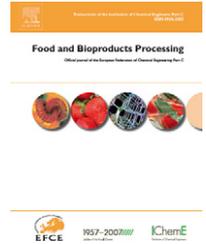


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Utilisation of spent brewer's yeast for yeast extract production by autolysis: The effect of temperature

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ABSTRACT

In this study, autolysis was induced by incubating cell suspensions of spent brewer's yeast at elevated temperatures of 45, 50, 55 and 60 °C with a reaction time ranging from 8 to 72 h. Contents and yields of solid, α -amino nitrogen, protein and carbohydrate were determined. It can be said that optimum temperature and time for the production of yeast extract was 50 °C for 24 h on the basis of α -amino nitrogen and protein contents. Sensory analysis was also done by yeast extract powder and control and samples with the addition of 0.5 and 1.0% yeast extract had the highest overall acceptance.

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1. Introduction

Yeasts are substantially beneficial in human culture and in particular they are widely used for the production of alcoholic beverages and leaven bread dough (Stewart and Russell, 1998; Walker, 1998). They are also a source for the production of yeast extracts which enhance or impart a meaty flavour to food products. Yeast extracts are mainly used in the food industry for the preparation of soups, gravies, meat products and sauces, and in the flavouring of snacks (Berry, 1982; Pepler, 1982; Erten and Tanguler, 2006).

Yeast extracts can be obtained by the methods of autolysis, plasmolysis and hydrolysis but the most frequently manufacturing practise is autolysis (Kim et al., 1999; Nagodawithana, 1992, 1994; Suphantharika et al., 1997; Shotipruk et al., 2005). Autolysis is a degradation process carried out by activating the yeast's own degradative enzymes to solubilize the cell components found within the cell (Babayan and Bezrukov, 1985; Dziezak, 1987; Nagodawithana, 1994; Erten and Tanguler, 2006). Degradative enzymes, generally compartmentalized within the living cell, are located in the general matrix of the cell. These hydrolytic enzymes, particularly protease and nuclease, break down insoluble macromolecules like proteins and nucleic acids to soluble products of peptides, amino acids (mainly glutamate), nucleotides and amino acid

derivates (Reed and Nagodawithana, 1991; Nagodawithana, 1994; Sommer, 1998).

Yeast extracts are commercially marketed as liquid, paste or powder (Nagodawithana, 1992; Sommer, 1998; Joseph, 1999). They are usually produced from baker's or spent brewer's yeast, *Saccharomyces cerevisiae*, by autolysis, but other yeasts, in particular, *Candida utilis* and *Kluyveromyces marxianus* are also sometimes used (Sommer, 1998). In general, since spent yeast in the brewing industry is relatively inexpensive, it is utilized largely in the production of extracts to meet the needs of food and fermentation industries (Reed and Nagodawithana, 1991; Stam et al., 1998). Although spent brewer's yeast is available in large quantities for a relatively cheap price, it is likely to contain undesirable flavour characteristics as a result of the carryover of hop resins and beer solids from beer fermentation (Nagodawithana, 1994; Sombutyanuchit et al., 2001).

The hop resins contain often a bitter taste and which they are primarily humulones and isohumulones which present as beer solids or as compounds firmly adsorbed at the surface of the yeast cells (Reed and Nagodawithana, 1991). This yeast cream requires a debittering before usage (Bridson and Brecker, 1970; Reed and Nagodawithana, 1991). In that process hop resins are dissolved by increasing the pH level and then, removed by further washing (Bridson and Brecker, 1970;

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Supphantharika et al., 1997). Hop resins are removed by rotary microfiltration (Shotipruk et al., 2005). Debittering is less costly and makes the final extract less bitter (Bridson and Brecker, 1970; Hough et al., 1982; Reed and Nagodawithana, 1991; Nagodawithana, 1994; Sombutyanuchit et al., 2001; Shotipruk et al., 2005).

Parameters which affect final flavour profile and extract yield in a process are temperature, pH, duration of autolysis, viability and autolysis promoters such as NaCl, ethyl acetate, amyl acetate and papain to facilitate cell lysis (Peppler, 1982; Reed and Nagodawithana, 1991; Champagne et al., 1999). Of these, temperature is the most important factor on autolysis. Although in a typical autolysis the temperature is adjusted to 45–60 °C (Conway et al., 2001; Nagodawithana, 1994), there is little information on the effect of elevated temperatures for the production of yeast extract by autolysis. The objective of this study was to examine the utilisation of spent brewer's yeast *Saccharomyces cerevisiae* (formerly *Saccharomyces carlsbergensis*) for the production of yeast extract by autolysis at elevated temperatures.

2. Materials and methods

2.1. Yeast

Yeast used in the experiments was spent brewer's yeast obtained as a by-product of lager production. It was kindly provided as slurry from Efes Pilsen Brewery, Adana, Turkey. After collection from fermentation vessel at the end of 4th fermentation, it was brought to laboratory as soon as possible for experiments.

2.2. Debittering of brewer's yeast cells

Brewer's yeast slurry was centrifuged at $11,000 \times g$ for 10 min at 4 °C to remove the beer liquor. Sterilized distilled water was added to yeast paste and the pH level was adjusted to 9 with NaOH. The suspension was stirred for 30 min at 4 °C and then washed two to three times with sterilized distilled water until the pH level became neutral. After neutralisation of pH, the suspension was stirred and then centrifuged at $11,000 \times g$ for 10 min at 4 °C to obtain debittered brewer's yeast cells (Sombutyanuchit et al., 2001). The pH was determined with an Orion Research 399 A Model.

2.3. Autolysis of spent brewer's yeast

Autolysis was performed in sterilized 5 L glass jars in duplicate. Six hundred grams of debittered spent brewer's yeast slurry were resuspended in 4 L of distilled water. The pH level was adjusted to 6.0 with 2N NaOH or 2N HCl. Autolysis was carried out at controlled temperatures of 45, 50, 55 and 60 °C in a water bath. During autolysis, samples were harvested at 8 h intervals, pasteurized at 80 °C for 30 min then cooled down to room temperature and finally, centrifuged at $11,000 \times g$ for 10 min at 4 °C for chemical analysis of the supernatant.

2.4. Production of yeast extract powder

The autolysis of debittered spent brewer's yeast for the production of yeast extract powder was carried out at 50 °C for 24 h. The process was terminated by heating at 80 °C for 30 min and then cooled down. The suspension was centri-

fuged to remove the cell debris. The supernatant was concentrated to 15% solids content by using water bath at 80 °C (Memmert, Germany). The concentrate was then spray dried with lab scale spray-drier (SD-04, UK) using an inlet air temperature of 180–190 °C, an outlet air temperature of 80–85 °C, a feeding rate of 30 mL/min, an air flow rate of 80 m³/h and an air pressure of 0.005 bar. The resulting powder was stored in a jar at 4 °C.

2.5. Chemical analysis

Dry solid content was determined in an oven at 105 °C for 24 h. Total nitrogen was analysed by using the Kjeldahl method. Protein was estimated by multiplying total nitrogen by 6.25 (Anon., 1990). α -Amino nitrogen was measured by the Nynhydrin method using glycine as a standard (Baker, 1991; Munch et al., 1997). Total carbohydrate was determined by means of phenol-sulphuric acid method (Catley, 1988; Amrane and Prigent, 1998). The pH was measured with Orion Research 399 A Model meter.

2.6. Calculation of percentage of yields of solid, protein and α -amino nitrogen

$$\text{Yield}(\%) = \frac{\text{Amount of dry solid in product}}{\text{Amount of total solids in raw material}} \times 100$$

Protein yield or α -amino yield (%)

$$= \frac{\text{Amount of protein or } \alpha\text{-amino nitrogen in nitrogen in product}}{\text{Amount of protein or } \alpha\text{-amino nitrogen in raw material}} \times 100$$

2.7. Sensory analysis

Yeast extract was added at the ratios of 0.5, 1.0, 1.5 and 2.0% to vegetable soup (Yonca Market, Adana) before boiling. Evaluation was done by a ranking test (Barillere and Benard, 1986), using a taste panel of 12 assessors. Vegetable soup without added yeast extract was used as a control. Samples were numbered from 1 to 5 and served in mixed order. They were ranked from the most preferred to least preferred one by assessor. Panelists were staff of the Department of Food Engineering.

2.8. Statistical analyses

Results obtained were evaluated for statistical significance by two-way analysis of variance (ANOVA) using the windows programme SPSS version 10.0 (Chicago, IL). If the analysis indicated a statistically significant difference ($P < 0.01$), Duncan Multiple Range test was applied to compare the differences ($P < 0.01$) (Ozdamar, 1999). For statistical analysis of sensory evaluation, data was analysed by Kruskal-Wallis test (Roessler et al., 1978; Barillere and Benard, 1986).

3. Results and discussions

3.1. Solid content and yield during autolysis

The influence of temperature on solid content and yield of yeast extract during autolysis is given in Figs. 1 and 2, respectively. Solid content and yield were affected by autolysis

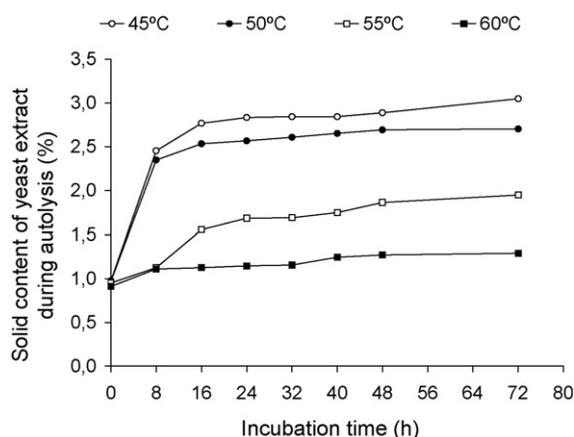


Fig. 1 – The influence of temperature on solid content of yeast extract during autolysis of spent brewer's yeast.

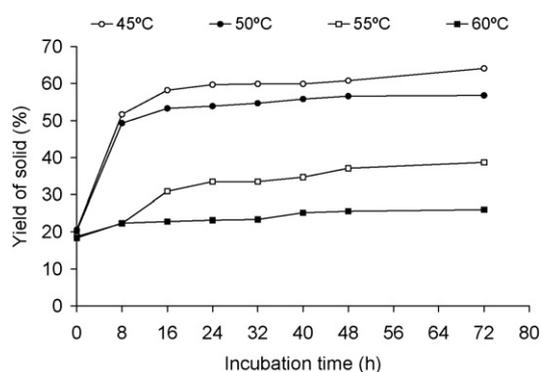


Fig. 2 – The influence of temperature on solid yield of yeast extract during autolysis of spent brewer's yeast.

temperature. The amount of solid released into liquid yeast extract from cells during autolysis considerably increased by 24 h of incubation when the process performed at temperatures of 45, 50 and 55 °C. After 24 h there was no appreciable increase. The influence of autolysis done at 60 °C was very slight. After 72 h of incubation period, the highest solid contents were obtained as 3.05% at 45 °C, and as 2.71% at 50 °C. The variance analysis (at $P < 0.01$) and Duncan multiple comparison tests also showed that the influence of elevated temperature on solid is significant.

Among the elevated temperatures, autolysis at 45 and 50 °C exhibited the highest yield of solid which reached 64.1 and 56.8%, respectively. Comparatively, yields obtained at 55 and 60 °C were lower than the above mentioned temperatures. The principal components of solids result from the release of intracellular components (Chae et al., 2001). They are mainly free amino acids (mainly glutamate), peptides, sugars and nucleotides (Stam et al., 1998; Erten and Tanguler, 2006). Supphantharika et al. (1997) stated that autolysis at 45–50 °C gave maximum yield. Results obtained in present study are in accordance with data presented by Supphantharika et al. (1997).

3.2. α -Amino nitrogen content and yield during autolysis

The effect of elevated temperature on α -amino nitrogen content during autolysis of spent brewer's yeast is shown in Fig. 3. There were marked differences in α -amino nitrogen content among the processes. The release of α -amino nitrogen

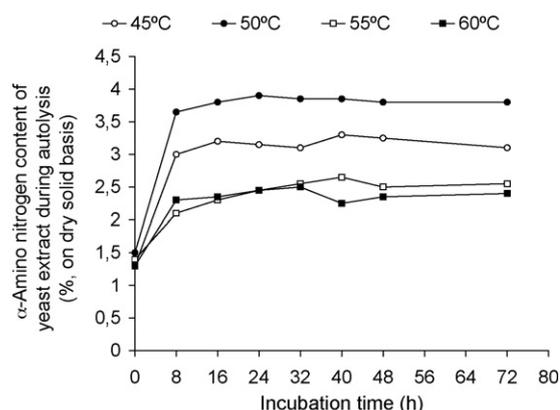


Fig. 3 – The influence of temperature on α -amino nitrogen content during autolysis of spent brewer's yeast.

into medium was found the highest at 50 °C. The highest amount of nitrogen reached 3.9% on dry solid basis in 24 h and then stayed constant during autolysis at 50 °C. Treatment at 45 °C gave maximum 3.2% of nitrogen on dry solid basis. Lower amount of α -amino nitrogen was determined with other treatments. In addition, significant differences were observed at four different temperatures with variance analysis ($P < 0.01$). According to the Duncan comparison test, the influences of 55 and 60 °C on amino nitrogen were not significant, but the differences between these temperatures and the temperatures of 45 and 50 °C were found significant ($P < 0.01$). The amount of α -amino nitrogen proposed by International Hydrolysed Protein Council in yeast extract produced by autolysis should be at least 3.5% on dry basis (Peppler, 1982). In our study, amino nitrogen content slightly exceeded minimum amount requested for autolyzed yeast extract, but did not fall within the range reported between 4.0 and 6.4% on dry solid basis for liquid yeast extract obtained from brewer's yeast by other studies (Gaudreau et al., 1999; Saksinchai et al., 2001).

With regard to yield of α -amino nitrogen as given in Fig. 4, the highest yield as 52% was observed at 50 °C and the lowest as 14.6% at 60 °C. These results suggest that there was an important breakdown of protein and peptides at 50 °C than other temperatures. It could be said that yeast proteases were inactive at 55 and 60 °C but active at 45 and 50 °C in agreement with the results of Supphantharika et al. (1997).

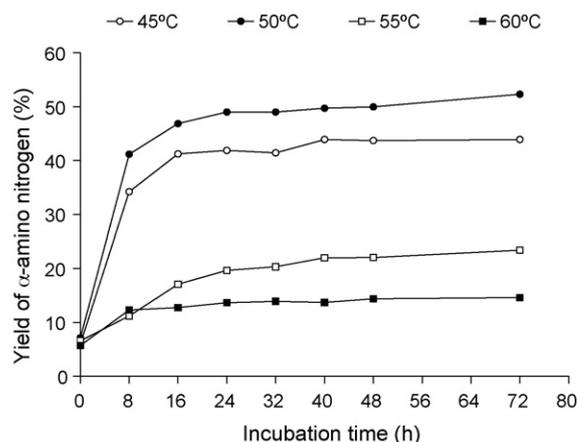


Fig. 4 – The influence of temperature on α -amino nitrogen yield during autolysis of spent brewer's yeast.

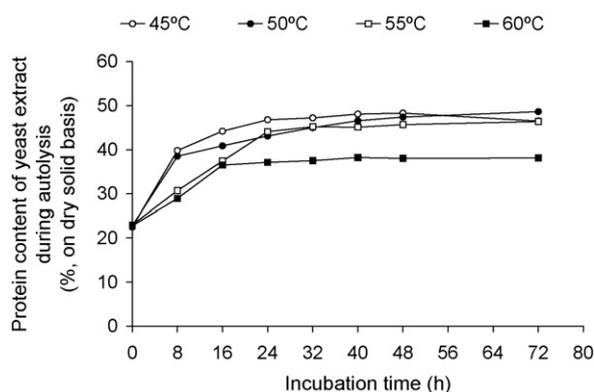


Fig. 5 – The influence of temperature on protein content of yeast extract during autolysis of spent brewer's yeast.

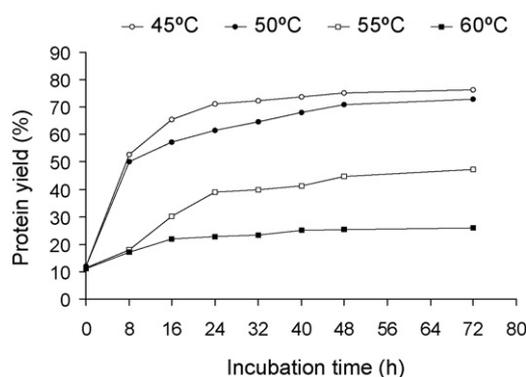


Fig. 6 – The influence of temperature on protein yield during autolysis of spent brewer's yeast.

Table 1 – Composition of yeast extract powder produced from spent brewer's yeast

	Liquid yeast extract		Yeast extract powder	
	Quantity (g)	%	Quantity (g)	%
Total amount	2612	65.3	51.09	1.95
Solid	64.51	2.47	46.75	91.5
Moisture	–	97.5	–	8.5
α -Amino nitrogen	2.45	3.8	1.82	3.9
Protein	27.54	42.7	21.31	45.6

3.3. Protein content and yield during autolysis

Protein was the principal compound in yeast extracts. Results presented in Fig. 5 show the influence of temperatures on protein content during autolysis. The level of protein on dry basis varied with autolysis temperature. It quickly increased within 24 h, furthermore, there was much lower increase until the end of the incubation time. The highest value was obtained as 48.7% on dry solid basis at 50 °C. There was slightly less release of protein from yeast cell as 46.5% at 45 and 55 °C than 50 °C. However, much less release was observed as 38% at 60 °C. Statistical analysis showed that a significant difference was obtained within the treatments ($P < 0.01$).

The hydrolysis of cellular proteins and release of degraded proteins, peptides and amino acids into yeast extract are main reactions of yeast autolysis (Lukondeh et al., 2003). Results in this experiment are in agreement with data published by several workers (Bridson and Brecker, 1970; Holder, 1977; Gaudreau et al., 1999; Chae et al., 2001; Saksinchai et al., 2001) who found 41.0–61.3% of protein content of yeast extract produced from brewer's yeast.

Protein yield of liquid yeast extract during autolysis is given in Fig. 6. It varied with elevated temperatures. The highest

yields were obtained as 76.4 and 73.1% at 45 and 50 °C, respectively. Increasing the temperature to 55–60 °C reduced the protein yield where the lowest yield was observed as 26% at 60 °C. Suphantharika et al. (1997), obtained the highest yield as 60% at 50 °C and the lowest yields as 26 and 30% at 55 and 60 °C, respectively.

3.4. Carbohydrate content

When the process was performed at temperatures of 45, 50, 55 and 60 °C, after 72 h of autolysis total carbohydrate was determined as 12.35, 13.9, 12.7 and 15.5%, respectively.

3.5. Production of yeast extract powder and sensory analysis

Yeast extract powder from debittered spent brewer's yeast was obtained at 50 °C for 24 h due to giving the highest content of α -amino nitrogen in this study (Section 3.2). Its composition is given in Table 1. Suphantharika et al. (1997) reported that solid and protein contents of spray dried yeast extract powder from spent brewer's yeast are 93.82 and 55.69%, respectively. In the present study, those amounts were observed slightly lower. In addition, drying yield was calculated as 72.46%, protein yield as 77.37% and α -amino nitrogen yield as 74.28%.

For sensory analysis, yeast extract powder produced from spent brewer's yeast was added to vegetable soup at the ratios of 0.5, 1.0, 1.5 and 2.0% (w/v). As a control, vegetable soup without yeast extract powder was used. Twelve panelists evaluated overall acceptability of vegetable soup with the addition of yeast extract powder at different ratios. The results are presented in Table 2. The control and samples which were added with 0.5 and 1.0% yeast extract powder ranked the best overall acceptance. Although sample with 0.5% yeast extract powder obtained the best result, significant differences were

Table 2 – Sensory analysis of vegetable soup with the addition of yeast extract powder at different ratios

	Samples ^a				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
The difference between points of classification ($P < 0.05$)	23 ^b	22 ^b	29 ^b	50 ^a	56 ^a

^a Sample 1: control; sample 2: addition of 0.5% yeast extract powder; sample 3: addition of 1.0% yeast extract powder; sample 4: addition of 1.5% yeast extract powder; sample 5: addition of 2.0% yeast extract powder. Different superscripts (a and b) indicate statistically significant differences at 5%.

not observed among them ($P < 0.05$). Samples with 1.5 and 2.0% yeast extract did not have an overall acceptance. All panelists stated that the addition of 1.5 and 2.0% yeast extract to vegetable soup led to unacceptable heavy taste. In addition, the samples with yeast extract powder were not notified as bitter coming from hops. Therefore it could be said that the debittering process in this study was sufficient.

4. Conclusions

The influence of autolysis at various controlled temperatures of 45, 50, 55 and 60 °C for the production of yeast extract from spent brewer's yeast by autolysis was investigated. Autolysis reaction time was ranged from 8 to 72 h. Among the experiments, autolysis at 50 °C for 24 h was desirable in terms of content and yield of α -amino nitrogen and protein. The levels of α -amino nitrogen and protein on dry solid basis were 3.9% and 48.7%, respectively, at 50 °C. Therefore, yeast extract powder was produced at that conditions for sensory analysis. It could be said that sample with the addition of 0.5% yeast extract powder showed the best mark but control and samples with the addition of 0.5 and 1.0% of yeast extract powder were not significantly different at $P < 0.05$.

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REFERENCES

- Amrane, A. and Prigent, Y., 1998, Effect of culture conditions of *Kluyveromyces marxianus* on its autolysis and process optimization, *Bioproc Eng*, 18: 383-388.
- Anon., 1990, In K. Hekrich (Ed.), *Official Methods of Analysis* (15th ed., pp. 1298-). Arlington, VA: Association of Official Analytical Chemists.
- Babayán, T.L. and Bezrukov, M.G., 1985, Autolysis in yeasts, *Acta Biotechnol*, 5(2): 129-136.
- Baker, C.D. 1991, *Recommended Methods of Analysis*. London: The Institute of Brewing p. 363.
- Barillere, J.M. and Benard, P., 1986, Exemples d'interpretation de resultats de degustation, *Connaiss Vigne Vin*, 20(3): 137-154.
- Berry, D.R. 1982, *The Biology of Yeast*. London: Edward Arnold Ltd. p. 57.
- Bridson, E.Y. and Brecker, A., 1970, Yeast extract, In Norris, J. R., & Ribbons, D. W. Eds. *Methods in Microbiology*. 3A (pp.229-295).
- Catley, B.J. 1988, In A. Yeast, I. Campbell & J. H. Duffus (Eds.), *Isolation and Analysis of Cell Walls*. (pp.289).
- Chae, H.J., Joo, H. and In, M.-J. 2001, Utilization of brewer's yeast cells for the production of food-grade yeast extract. Part I: Effects of different enzymatic treatments on solid and protein recovery and flavor characteristics, *Biores Technol*, 76: 253-258.
- Champagne, C.P., Barrette, J. and Goulet, J., 1999, Interaction between pH, autolysis promoters and bacterial contamination on the production of yeast extracts, *Food Res Int*, 32: 575-583.
- Conway, J., Gaudreau, H. and Champagne, C.P., 2001, The effect of the addition of proteases and glucanases during yeast autolysis on the production and properties of yeast extracts, *Can J Microbiol*, 47(1): 18-24.
- Dziezak, J.D. 1987, Yeast and yeast derivatives: applications, *Food Technol*, 41(2): 122-125.
- Erten, H., Tanguler, H., 2006, The production methods of yeast extract, *Feed Info News Service*, Web page: <http://www.feedinfo.com>, pp. 1-5 (dated 06/01/2006).
- Gaudreau, H., Champagne, C.P., Conway, J. and Degre, R., 1999, Effect of ultrafiltration of yeast extract on their ability to promote lactic acid bacteria growth, *Can J Microbiol*, 45: 891-897.
- Holder, M.G. 1977, Why yeast extracts are important, *Food Process Ind*, 38.
- Hough, J.S., Briggs, D.E., Stevens, R. and Young, T.W., 1982, (second ed.), *Malting and Brewing Science Hopped Wort and Beer* (vol. 2). London: Chapman and Hall p. 914.
- Joseph, R. 1999, Yeasts: production and commercial uses, In (Series Ed.) & K. Richard, Robinson, A. Carl, Batt, D. Pradip, & Patel (Vol. Eds.), *Encyclopedia of Food Microbiology*. vol. 3 (pp.2335-2341).
- Kim, J.-S., Kim, J.-W., Shim, W., Kim, J.-W., Park, K.-H., and Pek, U.-H. 1999, Preparation of flavor-enhancing yeast extract using a *Saccharomyces cerevisiae* strain with high RNA content, *Korean J Food Sci Technol*, 31(2): 475-481.
- Lukondeh, T., Ashbolt, N.J. and Rogers, P.L., 2003, Evaluation of *Kluyveromyces marxianus* as a source of yeast autolysates, *J Ind Microbiol Biotechnol*, 30: 52-56.
- Munch, P., Hofmann, T. and Schieberle, P., 1997, Comparison of key odorants generated by thermal treatment of commercial and self-prepared yeast extracts: influence of the amino acid composition on odorant formation, *J Agr Food Chem*, 45: 1338-1344.
- Nagodawithana, T. 1992, Yeast-derived flavors and flavor enhancers and their probable mode of action, *Food Technol*, 138-144.
- Nagodawithana, T. 1994, Savory flavours, In (Series Ed.) & A. Gabelman (Vol. Eds.), *Bioprocess Production of Flavour Fragrance and Color Ingredients*. (pp.135-168).
- Ozdamar, K. 1999, *Paket Programlar I'le I statistiksel Veri Analizi*. Eskişehir: Kaan Kitabevi p. 535.
- Peppler, H.J. 1982, *Yeast extracts*. *Economic Microbiology*, vol. 7, London: Academic Press pp. 293-312.
- Reed, G. and Nagodawithana, T.W., 1991, *Yeast Technology* (second ed.), New York: Avi Publishing p.765.
- Roessler, E.B., Pangborn, R.M., Sidel, J.L. and Stone, H., 1978, Expanded statistical tables for estimating significance in paired preference, paired-difference, duo-trio and triangle tests, *J Food Sci*, 43: 940-943.
- Saksinchai, S., Supphantharica, M. and Verduyn, C., 2001, Application of a simple yeast extract from spent brewer's yeast for growth and sporulation of *Bacillus thuringiensis* subsp. *kurstaki*: a physiological study, *World J Microbiol Biotechnol*, 17: 307-316.
- Shotipruk, A., Kittianong, P., Supphantharica, M. and Muangnapoh, C., 2005, Application of rotary microfiltration in debittering process of spent brewer's yeast, *Biores Technol*, 96: 1851-1859.
- Sombutyanuchit, P., Supphantharika, M. and Verduyn, C., 2001, Preparation of 5'-GMP-rich yeast extracts from spent brewer's yeast, *World J Microbiol Biotechnol*, 17: 163-168.
- Sommer, R. 1998, Yeast extract: production, properties and components, *Food Aust*, 50(4): 181-183.
- Stam, H., Hoogland, M. and Laane, C., 1998, Food flavours from yeast, In Wood, J. B. (Ed.). *Microbiology of Fermented Foods*. vol. 2 (pp.505-542).
- Stewart, G.G. and Russell, I., 1998, *An Introduction to Brewing Science and Technology: Brewer's Yeast*. London: The Institute of Brewing p.108.
- Supphantharika, M., Varavinit, S. and Shobsngob, S., 1997, Determination of optimum conditions for autolyzed yeast extract production, *Asian J Sci Tech Devel*, 14(1): 21-28.
- Walker, G.M. 1998, *Yeast Physiology and Biotechnology*. Chichester: John Wiley and Sons p. 350.