## Production of Single- Cell Protein from some Fruit peels

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#### Abstract

The bioconversion of various wastes such as fruit peels into some valuable products such as single-cell protein (SCP) has simultaneous potential to resolve nutritional protein deficiency worldwide. The shortage by obtaining an economical product for food and feed, as well as the mitigation of environmental pollutants to a large extent through the optimization of these residues, which serve as substrates for the production of a product with a high nutritional value. Therefore, the current study aimed to evaluate the feasibility of using fruit peels such as orange peel and pomegranate and their mixture through chemical treatment concentrations of dilute hydrochloric acid as well as thermal treatment by autoclave to obtain the highest concentration of sugars needed for yeast growth and the evaluation of biomass yields as a single-cell protein (SCP). The results showed that orange and pomegranate peels treated with different concentrations of 10, 20 and 30% hydrochloric acid had the highest yield of reducing sugars (4.89g/100g peel) obtained from orange peels treated with 10% acid concentration. After that, a comparison was made between the treatment with the best concentration of acid (HCl 10%) and the thermal treatment by autoclaving for orange peels, pomegranate peels and a mixture of them, so the treatment with acid (HCl 10%) for the mixture had the highest concentration of reducing sugars, followed by orange peels (5.90 and 4.87g/100g peels) respectively. But it was found that this treatment affects the growth of Saccharomyces cerevisiae later compared to the thermal treatment. After that, the media were prepared from orange peels and pomegranate peels, mixed with acid (HCl 10%) and heat treatment, and salts were added to one of them and the other without salts. The results were in favor of the thermal treatment. The orange peels thermal treated without salts gave the largest amount of protein, followed by the mixture without salts, then the mixture with salts that were thermal treated (9.39, 8.107 and 8.02 g/L) respectively.

Keywords: Single cell protein; Saccharomyces cerevisiae; orange peels; pomegranate peels; chemical and thermal treatments

#### Introduction

Frequent population growth in developing countries, this led to the search for alternative food sources rich in protein, whether for humans or animals. It is a major problem of protein deficiency in the world for humans because protein is an essential part of life as a dietary supplement. Many poor countries face food problems and nutrient deficiencies. The single- cell protein is also called microbial protein which can be produced by fungi, yeast and bacteria (Saeed et al. 2020). The different microorganisms used in the production of single- cell protein (SCP) are yeast (Saccharomyces, Candida, etc.), molds (Fusarium, Trichoderma, Rhizopus, etc.) algae (Chlorella, Spirulina, etc.), and bacteria (Alcaligenes, Silomonas, etc.). Microorganisms can use a variety of substrates such as agricultural waste which aid in the decomposition of pollutants (Nasseri et al. 2011).

This microbial protein that obtained from organisms are very rich in nutritional compounds such as protein,

minerals, carbohydrates, lipids, vitamins and amino acids and these are also used as good supplements of diet, it has high nutritional benefits to overcome the problems of protein deficiency in foods used for humans and animals. It is not seasonal production. This system is totally environment friendly and has a low cholesterol level and it is overall a better method to produce protein. Saccharomyces cerevisiae could be suitable for singlecell protein production from cheap waste products [Elrhman et al. 2016; Saeed et al. 2020]. Another advantage of SCP is the abundance of essential amino acids such as methionine and lysine, which are often limited in animal and vegetable food. Single cell protein is also an important mode in food replacement for some of the more expensive food sources such as fish, flour, beans and some meat. Single cell protein has become one of the most important topics and ways to solve some food problems (Zhou et al. 2017).

In recent years, *S. cerevisiae* has become a suitable plant the cells for the biosynthesis of many important bio-compounds (**Carsanba** *et al.* **2021**). *S. cerevisiae*, a

model organism in biology (**Fakult, 2012**). Some of the modified proteins can be used in the tumor mechanism through the regulation of Lysine protein alterations in cancer-related pathways. This indicates that it can work mutated proteins, new potential cancer drivers that could be used for diagnosis biomarkers in clinical practice (**Chen** *et al.* **2018**).

*S. cerevisiae* is a species of yeast and it is eukaryotic as a model organism that called baker's yeast. *S. cerevisiae* is the best studied eukaryote and a valuable tool for most aspects of basic research on eukaryotic organisms (**Parapouli** *et al.* **2020**).

Saccharomyces cerevisiae (Baker's yeast) is one of the most important and widely used cell factories for protein recombination production (**Huang et al. 2018**). S. cerevisiae is very important for single-cell protein production due to its ease of harvest, low amounts of nucleic acids, efficient mix of amino acids, and large size. This is considered one of the solutions in the third world countries and developing countries that suffer from food shortages and malnutrition problems (Sciences et al. 2011).

*S. cerevisiae* and some economically important fungi can be successfully grown in some fruit peels waste media such as mango wastes, Pineapple, spoilt tomato fruit, Papaya, carrot, green Banana, yellow Banana, orange, coconut wastes, pomegranate, sugarcanes waste, apple, tomato, wheat straws wastes and pea peels. Effect of substrate concentration revealed that biomass concentration an increased with increase in substrate concentration [Ahuja and Kumari, (2019); Khan *et al.* 2010; Milala *et al* 2018; Saeed *et al.* 2020].

These cellulosic substrates usually have a lot of lignin content which prevents the process of cracking by microorganisms. Thus, enzymatic, acidic, or basic methods of enzymatic treatment and dilute acid followed by sterilization were necessary to quantify these wastes and obtain higher yields from sugar reduction and hence higher ethanol production. Dilute HCl, sulphuric acid, alkaline treatment, and thermal hydrolysis (autoclaving), respectively, proved to give a good result where significant amounts of reducing sugars were obtained at the end of the saccharification process [Abdul and Ibrahim, (2017); John *et al.* 2017; Vázquez *et al.* 2017]. Thus, the single- cell protein production by yeast depends on the growth substrates or media composition (Mondal *et al.* 2012).

### **Materials and Methods**

Collection of fruit peels and Microorganism culture

Amr Salem Awad et al.

The pomegranate peels and or fruit peels were collected from the local markets of Albuhayra, Egypt in 2019. The microorganisms used to ferment fruit wastes was *S. cerevisiae* obtained from Department of Microbiology, Agriculture, Benha University. The yeast strain was grown on Yeast Extract Potato Dextrose Agar (YPDA) and sub-cultured every 3 weeks and incubated at 28-32°C. All Chemical were analytical grade and purchased from Sigma Company.

## Preparation of substrates and fruit peels hydrolysate

Pomegranate and orange fruit are collected and washed several times with water, to remove dust particles. Then were manually peeled and separated, then cut into small pieces with a knife. The pieces of peels are put in dark place until aerial dryness. The dry peel pieces were blended into smooth powder using a blender (model-brown) and sieved through a mesh screen. The samples were prepared, packed in transparent polythene bags and stored at room temperature until further study. *S. cerevisiae* lacks the enzyme system which hydrolyzes the polysaccharides into monosaccharide. The fruit peels were used as a substrate for production of single- cell protein production (SCP) after chemical and heat pretreatment. **Chemical pretreatment** 

# Determination of the best concentration of HCl for cellulose chemical hydrolysis

The peels were degraded to convert cellulose content into more available sugars by chemical treatments with little modification to the procedure of **(Lenihan et al. 2010).** Twenty mL of 10, 20 and 30% (w/v) HCl were added to each (10 g) of orange or pomegranate peels powder in the conical flasks. The mixtures were placed in a water bath at 100, 87 and 75°C for one hour, respectively. After being allowed to cool, it was filtered through Whatman No. 1 filter paper under vacuum. The filtrates were diluted with distilled water for 100 mL and determined the reducing sugars **(Saywell and Phillips, 1934).** 

#### **Thermal pretreatment**

The method (**Khan et al. 2010**) was used with some modifications. 200 mL of distilled water was added to the each 16 g from peels powder of orange, pomegranate and a mixture of them (1:1) in a conical flasks. The mixtures were autoclaved at 121°C for 30 mints. After being allowed to cool, they were filtered through Whatman filter paper under vacuum. A sample of filtrates was used to determine the reducing sugars (Saywell and Phillips, 1934).

#### S. cerevisiae inoculum preparation

For preparation of *S. cerevisiae* inoculum, sterilized potato dextrose broth media were inoculated with 3 days-old-culture of yeast and incubated at  $27^{\circ}$  C for 48 hr. The cell suspension was adjusted to a final concentration of 107 CFU.mL<sup>-1</sup>. The suspension inoculum was kept in a chiller at 4°C for further use.

## **Fermentation process**

Submerged fermentations were carried out in Erlenmeyer flasks using four types of media. The first and the second of them were designed by the fruit peels (orange, pomegranate or mix) hydrolyzate that was produced from chemical or thermal pretreatment. Third and fourth had the following composition a (NH4)<sub>2</sub>SO<sub>4</sub> (0.2 g), KH<sub>2</sub>PO<sub>4</sub> (0.1 g), MgSO<sub>4</sub>.7H<sub>2</sub>O (0.05 g), NaCl (0.01 g), CaCl<sub>2</sub> (0.01 g) made up to one liter with fruit hydrolysates (FH) that produced from chemical or thermal pretreatment (**Mondal** *et al.* **2012**).

In all the media, the initial pH was adjusted to 5.5 using 1N HCl and/or 1N NaOH. Each medium (98 mL) was transferred into 250 mL Erlenmeyer flask and then autoclaved at 121°C for 15 minutes. Inoculum of 2 mL from the suspension of *S. cerevisiae* was aseptically transferred into each medium. Fermentation was carried out at 28°C under shaker condition.

#### **Determination of Yeast Biomass:**

At the end of incubation time after 8 days all cultures were centrifuged at 10,000 rpm for 5 minutes. The supernatant was discarded, and the cell pellets were washed with sterile water. Before taking the weight of the biomass, it was transferred into an aluminum dish and was oven dried at 105°C for one hr. followed by cooling in desiccators to balance the temperature and weight (**Prosser and Tough, 1991**).

#### Statistical analysis

All the experimental results were presented by the average value of triplicates with mean  $\pm$  SD (standard deviation). The data were statistically analyzed using one-way analysis of variance (ANOVA) analysis and Duncan's multiple range tests (minimum significance threshold *P*=0.05) were used to compare the sets of means obtained from different experiments. *P*" values for three replicates of the investigated parameters and were calculated. Using (Costate- 2008 software version 6.400 for Windows), Excel software (Microsoft Office Excel Worksheet (.xlsx), USA, 2013) was used to draw charts.

### Results

#### **Reducing Sugar yield by Acid Hydrolysis**

Acid hydrolysis was applied to convert waste cellulose into monosaccharaides as reducing sugars that can be used as a good source of organic carbon for S. cerevisiae growth. Pretreatment by the acid hydrolysis was done at various concentrations of HCl (10%, 20%, and 30% w/v). The maximum reducing sugar obtained with pomegranate peel and orange peel hydrolyzed by 10% concentrated HCl was 3.959% and 4.88 % respectively. Table 1 show the release of reducing sugar from orange peel and pomegranate peel was found to decrease as the concentration of acid increases. It may be because further oxidation of reducing sugar was happened with increasing acid concentration. In this experiment, the powdered peels were hydrolyzed using hydrochloric acid at various temperatures 100° C, 87° C and 75° C respectively. The particle size of substrate, residence concentrations and temperature play a crucial role in hydrolysis. Small particle size increases the exposed surface area which is available to react with hydrochloric acid. The residence concentration increases the contact concentration between substrate and acid. The temperature plays a crucial role in any biochemical reaction as the rate constant of hydrolyzed reaction depends on temperature. If the temperature increases, the rate also increases.

**Table 1.** Reducing sugar concentration (%) produced from orange and pomegranate peels treated with various acid concentrations.

	The concentration of reducing sugars (%)		
Sample	10% HCl	20% HCl	30% HCl
Pomegranate Peel	$\textbf{3.959} \pm \textbf{0.09}$	$3.754 \pm 0.09$	$3.625\pm0.09$
Orange Peel	$\textbf{4.888} \pm \textbf{0.07}$	$4.615 \pm 0.07$	$4.330 \pm 0.07$

Significant *p* value <0.05. Data presented as mean  $\pm$  SD.

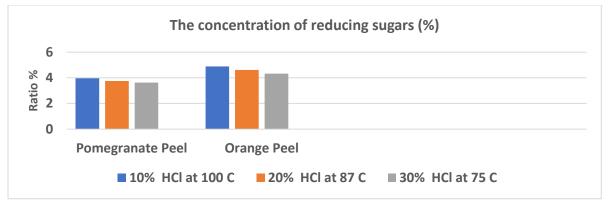


Figure 1. Reducing sugar production after acid treatment of orange and pomegranate peels.

Interestingly, under the same concentration of acid treatment and same temperature, orange and pomegranate peels differently influenced and gave a different amount of reducing sugar. However, by increasing acid concentration the oxidation of reducing sugar was noticed. The maximum reducing sugar (4.88% and 3.959%) was obtained from the orange peel and Pomegranate Peel respectively, which were produced by 10% HCl after one hour at 100° C. Hence, this condition is considered to be optimum.

## Reducing Sugar from thermal and acid treatments of orange and pomegranate peels and their mixture

A high level of reducing sugar was obtained after hydrolysis by thermal or autoclaved treatment. Therefore, thermal hydrolysis is able to extract reduced sugar even after acid hydrolysis of orange peel and pomegranate peel. The thermal treatment was used for further saccharification of lignocellulosic biomasses. The data in Table 2. show the maximum reducing sugar found during acid treatment were 5.90±0.09% and 4.86±0.07% of mix and orange peel respectively, while the maximum reducing sugar found during thermal treatment were 5.32±0.09% and 4.03±0.07% of mixture and orange peel respectively. The acid and thermal hydrolysis treatments of the samples were prepared in the optimum condition. However, acid hydrolysis is not normally recommended for saccharification due to liberation of furfural which turns down the next fermentation process. On the other hand, thermal hydrolysis is a biological process where the end products are environmentally friendly. These end products are less toxic which may negatively affect the next fermentation process.

	Concentration of reducing sugars (%)			
Sample	Pomegranate Peel	Orange Peel	Mix	
HCl 10%	3.946±0.05c	4.869±0.07b	5.902±0.09a	
Thermal	3.521±0.05c	4.030±0.07b	5.321±0.09a	

Table 2. Reducing sugar produced from Pomegranate, Orange Peels and mixture by acid and thermal treatments.

Significant *p* value <0.05. Data presented as mean  $\pm$  SD.

A high percentage of efficiency was achieved from the thermal hydrolysis of mixture peels. Therefore, this condition is considered as the optimum condition for thermal treatment. Additionally, data in Fig.2 explicate that acid conversion for production of reducing sugar from the two kinds of peels and their mixture were found to be higher as compared to the thermal hydrolysis. Therefore, the highest percentage of reducing sugar is produced from the mixture of two kinds of peels by acid hydrolysis followed by thermal treatment.

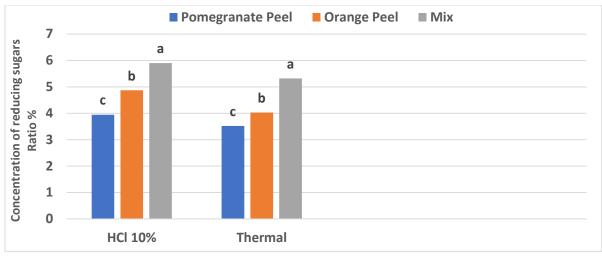


Figure 2. Reducing sugar production after acid and thermal treatments.

#### **Cell Proliferation and Protein Production**

Cell or biomass production of *S. cerevisiae* was performed based on the designed pattern (Table 3); 98 mL of sterile culture medium was added to each 250-mL shake flask. After inoculation with 2 mL of *S. cerevisiae* cell suspension, all the shake flasks were incubated in a shaker incubator and 28  $^{\circ}$ C for 8 days.

#### **Biomass weight:**

The maximum biomass weight of dried cell of *S. cerevisiae* at optimal condition was obtained equal to 9.388±0.091 g/L of (orange peel) thermal-hydrolysate media. Basically, *S. cerevisiae* has considerable amounts of protein with some minerals, soluble fiber,

and mono and poly saturated fatty acids (**Yamada and Sgarbieri, 2005**). While in this work all media prepared under the last same conditions Table 3 shows the results for all experiments of 1 to 12 were O.P HCl-hydrolysate with salts  $4.869\pm0.199$  g/L, O.P HCl-hydrolysate 3.642 $\pm$ 0.135 g/L, O.P thermal-hydrolysate with salts  $3.377\pm0.323$  g/L, O.P thermal-hydrolysate 9.388 $\pm$ 0.091 g/L, (pomegranate peel) HCl-hydrolysate with salts  $6.082\pm0.173$  g/L, P.P HCl-hydrolysate  $5.547\pm0.164$  g/L, P.P thermal-hydrolysate  $2.935\pm0.114$  g/L, Mix HCl-hydrolysate with salts  $3.983\pm0.069$  g/L, Mix HCl-hydrolysate 4.915 $\pm$ 0.099 g/L, Mix thermal-hydrolysate with salts  $8.021\pm0.124$  g/L and Mix thermal-hydrolysate 8.107 $\pm$ 0.153 g/L, respectively.

Table 3. weight of biomass produc	iced from different media.
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Ν	Sample name	Weight of biomass (g/L)
1	O.P HCl-hydrolysate with salts	4.869±0.199e
2	O.P HCl-hydrolysate	3.642±0.135g
3	O.P thermal-hydrolysate with salts	3.377±0.323g
4	O.P thermal-hydrolysate	9.388±0.091a
5	P.P HCl-hydrolysate with salts	6.082±0.173c
6	P.P HCl-hydrolysate	5.547±0.164d
7	P.P thermal-hydrolysate with salts	5.733±0.215d
8	P.P thermal-hydrolysate	2.935±0.114h
9	Mix HCl-hydrolysate with salts	3.983±0.069f
10	Mix HCl-hydrolysate	4.915±0.099e
11	Mix thermal-hydrolysate with salts	8.021±0.124b
12	Mix thermal-hydrolysate	8.107±0.153b

Significant *p* value <0.05. Data presented as mean  $\pm$  SD.

Data showed the evaluation of thermal treatment in biomass production was evaluated, produced biomass weight of O.P thermal-hydrolysate media  $9.388\pm0.091$  with thermal hydrolysis standard deviation, which confirmed the relative susceptibility of fraction of full factorial methodology to determine the optimal culture medium composition in the present work. The second high yield obtained cell biomass weight in the present work ( $8.107\pm0.153$  of mix thermal-hydrolysate at optimum conditions).

The variance of biomass yield between the differences media Fig. 3 shows that the O.P thermalhydrolysate was the better media that produced a high yield of a dry cell for *S. cerevisiae* as a single cell protein. The second media that produced high biomass was the mixture of pomegranate peel and orange peel (mix) without salts media treated with a thermal method, while the third highest yield produced from the Mix media with salts treated with thermal a method.

## Discussions

The data found from this study shows that the production of single cell protein by microorganisms depends on the treatments of the fruit wastes by chemical methods such as diluted hydrochloric acid and thermally treated (autoclaving) to increase the concentration of reducing sugars that necessary for microbial growth [Azam et al. 2014; Mondal et al. 2012; Puri et al. 2020; Vázquez et al. 2017]. Where the best treatments for fruit peels were thermal treatment by autoclaving, compared to treatment with different concentrations of acid, in terms of cost, as well as preparing the appropriate media for the growth of *S. cerevisiae* free from the presence of acid, which may cause interference that leads to the inhibition of yeast growth (Vázquez et al. 2017).

The growth of *S. cerevisiae* increases with the increase in the concentration of reducing sugars in the environment and thus the amount of protein produced by the yeast increases (**Hezarjaribi and Ardestani**, (2016).

The amount of protein produced from the different media prepared from acid-treated and thermally treated for *S. cerevisiae* growth was compared by dividing it into four environments for each residue of fruit peels. The result was the media without salts in which yeast grew more than compared the results with various other studies [Mondal *et al.* 2012; Uchakalwar and Chandak, 2014], especially the thermal treated media without salts. The amount of protein produced was greater for orange peels, followed by mix and pomegranate peels.

## Conclusion

It can be concluded that the most promising yield of single-cell protein production from S. cerevisiae by submerged fermentation of fruit peels. The degree of production of SCP depends on the type of substrate used and media composition. In addition to providing the carbon source available to living organisms represented in reducing sugars, and thus Promote sustainable consumption and production. The current study reveals that the fruit peels of oranges and pomegranates and their, combination can be used as a potential source product with a relatively high protein content using the various ingredients available in it, with the possibility of benefiting from the peels of the fruit in the production of valid proteins for feed and food. Hence, these peels must be used properly substrate for edible cellular biomass production from S. cerevisiae Instead of getting rid of it, and at the lowest financial cost, it can be used as a food supplement. Finally, the high yield and the growth of S. cerevisiae as single cell protein in the different media that prepared by thermal and acid treatment was produced from that media treated with thermal treatment for all wastes of orange peels, pomegranate peels and a mixture of them (1:1) with added salts and without salts. The results proved that autoclave/thermal treatment the best method to provide the optimum condition for microbial growth.

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**إنتاج البروتين أحادي الخلية من بعض قشور الفاكهة** عمرو سالم عوض، صلاح مصطفى سعد، إيناس مكاوى، نادية يحيى عطيه<sup>\*</sup> قسم الكيمياء الحيوية الزراعية، كلية الزراعة، جامعة بنها، مصر. Corresponding author: <u>amr.salem@fagr.bu.edu.eg</u>

التحويل الحيوي للنغايات المختلفة مثل قشور الفاكهة إلى بعض المنتجات القيمة مثل البروتين أحادي الخلية (SCP) لديه إمكانات متزامنة لحل نقص البروتين الغذائي في جميع أنحاء العالم. النقص من خلال الحصول على منتج اقتصادي للغذاء والأعلاف ، وكذلك التخفيف من الملوثات البيئية إلى حد كبير من خلال الاستفادة المثلى من هذه المخلفات ، والتي تكون بمثابة ركائز لإنتاج منتج ذي قيمة غذائية عالية. لذلك هدفت الدراسة الحالية إلى تقييم جدوى استخدام قشور الفاكهة مثل قشر البرتقال والرمان ومزيجهما من خلال المعالجة الكيميائية بتركيزات مختلفة لحمض الهيدروكلوريك المخفف وكذلك المعالجة الحرارية بالأوتوكلاف للحصول على أعلى تركيز من السكريات اللازمة لنمو الخميرة و تقييم إنتاج الكتلة الحيوية كبروتين وحيد الخلية (SCP) المعالجة الحرارية بالأوتوكلاف للحصول على أعلى تركيز من السكريات اللازمة لنمو الخميرة و تقييم إنتاج الكتلة الحيوية كبروتين وحيد الخلية (SCP) المعالجة الحرارية بالأوتوكلاف للحصول على أعلى تركيز من السكريات اللازمة لنمو الخميرة و تقييم إنتاج الكتلة الحيوية كبروتين وحيد الخلية الظهرت النتائج أن قشور البرتقال والرمان المعالجة بتركيزات مختلفة من حمض الهيدروكلوريك 10 و 20 و 20. كان لها أعلى محصول من السكريات المختزلة (4.89 جم / 100 جم قشر) تم الحصول عليها من قشر البرتقال المعالج بتركيز حمض 10. بعد ذلك تم عمل مقارنة بين المعاملة بأفضل المختزلة (10 HCl)، والمعالجة الحرارية بالاتوكلاف لقشر البرتقال وقشور الرمان ومزيج منها بحيث تكون المعاملة بالحصل المخليط أعلى تركيز من السكريات المختزلة ، يليه قشور البرتقال وقشور الرمان ومزيج منها بحيث تكون المعاملة بالحمض (لمان). لـ كان على نمو وللمان الحالم الحال المعالجة الحرارية بالاتوكلاف لقشر البرتقال وقشور الرمان ومزيج منها بحيث تكون المعاملة بالحمض (لمال ال الخليط على نمو ومان الخليط أعلى تركيز من السكريات المتالي وقشور الرمان ومزيج منها بحيث تكون المعاملة بالحمض (لموا الرمان على نمو وحلين أعل من ولالها منهما المعاملة بالمعالجة الحرارية. بعد ذلك تم تحضير البيئات من قشور الرمان والخليط منهما المعالجة بحامض (100 10 ٪) والمعالجة حرارية ، وأضيفت أملاح إلى إحداهما والأخرى بدون أملاح. كانت النتائج لصالح المعالجة والخليط منهما المعالجة بحامض (10 10 10 ٪) والمعالجة الحرارية. يلم والي وليها والمون والاح. كانت ا

الملمات الرئيسية: بروتين وحيد خلية ؛ خميرة Saccharomyces cerevisiae ؛ قشور البريقال؛ قشور الرمان؛ المعالجات الكيميائية والحرارية.