

Structural Complexity of the Nitrogen Source and Influence on Yeast Growth and Fermentation

Sandra Helena da Cruz,¹ Eduardo Maffud Cilli,¹ and José Roberto Ernandes^{1,2}

ABSTRACT

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The structural complexity of the nitrogen source strongly affects both biomass and ethanol production by industrial strains of *Saccharomyces cerevisiae*, during fermentation in media containing glucose or maltose, and supplemented with a nitrogen source varying from a single ammonium salt (ammonium sulfate) to free amino acids (casamino acids) and peptides (peptone). Diauxie was observed at low glucose and maltose concentrations independent of nitrogen supplementation. At high sugar concentrations diauxie was not easily observed, and growth and ethanol production depended on the nature of the nitrogen source. This was different for baking and brewing ale and lager yeast strains. Sugar concentration had a strong effect on the shift from oxido-fermentative to oxidative metabolism. At low sugar concentrations, biomass production was similar under both peptone and casamino acid supplementation. Under casamino acid supplementation, the time for metabolic shift increased with the glucose concentration, together with a decrease in the biomass production. This drastic effect on glucose fermentation resulted in the extinction of the second growth phase, probably due to the loss of cell viability. Ammonium salts always induced poor yeast performance. In general, supplementation with a nitrogen source in the peptide form (peptone) was more positive for yeast metabolism, inducing higher biomass and ethanol production, and preserving yeast viability, in both glucose and maltose media, for baking and brewing ale and lager yeast strains. Determination of amino acid utilization showed that most free and peptide amino acids present, in peptone and casamino acids, were utilized by the yeast, suggesting that the results described in this work were not due to a nutritional status induced by nitrogen limitation.

Key words: Amino acids, fermentation, nitrogen metabolism, peptides, *Saccharomyces*, yeast.

INTRODUCTION

Yeast are able to use a wide variety of compounds as a carbon and nitrogen source^{8,44,45}. Nitrogen is one of the main elements found in many macromolecules of living organisms, playing a central role in structure and function, and most organisms have elaborate control mechanisms to provide a constant supply of nitrogen^{8,21,22,37,45}. Thus, a

substantial proportion of cellular activity is concerned with procuring and assimilating nitrogen. It has been observed that ammonia, asparagine, glutamine and glutamate are preferentially used by yeast^{21,37}. When these primary nitrogen sources are absent, or present in concentrations low enough to limit growth, other nitrogen sources such as nitrite, nitrate, amides, amino acids and peptides can be used. The utilization of secondary nitrogen sources requires the synthesis of specific-catabolic enzymes and permeases, the expression of which is highly regulated by a process known as nitrogen catabolite repression. The latter is prevented in the presence of a preferred nitrogen source^{21,45}. Studies on the effect of nitrogen with only one compound as sole nitrogen and/or carbon source have been conducted as described by Large¹⁹. However in nature, carbon and/or nitrogen compounds occur in diverse and complex structural compositions, such as polysaccharides and proteins. Brewer's wort is an example of a typical complex environment where the yeast has to adapt its metabolism during the course of fermentation. Wort contains the sugars sucrose, fructose, glucose, maltose, maltotriose, dextrin material, as well as a complex mixture of amino acids, peptides, proteins, vitamins, ions, nucleic acids and other constituents³⁵. In order to select the best options out of the large diversity of available nitrogen and carbon sources, the yeast has developed molecular mechanisms of sensing and regulation, which include induction and repression of key systems³⁷. Nitrogen and carbon are the main nutrients in nature, and this implies that the mutual interaction of these nutrients plays an important role in the metabolism of living organisms³⁶. In this work we have studied the effect of the nature of the nitrogen and carbon source on the metabolism of the yeast *Saccharomyces*. It will be shown that the structural complexity of the nitrogen source, in correlation with sugar concentrations, greatly affects the fermentation performance of both baking and brewing yeast strains.

MATERIALS AND METHODS

Microorganisms

In this work we used a single colony isolate from a commercial baking yeast purchased from "Fleischmann e Royal Produtos Alimentícios Ltda do Brasil" (strain Fiso). Brewers' ale and lager yeast strains were kindly donated by Dr. Inge Russell, Labatt Brewing Company Limited, London, Canada. Yeast strains were maintained on

¹Departamento de Bioquímica e Tecnologia Química, Instituto de Química, Universidade Estadual Paulista (UNESP), P.O. Box 335, 14801-970 - Araraquara, SP, Brazil.

²Corresponding author: E-mail: ernandes@iq.unesp.br

Peptone-Yeast Extract-Dextrose slopes at 4°C, and subcultured monthly.

Chemicals and media components

Components for the growth media were from Difco Laboratories, including Yeast Nitrogen Base without amino acids and ammonium sulfate (Cat. n°. 0335-15-9) (referred to throughout this paper as Yeast Nitrogen Base), casamino acids (Cat. n°. 0230-01-1), peptone (Cat. n°. 0905-01-5), and yeast extract (Cat. n°. 0127-01-7). All others media constituents were obtained from commercial sources and were of the highest available purity.

Media and growth conditions

The media for yeast fermentations contained 0.17% (w/v) Yeast Nitrogen Base, glucose and maltose at various concentrations, supplemented with a 1% (w/v) nitrogen source (ammonium sulfate, casamino acids or peptone). The sugar solution was autoclaved separately, at twice the concentration of the experiment, and added before inoculation. An inoculum was prepared by suspending yeast cells from slopes in sterile water and this cell suspension was inoculated into the growth medium at 0.02 g (dry weight) per litre. Growth was carried out in 250-mL Erlenmeyer flasks, containing 50 mL of medium, or in 125-mL Erlenmeyer flasks with 20 mL of medium. The flasks were incubated in a shaker (250 rpm) at 30°C.

Analytical methods

At specified times during the fermentation an aliquot of cell suspension was withdrawn, centrifuged and the

supernatant frozen for subsequent analysis. Ethanol was analysed by gas chromatography (Model CG-37 equipped with an integrator-processor CG-300, CG Instrumentos Científicos, São Paulo, Brazil). Cell density was measured by turbidity readings at 570 nm and correlated to a dry weight/OD calibration curve. Cell viability was determined by methylene blue staining and plating. Carbohydrate analysis was carried out by colorimetric assay with 2-hydroxy-3,5-dinitrobenzoic acid²⁴. Amino acid content determinations of the commercial preparations were carried out, prior to and after fermentation, and before and after acid hydrolysis. Peptide hydrolysis (1 mg) was conducted with a mixture 6 N HCl (1 mL) and 5% phenol/water (0.08 mL) in Pyrex tubes with plastic teflon-coated screw caps (13 cm × 1 cm), in a nitrogen atmosphere, for 72 h at 110°C. Hydrolysed samples were speed-vac dried, diluted with 1.0 mL of citrate buffer pH 2.2 and 50 µl aliquots were analysed in a Beckman Amino Acid Analyser System 6300 and the results normalized, using an external standard amino acid calibration.

Reproducibility

All results presented in this work are the average of a minimum of three independent experiments.

RESULTS

To study of the effect of the structural complexity of the nitrogen and carbon sources on the metabolism of industrial yeast strains, media containing glucose or maltose, were supplemented with nitrogen in the form of

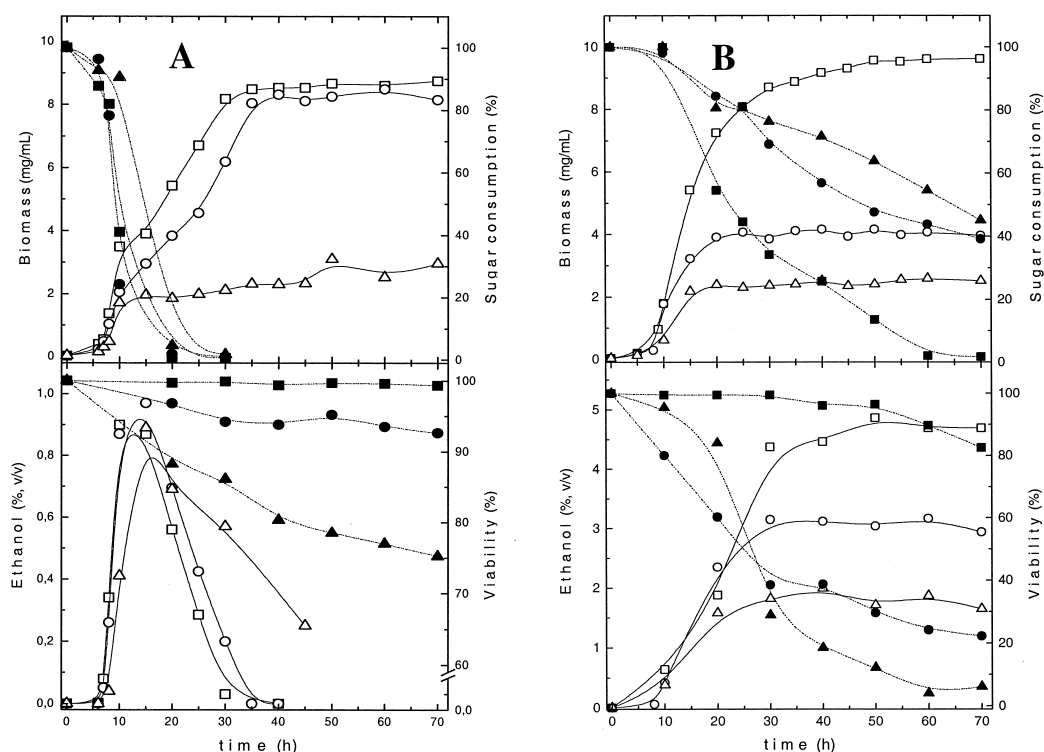


FIG. 1. Growth and ethanol production (open symbols), sugar utilization and yeast viability (closed symbols) measured during Fiso strain fermentation of YNB media containing 2% (w/v) glucose (A) and 15% (w/v) glucose (B) supplemented with 1% (w/v) peptone (square), casamino acids (circles) and ammonium sulfate (triangle). Fermentation at 30°C, initial pH 5.0, 250 rpm.

commercial enzymatic protein hydrolysates (peptone), acid hydrolysates of protein (casamino acids) or ammonium sulfate. Figs. 1A and 2A show biomass accumulation, ethanol production and consumption, sugar utilization and yeast viability during growth of *Saccharomyces cerevisiae* Fiso in the YNB media, containing 2% (w/v) glucose and 2% (w/v) maltose supplemented with different nitrogen sources. Higher biomass accumulation was observed in the media with peptone and casamino acids. Analysis of biomass accumulation, ethanol production and consumption, and sugar utilization clearly showed diauxie. The yeast initially utilized glucose to produce ethanol and biomass and after glucose exhaustion, ethanol was used as a carbon source. In the medium with ammonium sulfate, glucose was converted to ethanol and biomass, but ethanol was slowly utilized by the yeast reflected in the low biomass accumulation.

Figs. 1B and 2B show the same parameters and fermentations conditions of Figs. 1A and 2A, except that the media contained glucose and maltose at the 15% (w/v) level. Improved fermentation performance by the baking strain Fiso was observed in the medium supplemented with peptone when compared with casamino acids and ammonium sulfate, with higher biomass accumulation, faster fermentation and preservation of yeast viability. High sugar concentrations were found to induce significant loss of yeast viability in the media supplemented with casamino acids and ammonium sulfate, also causing suppression of the second growth phase, if we consider that the biomass accumulation in 15% (w/v) glucose is

similar to that detected in the first growth phase shown in Fig. 1A, in the media containing 2% (w/v) glucose. Fig. 3 compares the growth profile of the Fiso strain in various glucose concentrations in media supplemented with peptone (Fig. 3A) and casamino acids (Fig. 3B). Results indicate that at low glucose concentrations diauxie occurs and biomass accumulation is similar under peptone and casamino acids supplementation. However, the transition from oxido-fermentative (aerobic ethanol formation) to the oxidative metabolism (ethanol assimilation) occurred more rapidly in the presence of peptone. At higher glucose concentrations, diauxie was not easily observed and under casamino acids supplementation, the time for metabolic shift increased with the glucose concentration, together with a decrease in the biomass production. This drastic effect on glucose fermentation resulted in the extinction of the second growth phase, probably due to the loss of cell viability. The second growth phase was not observed in the presence of ammonium sulfate even at low glucose concentrations. Medium pH varied from 5.0 (initial pH) to 4.0, (in the medium supplemented with peptone and casamino acids) and dropped to values close to 3.0 (in the presence of ammonium sulfate). Control experiments using citrate buffer pH 5.0, showed that even under buffered conditions the results obtained under peptone and casamino acids supplementation were similar and slightly higher biomass production occurred in the fermentation medium with ammonium sulfate and 2% (w/v) glucose.

The amino acid content of the peptone and casamino acids used in this work is shown in Table I, before and

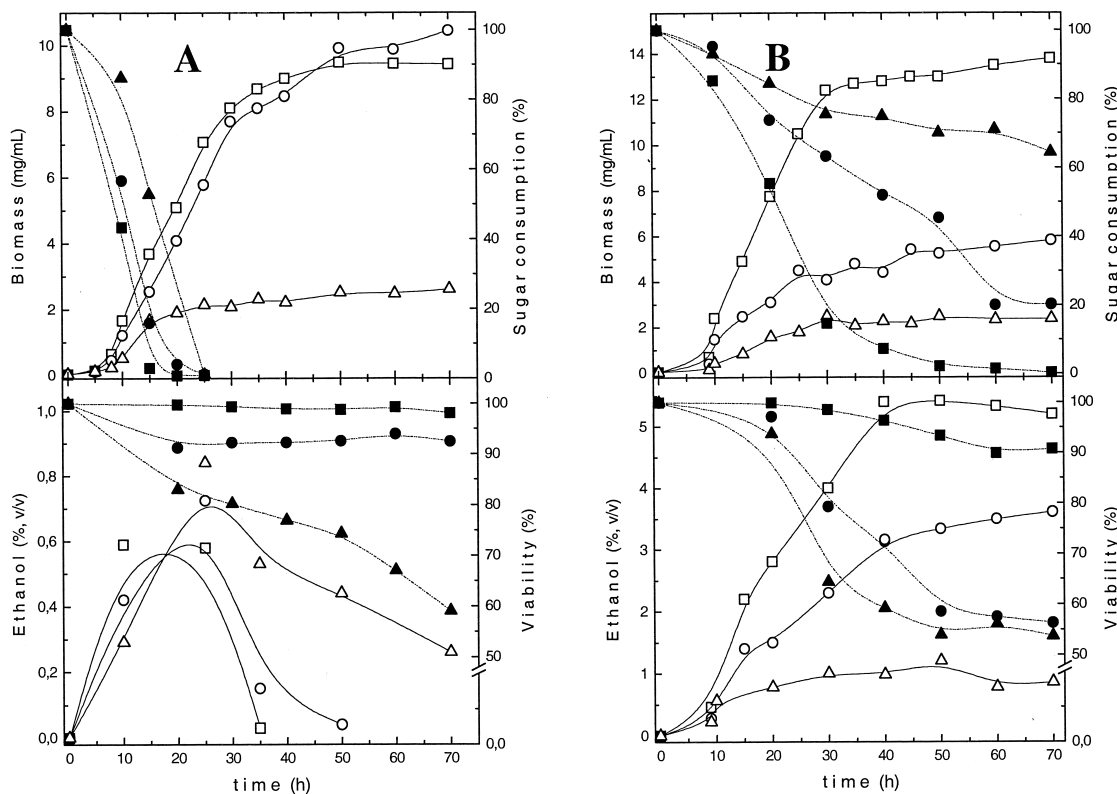


FIG. 2. Growth and ethanol production (open symbols), sugar utilization and yeast viability (closed symbols) measured during Fiso strain fermentation of YNB media containing 2% (w/v) maltose (A) and 15% (w/v) maltose (B) supplemented with 1% (w/v) peptone (square), casamino acids (circles) and ammonium sulfate (triangle). Fermentation at 30°C, initial pH 5.0, 250 rpm.

after acid hydrolysis, prior and after fermentation of 2 % (w/v) and 15% (w/v) glucose. In addition, Table I shows the amino acid content of a commercial preparation of peptone and casamino acids used in this work. The amounts of the different species of amino acids did not differ considerably in the nitrogenous supplement both in this study (Table I) and in previous work¹¹. Despite small differences in concentration, all species of amino acids were present in all commercial preparations, except for the presence of tryptophan in the casamino acids. Comparison of the relative amounts of free amino acids detected, before and after acid hydrolysis, indicated that in casamino acids, free amino acids predominated, and in peptone, nitrogen in the peptide form predominated. The baking strain Fiso utilized most of the nitrogen present in the form of free amino acids or peptides, in peptone and casamino acids, during fermentation of 2% (w/v) and 15% (w/v) glucose. The non-utilized amino acids in the free or peptide form varied from 0% to 10%, suggesting that the effect of the structural complexity of the nitrogen source was not due to a missing or limiting concentration of a particular amino acid in the preparation, but to more general aspects of nitrogen supplementation.

In order to further characterize the influence of the structural complexity of the nitrogen and carbon sources on yeast metabolism we also studied the effect of different nitrogen sources on the fermentation performance of industrial ale and lager brewing strains. Tables II and III show biomass, ethanol production and yeast viability results for ale and lager brewing yeasts fermenting 15% (w/v) glucose or maltose in media supplemented with peptone, casamino acids or ammonium sulfate. For all ale

and lager strains in 15% (w/v) glucose, peptone supplementation induced higher biomass and ethanol production, and preserved yeast viability when compared with casamino acids and ammonium sulfate, similar to the effect observed for the baking Fiso strain. Biomass, ethanol production and yeast viability were strain dependent (Tables II and III). It is worth noting that strain A11, under casamino acids supplementation, produced the same level of ethanol as in the peptone supplemented media, whereas ammonium sulfate always induced poorer fermentation performance. The results obtained with 15% (w/v) maltose also showed higher fermentation efficiency under peptone supplementation, when compared with casamino acids and ammonium sulfate, except for strains A3 and A11, which produced more ethanol and a comparable amount of biomass, under casamino acids supplementation. These results suggest that not only may the structural complexity of nitrogen and carbon sources interfere with yeast metabolism, but minor biochemical differences among yeast strains can also have a strong effect on fermentation performance.

DISCUSSION

The composition of the nitrogenous constituents of brewer's wort has a strong effect on yeast growth and fermentation and is an important parameter in the final quality of the beer. Brewer's wort contains the sugars sucrose, fructose, glucose, maltose, maltotriose, dextrin material, and a complex mixture of amino acids, peptides, proteins, vitamins, ions, nucleic acids and other constituents³⁵. Due to the influence of the amino acid spectrum

TABLE I. Amino acid composition of casamino acids and peptone, without and after acid hydrolysis, and before and after fermentation of glucose by *S. cerevisiae*.

Amino acid	Concentration in medium component (mg of amino acid g ⁻¹)											
	Casamino acids						Peptone					
	Without acid hydrolysis			After acid hydrolysis			Without acid hydrolysis			After acid hydrolysis		
	0 h	Cultivation		0 h	Cultivation		0 h	Cultivation		0 h	Cultivation	
	2% Glu ¹	15% Glu ¹		2% Glu	15% Glu		2% Glu	15% Glu		2% Glu	15% Glu	
Asparagine + Aspartate	61	1.34	0.1	62	3.2	0.6	6.5	0.7	0.3	68	10.4	5.1
Threonine	27	0.05	0.3	36	2.3	0.5	6.5	nd	nd	25	3.2	1.7
Serine	39	0.4	0.4	46	2.6	0.6	10	nd	nd	37	5.1	2.6
Glutamine + Glutamate	140	1	0.6	199	12	2.6	15	nd	nd	112	14.1	6.8
Proline	64	12	0.05	96	6	1.5	nd	nd	nd	114	6.2	9.7
Glycine	15	5	0.2	17	1.8	0.5	14.5	nd	nd	215	33.4	8.4
Alanine	21	0.3	0.4	25	3	0.7	24	nd	nd	80	10.2	2.5
Cysteine	... ²	nd	nd	... ²	nd	nd	... ²	nd	nd	... ²	nd	nd
Valine	33	1.8	0.6	54	5.5	1.3	18	0.7	0.3	34	3.1	0.5
Methionine	19	0.6	nd	26	0.4	0.03	8	nd	nd	10	nd	nd
Isoleucine	18	0.1	0.2	38	2.7	0.7	12	nd	nd	21	1.9	0.5
Leucine	44	0.6	0.3	60	3.6	0.8	32	nd	nd	43	3.1	0.8
Tyrosine	7	0.3	nd	17	0.9	0.2	10	nd	nd	9	0.2	0.1
Phenylalanine	18	nd	nd	25	0.9	0.1	20	nd	nd	22	0.04	0.4
Histidine	12	1.3	0.1	23	1.6	0.3	7	nd	nd	12	1.2	0.3
Lysine	47	1.2	0.2	73	4.6	0.9	24	nd	nd	69	4.3	1.1
Arginine	21	1.2	nd	29	1.1	1.1	47	nd	nd	75	3.6	1.1
Tryptophan	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total amino acids	586	27.2	3.45	826	52.2	12.4	254	1.4	0.6	946	100	41.6

¹ Fermentation conditions: 30°C, 250 rpm, initial pH 5.0, YNB medium, 70 h (Glucose 2%) and 45 h (Glucose 15%).

² Cysteic acid detected, nd: not detectable.

and composition, on yeast fermentation and beer flavor, the amino acid and peptide content and composition of wort and the removal of amino acids in a free and in a peptide form have been studied for many years^{2,7,16,28,38-42}. Recent publications have studied peptide utilization and the effect of nitrogen source and concentration on the uptake of peptides by brewing lager strains in a defined medium^{25,26}. During fermentation amino acids, free and in peptide form, are taken up from the medium by the cell. Free amino acids are incorporated directly without modification into proteins, or degraded by the cell and the nitrogen is used for the synthesis of other nitrogenous cell constituents and the amino acid derivative keto-acids may

be used by the cell for synthetic purposes¹⁹. The assimilation of wort amino acids is ordered and groups of amino acids have been identified on the basis of the rate of removal from the fermentation broth²⁸. Wort amino acids were also classified according to the essential nature of the keto-acid analogues in yeast metabolism¹⁶. In contrast, studies on wort peptides and their uptake and influence on brewing fermentations are sparse. Little is known about the range of peptides present, or the order in which they are removed from wort^{2,7,14,46}.

Additional concerns about the nutritional status of brewer's wort arose with the advent of high gravity brewing, a technological procedure that employs wort at higher

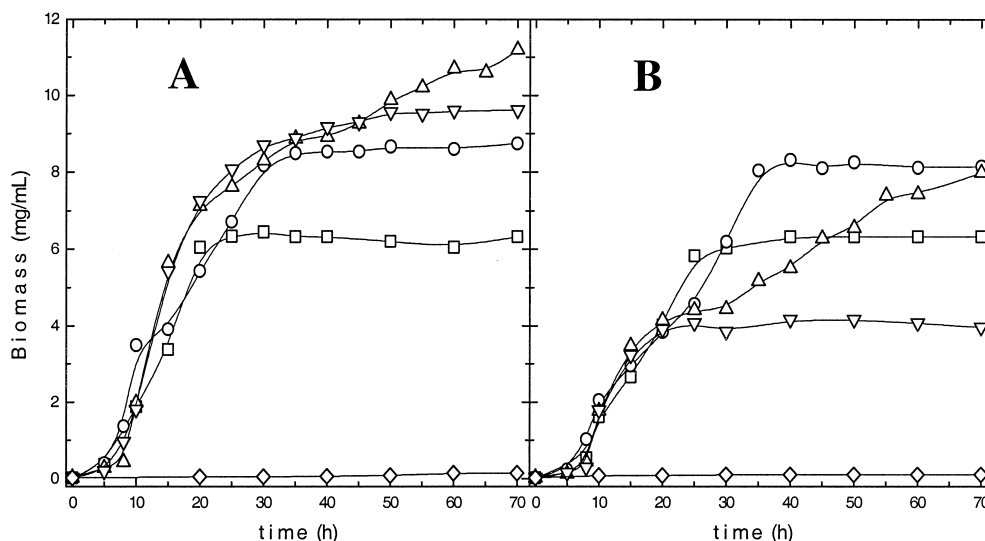


FIG. 3. Biomass production obtained during Fiso fermentation of YNB media in absence (\diamond), and presence of 1% (w/v) glucose (\square), 2% (w/v) glucose (\circ), 5% (w/v) glucose (\triangle), 15% (w/v) glucose (∇), supplemented with 1% (w/v) peptone (A) and 1% (w/v) casamino acids (B). Fermentation at 30°C, initial pH 5.0, 250 rpm.

TABLE II. Growth, ethanol production and yeast viability of industrial baking and brewing ale and lager strains. Fermentation of YNB media containing 15% (w/v) glucose supplemented with various nitrogen sources.

Strains	Time (h)	Peptone			Casamino acids			Ammonium sulfate		
		Biomass (mg/mL)	Ethanol (% v/v)	Viability (%)	Biomass (mg/mL)	Ethanol (% v/v)	Viability (%)	Biomass (mg/mL)	Ethanol (% v/v)	Viability (%)
A3	45	8.8	6.5	100	3.8	3.3	87	2.4	1.8	87
	70	9.2	5.1	93	4.1	3.7	65	2.5	1.7	78
A6	45	7.6	6.0	79	2.5	1.9	70	1.4	0.8	54
	70	8.1	6.8	38	2.3	2.5	63	1.6	0.8	39
A11	45	*	7.1	93	*	6.6	63	2.0	1.7	85
	70	10.0	6.5	89	5.0	7.1	67	2.4	1.9	69
A30	45	7.9	6.5	65	1.7	1.6	56	1.7	1.7	78
	70	8.6	5.8	15	1.8	2.0	40	1.9	2.0	27
L15	45	8.4	5.9	91	3.0	1.4	44	1.8	0.8	19
	70	9.2	6.5	43	3.2	3.2	44	2.3	0.7	10
L42	45	9.1	6.9	95	3.9	3.5	87	1.8	1.3	60
	70	8.5	5.7	94	4.3	4.4	82	2.0	1.3	39
L52	45	10.0	7.2	86	3.6	2.8	79	1.6	0.8	12
	70	9.7	6.2	65	3.7	3.8	69	1.7	0.4	7
L55	45	8.0	6.0	88	2.4	1.9	27	1.4	0.6	14
	70	8.0	6.2	28	2.4	2.0	14	1.4	0.5	5
F	45	9.3	4.7	96	3.9	3.1	35	2.3	1.8	15
	70	9.6	4.7	83	4.0	2.9	22	2.5	1.7	6

* Flocculation.

Fermentation conditions: 30°C, 250 rpm, initial pH 5.0, media supplemented with 1% (w/v) nitrogen sources.

gravities than traditional brewing concentrations. High gravity brewing has economic and operational advantages over traditional brewing methods. Despite its acceptance as a technique for increasing brewing capacity without significant capital expenditure, the achievement of an equilibrium between efficiency and quality relies on finding the ideal nutritional conditions for yeast growth and fermentation³⁴. In the initial attempts to ferment high gravity wort, the limiting factors implicated in many incomplete fermentations were attributed largely to ethanol toxicity, together with the inhibitory effect of high osmotic pressure. The critical ethanol concentration at which yeast ceases to grow depends on several factors⁴. High gravity worts were prepared by incorporation of corn syrups devoid of any nitrogenous nutrients thus inducing alterations in the nutritional status of the wort. It has been reported that the combination of increased pitching rates and nutritional supplementation, induced rapid fermentation of worts containing up to 28% dissolved solids. The factor limiting the production of high levels of ethanol by brewing yeast was attributed to nutritional deficiency^{3,5,6}. The studies showed that supplementation of high gravity brewing wort with complex lipidic compounds and nitrogen constituents avoided incomplete fermentations, thus allowing the production of high levels of ethanol and preservation of yeast viability. In addition to nitrogen supplementation, fermentation improvement was also dependent on the presence of the correct type of amino acids in the fermentation broth^{17,18}.

Since most industrial worts contain sugars and nitrogen compounds in diverse structural compositions³⁵, in this work the effect of the nature of nitrogen source on the metabolism of industrial baking and brewing strains was studied by employing media that contained glucose and maltose as a carbon source, and nitrogen compounds with differing levels of structural complexity. The sources of nitrogen varied from a single ammonium salt (ammonium

sulfate) to an acid protein hydrolysate (casamino acids) consisting predominantly of free amino acids and an enzymatic hydrolysate of protein consisting predominantly of peptides (peptone)¹¹ (this work Table I). The results presented in this work show that the structural complexity of the nitrogen source strongly affects yeast metabolism. Biomass accumulation and ethanol production, in addition to their dependence on the nature of the nitrogen supplement, were also affected by sugar concentration. At low glucose and maltose concentrations i.e., at 2% (w/v) sugar, diauxic growth was observed. Biomass production was similar with both peptone and casamino acids supplementation. In the medium with ammonium sulfate, sugar was converted to ethanol and the ethanol was slowly utilized by the yeast (Figs. 1A and 2A). It was observed that at higher sugar concentrations, diauxic was not easily observed and the transition from fermentative to oxidative metabolism occurred more rapidly in the presence of peptone. When the glucose concentration under casamino acid supplementation was examined, it was found that the time for metabolic shift increased with glucose concentration and concomitantly with the decrease in biomass production, inducing a drastic effect on yeast performance and resulting in the extinction of the second growth phase, probably due to the loss of yeast viability (Fig. 3).

In this work we also studied the fermentation performance of ale and lager brewing strains in YNB media containing glucose and maltose supplemented with various nitrogen sources. The data in Tables II and III indicates that biomass accumulation, ethanol production and yeast viability are strain dependent. In 15% (w/v) maltose or glucose for the baking strain and all the ale and lager strains tested, peptone induced higher fermentation performance when compared with casamino acids and ammonium sulfate. Strain A11 produced the same amount of ethanol under peptone and casamino acids

TABLE III. Growth, ethanol production and yeast viability of industrial baking and brewing ale and lager strains. Fermentation of YNB media containing 15% (w/v) maltose supplemented with various nitrogen sources.

Strains	Time (h)	Peptone			Casamino acids			Ammonium sulfate		
		Biomass (mg/mL)	Ethanol (% v/v)	Viability (%)	Biomass (mg/mL)	Ethanol (% v/v)	Viability (%)	Biomass (mg/mL)	Ethanol (% v/v)	Viability (%)
A3	45	11.1	4.1	100	10.4	5.8	99	2.7	1.7	89
	70	12.7	4.8	99	11.0	5.1	94	3.5	1.0	83
A6	45	7.3	5.1	98	2.8	2.6	93	1.5	1.0	79
	70	8.1	6.5	59	3.3	3.1	90	1.8	1.3	77
A11	45	*	2.9	99	*	4.5	100	3.6	0.8	94
	70	13.4	3.6	100	9.8	6.5	98	4.3	0.9	77
A30	45	9.2	5.6	80	2.1	1.8	88	1.6	1.4	74
	70	9.0	6.3	26	2.1	1.8	80	1.9	2.1	64
L15	45	5.2	5.1	97	5.7	2.3	85	2.0	0.6	42
	70	8.8	6.6	67	8.3	5.5	79	2.4	0.3	36
L42	45	9.1	3.8	99	6.7	3.5	96	1.5	0.7	77
	70	10	4.7	97	7.5	4.7	97	1.9	0.7	71
L52	45	11.2	5.0	97	6.7	3.0	92	2.7	0.8	45
	70	11.7	6.2	88	10.0	4.9	85	2.7	0.7	35
L55	45	7.5	4.0	96	3.8	2.0	82	1.6	0.7	50
	70	8.3	5.6	78	5.2	2.5	73	1.7	0.8	24
F	45	13	5.4	95	5.4	3.2	65	2.2	1.0	55
	70	13.8	5.2	91	5.8	3.6	56	2.4	0.9	54

* Flocculation.

Fermentation conditions: 30°C, 250 rpm, initial pH 5.0, media supplemented with 1% (w/v) nitrogen sources.

supplementation when fermenting glucose. In the fermentation of 15% (w/v) maltose, strains A3 and A11 produced more ethanol and a comparable amount of biomass under both peptone and casamino acids supplementation.

Lower biomass accumulation in the media supplemented with ammonium sulfate resulted from the yeast using the carbohydrate as a carbon and energy source. Additional energy and carbon were used by the cell to carry out the synthesis of its amino acids derived from both that carbon source and the ammonium sulfate in the media. The proton excess generated during amino acids synthesis must be pumped out of the cell to keep the internal pH constant, resulting in the acidification of the medium, which affects ammonium uptake and growth⁴⁵. The results obtained at the low sugar concentrations with glucose and maltose, in the presence of peptone and casamino acids, suggested that both nitrogenous supplements induced efficient conditions for yeast growth. At 15% (w/v) sugar, supplementation with casamino acids, in addition to inducing a lower production of biomass and ethanol, this also resulted in a loss of yeast viability. Casamino acids are a commercial acid protein hydrolysate with predominantly free amino acids¹¹ (Table I). Fermentation performance is influenced by the ability of cells to transport the amino acids across the plasma membrane, followed by either incorporation of amino acids into newly synthesized proteins or by catabolism, where they are used as nitrogen or carbon sources¹⁹. The uptake of amino acids by yeasts involves specific permeases for certain amino acids and a general amino acid permease of broad-spectrum specificity¹⁵. The utilization of nitrogen from a complex source is due the combination of the range of permeases present, their specificity, and feedback inhibition effects resulting from the composition of the yeast intracellular amino acids³⁵. Casamino acids contain large amounts of aspartic and glutamic acids, both of which are considered rich nitrogen sources and are used preferentially by the cell. These rich nitrogen sources significantly influence the uptake of other amino acids, for example, the general amino acid permease is nitrogen repressed by rich nitrogen sources¹⁵. However, this work showed that the yeast is able to utilize most of the nitrogen present in peptone and casamino acids (Table I), suggesting that the results obtained under casamino acids supplementation is not due to limitation of amino acid uptake.

This work has shown that peptone, a nitrogen source where amino acids predominate in a peptide form, was more efficient for yeast metabolism, inducing higher biomass and ethanol production and preserving yeast viability both in glucose and maltose media, for baking and brewing ale and lager strains. Since yeast do not secrete proteolytic enzymes, amino acids in the peptide form present in peptone and casamino acids should enter the cell as peptides, mediated by peptide permeases. Thus, the utilization of peptides relies on the ability of the yeast cell to transport them across the plasma membrane. Several peptide transport systems have been described³³, and these are distinct proteins from those involved in amino acid transport¹⁵. Results shown in Table I suggest that most amino acids, free and in the peptide form, from peptone and casamino acids are utilised by yeast. This suggests that both supplements are an efficient nitroge-

nous source for yeast growth and fermentation and at low sugar concentration, biomass production is similar in the presence of peptone and casamino acids. Differing metabolic behavior was observed at higher sugar concentrations. Only a small fraction of amino acids, free or in peptide form, from peptone and casamino acids were not taken up by the yeast at low and high sugar concentrations (Table I). The differing metabolic response to the structural complexity of the nitrogen source, in correlation with the carbon source, accounts in part for the results described in this work and the mutual interaction between nitrogen and carbon metabolism must also be considered.

Earlier studies reported on the effect of media composition complexity, in particular in reference to the nitrogen source on various metabolic processes^{10,12,20,27,31,32,43,45}. Supplementation of the medium with complex constituents always improved cellular metabolism. In addition to the studies already discussed in this paper, the complexity of the medium supplementation was reported to interfere with specific metabolic pathways, such as control of glycogen metabolism in yeast¹², secretion of enzymatic activities^{10,20,27,31,32,43,45}, primary¹ and secondary metabolites⁹. The effect induced by specific kinds of peptides on metabolic routes of nitrogen and carbon metabolism was also considered. It is worth noting the increasing interest in biologically active peptides released from proteins by enzymatic hydrolysis such as those obtained from milk proteins^{23,29}. In addition, recent studies have reported on the effect of specific peptides of casein pancreatic digestion on the production of tetanus toxin³⁰ and on the increased growth effect on lactic acid bacteria promoted by yeast extract filtrates¹³.

The results presented in this work show that the structural complexity of the nitrogen source interferes with the fermentation performance of industrial baking and brewing ale and lager yeast strains. Biomass accumulation, ethanol production and yeast viability were strain dependent. In general, it was observed that upon supplementation of the growth media, containing maltose or glucose, with a more complex structural nitrogen source such as peptone, that this induced higher biomass accumulation and ethanol production. However, a few brewing strains exhibited better fermentation performance in the medium supplemented with casamino acids. These results have industrial relevance since they suggest that not only the structural complexity of the nitrogen and carbon source may interfere with yeast metabolism, but also minor biochemical differences among yeast strains can have a strong effect on fermentation performance. These findings also suggest that in *Saccharomyces*, a complex structured nitrogen source is not submitted to the same control mechanisms as those involved in the utilization of simpler structured nitrogen sources.

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