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Uptake of Amino Acids During Beer Production: The Concept of a Critical Time Value

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ABSTRACT


According to M. Jones and J. Pierce in 1964, amino acid uptake occurs sequentially when Saccharomyces cerevisiae grows in a complex medium, whatever the individual concentration. The objective of the current work was to determine to what extent, under different conditions, the four previously defined amino acid families can still be distinguished. For this purpose, amino acid uptake was monitored during fermentation in the presence of one amino acid and two lager yeasts conducted at various scales (1-L tall EBC tubes and 1-L conical flasks) and temperatures (23 and 28°C). Industrial production (1-hL rectangular vessel) conducted with a co-culture of three ale yeasts was also investigated. A critical time (Tc), defined as the time it takes the amino acids of group A (named A' in the current work) to be totally consumed, emerged from all our experiments. This group coincides with the A class defined by M. Jones (aspartate, threonine, serine, glutamate, lysine, and arginine) plus methionine and minus arginine. Tc also corresponds with the beginning of consumption of an amino acid group (named C' in the current work) that includes only glycine and alanine. All other amino acids, defining the B' group, (named B' in the current work) are slowly and gradually taken up without any lag phase.

Keywords: Fermentation, Permeases, Saccharomyces cerevisiae

RESUMEN

Consumo de Aminoácidos Durante Producción de Cerveza: El Concepto de un Valor Crítico de Tiempo

Según M. Jones y J. Pierce en 1964, el consumo de aminoácido ocurre secuencialmente cuando Saccharomyces cerevisiae crece en un medio complejo, cual sea la concentración individual. El objetivo del trabajo actual era determinar a qué medida, bajo diversas condiciones, las cuatro familias previamente definidas de aminoácidos todavía pueden ser distinguidas. Para este propósito, el consumo de aminoácido se supervisó durante fermentación en presencia de una levadura de dos levaduras lager conducidas en varias escalas (tubos altos de 1-L EBC y frascos cónicos de 1-L) y temperaturas (23 y 28°C). Una producción industrial (recipientes rectangulares de 1-hL) conducida con co-cultura de tres levaduras de cerveza fue también investigada. Un tiempo crítico (Tc), definido como el tiempo que se tomaron los aminoácidos del grupo A (nombrado A' en el trabajo actual) para ser consumidos totalmente, emergió de todas nuestras experiencias. Este grupo coincide con la clase A definida por M. Jones (“aspartate, threonine, serine, glutamate, lysine, and arginine”) más “methionine” y menos “arginine”. El Tc también corresponde con el principio de consumo de un grupo de aminoácidos (nombrado C’ en el trabajo actual) que incluye solamente “glycine” y “alanine”. El resto de los aminoácidos, definido grupo B’, (nombrado B’ en el trabajo actual) lenta y gradualmente fueron consumidos sin ninguna retraso de fase.

Palabras claves: Fermentación, Permeasa, Saccharomyces cerevisiae

Saccharomyces cerevisiae toma aminoácidos desde la cerveza. Después de la degradación de Ehrlich, algunos se convierten en metabolitos como alcohol y ésteres, impartiendo los sabores frutales típicos a la cerveza (4). El control de estas aromas favorables podría ser mejorado al entender cómo se absorben aminoácidos en un medio complejo como la cerveza.

Un trabajó, la mayoría de los datos disponibles son laboratorios en condiciones de laboratorio que rara vez contienen una molécula de nitrógeno. Los aminoácidos son conocidos como siendo transportados en la levadura por permeasa del AAP (permeasa de aminoácidos) familia, que incluye 18 miembros (1). El trabajo también incluye un miembro único llamado Ssy1p (6,7), que proviene del resto de los demás miembros de la familia que poseen el nivel bajo expresión de su gen y un estado terminal N-terminal, con función como un sensor (10). El superproducción de un terminal dominante-negativo (2,8) (AGP1, BAP2, BAP3, TAT1, o TAT2) han sido conocidos para requerir Ssy1p por su función en la expresión (5,8). Según Forsberg et al (7), Ssy1p, Pmr3p, y Ssy5p son componentes de un sensor complejo (SPS) localizado en la membrana celular y han sido investigados como ser los que controlan el control de un gran número de permeasas (11).

Después de Ssy1p fue revelado como crucial para el consumo de aminoácidos en un medio complejo como la cerveza (13). Si el aminoácido Ssy1p-dependiente permeasas son expresado y son responsables por el ciclo de aminoácido en el interior de la célula. Según Regenberg et al (16), el de los aminoácidos del resto de los demás miembros de la familia pueden ser funcionales. Thorne (17) ha mostrado que el aminoácido individual, cuando se usan como la fuente principal de la cerveza, son absorbidos en una mezcla completa y que se absorben gradualmente y son presentes en la cerveza final.

En una mezcla compleja como la cerveza, los fabricantes de cerveza suelen distinguir cuatro grupos de aminoácidos (9,12,14). En el modelo usual, Grupo A, que incluye aspartato, threonine, serine, glutamate, lysine, y arginine, es reportado como ser inmediatamente absorbido y casi totalmente consumido después de unos pocos horas de fermentación. Grupo B, que incluye valina, metionina, isoleucina, leucina, histidina, es decir que no se retiene en una fase de la fermentación, pero se absorben gradualmente y son presentes en la cerveza final. Alanine, tyrosine, fenilalanina, tryptophane, and glycine define group C, which is characterized by a very long lag phase. These amino acids are used only when group A is totally depleted. Proline, finally, is known to be only slightly absorbed from wort under anaerobic conditions.

Of course, such a classification depends on the criteria used, either the time required to reach half concentration or the initial removal rate. Therefore, amino acids such as methionine (15) or glutamate and aspartate (12) have been sometimes removed from one group to the other to better fit the experimental data.

The objective of the current work was to refine this model by introducing a new factor and to check the validity of this parameter while varying experimental conditions such as temperature, strain, and vessel type. A comparison with an industrial fermentation was also included.

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EXPERIMENTAL

Yeast Strains
Saccharomyces cerevisiae strains BRAS291, BRAS12 (bottom fermentation yeasts), and BRAS212 (top fermentation yeast) were provided by the BRAS collection of the Université catholique de Louvain, Louvain-la-Neuve, Belgium. Three industrial strains (top fermentation yeasts) were provided by the Trappist Brewery of Rochefort, Belgium.

Media and Growth Conditions
Yeast were grown in YPS media (1% yeast extract, 5% peptone, and 10% sucrose) or in a 12°P industrial wort (12 g of extract per 100 g of wort).

Fermentations were carried out at 23 or 28°C, in agitated conical flasks or in 1-L tall EBC tubes, with a pitching rate of 10 millions cells per mL. The industrial brewery vessel (1 hL) was a rectangular stainless steel fermentor regulated at 23°C. The pitching rate was 3.3 millions cells per mL for each three strains.

Table I
Comparison Between the Experimental Conditions of Fermentation Used to Study Amino Acid Uptake

<table>
<thead>
<tr>
<th>Strains</th>
<th>Jones and Pierce (9)</th>
<th>Palmqvist and Ayrapaa (14)</th>
<th>Ramos-Jeunehomme et al (15)</th>
<th>This Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strains</td>
<td>Saccharomyces cerevisiae</td>
<td>S. carlsbergensis U15</td>
<td>S. cerevisiae 1278b and 2592, 59R</td>
<td>S. cerevisiae BRAS 291, BRAS 212 and BRAS 12 + industrial co-culture of 3 top fermentation yeasts</td>
</tr>
<tr>
<td>Stirring</td>
<td>Stirred (conical flask) and industrial vessel</td>
<td>Stirred and non stirred</td>
<td>Stirred</td>
<td>Stirred (conical flask), non stirred (EBC tube), and industrial vessel</td>
</tr>
<tr>
<td>Temperature</td>
<td>15.5 and 23°C</td>
<td>8-9°C</td>
<td>18°C</td>
<td>22 and 28°C</td>
</tr>
<tr>
<td>Medium</td>
<td>10-12°P</td>
<td>10°P</td>
<td>12°P</td>
<td>12°P</td>
</tr>
<tr>
<td>Group A</td>
<td>Glutamate, glutamine, aspartate, asparagine, serine, threonine, lysine, arginine</td>
<td>Asparagine, glutamine, serine, threonine</td>
<td>Glutamate, glutamine, aspartate, asparagine, serine, threonine, lysine, arginine, methionine</td>
<td>Aspartate, glutamate, serine, threonine, lysine, methionine (A' group)</td>
</tr>
<tr>
<td>Group B</td>
<td>Valine, methionine, leucine, isoleucine, histidine</td>
<td>Methionine, lysine, aspartate, leucine, glutamate, isoleucine, arginine</td>
<td>Valine, leucine, isoleucine, histidine</td>
<td>Valine, arginine, isoleucine, leucine, tyrosine, phenylalanine, tryptophane (B' group)</td>
</tr>
<tr>
<td>Group C</td>
<td>Glycine, phenylalanine, tyrosine, tryptophane, alanine</td>
<td>Valine, histidine, phenylalanine, alanine, tyrosine, tryptophane, glycine, proline</td>
<td>Glycine, phenylalanine, tyrosine, tryptophane, alanine</td>
<td>Glycine, alanine (C' group)</td>
</tr>
<tr>
<td>Group D</td>
<td>Proline</td>
<td>Proline</td>
<td>Proline</td>
<td>Proline</td>
</tr>
</tbody>
</table>

Fig. 1. Comparison of amino acid uptake by BRAS291 (lager yeast) between 1-L tall EBC tubes and 1-L conical flask fermentations conducted at 28°C. A, B, and C classes are as defined by Jones and Pierce (9). Because the experiments have been conducted on different batches of the same wort, slight differences can be observed in the initial concentration of some amino acids. Asp = aspartate, thr = threonine, ser = serine, glu = glutamate, lys = lysine, arg = arginine, val = valine, met = methionine, ile = isoleucine, leu = leucine, gly = glycine, ala = alanine, tyr = tyrosine, phe = phenylalanine, and trp = tryptophane.

Fig. 2. Amino acid uptake by BRAS12 (ale yeast) and BRAS12 (lager yeast) in a 1-L tall EBC tube fermentation conducted at 28°C. A, B and C classes are as defined by Jones and Pierce (9). Because the experiments have been conducted on different batches of the same wort, slight differences can be observed in the initial concentration of some amino acids. Asp = aspartate, thr = threonine, ser = serine, glu = glutamate, lys = lysine, arg = arginine, val = valine, met = methionine, ile = isoleucine, leu = leucine, gly = glycine, ala = alanine, tyr = tyrosine, phe = phenylalanine, and trp = tryptophane.
Amino Acid Uptake by Yeast

of the co-culture. Each sample was immediately centrifuged to discard particles and frozen at –20°C.

**Amino Acid Analysis**

One milliliter of sample was centrifuged at 10,000 rpm (12,062 g) in a Sorvall SS-34 rotor for 15 min at room temperature. A 3-mL mixture including 1 mL of the supernatant, 540 µL of ultra-pure water (Milli-Q water purification system; Millipore, Bedford, MA), 140 µL of a 1% (w/v) TFA solution, 120 µL of 2.5 mM norleucine (internal standard), and 1,200 µL of methanol was centrifuged at 10,000 rpm for 5 min at room temperature in an Eppendorf centrifuge. The supernatant was flushed through a C18 Sep-Pack cartridge (Waters, Milford, MA) preactivated with 20 mL of methanol, 20 mL of 0.1% (w/v) TFA, 10 mL of an 80/20 0.1% (w/v) TFA/methanol solution, and 30 mL of air. The fraction eluting between 1.5 and 2.4 mL was collected.

Amino acids were separated by cation-exchange HPLC, post-derivatized with orthophtaldialdehyde in presence of mercaptoethanol and quantified by fluorescence as previously described by Dethier et al (3). The variation coefficient is in all cases under 5%.

**RESULTS AND DISCUSSIONS**

*Saccharomyces cerevisiae* strains BRAS 291, BRAS212, and BRAS12 were pitched into EBC tubes containing 12°P wort. The wort amino acid composition was monitored by HPLC throughout the fermentations that were conducted at 28°C. Table I compares the experimental conditions used in this work with those previously applied by Jones and Pierce (9), Palmqvist and Ayrapaa (12), and Ramos-Jeunehomme et al (15).

As depicted in Figures 1 and 2, some rules in agreement with those previously described by Jones and Pierce (9) emerge from our data. A 'critical time' (Tc), can be defined for each strain as the time necessary for complete consumption of a first group of amino acids. This group coincides with the A class described by M. Jones (aspartate, threonine, serine, glutamate, lysine, and arginine), plus methionine and minus arginine. After 10 hr, this group was indeed completely depleted, while 30% of the amino acids belonging to other classes still subsisted. According to Ramos-Jeunehomme et al (15), methionine should be effectively consumed after a few hours of fermentation. Tc also marked the beginning of consumption of a third group, which according to our results should only include glycine and alanine. Phenylalanine, tryptophane, and tyrosine, although classified by Pierce (14) in group C, in this study turned out to be partially used before the critical time, especially by BRAS291 and BRAS212. The other amino acids, classified in the intermediate group, seem to be slowly and gradually taken up without any lag phase. The new groups, thus defined and named A’, B’, and C’ in this study, are depicted in Figure 3 for all three strains. Tc, close to 10 hr for BRAS291 and BRAS212, was much longer for BRAS12 (close to 20 hr).

![Fig. 3. Amino acid uptake by BRAS291 (lager yeast), BRAS212 (ale yeast), and BRAS12 (lager yeast) in a 1-L tall EBC tube fermentation conducted at 28°C. A’, B’, and C’ classes are as defined in the current paper.](image-url)

![Fig. 4. Amino acid uptake by BRAS212 (ale yeast) in a 1-L tall EBC tube fermentation conducted at 23°C. A’, B’, and C’ classes are as defined in the current paper.](image-url)
As for previous works done in this area, concentrations in the wort are the combination of the absorption and excretion mechanisms, especially in the case of aspartate and glutamate. Nevertheless, we can assume that excretion is quite negligible compared to the 2,100 ppm of amino acids found in the wort.

BRAS291 fermentations carried out in conical flasks gave results similar to those obtained with EBC tubes (Fig. 1). Of course, stirring increased the uptake kinetics, leading to a lower $T_c$ value (8 hr instead of the 10 hr observed in EBC tubes).

To understand why our results differed slightly from those published by Pierce (14), we also fermented BRAS212 at 23°C instead of 28°C. As shown in Figure 4, the temperature did not significantly influence the uptake sequence.

Finally, three yeast strains currently used in co-culture at a Belgian brewery were investigated in 1-L tall EBC tubes and industrial vessels. Figure 5 clearly shows that the pattern obtained with the co-culture was similar to those previously obtained with the BRAS291, BRAS212, and BRAS12 strains. Once more, a $T_c$ close to 10 hr could be defined.

Industrial fermentations were carried out in a rectangular vessel at 23°C. These experiments confirmed major divergences between groups A' and C'. As shown in Table II, 71 to 99% of the A' group was consumed in the first 15 hr, while as much as 50% of the C' group was still present at the end of this period. However, the A' group amino acids revealed were not completely consumed in the industrial vessel after 15 hr at 23°C, contrarily to what happened in the EBC-tube fermentations conducted at 28°C. The design of the fermentor vessel is probably responsible for the extra time of 3 hr needed to complete total depletion of the A' group. Surprisingly, arginine was consumed by the co-culture as a A' group amino acid in both EBC tube and industrial vessel. The uptake of this alkaline amino acid was previously depicted as very complex by Jones and Pierce (9), its evolution depending on flocculation abilities of the strains used.

In this study, the proposed critical time parameter should help brewers to better monitor their fermentation. However, molecular biology experiments are still needed to better understand the role of the sensors and permeases leading to this sequential assimilation of amino acids as seen in wort fermentation. Huge differences remain between molecular biology data on permeases and the model of sequential assimilation of amino acids. Even if it is now clear that GAP1p is not expressed in the brewery conditions (12) and that Ssylp has been revealed as a key sensor during

<p>| Table II |
| Amino Acid Concentration (ppm) in Fermented Wort (1-L Tall EBC Tube at 28°C or 1-hL Rectangular Vessel at 23°C) |</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>0 hr</th>
<th>15.75 hr</th>
<th>Difference (%)</th>
<th>0 hr</th>
<th>15 hr</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A'</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspartate</td>
<td>74.0</td>
<td>0.0</td>
<td>100.0</td>
<td>143.4</td>
<td>23.3</td>
<td>83.8</td>
</tr>
<tr>
<td>Threonine</td>
<td>135.4</td>
<td>3.4</td>
<td>97.5</td>
<td>331.9</td>
<td>74.4</td>
<td>77.6</td>
</tr>
<tr>
<td>Serine</td>
<td>50.8</td>
<td>0.0</td>
<td>100.0</td>
<td>136.7</td>
<td>30.9</td>
<td>77.4</td>
</tr>
<tr>
<td>Glutamate</td>
<td>32.4</td>
<td>2.4</td>
<td>92.6</td>
<td>132.1</td>
<td>7.5</td>
<td>94.4</td>
</tr>
<tr>
<td>Lysine</td>
<td>92.9</td>
<td>5.4</td>
<td>94.1</td>
<td>193.6</td>
<td>1.2</td>
<td>99.4</td>
</tr>
<tr>
<td>Methionine</td>
<td>32.9</td>
<td>0.0</td>
<td>100.0</td>
<td>76.1</td>
<td>21.9</td>
<td>71.3</td>
</tr>
<tr>
<td>B'</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>114.7</td>
<td>70.1</td>
<td>38.9</td>
<td>266.1</td>
<td>126.0</td>
<td>52.7</td>
</tr>
<tr>
<td>Arginine</td>
<td>119.9</td>
<td>0.0</td>
<td>100.0</td>
<td>284.3</td>
<td>89.9</td>
<td>68.4</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>67.3</td>
<td>14.5</td>
<td>78.4</td>
<td>160.4</td>
<td>66.6</td>
<td>58.5</td>
</tr>
<tr>
<td>Leucine</td>
<td>156.5</td>
<td>36.6</td>
<td>76.6</td>
<td>372.7</td>
<td>137.0</td>
<td>63.2</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>108.2</td>
<td>82.9</td>
<td>23.3</td>
<td>231.5</td>
<td>111.1</td>
<td>52.0</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>132.8</td>
<td>72.8</td>
<td>45.1</td>
<td>290.4</td>
<td>130.9</td>
<td>54.9</td>
</tr>
<tr>
<td>Tryptophane</td>
<td>72.2</td>
<td>60.9</td>
<td>15.6</td>
<td>90.5</td>
<td>45.0</td>
<td>50.3</td>
</tr>
<tr>
<td>C'</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>35.8</td>
<td>24.5</td>
<td>31.4</td>
<td>72.8</td>
<td>36.0</td>
<td>50.6</td>
</tr>
<tr>
<td>Alanine</td>
<td>109.5</td>
<td>53.3</td>
<td>51.4</td>
<td>230.6</td>
<td>114.8</td>
<td>50.2</td>
</tr>
</tbody>
</table>

Fig. 5. Amino acid uptake by a co-culture of 3 ale yeasts in 1-L tall EBC tubes-fermentations conducted at 28°C; A', B', and C' classes as defined in the current paper. Asp = aspartate, thr = threonine, ser = serine, glu = glutamate, met = methionine, lys = lysine, val = valine = ile = isoleucine, leu = leucine, tyr = tyrosine, phe = phenylalanine, trp = tryptophane, arg = arginine, gly = glycine, and ala = alanine.
fication (13), the role of each Ssy1p-dependent permease remains to be described.

CONCLUSIONS

Tc, defined as the time by which the amino acids of group A’ are totally consumed, emerged from all our experiments. Group A’ coincides with the A class described previously by Jones and Pierce (9) (aspartate, threonine, serine, glutamate, lysine, and arginine), plus methionine and minus arginine. Tc also corresponds with the beginning of consumption of a group (called C’ in the current work) that includes only glycine and alanine. All other amino acids, defining the B’ group, are slowly and gradually taken up without any lag phase.

LITERATURE CITED