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# Production of Extracellular Hydrolytic Enzymes by Yeast Extracts on Some Commercial Media

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## ABSTRACT

*The yeast Saccharomyces cerevisiae is a rich source of both intracellular and extracellular hydrolytic enzymes. In the present study yeast extract of three commercial media, corn meal, oat meal and sucker cane beside basal medium were studied to evaluate the extracellular hydrolytic enzymes which are invertase, lipase and protease. Results represented that corn meal medium was the better medium and gave the highest values of target enzymes: 10563 µg glucose/min/g extract, 74.06 U × $10^3$/g extract and 114.8 µg D, L-alanine /min/g extract, respectively.*

**Keywords:** Yeast extract, Commercial media, Invertase, Protease, Lipase

## INTRODUCTION

Enzymes are biological catalysts. There are about 40,000 different enzymes in human cells, each controlling a different chemical reaction, which increase the rate of reactions by a factor of between $10^6$ to $10^{12}$ times, allowing the chemical reactions to make life possible to take place at normal temperatures. They were discovered in fermenting yeast in 1900 by Buchner, and the name enzyme means "in yeast". As well as catalysing all the metabolic reactions of cells (such as respiration, photosynthesis and digestion), they may also act as motors, membrane pumps and receptors [1]. There are many factors that at once have an effect on the work of the enzyme as a result of its protein nature, together with the temperature and exchange of pH and different factors [2], which strongly suggest the high specialization of enzymes.

In the midst of the huge industrial development taking place in the world, enzymes with some special characteristics can be produced from microorganisms, especially from the baking yeast. A huge variety of enzymes useful at the medical, industrial and food level in the world, which helps in solving real problems [3]. Yeast fermentation has received unprecedented attention from scientists because of the beneficial transformations, including starch transformations into simpler sugars in the structure for easier use [2].

No doubt that microorganisms gave huge number of naturally produced enzymes which more than 100 enzymes with different industrial, medical and food applications especially yeast because its safety, economic coast, fast income in comparison with bacteria [4]. Also, it can be grown on several media to produce variety of secondary metabolites depending on the composition of the used medium [5].

The most important of these enzymes are hydrolytic enzymes that are capable of transitions of medical, nutritional and industrial significance. Invertase, which have the ability to convert sucrose to glucose, the importance of this enzyme lies in the fact that the energy sources of living organisms transform carbohydrates into simpler forms the importance of this enzyme lies in the fact that the sources of energy for living organisms transform carbohydrate into simpler images. On the other hand, monosaccharide such as glucose and fructose may have a direct and regulated role in the living cell.

One of these vital hydrolytic enzymes is lipase enzyme. It is one of the important enzymes in fat digestion which converting acyl glycerides into simpler fatty forms, in which the living organism can optimize and release the energy used by the organism in all walks of life [6,7]. The importance of this enzyme lies not only in food and medical uses,

but also in industrial uses, for example, in the presence of oil drops in water sources. The effectiveness of this enzyme lies in some clues to understand its activity of decomposition, interstitial activation and stereo selectivity of lipase [8]. This enzyme is spreading at the rate of 5% as the market value and with more efforts of scientists it will be present in the wide range all over the world with the highly variety of applications [9].

Proteases represent tone of the three largest groups of industrial enzymes, accounting for nearly 60% of the worldwide enzymes market [10]. Yeast extracellular proteases are of particular interest because of their direct commercial applications, and their potential use in expression systems for heterologous proteins production. Yarrowialipolytica XPR2 promoter and some parts of the XPR2 gene that provide secretory and processing signals have been used in constructs to direct the synthesis and secretion of pro chymosin, human anaphylatoxin C5a, porcinea1-interferon, invertase, tissue plasminogen activator, and hepatitis B virus middle surface antigen [11-14]. This enzyme can be produced extracellularly by several microorganisms such as *Candida albicans*, *Aspergillus* special attention for their role in pathogenesis [15].

So, the aim of the presented work is to get a high value activity of main hydrolytic enzymes from commercial media by using yeast backing of bread.

## MATERIALS & METHODS

### *Used media preparations*

Three commercial media was prepared by weighing of 30 g and cooked on hot plate then filtrated by cotton and sterilized in the autoclave at 121°C for 20 min, while basal medium was prepared. Yeast-peptone-dextrose (YPD) broth containing 0.5% (w/v) yeast extract, 1% peptone and 2% glucose by the same conditions of sterilization according to Ausubel et al. [16].

### *Culture of commercial yeast on different used media*

Active dry yeast baking-Helew El-Shame (*Saccharomyces cerevisiae*) (1g) was added on 100 ml broth of each sterilized media, shaking and incubated at 37°C for 48 h.

### *Extraction of the secondary metabolites from cultivated yeast media*

Each medium was centrifuged by using cooling centrifuge at 4°C, 10,000 rpm for 20 min to get on the clear supernatant and kept in the refrigerator up to use.

### *Lyophilization of obtained products*

All produced supernatant was lyophilized by using of Edwards Freeze Dryer, Modulyo instrument-freeze drier lab, Cairo University to get on lyophilized powder.

### *Protein determination*

The total protein content of yeast extracts produced by four used media was estimated spectrophotometrically at 280 nm. Bovine serum albumin (BSA) served as the standard [17].

### *Determination of extracellular hydrolytic enzymes*

#### Assay of invertase

According to Ishaaya and Swirski [18,19] invertase activity detection produced by four used media was occurred by spectrophotometric method which depended on the conversion of sucrose to free aldehyde groups of glucose by reduction of 3,5-dinitrosalicylic acid reagent at 550 nm. All samples were measured triplicate to perform statistical analysis.

#### Assay of protease

Protease enzymatic activity was measured according to Tatchell et al. [20] with some modifications, it depends on the splitting of albumin as a substrate into free amino acids, the obtained color was measured at 570 nm, D, L-alanine was used as standard and enzymatic activity was determined as ug alanine/min/g. b. wt. All samples were measured triplicate to perform statistical analysis.

**Assay of lipase**

Target enzymatic activity was measured according to Kwon and Rhee [21]. spectrophotometrically at 715 nm. This method depends on the emulsion of olive oil (20 gm) by using of phosphate buffer at pH 7.0 and $CaCl_2$ (10 mg) and polyvinyl alcohol (0.25%) in homogenizer for 30 min. Oleic acid used as standard to get on standard curve. each unit of activity expressed 1 µmol fatty acid/min. All samples were measured triplicate to perform statistical analysis.

## RESULTS

### *Total protein determination*

Figure 1 represented the amount of total protein of yeast extract of corn meal was significantly higher than which produced from other extracts. Total protein of corn meal yeast extract was 61.77 mg/ml while in basal medium, oat meal and sugar cane were 44.66, 25.61 and 15.03 mg/ml, respectively.

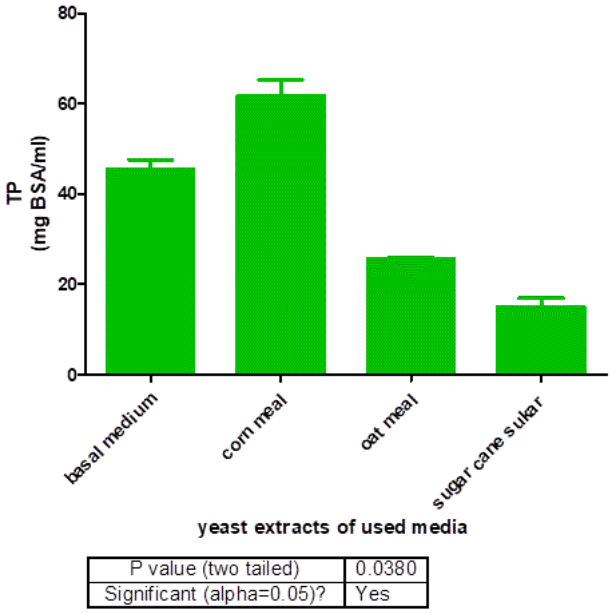



**Figure1:** Values of total proteins of yeast extracts by four used media

### *Determination of digestive enzymes of yeast extracts*

**Invertase activity of four yeast extracts**

Extracellular invertase enzyme which produced by corn meal yeast extract was significantly higher than which produced by other yeast extracts. Invertase activity of corn meal yeast extract was 10563 ug glucose/min/gm extract. Figure 2 illustrated the activity of invertase of other yeast extracts was ordered as sugar cane sucker (5753) > oat meal (2858) > basal medium (1997), respectively.

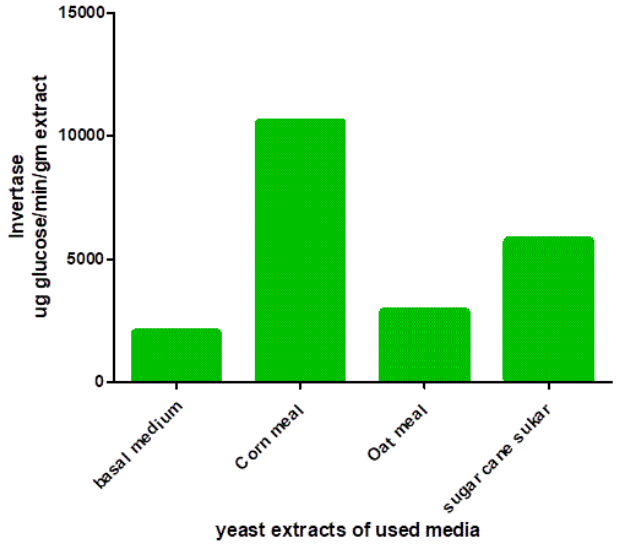
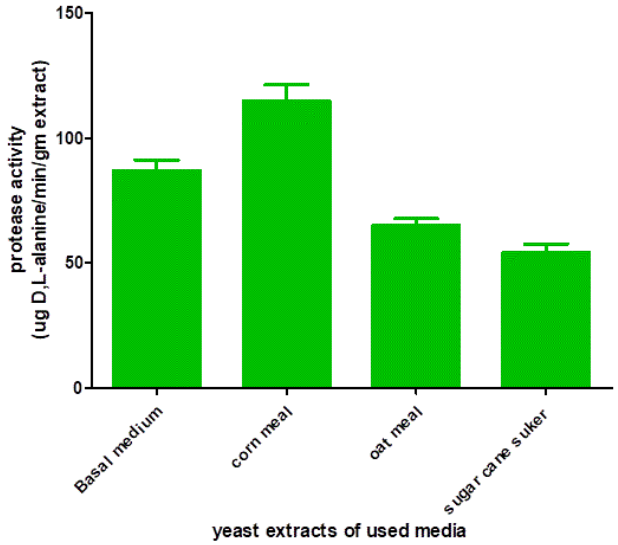



**Figure 2:** Invertase activity of yeast extracts

**Protease enzyme activity of yeast extracts**

Figure 3 represented protease activity of corn meal yeast extract was significantly higher than of other extracts. The activity of protease of corn meal was 114.8 µg D, L-alanine /min/gm extract while in basal medium, oat meal and sugar cane sucker was 87.133, 65.233 and 54.233 µg D, L-alanine /min/gm extract, respectively.




| | |
|---|---|
| P value (two tailed) | 0.0092 |
| Significant (alpha=0.05)? | Yes |

**Figure 3:** Protease activity of yeast extracts

**Lipase activity of four yeast extracts**

Figure 4 represented lipase activity of corn meal yeast extract was significantly higher than of other extracts. The activity of lipase of corn meal was 74.06 Ux103/gm extract. While in sugar cane sucker, oat meal and basal medium was 37.466, 21.93 and 21.33 Ux103/gm extract, respectively.

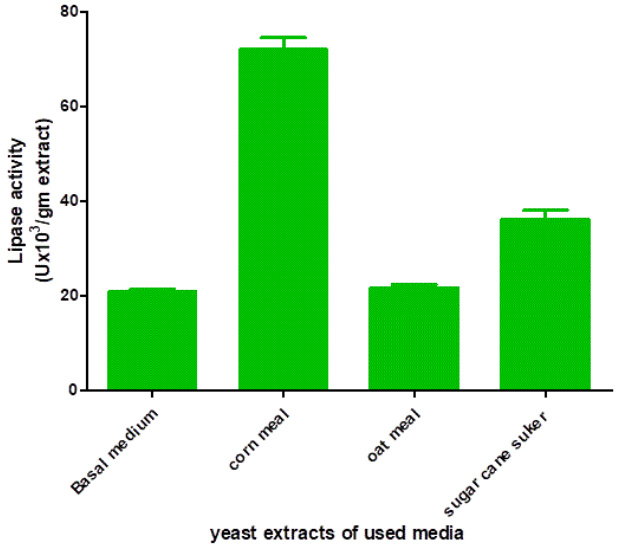



**Figure 4:** Lipase activity of yeast extracts

## DISCUSSION

The compositions of each nutritional medium used in this work assuming a vital partner chemically in enzymatic activities of each yeast extract. According to many references, which explain the different contents and different percentage of each gradient controlling on the enzymatic activity. USA-ARS, [22] mentioned that corn meal has Carbohydrate: 74.26%, Protein: 9.42%, Lipid: 4.74%, Total dietary fiber: 7.3%, Sugar: 0.64%, Energy: 365 kcal/100 g; while Oat meal has Carbohydrate: 66.27%, Protein: 16.89%, Lipid: 6.90%, Total dietary fiber: 10.6%, Sugar: 0%, Energy: 389 kcal/100 g. Abiose S.H. & Ikujenlola A. V. [23] found that common maize has Crude Fat (%) 4.50, Crude Protein (%) 9.80, Crude fiber (%) 2.60, Carbohydrate (%) 73.83, Energy (Kcal/100g) 375.00, Sodium (mg/100g) 61.65, Magnesium (mg/100g) 141.30, Potassium (mg/100g) 77.23, Calcium(mg/100g) 64.70, Zinc (mg/100g) 11.48, Iron (mg/100g) 1.10, Phytate (mg/100g) 1.22. In this work, corn meal was the better nutritional medium for yeast fermentation to get on the best extracellular hydrolytic enzymatic activity

Invertase enzyme converts sucrose to more simple forms of sugars: glucose and fructose. The proper concentration of carbon source is important for the optimum production of invertase enzyme. Chemohetrotrophic organisms are dependent on chemical energy sources and employ organic compounds as the principle carbon source. The lower concentration of carbon source might insufficient for the proper growth of yeast which results in less invertase production [24,25]. Strauss et al. [26] demonstrated that the presence of a readily utilizable nitrogen source repressed extracellular proteases so, it was expected that corn meal as a nutritional medium for yeast had a maximum nitrogen content more than other used nutritional media.

Many factors affecting on the activity and the production of extracellular lipases such as composition of used medium (nutritional requirements), environmental factors (temperature, pH, etc). So it can be expected that the better the environment and the surrounding environment, the more effective the enzyme is, because it is an enzyme that relies on induction [27-29].

A.J. et al., Zhang et al. and Hun et al. [30-32] could explain that formation of lipase in the producible microorganisms which were dependent on using of lipids as carbon source instead of carbohydrates or sugars.

## CONCLUSION

In the present work, four commercial media used for yeast fermentation to evaluate the extracellular hydrolytic enzymes: invertase, protease and lipase. From the showed data, we found that corn meal as microbial medium was better than other used media due to its highly content of all elements which needed for the enzymatic activity.

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