of ethyl acetate from yeast extracts obtained after 24 h of autolysis.

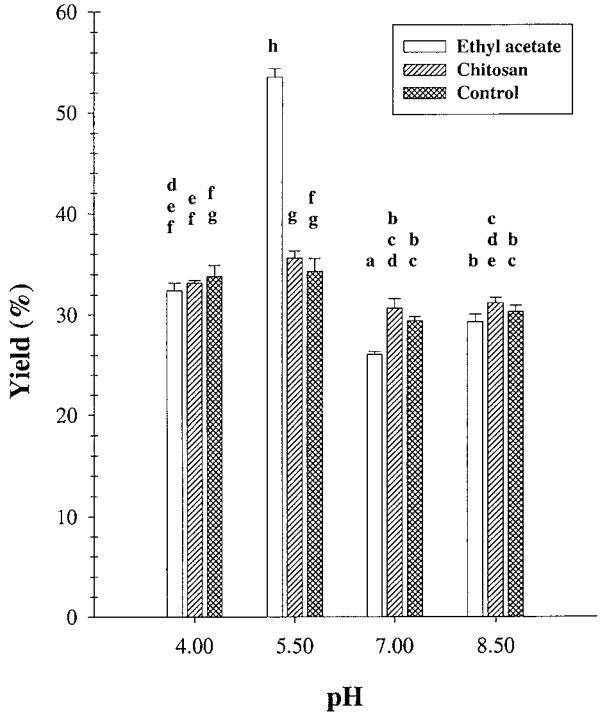

Fig. 2. Effect of chemical agents and pH on yeast extracts yields following 24 h of incubation at 48°C. Letters were added at the top of columns to indicate satistical differences. Columns that have the same letter are not significantly different ($P > 0.05$).

confirms the data of Slaughter and Nomura (1992). However, adjustment of pH alone is insufficient to obtain high yields within 24 h. This study shows that there is an interaction between pH and the autolysis promoters (Table 1). The highest yields were only obtained with a combination of pH 5.5 and the addition of ethyl acetate (Fig. 2). Interactions between enzyme addition, temperature and autolysis promoters have been reported (Behalova & Beran, 1979; Ryan & Ward, 1988; Sugimoto, 1974) and these data add to the literature on interactions between autolysis parameters.

The variance analysis shows that there is a significant effect of the autolysis promoter on yields (Table 1). This was related to the effect of ethyl acetate rather than that of chitosan. Although a patent (Origane & Sato, 1993) reports that chitosan is an effective autolysis promoter, presumably due to its effect on cell membranes (Young, Kohle & Kauss, 1982), our data do not support this (Fig. 2). Chitosan can be obtained from many sources and has various molecular weights or acetylation levels, and it remains to be determined if the type of chitosan used in this study or if the yeast strain used are responsible for this discrepancy.

3.6. Effect of autolysis parameters on total nitrogen content of yeast extracts

The total N content of the YE was mostly affected by pH (Table 2; Fig. 3). The effect of pH was much greater on N content than on total yields (Figs. 2 and 3).

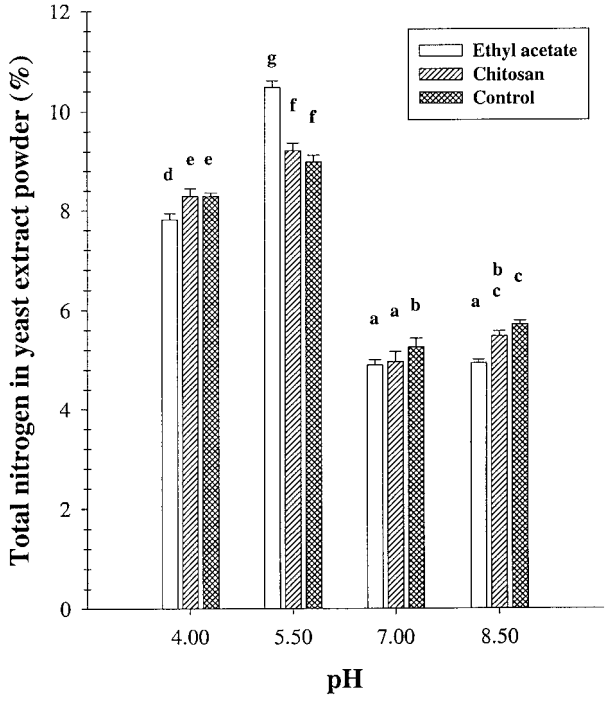

Fig. 3. Effect of chemical agents and pH on total nitrogen content of yeast extracts obtained after 24 h of incubation at 48°C. Letters were added at the top of columns to indicate satistical differences. Columns that have the same letter are not significantly different ($P > 0.05$).

Highest yields were obtained at pH 4.0 and 5.5, and the combination of pH 5.5 and EA gave the highest total N results (10.6% w/w dry product). This value falls into the high side of data found in the literature (7.1 to 11.6%; Kollar, Sturdik & Farkas, 1991; Peppler, 1982). The autolysis yield and total N data obtained with the process involving EA and a pH of 5.5 are thus in the range of commercial products and suggest that the pH and chemical agents are critical parameters in producing YE. It is difficult to establish what proteases were the most implicated. Proteinase yscB is reportedly the most active in the autolysis process (Slaughter & Nomura, 1992), but there are conflicting reports with respect to its optimum pH; thus, proteinase yscB is generally considered to be the most active at neutral values (Achstetter & Wolf, 1985), but values ranging from 4.5 (Hough & Maddox, 1970) to 7.0 (Lurton et al., 1989) are found in the literature. As mentioned previously, protease activities vary between strains. The analytical method also has been shown to influence results, as optimum values differed if the Lowry or TNBS methods were used to determine the contents of TCA fractions of yeast cell extracts (Slaughter & Nomura). Protease yscA being activated at acidic pHs (Lurton et al.), would thus presumably have some influence on autolysis at pH of 4.0 and 5.5.

There was no significant effect of autolysis promoter on total N contents of the YE (Table 2). This is in contrast with production yield data (Table 1). Therefore, protein hydrolysis was not greatly affected overall by the autolysis promoters. However, as was obtained with autolysis yields, there was an interaction between pH and the chemical agent (Table 2, Fig. 4). It can be assumed that ethyl acetate promoted the extraction of the enzymes from the vacuoles, which then acted under the influence of pH.

During autolysis, the appearance of soluble nitrogen compounds is generally correlated with the increase of total solids in the extract (Behalova & Beran, 1986). It was thus examined if a relationship existed between the total N values of the extracts and the autolysis yield. Correlation between yield and total N values only gave an R^2 value of 0.57, which suggests that there is only limited correlation between the action of the proteases and the autolysis yield when pH and autolysis promoters are simultaneously modified. Therefore, modifying the autolysis parameters influences the ongoing proteolytic activities, but may affect differently the other autolytic mechanisms. In addition to proteins, glycans and nucleic components are partially solubilized during autolysis (Behalova & Beran).

3.7. Effect of autolysis parameters on α-amino nitrogen content of yeast extract

There was a significant effect of pH as well as an interaction between pH and the autolysis promoters in

the levels of α-amino acids (Table 3, Fig. 4). A pH of 5.5 was best to obtain high levels of α-amino N. Interaction between pH and autolysis agents reflect the fact that at pH 4.0 the absence of an agent gave the highest value, while at pH 5.5 and 7.0 chitosan and EA, respectively, gave the best results.

The correlation between total N and α-amino N contents of the YE products obtained from the 12 treatments was better ($R^2 = 0.76$) than between yields and total N, but still is not very high. This suggests that, within the various proteases, peptidases do not react exactly the same as proteinases to the various autolytic conditions. The relationship between total N and α-amino N was further examined by calculating their ratios, which provided a picture of the protein and peptide hydrolysis level.

3.8. Effect of autolysis parameters on protein hydrolysis level of yeast extracts

The ratio of α-amino N to total N gave a statistical picture similar to that of total N or α-amino N alone, as significant effects of pH and interactions between pH and chemical agents were found (Table 4, Fig. 5). However, the high hydrolysis level at pH 7.0 was an original feature of this parameter. Obviously, the α-amino N content is dependant of the previous protein hydrolysis which explains the similar profiles of α-amino N (Fig. 4) and total N (Fig. 3). The calculated hydrolysis values obtained in this study suggest that the proteinases have less activity at pH values of 4.0, 7.0 and 8.5 than at pH 5.5, but that the peptidases have good activities at not only pH 5.5, but also at pH 7.0. It can be assumed that carboxypeptidase Y is responsible for this effect since its activity is good at slightly acidic to neutral pH values (Achstetter & Wolf, 1985).

3.9. Effect of autolysis parameters on turbidity of yeast extracts

A low turbidity of YE solutions can be considered as an advantage, as some applications of YE require clear solutions. The YE products had marked differences in turbidity levels, which was mainly affected by pH (Table 5, Fig. 6). Products obtained from autolysis at the lower pH levels had much lower turbidity. This effect of pH 4.0 differs from those of yield, total N and α-amino N, which were not so much benefitted by this pH level.

It was examined if there were correlations between the effect of the parameters on turbidity and the phenomena observed with the other variables. The R^2 values of first order equations between turbidity and yield, total N or α-amino N data were 0.13, 0.55 and 0.33, respectively. Thus, the best relationship seems to be with proteolysis. In this sense, YE solutions seem to have similar properties as

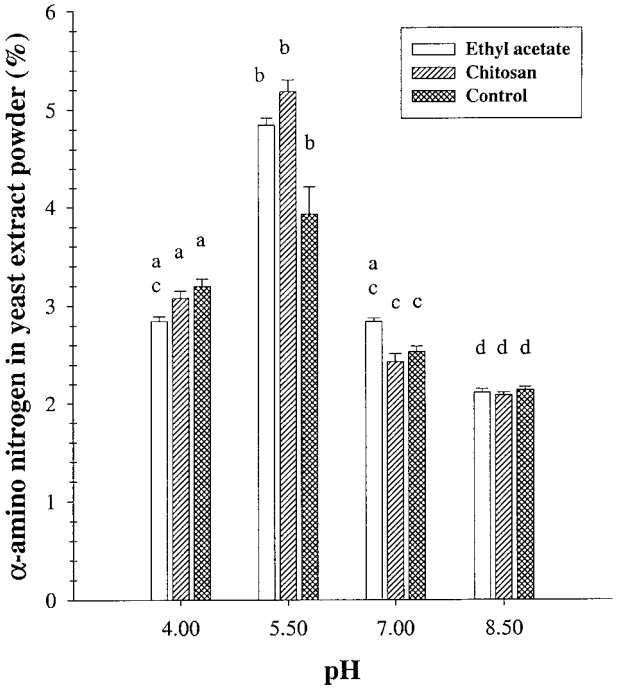

Fig. 4. Effect of chemical agents and pH on α-amino nitrogen content of yeast extracts obtained after 24 h of incubation at 48°C. Letters were added at the top of columns to indicate satistical differences. Columns that have the same letter are not significantly different ($P > 0.05$).

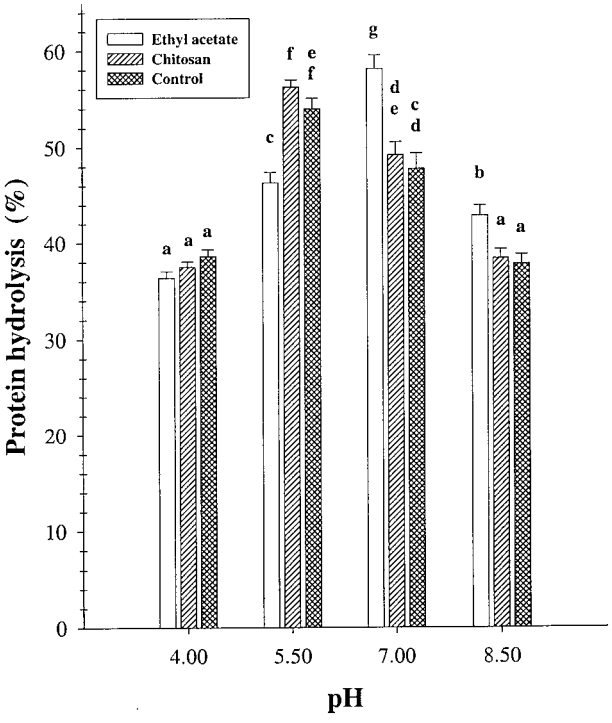

Fig. 5. Effect of chemical agents and pH on protein hydrolysis in yeast extracts obtained after 24 h of incubation at 48°C. Protein hydrolysis level was estimated by : (α-amino N/total N)×100. Letters were added at the top of columns to indicate satistical differences. Columns that have the same letter are not significantly different ($P > 0.05$).

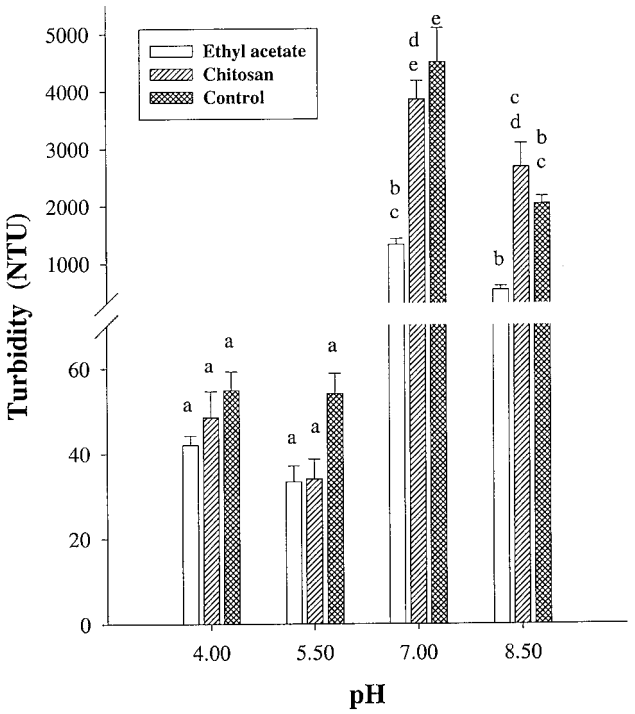

Fig. 6. Effect of chemical agents and pH on the turbidity of yeast extracts obtained after 24 h of incubation at 48°C. Letters were added at the top of columns to indicate satistical differences. Columns that have the same letter are not significantly different ($P > 0.05$).

beers, in which hazing is partially protein related (Hough, Briggs, Stevens & Young, 1982).

4. Conclusion

This study shows that pH is a major factor in the production of YE, affecting yields, proteolysis and turbidity of the products. Nevertheless, there are often interactions between pH and the autolysis promoters. Modifying the conditions of autolysis can generate different products, and results of this study provide insight on how to modify the composition of YE. Thus shifting the pH from 5.5 towards 4.0 during the course of the incubation could enable the production of clear YE solutions with limited α-amino N content. On the other hand a pH shift towards 7.0 during incubation may result in turbid solutions having high protein hydrolysis, which would seem desirable in YE destined as food ingredients for sauces. In this perspective, further studies are desirable on potential interactions between temperature and other autolysis parameters.

Yeast autolysis also generates RNA hydrolysis and nucleotides are important components of flavor enhancement (Nagodawithana, 1992) and can contribute to the microbial growth-promoting properties of YE (Desmazeaud & de Roissart, 1994). This initial study thus demonstrates that there are interactions between autolysis parameters on the chemical composition of YE, and point to the potential of producing YE with specific properties by choosing given autolysis parameters.

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