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The Effect of Different Temperatures on Autolysis of Baker's Yeast for the Production of Yeast Extract

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Abstract: This study aimed to determine the optimum autolysis conditions for the production of yeast extract, which is used to give a meaty flavor to food products and to increase their nutritional value. Autolysis was induced by incubating baker's yeast cell suspensions at different temperatures (45, 50, 55, and 60 °C) with a reaction time ranging from 8 to 72 h. Content and yield of total solids, α-amino nitrogen (α-AN), and protein were determined. Yeast extract powder was obtained by drying liquid yeast extract in a pilot scale spray drier. Sensory analysis was performed by adding the yeast extract powder at different concentrations (0.5%, 1.0%, 1.5%, and 2.0%) to vegetable soup. Optimum temperature and time for the production of yeast extract was 50 °C for 24 h, based on α-AN content, which was 3.7%. Furthermore, under the same conditions, protein content was 52.5% and total solids content was 1.98%. According to sensory analysis, the sample with 0.5% yeast extract powder and the control had the highest overall acceptance. According to variance analysis, differences between total solids, α-AN, and protein content determined at 4 different temperatures, and interactions between duration and temperature were statistically significant ($P < 0.01$).

Key Words: α-amino nitrogen, *Saccharomyces cerevisiae*, baker's yeast, temperature, yeast extract

Maya Ekstraktı Üretiminde Ekmek Mayasının Otolizi Üzerine Farklı Sıcaklıkların Etkisi

Özet: Bu çalışmanın amacı, çeşitli gıdalara et aroması vermek ve besin içeriğini arttırmak amacıyla ilave edilen maya ekstraktı üretimi için en uygun otoliz koşullarını belirlemektir. Çalışmada, 8 saatten 72 saate değişen reaksiyon aralıklarında 45, 50, 55 ve 60 °C sıcaklıklarda ekmek mayası hücre süspansiyonlarına otoliz işlemi uygulanmıştır. Maya ekstraktlarında kuru madde, α-amino azot ve protein içeriği ve verimi belirlenmiştir. Toz maya ektraktı püskürtmeli kurutucuda sıvı maya ekstraktını kurutarak elde edilmiştir. Ayrıca, sebze çorbasına farklı oranlarda (% 0,5, % 1,0, % 1,5 ve % 2,0) toz maya esktraktı ilave edilerek duyusal analiz gerçekleştirilmiştir. Maya esktraktı üretimi için optimum sıcaklık ve süre, α - amino azot içeriği dikkate alınarak 50 °C'de 24 saat olarak saptanmıştır. Bu sıcaklık ve sürede α - amino azot içeriği % 3,7 olarak bulunmuştur. Aynı koşullarda protein içeriği % 52,5 ve kuru madde içeriği % 1,98 olarak belirlenmiştir. Duyusal analizde, % 0,5 toz maya ekstraktı ilavesi ve kontrol örneği daha yüksek oranda tercih edilmiştir. Varyans analizine göre, değerlendirilen dört sıcaklık arasında α - amino azot, protein ve kuru madde yanında sıcaklık ve süre arasındaki interaksiyon da istatistiksel olarak önemli bulunmuştur ($P < 0,01$).

Anahtar Sözcükler: Otoliz, α-amino azot, *Saccharomyces cerevisiae*, ekmek mayası, sıcaklık, maya ekstraktı

Introduction

Yeasts are the most important and most extensively used microorganisms in the food industry, including the production of yeast extract (YE) (Walker, 1999). YE is a concentrate of soluble fractions of yeast cells. As a reliable economical source of peptides, amino acids, minerals, and B-complex vitamins, YE is widely used for improving the flavor of food, in order to satisfy consumer demand, and

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to increase the nutritional value of such foods as cheese spreads, sauces, meat products, soups, gravies, bakery products, seasoning, vegetable products, and seafood (Peppler, 1982).

There are 3 main methods for the production of YE: autolysis, plasmolysis, and hydrolysis (Nagodawithana, 1994). The most frequently used manufacturing practice is autolysis, which is essentially a degradation process carried out by activating the yeast's own degradative enzymes to solubilize cell components within the cell (Erten and Tanguler, 2006). These hydrolytic enzymes (particularly proteases and nucleases), which are located in the general matrix of the cell, are responsible for the degradation of yeast proteins and nucleic acids. Proteases break down yeast proteins into peptides and amino acid derivatives, whereas nucleases split nucleic acids, DNA, and RNA into nucleotides (Nagodawithana, 1994; Sommer, 1998).

YE is generally produced from *Saccharomyces cerevisiae* (baker's or spent brewer's yeast). Spent brewer's yeast must be debittered before use due to the carryover of hop resins and beer solids from beer fermentation (Walker, 1999); however, baker's yeast does not require debittering (Bridson and Brecker, 1970). The quality of YE in a well-controlled process is largely dependent on temperature, pH, duration, solubilizing aids, viability, and concentration of yeast (Peppler, 1982). Of these, temperature is the most important factor for autolysis. Data on the effect of elevated temperatures on the production of YE from baker's yeast via autolysis are scarce. The aim of the present study was to examine the influence of elevated temperature on the production of YE from baker's yeast, *S. cerevisiae,* via autolysis.

Materials and Methods

Yeast

Yeast used in the present study was commercial baker's yeast (*S. cerevisiae*) that was obtained as press yeast from Pakmaya (Turkey) and stored at 4 °C until used.

Autolysis of Baker's Yeast

Autolysis was performed in duplicate in sterilized 5-l glass jars. In 4 l of distilled water, 600 g of baker's yeast slurry was suspended. The pH level was adjusted to 6.0 with 2 N NaOH and/or 2 N HCl (Tanekawa et al., 1981). Autolysis was performed at controlled temperatures (45, 50, 55, and 60 °C) with a reaction time ranging from 8 to 72 h. During autolysis samples were harvested at 8-h intervals, pasteurized for 30 min at 80 °C in order to terminate the autolysis processes, then cooled down to room temperature and, finally, centrifuged (Eppendorf 5810) at 11,000× g for 20 min at 4 °C for chemical analysis of the supernatant (Sombutyanuchit et al., 2001).

Production of Yeast Extract Powder

Following autolysis, the suspension was centrifuged to remove cellular debris. The clear supernatant was concentrated to 15% w v^{1} of total solids content in an 80 °C water bath. The concentrate was subsequently spray-dried in a pilot scale spray drier (Lab-SD-04, UK) using an inlet air temperature of 180-190 °C, an outlet air temperatures of 80-85 °C, a feeding rate of 30 ml min^{1}, an air flow rate of 80 m^3 h^{1}, and an air pressure of 0.005 bar. The resulting powder was stored in a jar at 4 °C.

Chemical Analysis

Total solids contents of the yeast suspensions and YEs were determined by dry weights, following drying in an oven for 24 h at 105 ± 1 °C (Conway et al., 2001). Total nitrogen was measured by the Kjeldahl method via multiplication of total nitrogen by 6.25 (AOAC, 1990). α-Amino nitrogen (α-AN) was measured by nynhydrin, using glycine as a standard (Baker, 1991). Percentage yields of total solids, protein, and α-AN were determined according to Suphantharika et al. (1997).

Sensory Analysis

YE was added at the concentrations of 0.5%, 1.0%, 1.5%, and 2.0% to vegetable soup (Yonca Market, Adana) before boiling. Sensory evaluation was performed with a ranking test (Barillere and Benard, 1986) and a taste panel consisting of 12 individuals. The panelists were staff of the Department of Food Engineering and ranged in age from 35 to 64 years. Bran bread and water were given to the panelists in addition to the soup. Vegetable soup without YE powder was used as a control. Samples were numbered and served in mixed order, and were ranked from the most preferred to the least preferred by each panelist.

Statistical Analysis

The obtained results were evaluated for statistical significance with 2-way analysis of variance (ANOVA) using SPSS v.10.0 for Windows (Chicago, IL, USA). If an analysis indicated a statistically significant difference ($P < 0.01$), Duncan's multiple range test was used to compare the differences ($P < 0.01$) (Özdamar, 1999). For statistical analysis of the sensory evaluations, data were analyzed with the Kruskal-Wallis test (Roessler et al., 1978; Barillere and Benard, 1986).

Results

Effects of Temperature on Autolysis of Baker's Yeast

The influence of temperature on YE total solids content and yield during autolysis of baker's yeast is given in Figures 1 and 2, respectively. The amount of total solids released into liquid YE from cells during autolysis increased with time at all temperatures applied. After 72 h of autolysis, total solids content at 45, 50, 55 and 60 °C was 3.03%, 2.99%, 3.23%, and 2.74%, respectively. According to variance analysis, differences between total solids content determined at the 4 different temperatures applied were significant at $P < 0.01$. In addition, differences between the interactions of duration and temperature were also statistically significant ($P < 0.01$).

The total solids yield of YE increased as incubation time increased (Figure 2.). After 72 h of incubation, the highest and lowest yields were 56.1% at 50 °C and 50.7% at 60 °C, respectively. The lower yield at 60 °C may have been related to enzyme denaturation or to the fact that enzymes related to yeast autolysis were not very active at 60 °C.

In the present study the effect of autolysis temperature on the recovery of α-AN content from YE was studied. α-AN content and yield are given in Figures 3 and 4, respectively. Maximum α-AN content was 3.7% at 50 °C on a dry total solids basis at 24 and 32 h, and remained constant until 72 h. After 72 h of autolysis the lowest α-AN content was 2.4% on a dry total solids basis at 55 and 60 °C. The results obtained in the present study show that the best temperature for α-AN content was 50 °C. Differences between α-AN recovery at the 4 applied temperatures were statistically significant ($P < 0.01$). Our statistical analysis shows that differences between

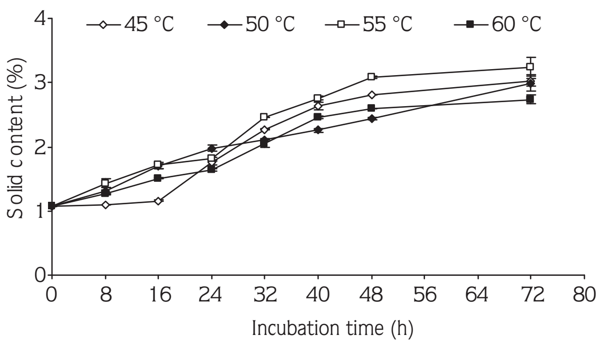

Figure 1. The effect of incubation temperature on total solids content of YE during autolysis.

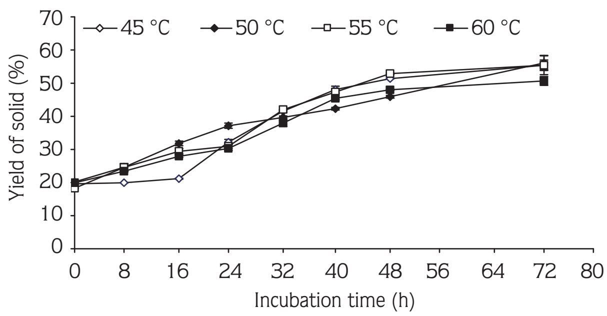

Figure 2. The effect of incubation temperature on YE yield during autolysis.

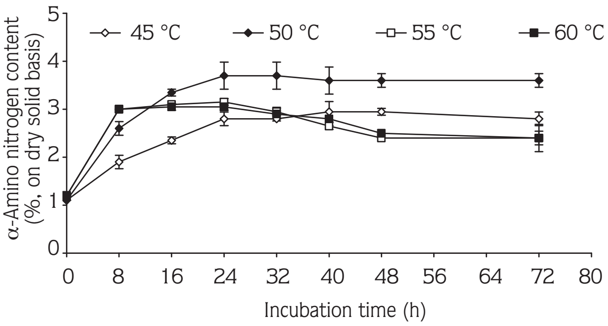

Figure 3. The effect of incubation temperature on α-AN recovery during autolysis.

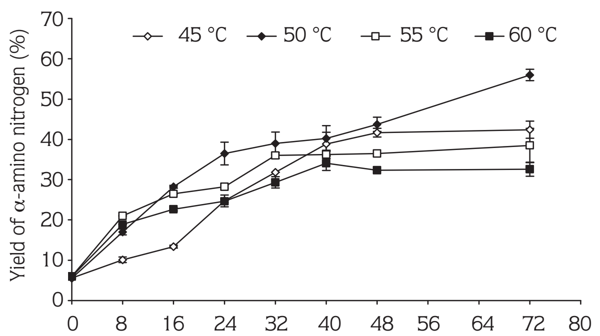

Figure 4. The effect of incubation temperature on α-AN yield of YE during autolysis.

interactions of duration and temperature were also statistically significant at $P < 0.01$.

The best α-AN yield (56%) was obtained at 50 °C at the end of autolysis (Figure 4). Among the evaluated temperatures, after an incubation period of 72 h the lowest α-AN yield was 32.59% at 60 °C. α-AN yields obtained at 45 and 55 °C after 72 h of autolysis were 42.42% and 38.5%, respectively.

The effects of the evaluated temperatures on protein recovery and yield during autolysis of baker's yeast are shown in Figures 5 and 6, respectively. Protein recovery quickly increased within the first 16 h, and then there was no significant change until the end of the incubation period (Figure 5). The highest protein recovery (59.1%) released into liquid YE from cells during autolysis was observed at 50 °C after 48 h of incubation. There was a slight difference in the release of protein from yeast cells at 45 °C after 72 h of incubation (58.9%). Autolysis performed at 55 and 60 °C resulted in the lowest protein recoveries–33.65% and 33.3%, respectively. Statistical analysis shows that there was a significant difference between treatments ($P < 0.01$). Moreover, differences between interactions of duration and temperature were statistically significant at $P < 0.01$.

With regard to protein yield, as shown in Figure 6, maximum yield was 44.9% within 24 h at 50 °C; protein yield then increased with time until the end of incubation, with a maximum of 76.2%. After 72 h of incubation, the highest protein yield was 77%, both at 45 and 50 °C. Comparatively, yields at 55 and 60 °C were 48.9% and 40.6%, respectively, and were lower than the yields obtained at 45 and 50 °C.

Production of YE Powder

Spray dried baker's YE powder was produced after 24 h of incubation at 50 °C, because it gave the highest α-AN content in this study. The general composition of autolyzed baker's YE powder obtained from a pilot scale experiment is given in Table 1. Total solids content of YE powder was 89.6%, α-AN content was 3.6%, and protein content was 56.4%.

Sensory Analysis

Results of the sensory analysis are given in Table 2. Based on taste and aroma, the samples panelists liked

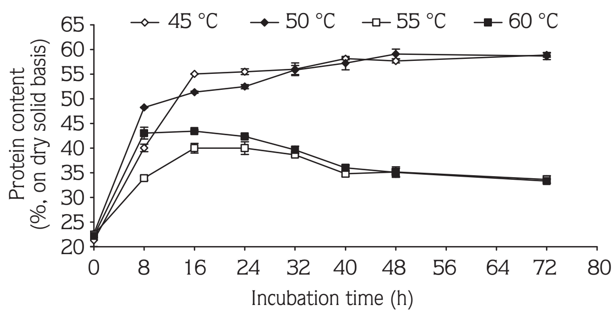

Figure 5. The effect of incubation temperature on protein recovery of YE during autolysis.

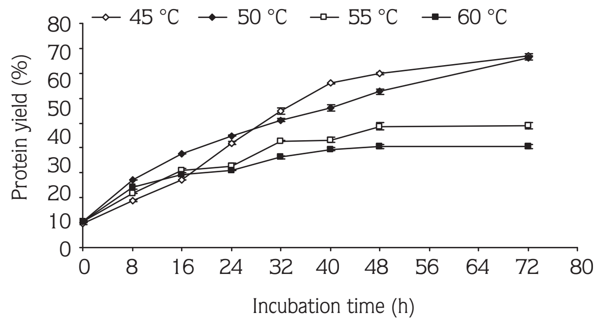

Figure 6. The effect of incubation temperature on protein yield of YE during autolysis.

Table 1. Composition of autolyzed YE powder produced via autolysis.

	Liquid YE Quality (Amount) (g)	%	YE Powder Quality (Amount) (g)	%
Total amount	2801	70	45.32	1.61
Total solids	56.02	2.0	40.6	89.6
α-AN	1.96	3.5	1.46	3.6
Protein	30.3	54.1	22.89	56.4

Table 2. Sensory analysis of vegetable soup with different concentrations of YE powder added.

	Samples*				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
The difference between points of classification ($P < 0.05$)	20^c	18^c	39^b	47^b	56^a

*Sample 1: Control; sample 2: addition of 0.5% YE powder; sample 3: addition of 0.1% YE powder; sample 4: addition of 1.5% YE powder; sample 5: addition of 2.0% YE powder. Different superscripts (a, b, and c) show statistical significance.

most were the control and the soup with 0.5% YE powder added. There was no significant difference between the control and 0.5% YE powder sample ratings, but significant differences were noted between these samples and those with 1.0%, 1.5%, and 2.0% YE powder ($P < 0.05$). Panelists stated that the addition of 0.5% YE powder to vegetable soup led to a slightly meaty taste. The panelists also noted an unpleasant yeast taste in the soup with 1.0%, 1.5%, and 2.0% YE powder. Göksungur (1993) added YE powder to vegetable soup at concentrations of 0.2%, 0.4%, 0.6%, and 1.5%, and reported that the most preferred sample was soup with 0.4% YE powder.

Discussion

In the present study autolysis temperature affected total solids content of YE. Tanguler and Erten (2008) also reported that when autolyzing spent brewer's yeast total solids released into YE from cells during autolysis increased with time at elevated temperatures of 45, 50, 55, and 60 °C.

Yield of solids is an important economic parameter in the autolysis processes (Champagne et al., 1999). In the present study YE yield increased with incubation time. The results obtained in the present study are in accordance with those published by Suphanthatika et al. (1997), who reported that maximum solids yield was obtained with autolysis at 45-50 °C.

α-AN content is one of the most important quality criteria for YE (Nagodawithana, 1992; Sommer, 1998). YE usually requires high α-AN content for food grade applications. Moreover, a short autolysis time would have significant economic benefit, preventing the development of contaminants and curbing the actions of enzymes (proteinases and peptidases) to yield the desired component (Nagodawithana, 1992). In the present study α-AN recovery changed with increasing incubation time. The amount of α-AN in YE produced by autolysis proposed by the International Hydrolyzed Protein Council should be at least 3.5%, on a dry solids basis (Peppler, 1982). The α-AN yield results obtained in the present study show that the breakdown of protein and peptides was considerably greater at 50 °C than at the other temperatures applied. It could be said that according to the results yeast proteases were more active at 50 °C. The α-AN content of YE obtained at 50 °C after 24 h of autolysis was higher than the level proposed by the International Hydrolyzed Protein Council. The results obtained in the present study are in agreement with the 3.0%-4.8%, on a dry solids basis, reported in the literature (Bridson and Brecker, 1970; Behalova and Beran, 1986; Tanguler and Erten, 2008).

Protein is an important compound in YE (Peppler, 1982; Sommer, 1998). Differences in protein content are most likely related to the active and inactive enzymes at different temperatures during autolysis. Tanguler and Erten (2008) reported that protein content on a dry solids basis varies with autolysis temperature of spent brewer's yeast. They also reported that protein content quickly increases within 24 h. Results obtained at 45 °C and 50 °C in the present study are in agreement with the 56.24% reported by Peppler (1982) and the 42.8%-49.3% reported by Behalova and Beran (1986). In addition, several researchers (Holder, 1977; Gaudreau et al., 1999; Chae et al., 2001; Saksinchai et al., 2001) reported that the protein content of YE produced from spent brewer's yeast was 41.0%-61.3% .

Spray dried baker's YE powder was produced in the present study after 24 h of incubation at 50 °C because it gave the highest α-AN content. Suphantharika et al. (1997) reported that total solids and protein contents of spray dried YE powder from baker's yeast after 48 h of incubation were 93.36% and 60.75%, respectively.

Göksungur (1993) reported that protein and α-AN contents of spray dried YE powder from baker's yeast were 46.8% and 2.5%, respectively. The results of the present study are slightly lower than those of Suphantharika et al. (1997), but slightly higher than those of Göksungur (1993).

Conclusions

In the present study the effect of elevated temperatures on the production of YE from baker's yeast via autolysis was investigated. The optimum temperature for the production of YE, with respect to α-AN content, was 50 °C for 24-32 h. At 50 °C, α-AN content was 3.7%, protein content was 52.5%, and total solids content was 1.98%. According to our sensory analysis, the 0.5% YE powder soup sample and the control ranked the best in overall acceptance.

Acknowledgements

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