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The Role of Magnesium and Calcium in Governing Yeast Agglomeration

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Summary

»Grit« formation by agglomerating cells of baker's yeast is an idiosyncratic phenomenon of irreversible cellular aggregation that is detrimental to yeast quality. Agglomeration results in failure of rehydrated dried yeast to evenly resuspend and has economic consequences for both yeast manufacturers and bakers. Several environmental factors are implicated in governing yeast agglomeration, but no significant differences between 'gritty' and 'non-gritty' yeast in terms of cell hydrophobicity or flocculence have been reported. In this study, analysis of cellular metal ions has revealed high levels of calcium in 'gritty' strains of *Saccharomyces cerevisiae*, which suggests that calcium ions may positively influence agglomeration. In contrast, it was found that cellular magnesium levels were higher in 'non-gritty' yeast. Furthermore, by increasing magnesium concentrations in molasses yeast growth media, a reduction in cellular calcium was observed and this concomitantly reduced the tendency of cells to agglomerate and form grit. Magnesium thus acted antagonistically against calcium-induced agglomeration, possibly by blocking calcium binding to yeast cell surface receptors. Results suggested that yeast agglomeration and metal ion bioavailability were inextricably linked and the findings are discussed in relation to possible measures of alleviating cellular agglomeration in the production of baker's yeast.

Key words: yeast, agglomeration, metal ions

Introduction

Agglomeration (or »grittiness«) of yeast is a phenomenon of cellular aggregation which is occasionally observed following the growth of baker's yeast (*Saccharomyces cerevisiae*) on molasses. The phenomenon is manifest when fresh or compressed yeast only partially resuspends when mixed in water, leaving most of the yeast as macroscopic clumps or cellular aggregates. Cellular agglomeration is undesirable since failure to evenly resuspend baker's yeast may adversely affect subsequent fermentation performance during the baking process. Although a type of cell adhesion, it is distinct from the reversible process of flocculation (*1,2*). Major differences exist between agglomeration and flocculation. For example, grit does not form during fermentation, only after pressing and re-hydrating yeast cakes (at >30 % solids); the settling profile of flocculating yeasts

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has two components (rapid and slow – see Ref. *3*) whereas in agglomerating yeasts only slow sedimentation is observed (*2*); and non-flocculent strains of *S. cerevisiae* have been shown to be prone to agglomeration (*2*).

Proposed mechanisms of agglomeration may be divided into two main components: non-specific and specific. Non-specific mechanisms involve yeast cell hydrophobicity, cell surface charge and cell wall composition. Guinard and Lewis (*2*) found no significant difference between 'gritty' and 'non-gritty' yeast in terms of cell hydrophobicity or flocculence. However, phosphate concentrations have been observed to be higher at the cell surface in 'non-gritty' cells compared with 'gritty' cells (*4*). Phosphate residues contribute to cell surface charge and physically interacting yeast cells need to overcome this negative charge. Therefore, lower phosphate levels in 'gritty' cells could be significant in effecting agglomeration by reducing the cell surface charge. The specific component of yeast agglomeration mechanisms involves cell envelope proteins. The yeast cell wall is a complex structure consisting of proteins, lipids and polysaccharides. Proteins in the matrixed mannan layer of the yeast cell wall comprise enzymes (*5*), sexual agglutinins (*6*) and lectins involved in flocculation (*7*). It is possible that branched α-mannan structures may act as recognition and/or binding sites in cell-cell interactions which lead to agglomeration (*2*). In comparing the structure, composition and cell surface properties of 'gritty' and 'non-gritty' cells, Guinard and Lewis (*2*) indicated that calcium ions, phosphates, proteins, carbohydrates and lipids were involved in agglomeration of *S. cerevisiae* cells.

Although magnesium and calcium ions have previously been shown to influence various cell-cell interactions in *S. cerevisiae*, including flocculation of brewing strains (*8*), quantification of metal ions in 'gritty' and 'non-gritty' yeast has not previously been determined. Guinard and Lewis (*2*) proposed that calcium ions act as cofactors in activating the binding capacity of surface proteins in 'gritty' yeasts. Since magnesium and calcium are known to be biochemical antagonists and compete for cellular binding sites (*9*), it may be possible to control agglomeration by altering the concentration ratios c(Mg)/c(Ca) available to yeast. For example, increasing magnesium ions concentration and/or reducing calcium ions concentration may concomitantly lower calcium-dependent binding between cells (by reducing the activation of proteins on the cell surface which are thought to be involved in agglomeration), thereby reducing grit. The aim of the present study was to elucidate the role of magnesium and calcium ions in the phenomenon of cellular agglomeration in baking strains of *S. cerevisiae*.

Materials and Methods

Yeast strain: Baking strains of the yeast *Saccharomyces cerevisiae* were kindly provided by Lallemand Inc., Montreal, Canada, supplied as either fresh liquid cream yeast (at 18 % solids) or as pressed yeast (at 30 % solids). Comparisons were made in experiments using 'gritty' and 'non-gritty' baker's yeast cultures: Lallemand industrial strain C variant 7258 and Lallemand industrial strain C variant 1619, respectively.

Fermentations: *Media* – A basal salts medium was employed prior to the establishment of fed-batch cultivations and consisted of $ZnSO_4$ (266 mg), $CaPO_4$ (67 mg), NaCl (51.4 g), $MgSO_4·7H_2O$ (4.16 g), H_3PO_4 (85 % v/v, 12 mL) and thiamine (106 mg) in approximately 5 litres of water (exact volume was dependent on recipe and moisture content of added yeast). The feed medium during fed-batch cultivation consisted of: 100 % cane molasses diluted to 40–45 °Brix (Reichert ABBE Mark II Digital Refractometer, Buffalo, NY. USA) and 5 % v/v aqua ammonia (NH_4OH). Antifoam and acid (H_2SO_4, 1M) additions to control foam and pH, respectively, were controlled by computer. Alterations to the metal ion content of the final medium were carried out depending on the experiment being performed (Table 1). Reduction of the molasses wort pH to 4.5 was accomplished using conc. H_2SO_4, and the wort was heat treated at 90–95 °C/15 min. After leaving the media to settle overnight, the clarified molasses (4 L) was then decanted into a sterile feed flask for the fermentation. Magnesium and calcium additions were made by adding sterile solutions of appropriate concentrated stock solutions of $MgSO_4·7H_2O$ and $Ca_3(PO_4)_2$, respectively.

Yeast Inocula – Each fermentation was inoculated with 261.08 g (dry mass equivalent) of fresh baker's yeast cream (18 % solids) or pressed yeast (30 % solids). Exact volumes or weights added depended on the percentage of solids of the source yeast being used.

Fed-batch fermentation – Fermentations were carried out in a 19-litre fermenter (Bioengineering AG, Switzerland; model D391) equipped with pH, antifoam and temperature probes. All fermentations were computer controlled using the Process Control Partner (PCP) programme (AlterSys Inc., Boucherville, Quebec) to control the additions of molasses and ammonium feeds and cultivation parameters. All fermentations were carried out at 32 °C over a period of 16 h. A 30-min maturation phase was included at the end of this period during

Table 1. Alteration of metal ion content of cane molasses employed in yeast propagations

Condition	Control	– Ca	+ Ca	1	2	3	4
c(Mg):c(Ca)	0.84:1	0.95:1(SA)	0.47:1	1.13:1	1.36:1	1.42:1	1.62:1
c(Mg, increase)	–	nt	–	5x	10x	15x	20x
c(Ca, increase)	–	nt	3x	–	–	–	–
$m(MgSO_4)$/g	0	nt	15g $CaCl_2$	3.5	7	10.5	14
m(Mg, total) or m(Ca, added)/g	0	nt	75	17.5	35	52.5	70

SA= addition of sulphuric acid to precipitate out calcium; nt = not tested

which agitation was maintained at 700 rpm and aeration at 20 L/min. Samples were taken at time zero and at the end of the fermentation. The final total fermentation volume was measured and yeast cells harvested by centrifugation at 7000 rpm for 5 min (Beckman Induction Drive Centrifuge, J2–21M: rotor JA10). The pellets were washed three times with water, resuspended and pooled, prior to vacuum filtration on No. 4 filter discs (Whatman). Yeast pressing was accomplished to >30 % solids using a pneumatic press and yeast cakes stored at 4 °C for further analysis.

Analysis of fermentation samples

Cell growth – Cell counts of both initial and final samples from fed-batch fermentations were determined using a haemocytometer (Improved Neubauer) under light microscopy. Percentages of budding and dead cells were calculated to give an indication culture viability using the vital staining technique of methylene blue staining (*10*). Samples (15 mL) from the start and end of fermentations were centrifuged and the volume of yeast pellet (packed cell volume) was recorded, as an estimation of growth.

Dry weight – Dry weight analysis of cream yeast or pressed yeast prepared during an experiment was carried out by weighing 2 g of pressed or cream yeast onto an aluminium tray of a moisture analyser (Thermo-Control, Sartorius GmbH). A minimal amount of water was used to resuspend yeast cakes, the drying cycle was initiated and the percentage dry weight recorded on completion. Percentage solids was calculated as follows:

$$\frac{Dry\,Weight\,(final)}{Initial\,Weight\,(yeast\,lake)} \times 100 = \%\ Solids$$

Dissolution test for yeast agglomeration – Pressed yeast cake (>30 % solids) was incubated at 30 °C overnight. Crumbled yeast (25 g) was added to 75 mL warm water in a 250 mL beaker and mixed well, left to settle for 3 min and mixed again. The solution/suspension was poured into a 100 mL measuring cylinder and left for further two minutes. The volume of »gritty« yeast deposit formed was measured in millilitres.

Formol test – Formaldehyde (37 % v/v, 15 mL) pH=8.6 (pH adjusted with 0.2 M NaOH), was added to 100 mL fermentation samples, also adjusted to pH=8.6 with 0.2 M NaOH. Once stabilised, the pH of the mixture was brought back up to pH=8.6 with NaOH and the volume of 0.2 M NaOH added by burette was noted. The level of formol was calculated as follows:

$$V(\text{NaOH added}) \times 0.2 = \text{Formol level}$$

An acceptable formol level was considered to be between 0.3 and 2.0 units.

Metal ion analysis – Magnesium and calcium levels were analysed by atomic absorption spectroscopy (AAS) by Analex Inc., Laval Quebec. Samples for AAS included: fermentation media, extra- and intra-cellular samples from fermentations, whole cell samples of »gritty« and 'non-gritty' cream yeasts, and dissolved and grit portions (following a dissolution test) of yeast cakes prepared from the fermentations. Cell wall fractions isolated following yeast cell disruption were also

Table 2. Comparison of magnesium and calcium levels of total cell and cell wall samples of cream yeast and dissolved and 'gritty' sections of 'gritty' and 'non-gritty' strains of *Saccharomyces cerevisiae*

Condition	w(Mg)/ppm		w(Ca)/ppm	
	'gritty'	'non-gritty'	'gritty'	'non-gritty'
Cream yeast				
Total cell	355.0	488.4	49.2	47.7
Cell wall	22.3	65.2	11.4	15.8
Dissolved section				
Total cell	164.7	417.8	58.6	38.5
Cell wall	33.9	84.0	5.0	5.9
Grit section				
Total cell	227.1	–	37.8	–
Cell wall	30.8	–	4.4	–

Table 3. Comparison of protein and phosphate levels between a 'gritty' and a 'non-gritty' strain of *Saccharomyces cerevisiae*

Condition	'gritty'	'non-gritty'
V(dissolution)/mL	51	2
w(protein)/%	53.98	44.97
w(phosphate)/%	3.12	2.96

Table 4. Magnesium and calcium levels in molasses feed during propagations with 'gritty' yeast

Culture code	Fermentation	Added γ(Mg)/(g/L)	Added γ(Ca)/(g/L)	Actual w(Mg)/ppm	Actual w(Ca)/ppm	N(Mg)/N(Ca)
1*	Control	–	–	1375.8	1631.9	0.84:1
2§	Control	–	–	1375.8	1631.9	0.84:1
3§	–Ca	–	SA	1625.4	1706.0	0.95:1
4§	Ca x3	–	15	1401.5	2985.1	0.47:1
5§	Mg x5	3.5	–	1645.4	1452.5	1.13:1
6§	Mg x10	7	–	2046.7	1503.7	1.36:1
7§	Mg x15	10.5	–	2269.3	1592.4	1.42:1
8§	Mg x20	14	–	2460.7	1513.4	1.62:1

* 'non-gritty' yeast (strain 1619);
§ 'gritty' yeast (strain 7258); SA= addition of sulphuric acid to precipitate out calcium

Table 5. Characteristics of 'gritty' yeast grown under conditions of altered Mg and Ca ion levels[1]

Fermentation	Control ('non-gritty')	Control ('gritty')	SA	Ca x3	Mg x5	Mg x10	Mg x15	Mg x20
Culture code	1*	2§	3§	4§	5§	6§	7§	8§
V(centrifuge)/mL								
initial	8	7	7.5	6	7	7.5	6	8
final	23	20	25	21	23	24	23	25
Viability (%)	100	98	100	99	99	100	100	100
Formol (units)	0.5	0.7	0.46	0.42	0.22	0.42	0.5	0.44
w(protein)/%	21.09	23.28	28.48	17.57	22.12	22.37	nt	28.95
w(phosphate)/%	1.72	1.73	1.71	1.43	1.56	1.50	nt	1.76
Yield/%	33.10	54.78	61.47	44.48	56.22	80.02	52.02	76.06
V(grit)/mL	0	30	25	35	19	12	22	32

* 'non-gritty' yeast (strain 1619); § 'gritty' yeast (strain 7258);
SA=sulphuric acid-treated molasses to precipitate out calcium; nt= not tested
[1] Refer to Table 4 for precise levels of Mg and Ca in growth media

analysed. Media and extra-cellular samples were supplied in liquid form and following the appropriate dilution were analysed directly. All other samples were supplied in pellet form and required digestion by nitric acid hydrolysis (2 mL conc. HNO_3 added to cell pellets and hydrolysed at 60 °C for 1 h) prior to dilution and analysis by AAS.

Analysis of compressed yeast samples – Yeast cakes were analysed for the level of 'grittiness' using the dissolution test, described earlier. Further analysis included metal ion determination by AAS and protein analysis by SDS-PAGE.

Yeast Cell Disruption – Harvested cells were washed twice with 1M Tris buffer pH=7.0 then resuspended into one volume of glass bead disruption buffer (Protease Inhibitor Cocktail Kit, ICN Biomedicals) and mixed with a volume of acid-washed glass beads (425–600 μm diameter, Sigma). Disruption was carried out by initially vortexing to ensure no air bubbles were trapped, then homogenizing for 5 min at 4 °C, using a Mini-Bead-Beater (Biospec Products). Glass beads were allowed to settle out and the supernatant decanted into a fresh Eppendorf tube. Two volumes of disruption buffer were added to the glass beads and mixed by inversion to wash beads; the washings were pooled with the initial supernatant. The broken cell preparation was centrifuged at 13000 rpm for 5 min at 4 °C (Eppendorf Microcentrifuge 5413) and samples separated. The cloudy supernatant containing cytoplasmic proteins and the resultant pellet contained the cell wall fraction (and membraneous debris).

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) – Protein analysis by SDS-PAGE was performed according to Laemmli (*11*), using a 10 % single concentration resolving gel with a 4 % stacking gel. SDS-PAGE was conducted on cytoplasmic extractions of whole cells. All fractions were vortexed with an equal volume of double-strength sample buffer (5 mL stacking gel buffer (0.5M TRIS, pH=6.8), 8 mL 10 % SDS, 4 mL glycerol, 2 mL β-mercaptoethanol, 500 mL bromophenol blue, double-distilled H_2O to a final volume of 20 mL) at 20 °C, before analysis. Electrophoresis was performed on minigels (Mini Protean II, Biorad Laboratories, CA, USA) at 100 mV for 1–2 h at room temperature (EPS 500/400; Pharmacia). Standard molecular weight marker proteins (SDS-PAGE Standard: Broad range, Biorad) were included on all gels. Resolved proteins were silver stained (Silver Stain Plus, Biorad) at room temperature on a rotary shaker (Gyrotory Shaker model G2; New Brunswick Scientific, NJ, USA). Gels were air dried between sheets of pre-wetted cellophane, held in a gel clamp (Biodesign NY, USA) overnight. Water used in all stages of protein analysis was double-distilled.

Removal of cell surface metal ions in cream yeast: 200 mL volumes of 'gritty' cream yeast (18 % solids approx.) were centrifuged at 7000 rpm for 5 min and the supernatant discarded. The yeast pellet was then resuspended in a 200 mL volume of the appropriate concentration of chelating agent (Table 6) and mixed well to ensure complete resuspension for 20 min, with a Teflon-coated magnetic stirrer. The cell suspension was then vacuum filtered through a No. 4 filter disc (Whatman) and pressed to >30 % solids. Resulting yeast cakes were incubated overnight at 30 °C, prior to the analysis of the level of agglomeration using the dissolution test described above.

Results and Discussion

The ability of yeast to agglomerate is known to be strain-dependent. However, the *S. cerevisiae* genotype only partially explains the reasons why this yeast agglomerates or the mechanisms that cause it. As with yeast cell flocculation, metal ions are thought to be involved in both non-specific and specific mechanisms of yeast agglomeration (*2*). This involvement is based either on the effects of surface charge of the individual yeast or a specific protein activation step.

Yeast cell walls are rich in mannan and glycoprotein residues that possess a myriad of binding sites. The activation of some of these sites only occurs following the binding of a cation, forming a metalloprotein complex, which alters the conformation of the protein. In the flocculation of yeast cells, Miki *et al.* (*3,12*) have proposed a lectin-like adhesion mechanism, involving Ca^{2+} activation of α-mannan which binds saccharide groups of adjacent cells. Agglomeration is also thought to involve protein-metal ion interactions at the yeast cell surface

Table 6. Effect of metal ion chelators on grit formation in a commercial strain of *S. cerevisiae* (strain 7258)

		Compressed Yeast		
Chelator	*c*/M	*w*(solids)/%	*V*(grit)/mL	Reduction in grit/%
Control	–	33.1	33	–
EDTA	0.025	32.0	19	42.4
	0.05	35.2	31	6.06
	0.10	36.9	33	0.00
	0.25	39.3	24	27.3
	0.50	44.0	27	18.0
Sodium Citrate	0.01	32.3	32	3.03
	0.025	33.7	36	+9.09
	0.05	34.0	25	24.2
	0.10	36.1	25	24.2
Sodium Pyrophosphate	0.01	30.6	30	9.09
	0.025	34.2	27	18.2
	0.05	35.5	25	24.2
	0.10	35.6	26	21.2

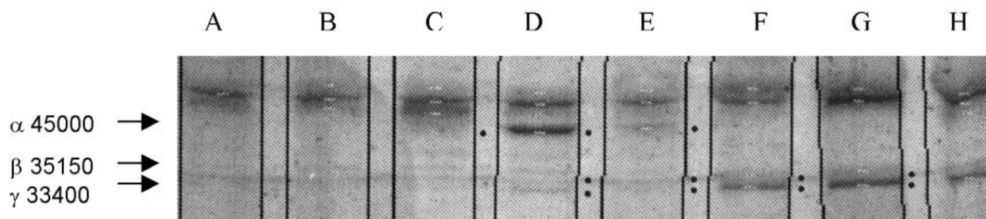

Fig. 1. Comparison of protein profiles of both 'gritty' (strain 7258) and 'non-gritty' (strain 1619) strains of the yeast *S. cerevisiae*. Cytoplasimc proteins were extracted from cells and seapated by ployacrylamide gel electrophoresis.
Lanes: A- Non-gritty cream yeast (strain 1619), B- Dissolved section of non-gritty yeast cake 30 % solids (strain 1619), C- Gritty cream yeast (strain 7258), D- Dissolved section of gritty yeast cake 30 % solids (strain 7258), E- Grit section of gritty yeast cake 30 % solids (strain 7258), F- Gritty cream yeast (strain 7258), G- Dissolved section of gritty yeast cake 30 % solids (strain 7258), H- Grit section of gritty yeast cake 30 % solids (strain 7258). α, β, and γ refer to proteins identified by Guinard and Lewis (2).

and Guinard and Lewis (*2*) have demonstrated a significantly positive correlation between cell calcium and yeast sedimentation characteristics.

Magnesium and calcium levels in gritty and non-gritty yeast strains were measured and the results in Table 2 show that calcium levels were higher in gritty strains of compressed yeast, whereas magnesium levels were higher in non-gritty strains. Total cell calcium levels of the dissolved sections of compressed yeast were observed to be higher in gritty strains. However, the magnesium levels of both the total cell and cell wall fractions were significantly higher in 'non-gritty' strains (both in cream yeast and the dissolved section of compressed yeast samples). These results suggest that magnesium may antagonise calcium-activated agglomeration. Cell envelope proteins have previously been implicated in yeast cell adhesion (*3,13,14*). For example, flocculation behaviour can be inhibited by treating yeast cells with mercaptoethanol or cysteine (chemicals which disrupt inter-peptide disulphide bonds) or proteinase enzymes (*13*). Similarly, cell-surface proteins may influence agglomeration since proteinase treatment of a 'gritty' yeast strain reduced grit formation (*15*). Table 3 shows that protein levels in 'gritty' yeast are higher than in 'non-gritty' yeast (54 % compared to 45 %). However, phosphate levels were not significantly different between the two yeast types (Table 3). The high cell calcium levels in compressed yeast samples of gritty strains indicate that a close linkage between calcium ions and proteins in the cell wall may provide a plausible explanation for the mechanisms of yeast agglomeration.

Electrophoretic profiles of yeast cytoplasmic proteins extracted from cream yeast and the grit as well as dissolved sections of compressed yeast are shown in Fig 1. When gritty and non-gritty strain proteins were compared, three bands were absent in the non-gritty strain. This supports the results of Guinard and Lewis (*2*) who similarly observed three bands specific to gritty strains: α:45000D, β:35150D and γ:33400D. The gritty cream yeast possessed the β and γ bands, whereas the profiles of dissolved and grit sections of this strain exhibit all three bands. High levels of magnesium were observed in cell wall samples of non-gritty yeast. This combined with lower protein levels and the specific absence of these 3 bands leads us to hypothesise that it is possible that magnesium ions saturate calcium-specific binding sites in the cell wall, preventing calcium activation of proteins implicated in agglomeration (*2,15*). Magnesium may thus reduce yeast grit formation through antagonistic interaction with calcium (*9*).

Expression of the 'gritty' phenotype during yeast cultivation was assessed under conditions of altered Mg:Ca concentration ratios (Table 4). Table 5 summarises the general fermentation characteristics and data on formol, protein and phosphate content of cells grown under such conditions. Yeast growth was promoted in media containing higher magnesium levels with Mg:Ca ratios in the range 0.84–1.62:1. Grit levels varied depending on media ionic composition. For example, augmenting calcium levels increased grit levels by 16.67 %, suggesting that calcium promoted yeast agglomeration. Excessive increases in media c(Mg) levels (1.62:1 c(Mg)/c(Ca)) also led to an increase in yeast grit formation, perhaps due to a non-specific involvement of high levels of divalent cations in agglomeration. Stratford and Brundish (*8*) stated that in flocculation of *S. cerevisiae*, mineral salts at low concentrations were essential for flocculation to occur, at moderate concentrations salts enhanced flocculation and at high concentrations they caused reversible inhibition of flocculation. In the present study, high magnesium levels of 2461 ppm in molasses may be sufficient to promote agglomeration, possibly by overcoming cell surface charge and therefore promoting strong cell-cell adherence.

Altering magnesium bioavailability in fermentation media directly influences yeast cell calcium levels. For example, an increase of magnesium concentrations in molasses correlated with a reduction in calcium levels in compressed yeast. Since metal ions are implicated in the mechanisms of agglomeration (*2*) and are closely involved in flocculation (*16*,*8*), the effect of metal ion chelating agents on yeast grit formation was studied. It was found that the chelators sodium citrate and sodium pyrophosphate were particularly effective in reducing grit formation (Table 6) and this supported previous data implicating divalent cations in the phenomenon of yeast agglomeration.

Conclusions

Yeast agglomeration is known to be dependent on a number of variables including strain, fermentation conditions and media composition. No significant differences in ion levels were observed in the grit and dissolved sections of a gritty pressed yeast following a dissolution test. This suggested that the phenomenon is one of the whole yeast population and argues against there being are two types of yeast cells, which would subsequently separate into a particular section. Cell magnesium levels were found to be higher in non-gritty cream yeast and the dissolved sections of compressed yeast, whilst the levels of calcium were higher in compressed gritty strains. This suggested a direct involvement of calcium ions in promoting agglomeration in resuspended compressed yeast. If 'gritty' strains were able to bind higher amounts of calcium, then the activation of calcium-specific proteins implicated in agglomeration would be enhanced in high calcium media. Magnesium counteracted calcium promotion of agglomeration and it was found that high c(Mg)/c(Ca) ratios in molasses reduced levels of yeast grit by 36–60 %. By increasing magnesium concentration in molasses growth media, a reduction in cellular calcium was observed and this concomitantly reduced the tendency of cells to aggregate and form grit. Such manipulation of metal ion levels in growth media represents a relatively simple approach to minimising grit formation in the production of baker's yeast. However, excessively high magnesium levels promote grit formation due of the non-specific action of a large number of cations reducing the net negative charge on yeast cell surfaces and permitting cell adherence. It is important to note in this context that the mineral content of molasses, although highly variable, generally favours calcium, and c(Mg)/c(Ca) ratios as low as 0.1:1 have been reported (*17*). Additionally, levels of calcium in cane molasses are around seven times higher than those found in beet molasses (*17*). Such ionic conditions would, on the basis of results presented here, tend to promote grit formation in propagated baker's yeast. Overall, our findings suggest that interactions between calcium ions and cell surface proteins are closely associated with the phenomenon of yeast agglomeration and that magnesium strongly influences such interactions. Magnesium thus acted antagonistically against calcium-induced agglomeration, possibly by blocking calcium binding to yeast cell surface receptors. As such, it is suggested that yeast agglomeration and metal ion bioavailability are inextricably linked. By re-establishing an ionic balance favouring magnesium in complex media the idiosyncratic problem of yeast agglomeration could be addressed.

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References

1. G. G. Stewart, I. Russell: *Brewing Science*, Chpt 2, J. R. A. Pollock (Ed.), Academic Press Toronto, Ontario, Canada (1981).

2. J-X. Guinard, M. J. Lewis, *J. Inst. Brewing. 99* (1993) 487–503.

3. B. L. A. Miki, N. H. Poon, A. P. James, V. L. Seligy, *J. Appl. Bacteriol. 150* (1982a) 878–889.

4. D. E. Amory, J. P. Dufour, P. G. Rouxhet, *J. Inst. Brewing, 94* (1988) 79–84.

5. H. J. Phaff: Structure and biosynthesis of the yeast cell envelope. In: *The Yeasts*, *Vol. 2*, A. H. Rose, J. S. Harison (Eds.), Academic Press, New York (1971) pp. 135–210.

6. D. Burke, L. M. Previato, C. E. Ballou, *Proc. Natl. Acad. Sci. USA, 77* (1980) 318–322.

7. B. L. A. Miki, N. H. Poon, A. P. James, V. L. Seligy: Flocculation in *Saccharomyces cerevisiae*: mechanisms of cell-cell interactions. In: *Current Developments in Yeast Research*, G. G. Stewart, I. Russell (Eds.), *Adv. Biotechnol. 30* (1980) 193–198.

8. M. Stratford, H. M. Brundish, *Yeast*, *6* (1990) 77–86.

9. G. M. Walker, *Magnesium Res. 12* (1999) 303–309.

10. D. R. Mills, *Food Res. 6* (1941) 361–371.

11. U. K. Laemmli, *Nature*, *227* (1970) 680–685.

12. B. L. A. Miki, N. H. Poon, A. P. James, V. L. Seligy, *J. Appl. Bacteriol. 150* (1982b) 890–899.

13. N. B. Trivedi, G. K. Jacobson, W. Tesch, *CRC Crit. Rev. Biotechnol. 4* (1986) 75–110.

14. H. Nishihara, T. Toraya, *Agric. Biol. Chem. 51* (1987) 2721–2726.

15. R. M. Birch: The role of selected metal ions in the growth and physiology of wine yeasts, *PhD Thesis*, University of Abertay Dundee, Dundee, UK (1997).

16. H. Kuriyama, I. Umeda, H. Kobayashi, *Can. J. Microbiol. 37* (1991) 397–403.

17. G. M. Walker, R. M. Birch, G. Chandrasena, A. I. Maynard, *J. Am. Soc. Brew. Chem. 54* (1996) 13–18.

Uloga magnezija i kalcija u reguliranju aglomeracije kvasca

Sažetak

Stvaranje »zrnaca« aglomeracijom stanica pekarskoga kvasca očituje se u pojačanoj osjetljivosti pri ireverzibilnoj agregaciji stanica, i to na štetu kakvoće kvasca. Posljedica aglomeracije je nemogućnost rehidriranog suhoga kvasca da se ravnomjerno raspodijeli, što se ekonomski negativno odražava kako na proizvođače kvasca, tako i na pekare. Nekoliko činitelja okoline utječe na aglomeraciju kvasca. Međutim, dosada nisu potvrđene bitne razlike što se tiče hidrofobnosti i flokulacije stanica između »zrnatog« i »nezrnatog« kvasca. U ovom je radu analizom metalnih iona u stanici otkriven veliki udjel kalcija u »zrnatim« sojevima *Saccharomyces cerevisiae*, što pokazuje da kalcijevi ioni pozitivno utječu na aglomeraciju. Nasuprot tome, u »nezrnatom« kvascu nađen je veći udjel magnezija u stanicama. Nadalje, povećavajući koncentraciju magnezija u melasi koja je podloga za rast kvasca, opaženo je snizivanje kalcija u stanicama, čime je istodobno snižena njihova tendencija da se aglomeriraju i stvaraju zrnca. Prema tome, magnezij djeluje antagonistički na aglomeraciju induciranu kalcijem, vjerojatno blokirajući vezanje kalcija na receptore koji se nalaze na površini stanica. Rezultati pokazuju da između aglomeracije kvasca i prisutnosti metalnih iona u podlozi postoji čvrsta povezanost te su razmatrane moguće mjere olakšavanja stanične aglomeracije tijekom proizvodnje pekarskoga kvasca.