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Research Article

Acceleration of Yeast Autolysis by Addition of Fatty Acids, Ethanol and Alkaline Protease

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Background: Autolysate products from yeast origin are very interesting for food, feed, cosmetic, pharmaceutical, and fermentation industries. The lysis process greatly influences the quality and efficiency of the final autolysates.

Objectives: Here, we have compared four lysis methods based on autolysis, plasmolysis (with ethanol 1.5% (v/v) and coconut fatty acids 1% (w/w)) and hydrolysis (with alkaline protease 0.4 % (v/w)) on degrading the baker's yeast *Saccharomyces cerevisiae*.

Materials and Methods: The efficiency of processes was evaluated according to the recovered solid and protein contents, release of intracellular materials, cell viability, microscopy imaging, degree of hydrolysis and electrophoresis studies.

Results: Results showed that the increased recovered solids and proteins, as well as a higher degree of hydrolysis (DH) were obtained for the enzymatic hydrolyzed cells using alkaline protease. SDS-PAGE analysis also confirmed the results. Further, functionality of the final products by agglutination test showed that the hydrolyzed cells could effectively bind pathogenic bacteria compared to the other cell lysates.

Conclusions: In conclusion, this work provides adequate evidence for efficiency of alkaline protease for producing the nutritional cell lysates from baker's *S. cerevisiae* used in food, feed, cosmetic, and pharmaceutical applications. Moreover, this was the first report on using coconut fatty acids and alkaline protease in lysis of baker's yeast.

Keywords: Autolysis; Enzymatic Hydrolysis; Plasmolysis; *Saccharomyces cerevisiae*; Yeast Autolysate

1. Background

Yeast autolysate is being used as a rich source of nutrients such as proteins, carbohydrates, vitamins, fiber, nucleotides, minerals and other micronutrients (1). It is widely utilized to enhance the color and flavor in food production, and additionally has found many applications in animal feed, cosmetic, pharmaceutical, and fermentation industries (2). The autolyzed and hydrolyzed yeast products contained both the soluble (extract) and insoluble (cell wall) fractions of yeasts. The cell wall fraction can be used in food industry as dietary fibers, fat replacers and emulsifiers due to their holding water and oil binding capacities. Their incorporation in food helps in lowering blood sugar and cholesterol. The soluble fraction (extract) of the cells, providing nucleotides, nutritional and antioxidant peptides, and it is also a rich source of vitamins. There are many industrial food applications for yeast auto lysates or hydrolysates, or their soluble or insoluble fractions. For example, functional beverage, bread, ready to serve soups, snack foods and variety of sauces and other food products. Among all possible applications, yeasts have been commonly used in animal feed as protein supplements for more than 100 years (3). Based on many studies and experiences, feed supplements with yeasts are investigated not only for their protein,

mineral, lipid and vitamin-rich compositions, but also for their bioactive effects on animals health and immunity (3). Accordingly, yeast derivative products have found increased demand for novel feed ingredient products mainly due to their health positive cellular components as immune modulators and their beneficial effects on growth, reproduction and mucosal surfaces in animals (4).

The autolysis process is of great value to biochemical researchers, as well as industrialists since it greatly influences the quality and efficiency of the final auto lysate product (odor, color, hydrolysis degree, bioactivity, bioavailability, bio absorption, and so on) based on special applications. The term "autolysis" was firstly defined by Salkowski (1875) as a lytic event in cells, which is induced by the action of the cell's own intracellular enzymes (5). Autolysis is an irreversible process, which naturally occurs in the non-viable yeasts (at the end of the stationary phase of growth) by the yeast' hydrolase enzymes, and consequently formation and releasing low molecular weight substances into the extracellular space (6,7). Natural autolysis is a slow process, thus generally takes more than one week. Accordingly, scientists usually try to accelerate autolysis in yeasts by some famous inducers and/or other biochemical and physical-based methods. Accordingly, the induced autolysis has been widely developed for industrial applications (5). For example, under a process called "plasmolysis", by using some accelerators (as plasmolysers) such as sodium chloride, toluene, ethyl acetate and ethanol can efficiently accelerate autolysis in yeasts (1). The nature of the solvent or chemical, its concentration and the duration of the process can actually influence the efficiency of the final product (8). Natural hydrolytic enzymes (proteinases, glucanases, and nucleases) are responsible for autolytic degradation of most yeast cell polymers during autolysis (9). Although yeast proteins are solubilized and hydrolyzed during the autolysis process by the natural yeast enzymes, using exogenous enzymes can significantly improve the proteolytic system inside the cells and increase the final yield (5,10).

Proteases cleave peptide bonds of proteins and they are extracted from different sources such as plants, animals, and microbes. They are classified as exopeptidases and endopeptidases based on their sites of action (11). The choice of enzymes for the proteolytic reaction is dependent to the protein source and the degree of hydrolysis. Microbial proteases have dominated applications in industrial sectors compared to plant and animal proteases in particular in food industries as they are more stable than plant and animal enzymes (12). Proteases from bacteria and fungi are produced on a large scale and usually only require simple purification steps, which can be used for an industrial application (13). Fungal proteases belonging to the genera *Aspergillus, Penicillium, Rhizopus, Mucor* and *Humicola* are being used for hydrolyzing protein and other components of soy bean and wheat in soy sauce production. The Fungal proteases of *Aspergillus* sp., in particular are known for their capacity to secrete high levels of enzymes in their growth environment (14). Among the industrial bacterial sources, *Bacillus* sp. are the most popular microorganisms for production of alkaline proteases in a relatively shorter period of time, exhibiting high substrate specificity and catalytic activity. In contrast, the produced proteases by plants and animals are more labor-intensive and time-consuming and the number of industrially employed enzymes of plant origin is still small (15). Besides, the production of proteases from animal sources, in particular from pigs, is insufficient to fulfill the industrial demand worldwide (16). The data for comparison of different sources of enzymes has been summarized in **Table 1**.

2. Objectives

The aim of the present work was to investigate the effect of three treatments including two plasmolysis methods, using ethanol and PALMERA® B1210 coconut fatty acids, and a hydrolysis process, by alkaline protease as a proteolytic enzyme, on accumulation of lysate products of the commercial strain *Saccharomyces cerevisiae*. Autolysis was also used as a basic method for cell lysis.

3. Materials and Methods

3.1. Chemicals and Bacterial Strains

The dried baker's yeast *S. cerevisiae* (PTCC 5269) used as the original substrate was obtained from Razi Yeast and Alcohol Co., Iran. Alkaline protease (EvaTase 180G, India) was purchased. PALMERA® B1210 coconut fatty acids were purchased from KLK OLEO (Malaysia). 2,4,6-Trinitrobenzene sulfonic acid (TNBS), 3,5-Dinitrosalicylic acid (DNS), and Trypan Blue were purchased from Sigma Aldrich (St. Louis, MO, USA). Tris, glycine, sodium dodecyl sulfate (SDS), and bacto agar were obtained from Bio Basic Inc. (Markham, ON,

Table 1. Classification of enzymes used for industrial applications

No.	Source	Example	Production Advantage/Disadvantage	Applications
1	**Microbial Proteases** (Alkaline, Acidic, Neutral Proteases)	- Serine proteases - Pepsin - Aspartic proteases - Neutrases - Thermolysin	- High yield of production - Rapid process - Limited space requirement - Easy genetically manipulation - Cost-effectiveness - A wide range of physical and chemical characteristics	- Detergent and leather - Pharmaceuticals - Textile - Protein hydrolysis - Food industry and baking - Feed industry - Brewing industry - Beverage industry
2	**Plant Proteases**	- Bromelain - Keratinases - Ficin - Papain - Actinidin - Zingibain - Cardosins - Cucumisin - Oryzasin	- Time-consuming process	- Dairy processing - Meat tenderization - Protein hydrolysis - Baking industry
3	**Animal Proteases**	-Pancreatic - Trypsin - Pepsin - Chymotrypsin - Renin - Carboxylpeptidases - Aminopeptidases	- Insufficient to fulfill the industrial demand worldwide	No industrial application

Canada). DNA gel stain was purchased from Pishgam Biotech (Tehran, Iran). Ethanol, bovine serum albumin (BSA), potassium bromide (KBr) and all other chemicals were obtained from Merck (Darmstadt, Germany).

Escherichia coli ATCC 35218 were provided by Faculty of Veterinary Medicine, University of Tehran, Iran (**Table 2**). The strain was transferred to Brain Heart Infusion broth (BHI) (Merck, Darmstadt, Germany) and sub-cultured in the same medium. Cells were incubated overnight at 37 °C under aerobic conditions, then transferred to fresh medium and harvested for 36 h.

3.2. Autolysis, Plasmolysis and Hydrolysis Preparations

Autolysis Process. 15 g dried yeast powder (dry matter content of 94%) was placed in a 250 mL Erlenmeyer flask, and water was added (until a final volume of 100 mL is reached). pH was adjusted to 5.5 with HCl and the autolysis was conducted at 55 °C with agitation at 150 rpm for 36 h.

Plasmolysis I Process. 15 g dried yeast powder was placed in a 250 mL Erlenmeyer flask, then water was added (until a final volume of 100 mL is reached). Ethanol (1.5% (v/v)) was added and pH was adjusted to 5.5 with HCl. The plasmolysis was conducted at 55 °C with agitation at 150 rpm for 36 h.

Plasmolysis II Process. 15 g dried yeast powder was placed in a 250 mL Erlenmeyer flask, then water was added (until a final volume of 100 mL is reached). PALMERA® B1210 coconut oil (1% (w/w)) was added and pH was adjusted to 5.5. The plasmolysis was conducted at 55 °C with agitation at 150 rpm for 36 h.

Hydrolysis Process. 15 g dried yeast powder (dry matter content of 94%) was placed in a 250 mL Erlenmeyer flask, then water was added (until a final volume of 100 mL is reached). Alkaline protease (0.4% (v/w) was added and pH was adjusted to 7.0 with NaOH. The hydrolysis was conducted at 55 °C with agitation at 150 rpm for 36 h.

After all treatments, the flasks were boiled at 100 °C for 5 min to terminate the lysis processes. Besides, the clear supernatants were collected after centrifugation (8,000 × g for 20 min) and stored at -20 °C for further analysis.

3.3. Analytical Methods

Solid and Protein Contents. The total solid contents of each lysate supernatant (after centrifugation at 8,000 ×

Table 2. Characterization of total ash, total released carbohydrate, and pH changes values provided by different lysis methods. Data for the carbohydrate content was provided from the supernatant of the centrifuged lysates. Plasmolysis and hydrolysis were performed with 1.5% (v/v) ethanol (Plasmolysis I), 1% (w/w) PALMERA® coconut fatty acids (Plasmolysis II) and hydrolysis with 0.4% (v/w) alkaline protease.

	Time (h)	**24**	**36**
Autolysis	pH	5.92	4.98
	Ash (%)	-	5.8
	Carbohydrates ($mg.mL^{-1}$)	1.25	1.5
Plasmolysis 1	pH	6.08	5.06
	Ash (%)	-	5.7
	Carbohydrates ($mg.mL^{-1}$)	1.13	1.66
Plasmolysis 2	pH	5.94	4.79
	Ash (%)	-	5.9
	Carbohydrates ($mg.mL^{-1}$)	1.25	1.56
Hydrolysis	pH	6.0	4.78
	Ash (%)	-	6.3
	Carbohydrates ($mg.mL^{-1}$)	1.88	2.63

g for 20 min), as the recovered solid, were determined following drying the samples at 100 °C (Sanat Ceraam MA, Tehran, Iran) until a constant mass was attained. The yield of solid was expressed as a percentage of the solids recovered in the supernatant with respect to the total yeast solid (supernatant and pellet). The soluble protein contents ($mg.mL^{-1}$) in the lysate supernatants were also determined by the Biuret method with BSA as standard (8).

Absorbance Measurements. For absorbance measurements at 260 (A_{260}) and 280 (A_{280}) nm, the lysate supernatants were taken during each lysis process (24 and 36 h). The supernatants were diluted using 25 mM Tris-HCl buffer, pH 8.0, and the buffer was used as blank. All absorbances were read using a microplate spectrophotometer (µQuant, BioTek, USA).

Ash Content, Carbohydrate Content, and pH Measurement. Ash content of the cell lysates was determined by the standard method (18). The total amount of released carbohydrates in the lysate supernatants was determined using reducing sugars assay with DNS (19). The yield of released carbohydrates was expressed as a percentage of the carbohydrates recovered in the supernatant with respect to the original lysate solid. In addition, pH changes were checked with a pH meter (Metrohm, model 913, Switzerland).

Degree of Hydrolysis (DH), SDS-PAGE Analysis and Nucleic Acids Detection. The TNBS method was used to measure DH as described by Adler-Nissen (20). The TNBS reagent consisted of 0.1% (w/v) TNBS in water. All samples and standard solutions were prepared in 1% (w/v) SDS. Total soluble protein fractions of the lysate supernatants were separated using 15% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) under a reducing condition by the method of Laemmli (21). Live yeast cells were also fully disrupted by agitating glass beads following their sonication (15 cycles consisting of 40 s of sonication and a 20 s break at 0 °C). The suspension was centrifuged (8000 × g for 15 min) and the supernatant was run as control. Further, the nucleic acid content of cell lysates was detected using 1% agarose gel. 5 µL of each cell lysate suspension was run on a 1% agarose gel and visualized using a UV trans illuminator (SMOBio B-BOX™) after staining with DNA Safe Stain Dye.

Cell Viability Analysis and Microscopic Imaging. Yeast cell viability was checked after 36 h of the lysis by harvesting yeast lysate suspensions on yeast extract-

peptone-dextrose (YPD) agar plates at 30 °C. Moreover, to check microbial contaminations, the samples were cultivated on Luria-Bertani (LB) agar plates followed by incubation overnight at 37 °C. As control, the plate with the live yeast cells were inoculated. The morphology and viability of the yeast suspensions were further assessed microscopically. Preparations were stained with Trypan Blue 1%. Microscopic observations were carried out under a light microscope (Eclipse TE2000-S, Nikon Inc., Tokyo, Japan) at 400 × magnification.

FTIR Analysis. The Fourier transform infrared spectroscopy (FTIR) using the KBr-disk method was recorded on a FTIR spectrometer (PerkinElmer Spectrum One, PerkinElmer Inc., Waltham, MA, USA) in the range of 800 to 4000 cm^{-1} with 32 scans and a resolution of 4 cm^{-1}.

Agglutination Assay. The ability of the yeast lysates to bind pathogenic bacteria was checked by an agglutination test (22). Accordingly, 20 µL of the cell lysate suspension were mixed with 1500 µL of cultured *Escherichia coli* ATCC 35218 bacteria (OD_{690}= 0.7). Agglutination was observed by eye and subjectively evaluated by checking the extent of the formed clumps 30 s after mixing the microorganisms with the cell lysates.

4. Results

4.1. Recovery of Solids and Proteins

The effect of different cell treatments on the content of released solids and proteins have been shown in **Figure 1A, B**. In autolyzed cells, the autolysis was accelerated by a high temperature (55 °C) and a low pH (5.5). The recovered soluble solids and proteins in the autolysates

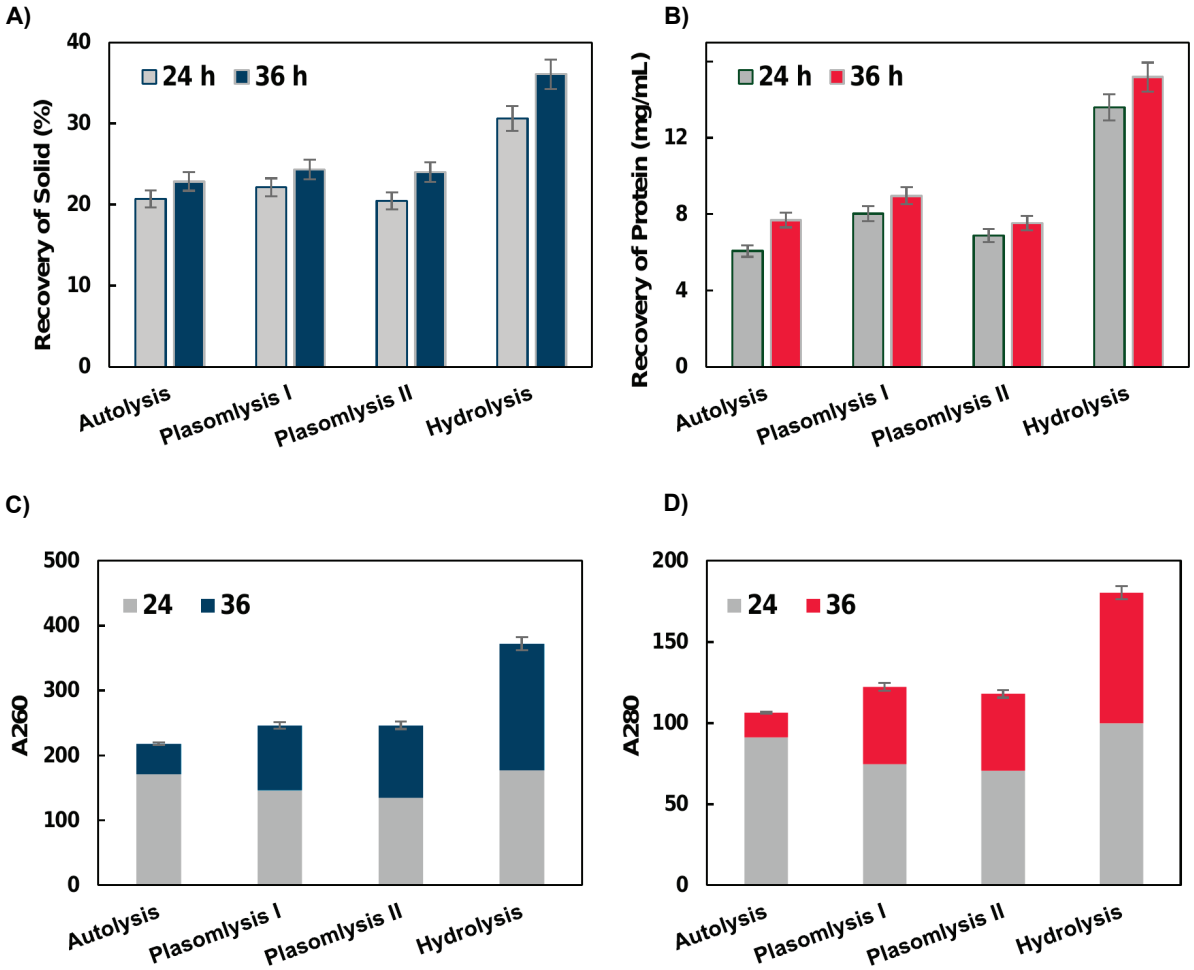


Figure 1. A, B) The soluble solids and proteins content of yeast extract during lysis processes. The recovery of solids **A)** and proteins **B)** during autolysis (55 °C, pH 5.5), and induced autolysis with 1.5% (v/v) ethanol, 1% (w/w) PALMERA® coconut fatty acids, and hydrolysis with 0.4% (v/w) alkaline protease. **C, D)** Monitoring cell leakage of intracellular substances.

reached to about 23% and 7.67 mg.mL^{-1} respectively, after 36 h (**Fig. 1A,B**). In the plasmolyzed cells, either by ethanol (plasmolysis I) or by PALMERA® coconut fatty acids (plasmolysis II), the released solids and proteins slightly increased during time compared to the autolyzed cells. Considerably, in hydrolyzed cells by alkaline protease, the content of solid and protein amounted to about 36% and 15.18 mg.mL^{-1}, respectively, after 36 h of the hydrolysis process (**Fig. 1A,B**).

4.2. Monitoring Releasing Intracellular Substances

The changes in absorbances at 260 nm (**Fig.1C**) and 280 nm (**Fig.1D**) were determined to monitor the leakage of intracellular contents, in particular nucleic acids, nucleotides, proteins and peptides, outside the cells. According to **Figure 1C, D**, the changes in A_{260} and A_{280} were insignificant between autolyzed, and plasmolyzed cells either by 1.5% ethanol or 1% PALMERA® coconut oil. In comparison, hydrolysis using alkaline protease showed a higher A_{260} and A_{280} revealing a higher degree of leakage of intracellular substances during hydrolysis process (**Fig. 1C, D**).

4.3. Ash and Carbohydrate Contents and Monitoring pH Changes

The content of total ash, and the released soluble carbohydrates have been compared in **Table 2**. The amounts of released carbohydrates increased during time and the highest value was obtained for the hydrolyzed cells (2.63 mg.mL^{-1}). pH changes were also monitored for the cell lysates. The initial pH value of autolyzed and plasmolyzed cells were adjusted to 5.5 and data showed that after 24 h of lysis, it increased to about 6.0 for all samples, followed by a drop to approximately 1 unit after 36 h. For the hydrolyzed cells, pH was initially adjusted to 7.0, and it reached to about 6.0 after 24 h of the hydrolysis process. Then after 36 h, the hydrolyzed cells exhibited a significant decline in pH to 4.7 (**Table 2**).

4.4. Monitoring Cell Viability and Morphology

Further, the viability of yeast cells in the cell lysates was checked after cultivating suspensions of the lysates on YPD agar. Results showed that there were no viable colonies in autolyzed, plasmolyzed and hydrolyzed cells after 36 h of each lysis process (**Supplementary Fig. S1 A**). Moreover, the microbial test was performed and it was shown that there were no any viable bacterial cells on LB agar plates for the cell lysates (**Supplementary Fig. S1 B**). The light microscopy imaging before and after staining with TB 1% revealed that the cell lysates were damaged after 36 h of lysis and there wasn't any intact cell (**Fig. 2**). In comparison, the intact cells, as control, showed a clear cytoplasm.

4.5. Quantification of DH by TNBS and SDS-PAGE Analysis, and DNA Hydrolysis

After 36 h hydrolysis, DH values of 20.3%, 18.8%, 17.2% and 14.15% were calculated for the hydrolyzed (with 0.4% alkaline protease), plasmolyzed I (with 1.5% ethanol), plasmolyzed II (with 1% coconut fatty acids), and autolyzed cells, respectively (**Fig. 3A**). According to SDS-PAGE results, smaller protein fragments and peptides were detected in all samples (lanes 1-4 in **Fig. 3B**). The content of small peptides (lower than 14 kDa) seemed to be higher in the hydrolyzed cells compared to the other cell lysates (**Fig. 3B**, lane 4). In live yeast cells, proteins mostly presented with higher molecular weights (**Fig. 3B**, lane 5). Hydrolysis of nucleic acid contents of cell lysates were also detected by 1% agarose gel (**Fig. 3C**). The results indicated that the hydrolyzed cells by alkaline protease (lane 4 in **Fig. 3C**) showed a higher degree of nucleic acid hydrolysis.

4.6. FTIR Analysis

The FTIR spectra of the lysates have been shown in **Figure 3D**. In all samples, the spectrum regions characteristic of polysaccharides (950–1200 cm^{-1} and 750–950 cm^{-1} were monitored (23). Moreover, the presence of proteins and chitin (the bands at 1600–1800 cm^{-1}) was confirmed (24,25). The bands in the spectral region of 3650–3200 cm^{-1} and 2985–3015 cm^{-1} mainly represent OH stretching vibration that are caused by hydroxyls and lipids, respectively (26).

4.7. Agglutination of Yeast Cell Lysates

Results showed that only hydrolyzed cells were able to bind *E. coli* and form clumps with the bacteria (**Fig. 4d**). The other cell lysate products indicated a very weak degree of agglutination (**Fig. 4A,B,C**).

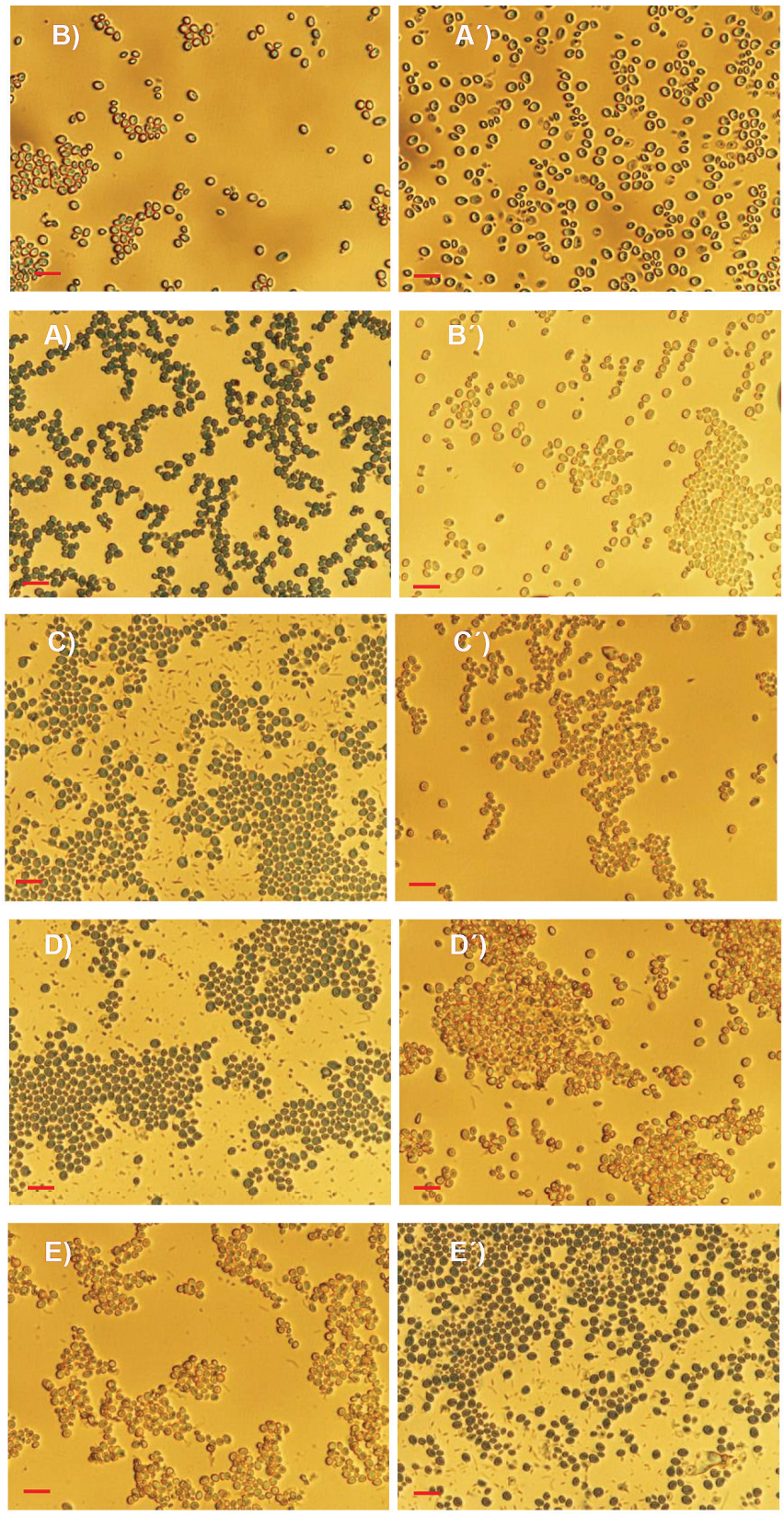


Figure 2. Optical micrographs of the lysed yeast cells. **A)** Intact, **B)** autolyzed, **C)** plasmolyzed I, **D)** plasmolyzed II and **E)** hydrolyzed cells before **(A-E)** and after **(A´-E´)** staining with Trypan blue 1% (magnification × 400). Bar: 20 µm.

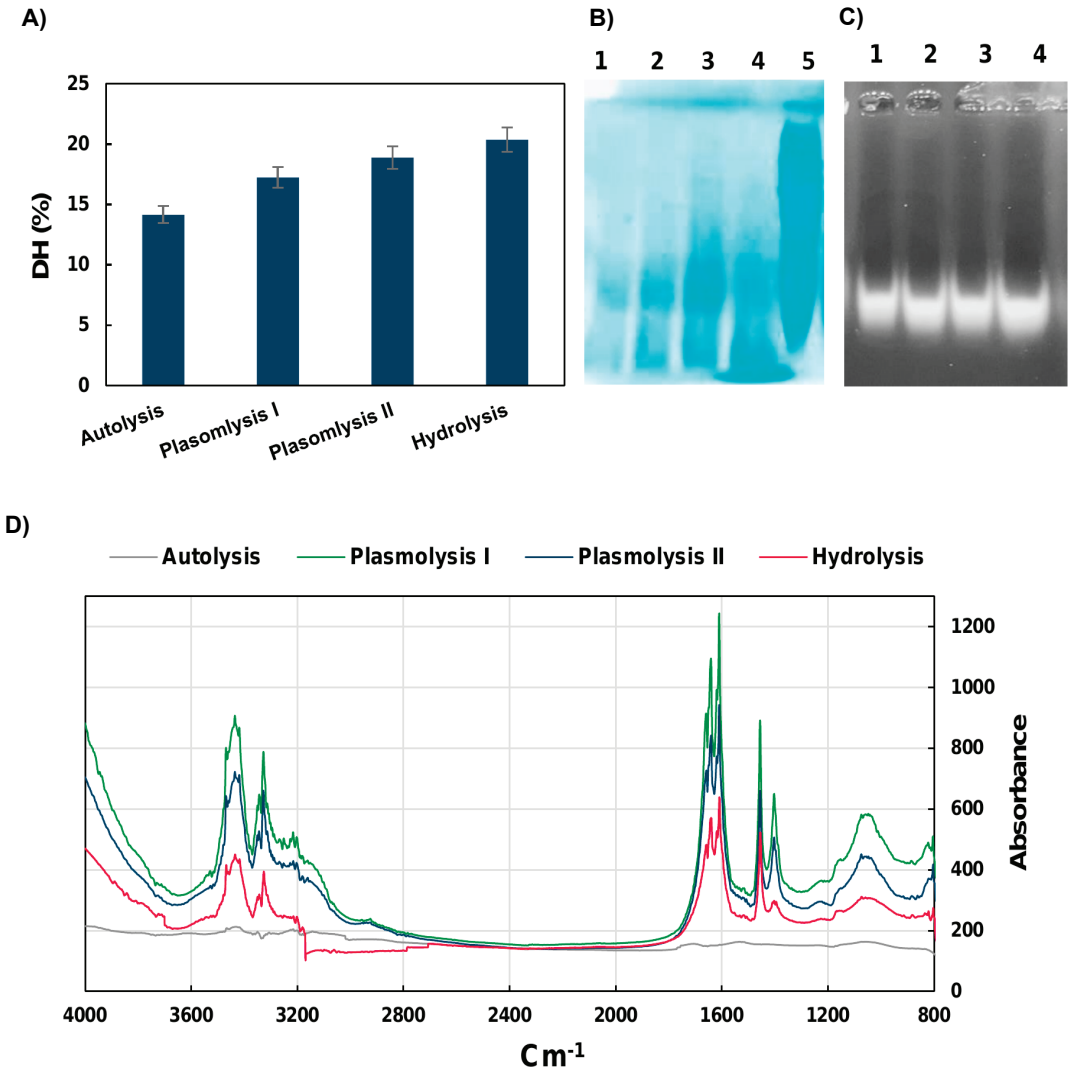
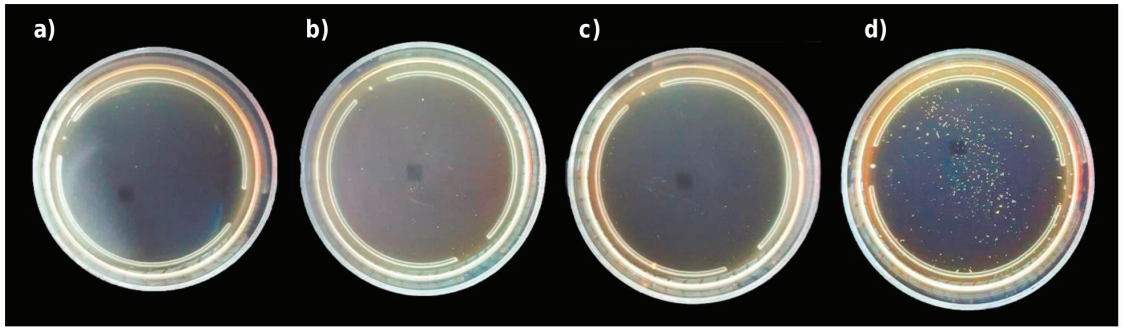


Figure 3. DH (%) values **(A)** and analysis of hydrolyzed protein and nucleic acid contents using 12.5 % SDS-PAGE **(B) and** 1% agarose gel **(C) f**or the autolyzed, plasmolyzed, and hydrolyzed cells. **A)** DH values were determined by the standard TNBS assay. Values are means of duplicate determinations. Error bars show standard deviations. **B)** SDS-PAGE analysis of lysates. Lane 1: the autolyzed cells, Lane 2: the plasmolyzed cells I, Lane 3: the plasmolyzed cells II, Lane 4; the hydrolyzed cells, and Lane 5; the intact cells as control. **C)** The nucleic acid analysis of autolyzed (1), plasmolyzed cells I (2), plasmolyzed cells II (3), and hydrolyzed cells (4). **D)** FTIR spectra of the dried autolyzed cells (gray line), plasmolyzed cells I (green line), plasmolyzed cells II (bue line), and hydrolyzed cells (red line).

5. Discussion

Processing of yeast cells results in increased levels of available soluble protein and nutrient contents in the yeast lysate powders as well as high levels of flavoring components used for food, feed, cosmetic and pharmaceutical industries. The demand for using yeast derivative products as animal feed additives and food supplements is greatly increasing as the concerns associated with providing other protein sources and plant-based meals have been increased (27). Aquaculture feeds, for example, are generally formulated with various types of ingredients to improve the normal biological functions of fishes, immune system, growth, and reproduction (4). Yeast cells contain about 40–55% crude proteins, and also other bioactive components including β-glucan and mannanoligosacharids (MOS) beneficial for gut health, immune responses, and fish growth (28,29). Moreover, yeast extracts are used to



Samples	a	b	c	d	
	Autolysis	Plasmolysis I	Plasmolysis II	Hydrolysis	Control*
E. coli clumps	+	+	+	+++	-

* BHI medium

Figure 4. Agglutination analysis of pathogenic bacteria by different cell lysates. Agglutination was checked by eye after mixing *E. coli* ATCC 35218 bacteria with the autolyzed cells (a), plasmolyzed cells with 1.5% ethanol (b), plasmolyzed cells with 1% PALMERA® coconut fatty acids (c), and hydrolyzed cells with 0.4% alkaline protease (d).

enrich pet food and recommended as an anti-obesity functional feed source for dogs (30). In these cases, releasing the maximum content of yeast ingredients during lysis processes is important to access the final product with the highest bioavailability.

In comparison, due to the rather high nucleic acid content (7–12% dry weight), mainly ribonucleic acid, the consumption of yeasts in food products is generally limited. Ribonucleic acid is metabolized by uricase to uric acid in humans and may increase the risk of developing kidney stones or gout (31). Accordingly, the nucleic acids content should be less than 3% in yeast derivative products before they can be used in human diets. Using enzymes during processing can reduce the nucleic acid contents, and the produced molecules can be converted into flavoring agents (31). In addition, before processing, yeasts may have low digestibility and a mild taste. Then, yeast processing is necessary for producing a product with a low nucleic acid content, high digestibility, and strong flavoring properties. Moreover, as the yeast cells are disrupted or degraded by enzymes, heat or chemicals during the lysis process, there are no any viable cells, and the risk of opportunistic infection is minimized for human.

The yeast cell has a very thick cell wall, which is relatively resistant to digestive enzymes or chemicals (32). Thus, the choice of cell disruption is critical for releasing the intracellular components and the extraction of the cell wall proteins as well. We have previously checked the efficiency of ethyl acetate as a plasmolyser and Alcalase as a hydrolytic enzyme on lysis of the baker's yeast *Saccharomyces cerevisiae* (1). Our data indicated that Alcalase was an efficient enzyme to degrade the yeast cells. Further, in the present work, we have compared three other lysis methods on degrading the baker's yeast including plasmolysis, with ethanol and coconut fatty acids, and enzymatic hydrolysis by alkaline protease (EvaTase) with respect to autolysis as the basic method. Although there have been several studies in which the commercial lytic enzymes have been used (5,10), the enzymatic hydrolysis of yeasts by EvaTase has not been reported to date. Besides, it has been shown that using fatty acids having from 4 to 14 carbon atoms and their mono-, di- and tri-glyceride esters, in particular caprylic and capric acids, could significantly enhance the rate of autolysis in *Candida utilis*, *Saccharomyces cerevisiae*, *Saccharomyces fragilis* and *Saccharomyces carlsbergensis* (33). These compounds like the other organic solvents such as ethanol, ethyl acetate, toluene and chlorinated hydrocarbons function by the labilization of the cell membranes and supporting the autolysis. So, here, we used the coconut oil as a rich source of fatty acids (4-14 carbons) to induce the autolysis in the baker's yeast. It

is noteworthy to mention that this is the first report on using EvaTase and coconut fatty acids in hydrolysis of baker's yeast.

Based on the analysis of the recovery of solids and proteins, as well as changes in absorbance at 260 nm and 280 nm (**Fig. 1**), the release of solids, proteins and nucleic acids into the extracellular space significantly increased during the first 24 h of the incubation. Activation of the proteolytic yeast enzymes are responsible for starting autolysis in yeasts, breakage of the intracellular as well as the cell wall proteins, and finally disturbing the cell membranes and releasing the cell contents into the surrounding medium (1). In hydrolyzed cells by alkaline protease, the content of solid and protein were 1.5 (for solids) and 1.9 (for proteins) times higher than the amount obtained for the autolysate, respectively. These data confirmed that the release of soluble solids, proteins and nucleotides was more rapid and effective during the enzymatic hydrolysis by alkaline protease.

pH decreased for all samples after 36 h (**Table 2**). A decreased pH for the autolyzed cells was previously reported by others as a result of the releasing acidic substances, such as nucleic acids or intracellular hydrolyzed products from the inside of cells (34). Monitoring the cell viability was further performed with Trypan blue 1% (**Fig.2**). TB is a diazo dye used to quantify live cells by coloring dead cells selectively. Because live cells have an intact cell membrane, TB cannot interact with cells unless the membranes are damaged and then enter the cytoplasm. Therefore, all the cells that exclude the dye are considered viable. In contrast, in dead cells, TB passes through the damaged cell membrane and enters the cytoplasm, which creates a distinctive blue color readily observed by a light microscope (35). Here microscopy imaging clearly indicated that the cells were damaged after 36 h of lysis.

The DH values obtained for the four methods were different, with values for hydrolysis > plasmolysis II > plasmolysis I > autolysis (**Fig. 3A**). Electrophoresis analysis confirmed stronger hydrolysis of protein fragments and nucleic acid into small peptides and nucleotides in the hydrolyzed cells (**Fig. 3B, C**). The hydrolysis of DNA and RNA generally occurs from the activity of the yeast intracellular nucleases during the lysis process. DNases are mainly involved in the autolytic and hydrolytic degradation and the diversity of the degradation products suggests that a wide range of DNase, nucleotidase and nucleosidase enzymes are probably active in this process (36). The FTIR spectra showed that there exist very slight differences between the spectra of different cell lysates (**Fig. 3D)** as it was also previously reported by others (24,37).

The cell wall of yeast has the ability of binding enteropathogenic bacteria such as *E. coli* and *Salmonella* spp., and therefore is able to prevent intestinal attachment of these pathogens and subsequently minimize their colonization in the animals' gut (38,39). β-glucans (20–55%) and MOS (also as mannoproteins) (30–60%) are two major components of the cell wall of *S. cerevisiae* (37). MOS are the responsible substances, which are known for their ability to bind pathogens (40). Our data from agglutination test indicated a strong binding to bacteria and form visible clumps for the hydrolyzed cells (**Fig. 4**).

6. Conclusion

In conclusion, the lysis methods reported here were used to produce nutritional yeast cell lysates from the baker's *S. cerevisiae*. The choice of yeast lysis process appeared to be important as it affects the degree of hydrolysis and providing more active and nutritional substances from the yeast. As a point, enzymatic hydrolysis of yeast cells by the alkaline protease enzyme (EvaTase) has not been reported to date. Moreover, this was the first report for using coconut fatty acids as a plasmolyser to induced autolysis in yeasts. Totally, the hydrolysis process by alkaline protease used in this study can be applied to produce yeast lysates from *S. cerevisiae* in a large scale, and in a safe and easy way, which has great potential in food, feed, cosmetic, and pharmaceutical applications.

Author's Contributions

ZT proposed the original idea and designed the experiments, analyzed data, wrote and revised the manuscript. RN was involved in performing the experiments and analyzing data. MK contributed to performing the experiments and analyzing data. HG was involved in the bacterial culture and agglutination test. RHS supervised the experiments, provided intellectual input, and revised the manuscript.

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