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EFFECTS OF CU, MN, PB AND CD ON THE TECHNOLOGICAL PROPERTIES OF BAKER'S YEAST (*IN VITRO* STUDY).

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ABSTRACT

Effect of different concentrations of heavy metals (Cu, Mn, Pb and Cd) on four parameters of baker's yeast (yield, total viable cells, protein content and fermentation power) representing their technological properties were tested. Increasing the concentrations of Cu up to 2 ppm and Mn up to 20-25 ppm, increased the studied parameters, while higher concentrations of both elements resulted in a negative effect of these parameters. On the other hand, the presence of Pb or Cd in growth medium decreased baker's yeast properties, this effect was increased as the Pb or Cd increased. Above all, as a conclusion Pb and Cd were classified as toxic metals for yeast growth at any concentration, meanwhile the optimum concentrations of Cu and Mn in growth medium of baker's yeast which gave the highest level of all parameters were 2 and 20-25 ppm, respectively.

Keywords: Baker's, *Saccharomyces cerevisiae*, copper, manganese, lead, cadmium, yield, total viable cells, protein content and fermentation power.

INTRODUCTION

Baker's yeast (*Saccharomyces cerevisiae*) is widely used in bread making, also it can be used directly as medicinal tablets or consumed as fresh because it is a good source for proteins, essential amino acids, vitamins and also many minerals, i.e., calcium, phosphorous, magnesium and iron. (Iatskovskaia *et al*., 1992). The yeast can be added to animal feeds to support a high nutritive value and to suppress the toxic effects of some mycotoxins on animals, in addition to increase the weight gain. (Stanley *et al*., 1993).

The micro nutrients become toxic when intracellular concentration rise above the physiologically required level (Soares *et al*., 2003). Heavy metals have effects on the fermentation power and yeast production when growing on cane molasses. Heavy metals also have serious effects, when its levels increased inside yeast cells, on human and animals by using yeast as food (Ozer and Ozer, 2003)

Although some metals are essential nutrients in low concentrations, excess concentrations of all heavy metals lead to various toxic effects such as oxidative stress and inhibition of enzymes (Drost *et al*., 2007). Metals, such as lead, mercury, cadmium and arsenic constitute a significant potential threat to human health. Several metals are known to be human carcinogens, including arsenic, chromium and nickel (Kakkar and Jaffery 2005; Son *et al*., 2007).

Manganese is an essential trace element in biological systems. Manganese dependent enzymes are found within diverse locations in the cell including the Golgi, mitochondria and cytoplasm. However high concentrations of manganese are potentially toxic (Yang *et al*., 2005). Baker's yeast is sensitive to high concentrations of lead with an extension of lag phase and duration of the fermentation and an overall decrease in final cell biomass production. Increasing lead concentration resulted in a negative effect on growth rate and maximum dry cell concentration in the aerobic fermentation of molasses (Skountzou *et al*., 2003).

Certain heavy metals, such as copper, nickel, and zinc are essential trace elements for normal growth and metabolism of microorganisms (Borst-Pauwell, 1981), however these metals become toxic when intracellular concentration rise above physiologically required levels. Metal toxic effects, including the blocking of functional groups, displacement and/or substitution of essential metal ions from biomoleculs, conformational modifications, denaturation and inactivation of enzymes, and disruption of cellular and organellar membrane integrity (Gadd, 1993).

Therefore, this research was initiated to evaluate the cell biomass, protein content, fermentation power and total viable cells of baker's yeast as affected by different concentrations of heavy metals (Cu, Mn, Pb and Cd).

MATERIALS AND METHODS

Materials:

1. Yeast strain.

Yeast strain (*Saccharomyces cerevisiae F.707*) was obtained from El-Hawmdia for Chemicals Factory (Sugar and Integrated Industry Company) - Giza, Egypt.

2. Maintaining medium.

Yeast extract peptone glucose agar (YPGA) medium (Merck, Darmstadt, Germany) was used for maintaining of *S. cerevisiae* strain.

3. Cultivation medium.

Yeast extract peptone glucose broth (YPGB) medium (Merck, Darmstadt, Germany) was used for growth of *S. cerevisiae* strain.

4. Total viable yeast cells count medium.

Acidic dextrose agar (ADA) medium (Merck, Darmstadt, Germany) was used to determine the total viable yeast cells count

Methods:

1. Maintaining Yeast strain.

Yeast strain was maintained on yeast extract peptone glucose agar medium (YPGA) at 4°c and transferred monthly.

2. Inoculum preparation.

Hundred mL of YPG broth medium (pH 4.5) were placed in 250 ml conical flasks, then autoclaved (30min at 121°C). The sterile medium in each flask was inoculated by a loopful of 24 hr slant YPG agar culture of yeast. Then incubated for 24 hr at 30°C under shaking condition (150 rpm)

3. Cultivation.

Four hundred mL of the cultivation medium were placed in 1L Erlenmeyer flasks. The flasks were autoclaved (30min at 121°C), 16mL of this previously prepared inoculum were used to inoculate each flask. Growth was aerobically carried out at 30°C under shaking condition (150 rpm) for 24 hr. To study the effect of heavy metals on baker's yeast (*S. cerevisiae*) properties, Cu, Pb, Mn and Cd were added separately to cultivation medium to give final concentrations as presented in Table (1), growth was carried out as previously described. At the end of incubation period, yeast was recovered by centrifugation at 4500 rpm for 5min (Soares *et al*., 2003). Biomass (yield), protein content, total viable cells and fermentation power were determined.

Table (1): Concentrations (ppm) of metals in baker's yeast growth medium.

Metals	Concentrations (ppm)							
Cu	0	2	4	6	8	10	12	14
Mn	0	5	10	15	20	25	30	35
Pb	0	2	4	6	8	10	12	14
Cd	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5

4. Estimation of yeast yield.

After centrifugation, yeast cells were washed twice with distilled water and the precipitated layer was weighed (Egyptian Standard, 191/2000).

5. Determination of crude protein.

Crude protein was determined in the produced baker's yeast using kjeldahl method (AOAC 2000).

6. Determination of total viable yeast cells count.

Yeast solution (1:10) was prepared using saline solution. Samples were then serially diluted and plated on acidic dextrose agar medium using pour plate technique according to Egyptian Standard (191/2000). The inoculated plates were incubated at 30^{o}c for 2 days. The developing colonies were counted and the total viable yeast counts were expressed as colony forming unit (CFU) per gram of samples.

7. Evaluation of fermentation power.

The Fermentation power of baker's yeast was determined using an SJA-Fermentograph NASSJO-Sweden. The analysis was conducted to determine the total carbon dioxide evolved during yeast fermentation in normal dough in one hour. The composition of the prepared dough was 160mL water, 4g NaC1, 10g (wet weight) of baker's yeast and 280g wheat flour. Mixing of the dough (35^{o}C for 5min) was carried out using a Diosna D-4500 mixer. After mixing, the dough was transferred to a plate and placed in the chamber of the Fermentograph (35^{o}C). The chamber was closed and the recorder was allowed to trace the curve of CO_2 formation for 1 hr. The fermentation power was recorded as CO_2 volume after 1 hr. (Suihko and Mfikinen 1981; Egyptian Standard, 191/2000).

8. Statistical analysis.

Results were subjected to one-way analysis of variance (ANOVA) of the general liner model (GLM) using SAS (1999) statistical package. The results were the average of three experiments.

RESULTS AND DISCUSSION

Microelements play an important role in the cellular metabolism, primarily due to their requirements as cofactors for a large number of enzymes. However, excess amounts of the same metal ions are toxic and can cause damage to the function that they serve (Tomas *et al*., 2004). To study the effect of heavy metals on baker's yeast properties, four heavy metals as Cu, Mn, Pb and Cd were investigated to evaluate their effect on yeast yield, protein content, total viable cells and fermentation power.

1. Effect of copper.

Different Cu concentrations (up to 14 ppm) were tested in order to determine their effects on *S. cerevisiae* (baker's yeast) properties as shown in Figure (1). Results indicate that all studied parameters i.e., yeast yield, protein content, total viable cells and fermentation power increased by adding Cu in the growth medium up to 2 ppm then decreased by increasing the Cu concentration. The detected values at 2 ppm of Cu were 34.13 g/L, 49.52 %, 12.13×10^9 CFU/g and 800 $Cm^3CO_2/1^{st}$ h. for yeast yield, protein content, total viable cells and fermentation power, respectively.

These results are in agreement with those obtained by Ismail (2006) who reported that, yeast gave the best yield and fermentation power at 2 ppm Cu concentration added as Cu sulphate. Also Jones and Gadd (1990) reported that Cu concentrations 1–10µM was optimum for the yeast growth and fermentation activity. Jones and Greenfield (1984) stated that Cu concentration above 1µM inhibited glycolytic flux, inhibited growth (above 10µM), and induced yeast cell death in the logarithmic growth phase (at 0.8mM).

Moreover Soares *et al*., (2003) stated that addition of Cu (50µM) to yeast cell suspension resulted in a decrease of about 50% of the viability in the first 40 min and no further loss of viability was observed until 120 min. The increase of Cu concentration up to 200µM recorded a rapid loss of viability by 50% in the first 5 min and dramatic loss of viability (more than 99% after 60 min). No loss of viability was detected with the addition of 10µM Cu, until 60 min.

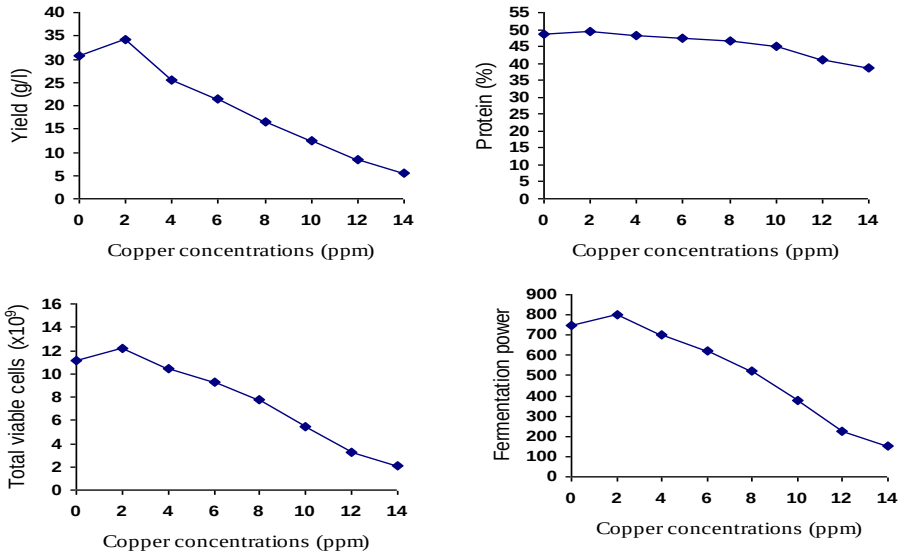

Fig (1). Effect of copper on baker's yeast properties

The decrease in the studied *S. cerevisiae* at high Cu concentrations may be due to a rapid decline in membrane integrity, which is generally manifested as leakage of mobile cellular solutes (e.g., K^+) and cell death. At toxic concentrations, Cu interacts with cellular nucleic acids and enzyme active sites, although a principal initial site of Cu action is considered to be at the plasma membrane (Avery *et al*., 1996). Also Bitton *et al*. (1984) reported that the effective concentration of Cu which inhibits 50% of the *Saccharomyces cerevisiae* cells (EC_{50} value) was 5.6mg/L.

2. Effect of manganese.

The results of *S. cerevisiae* biomass, protein content, total viable cells and fermentation power as affected by different Mn concentrations namely 0, 5, 10, 15, 20, 25, 30 and 35 ppm are shown in Figure (2). Data disclose that, the optimum Mn concentration for the four studied properties was ranged between 20-25 ppm. The highest values of yeast yield, protein content, total viable cells and fermentation power were confirmed with the growing of yeast strain under the same concentration being 44.57 g/L, 52.63 %, 14.00×10^9 CFU/g and 925 $Cm^3CO_2/1^{st}$ h., in succession. Low values in this respect were observed either above or below the optimum Mn concentration with special reference to the highest ones.

In this concern, Liu *et al.* (1997) reported that transition metals such as Mn serve as essential cofactors for a variety of enzymatic reactions and play important structural and functional roles in cell metabolism. However, these ions can be toxic when present at elevated levels, and this may explain that increasing concentrations of Mn above 20-25 ppm result in an inhibition of yeast growth, which result in lowering of protein content, fermentation power and total viable cells.

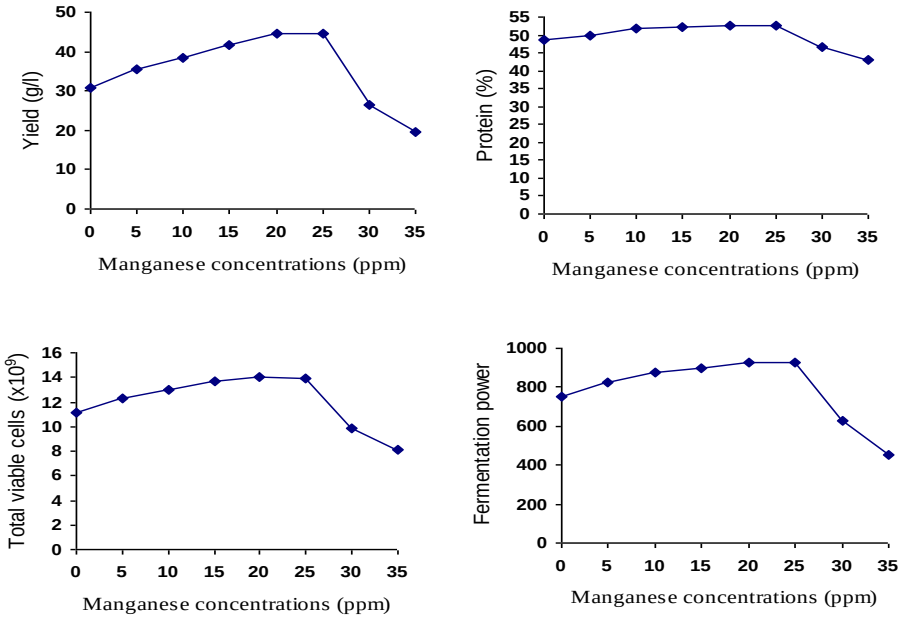

Fig (2). Effect of manganese on baker's yeast properties

Also, Jones and Gadd (1990) reported that Mn ions are very important because they have a positive effect on the respiratory activity and the growth rate of *Saccharomyces cerevisiae*, they added that yeast cells require Mn as an essential trace element at a concentration of 2–10 µM for optimal yeast growth. Moreover Tomas *et al*. (2004) reported that the specific growth rate of *Saccharomyces cerevisiae* was higher in a continuous batch culture, if Mn^{2+} ions were present in optimal concentrations in the medium. Mn has an important role in the metabolism of *S. cerevisiae* as a part of some enzymes, *e.g.* pyruvate carboxylase.

3. Effect of lead.

The growth medium was adjusted to Pb concentrations ranged between 0 to 14 ppm. Results (Figure 3) show the significant gradual decreases in yield as well as protein content, total viable cells and fermentation power by increasing Pb concentration in the growth medium. The decrease of all parameters under investigation may be due to the high toxicity of Pb, which considered non essential element for baker's yeast growth. Throughout the range of applied Pb concentrations (0-14 ppm) the yield of baker's yeast reduced from 30.67 to 5.80 g/L, protein reduced from 48.48 to 39.78 %, total viable cells reduced from 11.13 to 1.75 CFU/g×10^9 and fermentation power decreased from 750 to 125 Cm^3 CO_2/1st h.

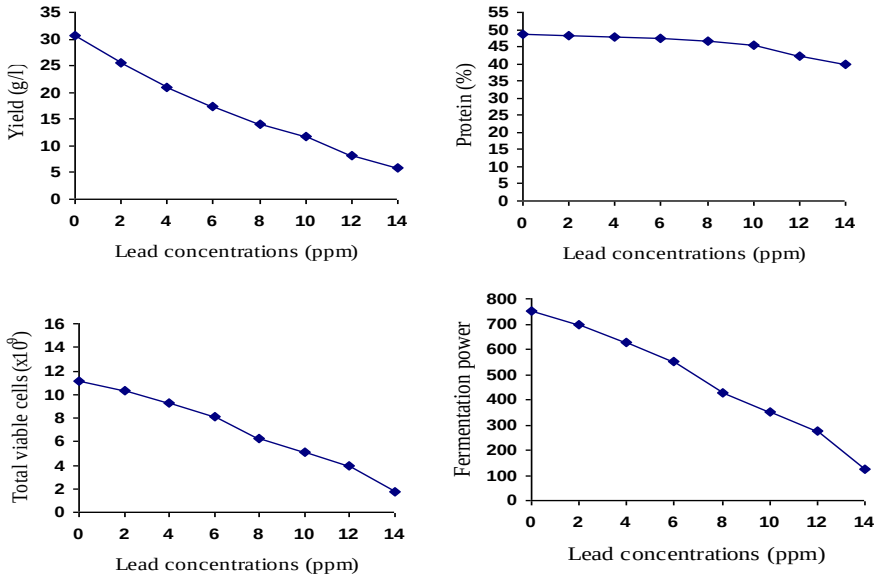

Fig (3). Effect of lead on baker's yeast properties

These results are coincide with Soares *et al*., (2003) who revealed that addition 200µM Pb to yeast cell suspension resulted in a decrease of about 15% of the viability in the first 20min. Baker's yeast is sensitive to any concentrations of Pb with an extension of lag phase and duration of the fermentation and an overall decrease in final cell biomass production. Similar findings were noticed by Skountzou *et al*. (2003) who indicated that baker's yeast growth was affected by adding Pb (10mg/L), resulted in a negative effect on growth rate and maximum dry cell concentration in the aerobic fermentation of molasses. Moreover a Pb had a toxic effects, reduced final biomass when compared to the control, increased fermentation times and decreased cell growth rates up to 50% of that in the control.

4. Effect of cadmium.

Different Cd concentrations ranged between 0 to 3.5 ppm were tested in order to determine their effect on *S. cerevisiae* properties (yield, protein content, total viable cells and fermentation power). Data presented in Figure (4) show a significant gradual decrease in all tested parameters by increasing Cd concentration in the growth medium. Inhibition was variable depending upon Cd concentrations. On the other hand, Cd at 0.5 ppm, did not have a significant effect on baker's yeast properties. Throughout the range of applied Cd concentrations (0 - 3.5 ppm) the fresh yield of yeast reduced from 30.67 to 6.20 g/L, protein reduced from 48.48 to 38.53 %, total viable cells declined from 11.13 to be 2.50 CFU/g × 10^9 and fermentation power reduced from 750 to 175 Cm^3Co_2 /1st h.

These results are in agreement with those reported by Hassen *et al*. (1998) who found a variable inhibition effect of Cd on *Pseudomonas aeruginosa* and *Bacillus thuringiensis* depended on the Cd concentrations in the medium. Generally, higher concentrations of metal caused a higher inhibition, in this respect, Ross (1975) and Gadd (1993) stated that Cd is nonessential for biological functions and strong inhibitors of microbial metabolism even at low concentrations. Moreover, Mapolelo *et al*. (2005) reported that Cd strongly binds to functional groups on yeast cell walls. Hence this result in formation of a Cd-complex with a high formation constant, which facilitates the displacement of other metals.

Cd toxicity depends on its ability to form complexes with some biological anti-oxidant defense. In support of this hypothesis, a major effect of Cd is to cause oxidative stress, particularly lipid peroxidation Gomes *et al*. (2002). Toxicity of Cd may be also caused by depletion of glutathione (GSH), considered as a major antioxidant in yeast cells (Schmitt *et al*., 2004).

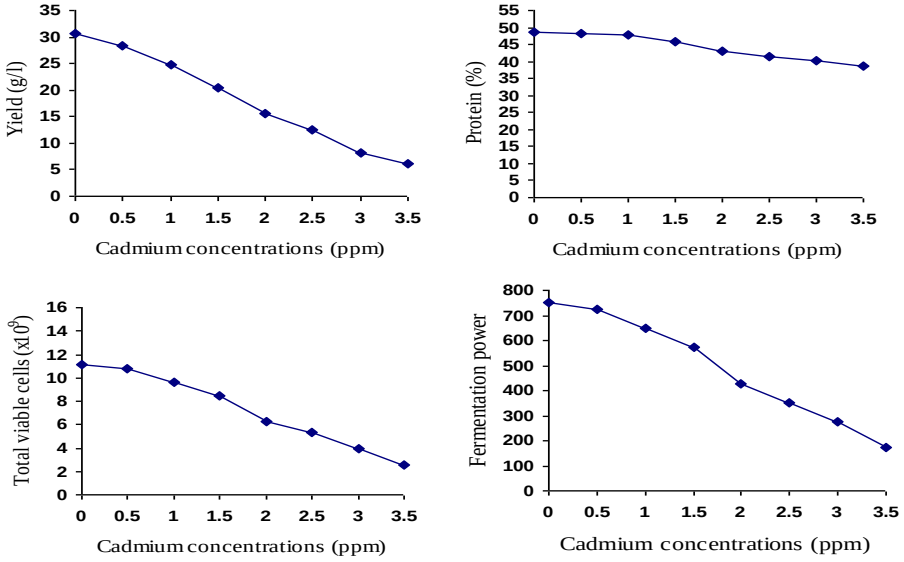

Fig (4). Effect of cadmium on baker's yeast properties

CONCLUSION

From the previous presentation of the results, it can be found that adding Cu metal to the growth media of *S. cerevisiae* at concentrations ranged between 0 to 14 ppm, the yield, total viable cells, protein content and fermentation power were increased at 2 ppm Cu. When the Cu concentrations increased more than 2 ppm, the values of four previous parameters were decreased. Similar trend was seen with Mn, the values of previous parameters increased when Mn concentration increased from 0 to 25 ppm, then decreased at Mn concentrations above 25 ppm. On the other

hand, Pb and Cd have negative effect on the yield, total viable cells, protein content and fermentation power at all applied concentrations. This is because they are non essential metals for biological function of the yeast and they are strong inhibitors of its metabolism.

It can be concluded from the obtained results that Cu concentration of 2 ppm and Mn concentrations ranged between 20-25 ppm were recommended to be the optimum concentrations in growth media of *S. cerevisiae*. In addition, the growth media must be free from Pb and Cd as they inhibited the activity and the growth of baker's yeast at any concentrations. Generally, higher concentrations of metals caused a higher inhibition and the effect of metal ions on yeast growth depended on the metal species.

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تـأثير النحـاس والمنجنيـز والرصـاص والكـادميوم علـى الخصـائص التكنولوجيـه لخميرة الخباز

نجـوى محمـد الشـيمى* ، أحمـد محمـد أهـاب* ، مجـدى محـب الـدين سـعد و جمعه نور الدين عبد الرحمن****

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**** قسم سموم وملوثات الغذاء – المركز القومى للبحوث – الدقى – الجيزة – مصر**

فى هذه الدراسه تم تقييم تأثير تركيزات مختلفة من العناصر المعدنية الثقيله (النحاس – المنجنيز – الرصاص – الكادميوم) على أربعة صفات لخميرة الخباز (ناتج الخميرة – عدد الخلايا الحية – محتوى البروتين – قوة التخمير) التى توضح الخصائص التكنولوجية لخميرة الخباز.

وقد أظهرت النتائج أن زيادة تركيز النحاس إلى ٢ جزء فى المليون والمنجنيز إلى ٢٠ – ٢٥ جزء فى المليون أدى إلى زيادة الصفات التى تم دراستها ، فى حين أن التركيزات الأعلى من تلك العناصر أحدثت تأثير سلبى على صفات الخميره . وعلى الجانب الأخر وجود كلا من الرصاص أو الكادميوم فى بيئة النمو أدى إلى إنخفاض خصائص خميرة الخباز ، وزاد هذا الإنخفاض بزيادة تركيز الرصاص أو الكادميوم . وقد اتضح مما سبق أن عنصرى الرصاص والكادميوم يصنفان كعناصر سامه لنمو الخميرة عند أى تركيز لهما فى البيئة ، فى حين أن التركيزات المثلى للنحاس والمنجنيز فى بيئة نمو الخميرة والتى أعطت أعلى قيم لكل الخصائص التكنولوجية للخميرة التى تم دراستها كانت ٢ جزء فى المليون و ٢٠ – ٢٥ جزء فى المليون، على التوالى .