Zeszyty Problemowe Postępów Nauk Rolniczych
nr 596, 2019, 13–21
DOI 10.22630/ZPPNR.2019.596.2

RESEARCH ON THE QUALITY OF BAKER'S YEAST ENRICHED WITH CHROMIUM

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Summary. Current study investigated the effect of Cr(III) applied in doses of 0.01 and 0.05 g per 1 dm^3 of culture medium on its absorption and effects on growing *Saccharomyces cerevisiae 2200* yeast. Cr(III) originated from its two inorganic salts: $CrCl_3 \times 6H_2O$ and $Cr_2(SO_4)_3 \times 18H_2O$. Yeasts were cultured using a biofermenter, on a molasses wort, for 24 h, at a temperature of 28°C and pH 5,0. Irrespective of type of chromium salt applied for yeast enrichment, a decrease was observed in biomass yield compared to the control group, in the case of both Cr(III) doses, i.e. 0.01 and 0.05 $g \cdot dm^{-3}$. The working yeasts samples were characterized by a higher content of protein as compared to control groups irrespectively on the source and dose of added chromium. Conversely, the amount of chromium bounded in working samples depended on both dose and source of chromium. The fermentation activity of the yeast supplemented with chromium was lower than the value stipulated in the Polish Standard.
Key words: yeast, *Saccharomyces cerevisiae*, chromium (III), enrichment

INTRODUCTION

Chromium is considered microelements – the so-called trace elements, the demand for which reaches <100μg/day. An indispensable component of men' and animals' diet is trivalent chromium ion [Pechova and Pavlata 2007, Gupta and Gupta 2014, Lewicki et al. 2014].

When combined with nicotinic acid, glutamic acid and with glycine and cysteine, chromium (III) forms a compound called Glucose Tolerance Factor (GTF), which aids the action of insulin [Tulasi and Jayantha Rao 2014]. It controls the blood level of choleste-

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rol, reduces atherosclerosis of blood vessels, and increases immunity to infections [Wang et al. 2007, Kośla et al. 2018]. Some hypotheses exist that chromium enhances glucose accumulation in muscles and stimulates glycogen synthesis, thus reduces fat deposition and, eventually, prevents obesity. In addition, chromium plays some role in the metabolism of selected proteins. It occurs, beside others, in the active centre of trypsin – one chromium atom per each molecule of this enzyme [Kośla et al. 2018]. This element is also present in RNA and is claimed to stabilize its structure [Pas et al. 2004].

The recommended daily allowances of chromium depend on age and gender, and the demand for this element is increasing along with an increasing physical effort due to its higher urination [Krejpcio 2001].

The bioavailability of chromium to a body is affected by the form it is administered in. Chlorides, sulfates or chromates of trivalent chromium, i.e. its inorganic compounds, are hardly absorbable (ca. 1%). In contrast, when administered in the GTF form, e.g. from liver or yeast (brewer's yeast), it is absorbed in 15–20 % [Kośla et al. 2018].

The transformation of inorganic chromium into a biologically-active form is of significance to its physiological effects [Batic and Raspor 2000, Berner et al. 2004]. Investigations on the role of bioelements in metabolic processes in animals and men have demonstrated that they are better assimilable, more effective and less toxic in the organic form bound with protein, i.e. metalloproteins - the so-called bioplexes [Dębski et al. 2004, Ohh and Lee 2005, Dominguez-Var et al. 2009]. The natural capability for bioplexes formation has been reported in the case of baker's yeast, hence a possibility exists for them to be used as a carrier of essential trace elements, including chromium [Błażejak et al. 2003].

Baker's yeast are commonly accepted by consumers and included into the GRAS group (Generally Recommended as Safe), meaning that they are safe to human. They are a source of both E and H vitamins, and protein of a high nutritive value that is one constituted by many exogenous amino acids (with a particularly high content of lysine). For this reason, they are a perfect food additive that increases the nutritive value of bakery products, soups, and concentrates; they may as well be used for the production of bioplexes, e.g. chromium ones. Literature data demonstrate that yeast of the species *Saccharomyces cerevisiae* are capable of absorbing up to 30 mg Cr(III)·g^{-1} CDW (CDW – cell dry weight) [Kaszycki et al. 2004].

It should be emphasized that in the yeast industry valuable are those yeast strains that are characterized by fast proliferation, assure high stability of the finished product during storage and a high force of dough raising [Lipińska 2010]. Hence, considering chromium yeast production, it is of chief point to adjust conditions of cell enrichment that would allow the biomass to display traits desirable by the yeast industry, with simultaneously the highest possible absorption of chromium (III). Due to economic concerns, no decrease should occur in biomass yield, whereas protein content of biomass should not increase, because it could deteriorate their stability. In addition, a key problem is posed by the selection of the appropriate dose and source of chromium (III), as in high doses it may inhibit yeast growth [Liu et al. 2001, Jianlong et al. 2004, Pas et al. 2004, Ksheminska et al. 2005].

The objective of current study was to examine the absorption of trivalent chromium from selected inorganic compounds by an industrial strain of baker's yeast cultured in molasses wort.

MATERIALS AND METHODS

In this study, we used industrial strain of baker's yeast of the *Saccharomyces cerevisiae 2200*, obtained from the Museum of Pure Cultures of the Department of Biotechnology and Food Microbiology, Warsaw University of Life Sciences.

The yeasts were stored at 4 °C on solid YPD medium and transferred every four weeks to fresh medium.

The following inorganic salts of chromium (III) were used in order to enrich yeast cells in chromium: $CrCl_3 \times 6H_2O$ and $Cr_2(SO_4)_3 \times 18H_2O$. Two doses: 0.01 and 0.05 $g \cdot dm^{-3}$ of culture medium for every studied salt were applied, respectively.

Inoculation and yeast propagation were performed in a medium containing molasses. Molasses (81°Blg) was diluted to 10°Blg, and afterwards two salts were added: $NH_4H_2PO_3$ 1,9 $g \cdot dm^{-3}$ (POCH) and $(NH_4)_2SO_4$ 8,90 $g \cdot dm^{-3}$ (POCH). The pH value was adjusted to 5.0 ±0.2 using of 25% H_2SO_4. The inoculum culture was run at 28°C, for 24 h on a reciprocating shaker (SM-30 Control E. Büchler, Germany) at a vibration amplitude of 200 cycles/min. The resultant inoculum was used to inoculate the medium in the biofermenter in a dose determined based on the measurement of optical density (OD) in a Spectronic 20 Genesys spectrophotometer, at a wavelength of 600 nm. The inoculation was planned so as to assure the OD of mother yeast at a level of 1.6. The biomass was cultured in a Bioflo 3000 biofermenter (New Brunswick Scientific, USA) with a working volume of 4000 cm^3.

After 24 h culture in a biofermenter, the cell biomass was separated from the medium by 10-minute centrifugation (SIGMA centrifuge, $3000 \times g$, +4°C).

After preliminary separation of biomass from the medium, it was rinsed three times with sterile distilled water and re-centrifuged after each rinsing (parameters the same as given above). The stage of yeast cells rinsing with sterile distilled water was aimed at removing chromium that was not permanently bound with a cell. In addition, the rinsing enabled elimination of contaminants and molasses mucilages from biomass that could adulterate the quantity of chromium actually bound by cells of *Saccharomyces cerevisiae 2200* yeast.

The yield of resultant yeast biomass was determined with the gravimetric method. Protein content of biomass was assayed with the Kjeldahl's method [AOAC 1995].

The fermentation activity was determined based on the measurement of the volume of carbon dioxide emitted from dough fermenting for two hours at a temperature of 35°C in a fermentograph's chamber (N 434, SJA, Sweden). Results were read out after 60 and 120 minutes. The dough was prepared from: 5 g of baker's yeast, 160 cm^3 of a 25% solution of table salt, and 280 g of wheat flour type 500.

The quantity of chromium bound by yeast biomass was determined with the method of atomic emission spectrometry with inductively coupled plasma (ICP-AES) at three wavelengths: 267.7; 283.5 and 284.3 nm. Results were expressed per mg Cr(III)·100 g^{-1} cell dry weigh (CDW) of yeast.

Statistical analysis of results

Results achieved were analyzed statistically with Statgraphics Plus ver. 4.1 software, using the two-way analysis of variance and Tukey's test, at a significance level of $\alpha = 0{,}05$.

RESULTS AND DISCUSSION

The possibility of applying baker's yeast on the industrial scale to produce chromium yeast and natural protein-mineral preparations is determined by both the absorption of significant quantities of deficient elements, including chromium (III), by yeast cells, and also by obtaining a good yield of biomass characterized by an appropriate protein content and stability.

One of the basic assumptions of this study was to investigate whether the addition of chromium (III) from $CrCl_3 \times 6H_2O$ and $Cr_2(SO_4)_3 \times 18H_2O$ to a culture medium (molasses wort) in doses of 0.01 and 0.05 $g \cdot dm^{-3}$ affects the yield of *Saccharomyces cerevisiae 2200* yeast biomass produced after 24-hour culture in a biofermenter. Results of the yield of yeast biomass from the culture on the control medium and medium containing both sources of Cr(III) in two doses were presented in Table 1. They demonstrate that the presence of chromium salts in the culture medium, irrespective of their type and dose, reduced the amount of biomass produced. In samples with $CrCl_3 \times 6H_2O$, at doses of 0.01 and 0.05 $g \cdot dm^{-3}$ Cr(III), the biomass yield was 92.3 and 96,5% of control sample yield, respectively. In the case of the same doses of chromium sulfate biomass yield accounted for 96,5 and 97,5%, respectively. The statistical analysis of results demonstrated that biomass yield was affected only by the dose of chromium, irrespective of its source.

Table 1. Yield of yeast biomass and content of protein and chromium depending on the source and dose of Cr(III)

Tabela 1. Wpływ dawki Cr(III) oraz jego źródła na plon biomasy drożdży oraz zawartość chromu i białka

Source of Cr(III) Źródło Cr(III)	Dose of Cr(III) [$g \cdot dm^{-3}$] Dawka Cr(III)	Yeast cell biomass yield [$g_{CDW} \cdot dm^{-3}$] Plon biomasy drożdży	Protein [$g \cdot 100g_{CDW}^{-1}$] Białko	Content of Cr(III) [$mg \cdot g_{CDW}^{-1}$] Zawartość Cr(III)
	[$g \cdot dm^{-3}$]	[$g_{s.s.} \cdot dm^{-3}$]	[$g \cdot 100g_{s.s.}^{-1}$]	[$mg \cdot g_{s.s.}^{-1}$]
control sample próba kontrolna	0	11.10 ±0.35^c	51.0 ±0.72^c	0.14 ±0.02^a
$CrCl_3 \times 6H_2O$	0.01	9.10 ±0.15^a	53.2 ±1.1^a	0.58 ±0.07^d
	0.05	10.15 ±0.47^b	53.5 ±0.9^b	0.47 ±0.04^c
$Cr_2(SO_4)_3 \times 18H_2O$	0.01	9.30 ±0.43^a	52.4 ±0.81^a	0.21 ±0.05^b
	0.05	10.41 ±0.38^b	54.5 ±1.38^b	0.60 ±0.03^d

a, b, c – values in the columns with different letters are significantly different at $\alpha = 0,05$

a, b, c – wartości w kolumnach oznaczone niejednorodnymi literami są statystycznie różne przy $\alpha = 0,05$

Liu et al. [2001] observed a descending tendency in biomass yield along with an increasing concentration of chromium in the culture medium, above 0.4 $g \cdot dm^{-3}$, yet they noted a simultaneous increase in the content of chromium in yeast cells. In the case of lower concentrations of Cr(III) in the culture medium, the yield of cell biomass was found to increase. It ought to be emphasized, however, that investigations of the above-mentio-

ned authors were conducted under different culture conditions, namely: with YPG culture medium. This medium differs substantially from molasses medium apart from mineral salts contains saccharose.

Jianlong et al. [2004] were enriching *Saccharomyces cerevisiae* yeast with Cr(III) that originated from chromium (III) chloride. They observed that growth curves of Cr(III)-enriched yeasts were characterized by a longer lag phase compared to the control. It means that in presence of $CrCl_3$ salt, the yeasts were adapting for a longer time and thereby time of generation was longer. The extended generation time of yeasts cultured in the medium with addition of $CrCl_3 \times 6H_2O$, compared to the control culture, was the reason behind a lower biomass yield reached after the same time of culture in control and enriched samples.

The content of protein in yeast is strictly linked to stability and fermentation activity of yeast cells. An increase in protein content of yeast cells is accompanied by an increase in their fermentation activity and, simultaneously, by a significant decrease in their stability. According to Lipińska [2010], the recommended content of protein in yeast biomass is between 38 and 42% d.w.

Results of protein content determination in yeast biomass from the culture on the medium containing Cr(III) from $CrCl_3 \times 6H_2O$ and $Cr_2(SO_4)_3 \times 18H_2O$ in doses of 0.01 and 0.05 $g \cdot dm^{-3}$, are presented in Table 1. Both in the control and working samples the content of protein was high and reached over 50%. Simultaneously, a tendency was observed for an increasing protein content along with an increasing concentration of chromium in the culture medium. The highest protein content (54.5 %) was determined in the case of yeast cultured in the medium with $Cr_2(SO_4)_3 \times 18H_2O$ in a dose of 0.05 $g \cdot dm^{-3}$. The statistical analysis of study results showed that protein content of yeast biomass was influenced only by the dose of chromium, irrespective of its source. In turn, Jianlong et al. [2004] in their study on baker's yeast *Saccharomyces cerevisiae* enrichment in Cr(III) originating from chromium chloride, observed that at a dose of 15 μM $Cr \cdot dm^{-3}$ the content of protein was decreasing compared to the control sample.

Inorganic salts of chromium penetrate with difficulties through cell membranes, therefore the transformation of inorganic Cr(III) to a biologically-active form is of significance to its physiological effects [Lewicki et. al. 2014]. In view of that, use can be made of the natural capability of baker's yeast to bioaccumulate elements [Jianlong et al. 2004, Mapolelo et al. 2004, Pas et al. 2004]. Literature data indicate that the yeast of *Saccharomyces cerevisiae* species are capable of absorbing up to 30 mg Cr(III)$\cdot g^{-1}$ CDW [Kaszycki et al. 2004].

This study investigated which of Cr(III) inorganic salts and in what doses would allow to achieve a greatest quantity of this element bound by yeast cells, without having a negative impact on biomass yield. Results of the determination of Cr(III) quantity bound by cells of baker's yeast from the culture on the media containing Cr(III) from $CrCl_3 \times 6H_2O$ and $Cr_2(SO_4)_3 \times 18H_2O$ in doses of 0.01 and 0.05 $g \cdot dm^{-3}$, are presented in Table 1. The results demonstrate that the yeast cultivated on the molasses medium without the addition of chromium salt (control sample) were characterized by a low level of this bioelement in cells reaching 0.14 $mg \cdot g^{-1}$ CDW, whereas those cultivated in the medium enriched with Cr(III) were observed to bound it relatively well. The best Cr(III) bounding by the investigated yeast was achieved using $CrCl_3 \times 6H_2O$ in a dose of 0.01 $g \cdot dm^{-3}$ (0.58 $mg \cdot g^{-1}$

CDW) and $Cr_2(SO_4)_3 \times 18H_2O$ in a dose of 0.05 g Cr·dm^{-3} (0.60 mg·g^{-1} CDW). In both cases, analyses showed the highest content of protein (Table 1). In addition, after the culture in the medium with $Cr_2(SO_4)_3 \times 18H_2O$ in a dose of 0.05 g·dm^{-3} Cr(III), a relatively small reduction was observed in biomass yield, compared to the control sample.

The statistical analysis of results demonstrated that the quantity of chromium bound by yeast cells was affected by both: dose and source of chromium. Differences noted between particular salts in the quantity of chromium bound by yeast may be due to the effect of an acid residue of the chromium salt used in the study.

The technological usability of baker's yeast is determined, most of all, by their fermentation activity. The value of the fermentation activity of the studied yeast, expressed by the volume of emitted CO_2, was in each case lower than the requirements of the Polish Standard for baker's yeast, according to which the emission should not be less than 600 cm^3 after one hour of dough fermentation and 1400 cm^3 after two hours (Table 2).

Table 2. Fermentation activity of *Saccharomyces cerevisiae 2200* yeast enriched with Cr(III)

Tabela 2. Aktywność fermentacyjna drożdży *Saccharomyces cerevisiae 2200* wzbogaconych Cr(III)

Source of Cr(III)	Dose of Cr(III)	Emitted CO_2	
		after 60 min	after 120 min
	Dawka Cr(III) [g·dm^{-3}]	Wydzielony CO_2 [cm^3]	
		po 60 min	po 120 min
control sample próba kontrolna	0	280 ±23	1025 ±21^b
$CrCl_3 \times 6H_2O$	0.01	250 ±21	1027 ±25^b
	0.05	230 ±34	850 ±29^a
$Cr_2(SO_4)_3 \times 18H_2O$	0.01	212 ±28	937 ±31^a
	0.05	200 ±30	905 ±25^a

a, b, c – values in the columns with different letters are significantly different at $\alpha = 0.05$

a, b, c – wartości w kolumnach oznaczone niejednorodnymi literami są statystycznie różne przy $\alpha = 0.05$

Worthy of attention is, however, the fact that the yeast analyzed in this study were cultured under laboratory conditions, hence should be compared to a control sample – yeast cultured in a medium without Cr(III) addition. The best fermentation activity was determined for the yeast cultured on the control medium and on the medium with the addition of 0.01 g·dm^{-3} Cr(III) originating from $CrCl_3 \times 6H_2O$. The other samples displayed a significantly poorer fermentation activity. As it results from data presented in Tables 1 and 2, the high content of protein had no effect on the fermentation activity of the investigated yeast.

Little literature data is available on the fermentation activity of biomass of baker's yeast enriched with Cr(III). Cervantes et al. [2001] reports that the rate of fermentation is inhibited at a chromium concentration in the medium above 30 mM. During assay of the fermentation activity of the yeast samples examined, this concentration was not exceeded.

CONCLUSIONS

Based on the results obtained, the following statements and conclusions were formulated:

- The enrichment of culture medium with Cr(III) salts causes a decrease in biomass yield compared to the control sample, irrespective of the dose and source of Cr(III).
- The $CrCl_3 \times 6H_2O$ and $Cr_2(SO_4)_3 \times 18H_2O$ salts affect an increase in protein content of yeast cells proportionally to an increasing concentration of chromium in the culture medium, irrespective of the type of salt.
- Out of the two salts examined, a better source of Cr(III) seems to be $Cr_2(SO_4)_3 \times 18H_2O$ in a dose of 0,05 $g \cdot dm^{-3}$ Cr(III), in the case of which the increase in Cr(III) content in yeast cells was the highest and accompanied by a relatively small decrease in biomass yield, compared to the control sample.
- The best fermentation activity was noted for the yeast cultured in the medium with the addition of 0.01 $g \cdot dm^{-3}$ Cr(III) from $CrCl_3 \times 6H_2O$.
- Results of Cr(III) binding by the investigated industrial strain of *Saccharomyces cerevisiae* yeast show the feasibility of their application as a chromium preparation.

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OCENA WARTOŚCI TECHNOLOGICZNEJ DROŻDŻY PIEKARSKICH WZBOGACONYCH CHROMEM

Streszczenie. W pracy zbadano wpływ Cr(III) stosowanego w dawkach 0,01 i 0,05 g·dm^{-3} na wzrost drożdży *Saccharomyces cerevisiae 2200* oraz wybrane parametry jakościowe uzyskanej biomasy (zawartość chromu związanego przez komórki, zawartość białka, siła pędna). Źródłem Cr(III) były jego dwie nieorganiczne sole: $CrCl_3$ i $Cr_2(SO_4)_3$. Hodowlę drożdży prowadzono w bioreaktorze w podłożu melasowym, przez 24 h, w temperaturze 28°C. Bez względu na rodzaj użytej soli Cr zaobserwowano spadek wy-

dajności biomasy w stosunku do próby kontrolnej, zarówno w przypadku dawki 0,01 jak i 0,05 g·dm^{-3}. Suplementacja podłoża Cr(III) skutkowała uzyskaniem biomasy o większej zawartości białka oraz chromu. Ilość związanego chromu zależała zarówno od dawki jak i źródła. Siła pędna badanych drożdży nie spełniała wymagań PN.

Słowa kluczowe: drożdże, *Saccharomyces ceresisiae*, chrom (III), wzbogacanie