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EFFECT OF DOWNSTREAM PROCESSING ON CONTENT OF GLUTATHIONE AND CELL VIABILITY DURING PRODUCTION OF INACTIVE DRY YEAST

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Glutathione (GSH) is a tripeptide thiol with important protective activity in living organisms. Due to its ability to prevent oxidation, this substance is commonly used in biotechnological products. The fed-batch process with yeasts of the genus *Saccharomyces* coupled with downstream processing to obtain inactive dry yeasts with high GSH content is the common method for industrial production. Short-term high-temperature treatment (1 minute at 105 °C) emerged as potentially optimal, achieving CFU/g is less than 10² while maintaining relatively high GSH levels. Another approach showed the promise of adjusting pH changes to levels of 2.4–3.0 without altering inactivation conditions (95 °C/30 min) as an alternative to the previous one. Further studies are needed to explore the complex interplay between acidic conditions, duration of inactivation, and temperature parameters for simultaneously maximizing glutathione retention while controlling microbial viability.

Keywords: glutathione, inactive dry yeasts, CFU.

Introduction

Glutathione (GSH, γ -L-glutamyl-L-cysteinylglycine) is a tripeptide thiol compound that plays a crucial role in cellular defense against oxidative stress [8, 13]. Due to its potent antioxidant properties, GSH has attracted significant attention for applications in pharmaceuticals, food supplements, and cosmetics [4, 7, 11]. Yeast-derived GSH is particularly valuable due to its natural origin and the well-established status of yeast as Generally Recognized As Safe (GRAS) [1, 9].

The production of inactive dry yeast with high GSH content represents a significant challenge in industrial biotechnology. While substantial progress has been made in optimizing fermentation conditions to enhance intracellular GSH accumulation in *Saccharomyces cerevisiae* [6, 11], considerably less attention has been directed toward downstream processing stages, which can result in GSH losses exceeding 40–60 % of the initial content [9, 14].

The preservation of GSH during downstream processing faces multiple challenges due to its chemical instability. The tripeptide's thiol group (-SH) is highly susceptible to oxidation, particularly

under aerobic conditions commonly encountered during processing [7]. Additionally, GSH can undergo hydrolysis of its peptide bonds and form mixed disulfides with cellular proteins [3, 12].

Temperature and pH represent critical factors affecting GSH stability during downstream processing. Studies have demonstrated that GSH degradation accelerates significantly with increasing temperatures, particularly above 60 °C [7, 15]. At temperatures commonly used for thermal inactivation (70–80 °C), GSH half-life decreases to less than 30 minutes [11]. Regarding pH, GSH exhibits maximum stability within the narrow range of 5.0–7.0. Deviations toward alkaline conditions (pH > 7.5) accelerate auto-oxidation of the thiol group, while strongly acidic conditions (pH < 3.5) promote hydrolysis of peptide bonds [11, 18].

Despite the recognized importance of these parameters, significant knowledge gaps persist in our understanding of GSH preservation during downstream processing, particularly in industrial-scale production environments. Industry relies on general knowledge of yeast biology and consultants. The latter ones usually operate under strict non-disclosure

agreements which further limits data availability. It is worth noting that the combined and interactive effects of temperature and pH throughout the entire downstream processing chain remain largely unexplored. Although individual effects have been documented, the synergistic impacts when unfavorable temperature and pH conditions coincide are poorly characterized. For example, GSH degradation at elevated temperatures was substantially higher at alkaline pH compared to neutral conditions [9]. This suggests complex interaction mechanisms that warrant further investigation [2].

Therefore, the **goal of this work** is to establish how downstream processing can change the GSH content in the yeast.

Materials and research methods

The experimental part of the work was carried out at the laboratory of PRJSC ENZYM COMPANY. The commercial strain of *S. cerevisiae* with high potential to produce GSH was grown similarly to regular baker's yeast and was processed to obtain yeast cream. The exact name of the strain and cultivation regime details are undisclosed because such information had been considered a commercial secret.

Moisture content was measured via RADWAG MA50.R moisture analyzer.

pH of yeast cream was measured by a pH-meter and was changed by the means of addition of 10 M KOH and 75 % H_3PO_4 solutions. The addition of mentioned components occurred in minimal quantities necessary for pH adjustment. The choice of concentrations was determined by the applied part of these studies, as these specific concentrations of acids and bases are used in production conditions at the biotechnological enterprise. The use of reagents with high concentrations is due to the minimization of introducing additional water into the product before drying in terms of energy consumption optimization.

Inactivation procedures were carried out using Memmert WTB15 water baths and shake flasks. 200 ml of yeast creme were poured into 500 ml shake flasks and heated during different times at the required temperatures.

For colony-forming unit measurement, 1-g samples were diluted in 100 mL of 0.31 mM phosphate buffer (pH 7.2) and homogenized for 5 min by using a magnetic stirrer. This solution was serially

diluted (1:10) and appropriate dilutions were inoculated on YPD plates. Plates were incubated at 30 °C for 3 days before yeast colonies were counted.

Subsequent drying of yeast cream was conducted by the means of an ESDT1 spray dryer.

GSH production was measured according to [15].

Results and discussion

Typical commercial GSH-rich inactive yeasts production consists of cultivation stage while biomass was grown and downstream processing stage in order to obtain actual product in form of packaged dry yeasts. During the downstream processing stage yeast biomass undergoes separation from the medium remnants and subsequent washing out until yeast cream with 18–22 % of moisture content is received. Such yeast cream can also be acid-treated to keep the pH relatively low to make bacterial contamination impossible. Following heat treatment necessary for yeast inactivation and inactivated yeast cream then dried to obtain dry yeasts.

The GSH-reducing steps of downstream processing (pH change, heating and drying) were mimicked at the down-scale format. At first, it was necessary to look after pH change and its effect on the GSH content and CFU in dried yeasts (Table 1). Three different pH values were tested (2.4 and 10) whereas untreated yeast cream was used as a control (pH value 5). All samples undergo heat treatment at 95 °C for 30 min and subsequently spray dried. Dry yeasts were diluted in distilled water to obtain a mixture with 5 % dry matter in order to check the possible pH change after the drying process.

There was an insignificant change of GSH levels after pH treatment. pH shift strategy can lead to either GSH accumulation or loss, depending on conditions and strain [5, 10]. However, there is a lack of evidence that this methodology was used during post-fermentation processing, rather than only during cultivation. Under extreme alkaline or acidic conditions, yeast cells rapidly accumulate reactive oxygen species (ROS). This oxidative burden triggers the upregulation of oxidative stress response genes, which encode enzymes critical for glutathione biosynthesis [16]. Lack of significant change in GSH content can be attributed to the short duration of inactivation thus the mechanism described was not able to

start up. Inactivation process caused loss of 0.4–1.6 % of GSH with the highest loss in the probe 8. Following drying led to loss of 0.5–0.6 % GSH. The duration of heating during inactivation is longer than during drying thus we suggested that it caused bigger GSH loss.

It is worth noting that the CFU of the dry product was quite different in all samples and especially low in the 8 probe. It is generally known at yeast factories that high pH is detrimental to yeast growth which could explain such results. There is a common practice at GSH market to keep CFU equal or less than 10^2 whereas we obtained such results only in sample 10.

Inconclusivity of data led us to further investigate the relationship between GSH, CFU and pH treatment.

There was also a technology demand to keep yeast cream aseptic during downstream processing and acidic treatment is quite a common way to minimise bacterial contamination. Another experiment was conducted to evaluate the effect of an acidic pH treatment and inactivation on GSH content and quantity of CFU (Table 2).

According to our data, the acidic treatment caused an insignificant effect on GSH content, similar to the results from Table 1. Inactivation procedure led to loss of 0.5–0.6 % of GSH, contrary to the 0.4–1.6 % change from the first experiment. The difference between the magnitude of GSH content losses, as well as changes due to acid/base treatment, could be linked to the individual strain characteristics. GSH losses during drying were 0.14 % for control samples and 0.67–0.7 %.

Table 1

Effect of pH change and heating on GSH content and quantity of colony-forming units

No.	Description	GSH, %	CFU/g
1	Control sample	4.52 ± 0.15	N/A
2	Before inactivation. pH=2.4	4.57 ± 0.16	N/A
3	Before inactivation. pH=4	4.73 ± 0.1	N/A
4	Before inactivation. pH=10	4.71 ± 0.14	N/A
5	Control sample. After inactivation	4.10 ± 0.1	N/A
6	After inactivation. pH=2.4	3.94 ± 0.13	N/A
7	After inactivation. pH=4	3.99 ± 0.15	N/A
8	After inactivation. pH=10	3.22 ± 0.09	N/A
9	Dry yeasts. Control sample	3.48 ± 0.09	$3 \cdot 10^4$
10	Dry yeasts. pH=2.4	3.39 ± 0.1	$2 \cdot 10^2$
11	Dry yeasts. pH=4	3.52 ± 0.11	$3.6 \cdot 10^4$
12	Dry yeasts. pH=10	3.05 ± 0.12	0

Table 2

Effect of acidic treatment and heating on GSH content and quantity of colony-forming units (CFU)

No.	Description	GSH, %	CFU/g
1	Control sample	4.64 ± 0.2	N/A
2	Before inactivation. pH=3	4.98 ± 0.19	N/A
3	Before inactivation. pH=4	4.91 ± 0.21	N/A
4	Control sample. After inactivation	4.03 ± 0.15	N/A
5	After inactivation. pH=3	4.47 ± 0.15	N/A
6	After inactivation. pH=4	4.58 ± 0.18	N/A
7	Dry yeasts. Control sample	3.89 ± 0.16	$3 \cdot 10^4$
8	Dry yeasts. pH=3	3.8 ± 0.14	$3 \cdot 10^2$
9	Dry yeasts. pH=4	3.8 ± 0.2	$3.5 \cdot 10^6$

Table 3

Effect of the duration of inactivation on GSH and content quantity of colony-forming units (CFU)

No.	Temperature of inactivation, °C	Time of inactivation, min	GSH in dry product, %	Losses of GSH, %	CFU/g
1	N/A	0	3.8 ± 0.19 (in yeast cream)	N/A	>10 ⁹
2	N/A	0	3.4 ± 0.18	0.4	>10 ⁶
3	95	5	2.9 ± 0.14	0.9	2·10 ⁶
4	95	10	2.8 ± 0.1	1.0	2·10 ⁵
5	95	20	2.8 ± 0.1	1.1	4·10 ⁴
6	95	30	2.7 ± 0.13	1.0	3·10 ⁴
7	100	5	2.9 ± 0.12	0.9	<10 ³
8	100	10	2.5 ± 0.11	1.3	<10 ³
9	105	1	3.2 ± 0.15	0.6	<10 ²
10	105	5	2.7 ± 0.09	1.1	<10 ²

Notably, yeast cream used at control samples also had unequal GSH content despite a similar cultivation regime. Replicability issues are the common problem during GSH yeast production using complex media. CFU was almost identical to the results from the previous experiment. Yet the interest to keep CFU equal or less than 10² led to further investigation of the effect of thermal parameters and duration of inactivation.

The results of testing different time and temperatures during inactivation are shown in Table 3.

The obtained results showed that both CFU and GSH content in dry yeasts significantly depend on the duration of inactivation at 95 °C. Minimal values observed at the maximal duration of 30 min clearly indicate that extensive heating negatively affects the GSH content and cell viability. This result goes along with a report that proposes that GSH plays an important role mainly at the first 30 minutes of thermal-induced cell death [17]. Also, the conditions of some shorter inactivation regimes were tested. Inactivation for 5 minutes at 100 °C caused a drop of CFU by the order of 10 and inactivation for 10 minutes at the same temperature resulted in significant loss of the GSH. Short-term inactivation (1 min) at 105 °C led to the lowest CFU among the samples while GSH content was relatively high.

Conclusions

Our findings have been shown that high pH value of yeast cream and prolonged inactivation before drying can negatively affect both GSH content and CFU. The magnitude of GSH and CFU can be

attributed both to individual strain characteristics and possible replicability issues during cultivation due to the use of complex mediums. The latter one is extremely important to the correct interpretation of the results. The obtained results have practical application and were used for industrial production of inactive yeast with high glutathione content.

The optimal CFU/g is less than 10² and can be reached by the 1–5 minutes inactivation at the temperature of 105 °C. Acidic treatment of yeast cream to the pH = 2.4–3.0 led to the similar results. The relationship between acidic treatment and duration of inactivation presents a promising area for future studies. Specifically, further research could investigate the combined effects of acidic pH conditions and varying inactivation times and temperatures to optimize the process for both low CFU and minimal GSH loss.

Prolonged high-temperature inactivation has been shown to lead to significant GSH losses, highlighting the importance of carefully controlling these parameters. Future studies could focus on understanding this relationship more deeply to develop inactivation strategies that maximize yeast inactivation while preserving GSH content, simultaneously exploring the interplay between pH, temperature, and time.

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ВПЛИВ ПОСТФЕРМЕНТАЦІЙНОЇ ОБРОБКИ НА ВМІСТ ГЛУТАТІОНУ ТА ЖИТТЄЗДАТНІСТЬ КЛІТИН ПІД ЧАС ВИРОБНИЦТВА НЕАКТИВНИХ СУХИХ ДРІЖДЖІВ

Глутатіон (GSH) – це трипептидний тіол із важливою захисною активністю в живих організмах. Завдяки своїй здатності запобігати окисненню цю речовину широко використовують у біотехнологічних продуктах. Процес періодичного культивування з підживленням дріжджів роду *Saccharomyces* у поєднанні з подальшою постферментаційною обробкою для отримання неактивних сухих дріжджів з високим вмістом GSH є поширеним методом промислового виробництва. Короткотривала високотемпературна обробка (1 хвилина при 105 °C) виявилася потенційно оптимальною, забезпечуючи КУО/г менше 102, зберігаючи при цьому відносно високий рівень GSH. Інший підхід – це корекція рН до рівня 2,4–3,0 без зміни умов інактивації (95 °C/30 хв) як альтернатива до попереднього. Подальші дослідження необхідні для вивчення складної взаємодії між кислотними умовами, тривалістю інактивації та температурними параметрами для одночасної максимізації збереження глутатіону при контролі мікробної життєздатності.

Ключові слова: глутатіон, інактивні сухі дріжджі, КУО.