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Amino Acid Permeases and their Influence on Flavour Compounds in Beer

Amino acids are the chief source of assimilable nitrogen in wort. Beer flavour is highly influenced by wort nitrogen content and their utilization by yeast. Amino acid permeases that transport amino acids into the yeast are regulated by two mechanisms namely SPS-sensor complex (Ssy1-Ptr3-Ssy5) and nitrogen catabolite repression (NCR). Various by-products and metabolites like alcohols, esters, organic acids, aldehydes and ketones from beer fermentation contribute to beer flavour. Sufficient FAN amounts, nitrogen combinations in the wort and a good understanding of the regulation of amino acid permeases help promote adequate growth and a good flavour profile during alcoholic fermentations.

Descriptors: fermentation; nitrogen; amino acid permeases, flavour

1 Nitrogen assimilation in yeast

Nitrogen availablity is an essential factor needed for growth and various metabolic activities in yeast. It utilizes nitrogen in the form of ammonium salts, amino acids and small peptides (dipeptides and tripeptides) which are found e.g. in brewing wort [5]. These assimilated nitrogen sources are then converted to ammonia, glutamate and glutamine which play a vital role in the formation of higher alcohols, organic acids, esters and diketones that are responsible for the characteristic flavour profiles of beer [29].

Wort consists of 19 essential amino acids and small peptides which are collectively known as free amino nitrogen (FAN) [23]. Its concentration greatly determines the quality and efficiency of the beer fermentation. However excess of FAN is quite disadvantageous as it may result in high concentrations of fusel alcohols in beer. Therefore for a standard malt-wort with 12 °P, the ideal FAN concentration ranges between 200–240 mg/l [10]. Once assimilated, amino acids pass via the transaminase system in yeast where the amino groups are removed and are utilized as building blocks for the synthesis of various amino acids and proteins.

Although wort contains a wide range of 30 distinct nitrogen sources, not all of them support the growth of yeast to the same extent. For this reason, the uptake of amino acids in yeast is a highly regulated process through various amino acid permeases whose transcriptional control takes place either by nitrogen catabolite repression (NCR) or by SPS (Ssy1p-Ptr3p-Ssy5) plasma membrane amino acid sensor system [4]. Through these mechanisms, yeasts select preferred nitrogen compounds that support fast growth with doubling times of 2 h (asparagine, glutamine, and ammonium) or minor preferred ones leading to doubling times < 3 h (aspartate, alanine, serine, arginine, glutamate, phenylalanine and valine) over non-preferred ones that support slower growth with doubling time > 4 h (leucine, isoleucine, methionine, threonine, tryptophan, and tyrosine) [14]. Those amino acids that support fast growth are consumed early compared to specific permeases under SPS mediated control mechanism. On the other hand, those amino acid sources that support slow growth are consumed at a later stage under the control of NCR mechanism.

Based on the order of uptake of the amino acids into yeast, they are classified into four groups as listed in table 1 [10].

Table 1 Classification of amino acids based on their order of uptake into yeast

Classification	Quality of nitrogen source	Amino acids
Group A	Very good	glutamine, glutamate, asparagines, aspartate, serine, threonine, lysine
Group B	Good	valine, methionine, leucine, isoleucine, histidine
Group C	Poor	glycine, phenylalanine, tyrosine, tryptophan, alanine
Group D	Poor/least preferred	proline

1.1 Nitrogen Catabolite Repression (NCR)

To prevent the uptake of non-preferred nitrogen sources at the start of fermentation, yeast uses the nitrogen catabolite repression mechanism. The molecular mechanism includes sensing of the available nitrogen sources and induction of the required systems while repressing the unfavourable systems. NCR enables transcriptional activity of amino acid permeases involved in the uptake of amino acids that are poor nitrogen sources to be repressed as long as preferred nitrogen sources (Table 1) are available [36].

NCR-mediated transcriptional repression is modulated by the activity of four DNA-binding GATA transcription factors namely *GLN3* (Glutamine metabolism), *GAT1* (Transcriptional activator with GATA-1-type zinc finger DNA-binding motif), *DAL80* (Deg-

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radation of Allantoin), and *GZF3* (Gata Zinc Finger protein) [17]. While *GLN3* and *GAT1* are transcriptional activators *DAL80*, and *GZF3* are repressors of Gln3p- and Gat1p- mediated transcription. Under conditions of poor nitrogen source availability, Gln3p and Gat1p accumulate in the nucleus leading to the activation of NCR controlled gene transcription. But in the presence of good nitrogen source, these transcriptional activators are restricted to the cytoplasm where they interact with Ure2p (a Gln3 inhibitor) causing a rapid decrease in the expression of genes encoding and transport systems required for uptake and degradation of poorly used nitrogen sources [6].

Furthermore, the activation of *GLN3* and *GAT1* transcription factors is controlled by their interactions with TOR (target of rapamycin) proteins [3]. During nitrogen starvation, TOR proteins are inhibited by rapamycin proteins resulting in the dephosphorylation and accumulation of the Gln3p and Gat1p in the nucleus. On the other hand upon availability of good nitrogen sources, interaction of TOR protein with Gln3p causes its phosphorylation and retention in the cytoplasm. All these interactions work together to control the expression of the NCR genes.

The various amino acid transporters involved in the uptake of non-preferential nitrogen sources, that are subjected to NCR control are unspecific permeases like Gap1p (general amino acid permease) and Agp1p (affinity glutamine permease), high specific permeases like Put4p (proline permease) and Mep1p, Mep2p, and Mep3p (ammonium permeases). Once induced, the amino acid permeases are localized at the plasma membrane and are involved in the active transport of the available poor nitrogen sources into yeast [7].

Table 2 Amino acid permeases and their transport function in yeast. (Yeast Transport Protein database: YTPdb) [1]

Regulation mechanism	Gene	Description/function
Nitrogen catabolite repression (NCR)	GAP1 YKR039W	General amino acid permease
	CAN1 YEL063C	Arginine permease
	DAL5 YJR152W	Allantoate permease
	MEP2 YNL142W	Ammonium permease
	UGA4 YDL210W	GABA permease - also involved in delta-aminolevulinate transport
	PUT4 YOR348C	Proline permease
Ssy1-Ptr3-Ssy5 (SPS)	HIP1 YGR191W	Histidine permease
	DIP5 YPL265W	Glutamate and aspartate permease
	LYP1 YNL268W	Lysine permease
	AGP1 YCL025C	Broad-specificity amino-acid permease – inductible by most neutral amino acids
	GNP1 YDR508C	Broad-specificity amino-acid permease
	TAT1 YBR069C	Tyrosine and Tryptophan Amino acid Transporter
	TAT2 YOL020W	Tryptophan Amino acid Transporter
	BAP2 YBR068C	Branched-chain Amino acid Permease
	BAP3 YDR046C	Branched-chain Amino acid Permease (paralog of BAP2)
	SSY1 YDR160W	Permease-like sensor of external amino acids
	MMP1 YLL061W	S-methylmethionine permease
	SAM3 YPL274W	S-adenosylmethionine permease
	AGP2 YBR132C	Carnitine permease
	MUP1 YGR055W	High-affinity methionine permease
	MUP3 YHL036W	Low-affinity methionine permease
	HNM1 YGL077C	Choline permease
	BIO5 YNR056C	7-keto 8-aminopelargonic acid permease
	TPO1 YLL028W	Vacuolar polyamine-H+ antiporter
	DTR1 YBR180W	bisformyl dityrosine-H+ antiporter of the plasma membrane involved in excretion of bisformyl dityrosine to the maturing spore wall
	SIT1 YEL065W	Transporter of the bacterial siderophore ferrioxamine B
	ENB1 YOL158C	Transporter of the siderophore enterobactin
	ARN2 YHL047C	Transporter of the fungal siderophore triacetylfusarinine C
	ARN1 YHL040C	Transporter of ferrirubin, ferrirhodin and other ferrichromes

1.2 SPS amino acid sensor system (Ssy1p-Ptr3p-Ssy5)

Yeast detects available amino acids in the medium using the SPS-sensor complex (Ssy1-Ptr3-Ssy5) situated in the plasma membrane of yeast [4]. Ssy1 is the amino acid sensor on the plasma membrane that transmits intracellular signals to activate the amino acid permease genes involved in the transport of the respective amino acids. Ssy1 devoid of transport activity works together with two other intracellular proteins Ptr3 and Ssy5 and transmits signals causing the activation of transcription factors Stp1, Stp2 and Uga35/Dal81. The expression of these transcription factors is regulated by the yeast amino-acid sensor independent (ASI) complex (Asi1-Asi2-Asi3) which is involved in preventing illegitimate expression of genes in the absence of amino acid signalling [13].

When activated, these transcription factors bind to SPS-sensor regulated promoters and induce transcription of amino acid permeases. The amino acid transporters are then transferred to the plasma membrane using Shr3 (a membrane-localized chaperone) resulting in increased amino acid uptake [25]. However when there is nitrogen-depletion in the medium, the SPS-sensor signals for localization of transcription factors Stp1 and Stp2 to the cytosol which in turn results in the repression of amino acid permeases controlled by the SPS-sensor complex. This repression of the SPS-regulated genes is followed by activation of NCR-sensitive genes which is reversible by re-addition of good nitrogen sources to the medium [24].

Various amino-acid permeases that are activated by SPS-signalling mechanism includes branched chain amino acid permeases (Bap2p and Bap3p), the high-affinity glutamine transporter Gnp1p, the tyrosine and tryptophan permeases Tat1p and Tat2p, the dicarboxylic amino acid permease Dip5p, and the high-affinity methionine permease Mup1p. In general, permeases involved in the uptake of amino acids that are taken up during the early stages of fermentation (Asp, Thr, Glu, Leu, His, Met, Ile, Ser, Gln, and Phe) are encoded by genes that are subjected to Ssy1p-mediated regulation.

2 Aminoacidpermeasesin yeast

Amino acid permeases in yeasts include a family of 23 members [27, 8]. These amino acid permeases are accommodated in the yeast plasma membrane whereby a wide range of amino acids are transported into the cells. Based on their regulation the family of amino acid permeases in yeast fall into two different classes. The expression of most of the permeases are constitutive where as some of them are regulated by sensing the availability of nutrients. Most permeases are involved in the import of specific amino acids while some import a broad range of substrates. The list of various amino acid permeases in yeast along with their function are provided in table 2.

3 Higher alcohols in beverage flavour production

Flavour production in yeast is a combination of yeast activity during brewing process and wort composition [31]. Various yeast metabolites including aromatic and aliphatic alcohols, esters, organic acids and sulphur compounds make up more than 200 flavour components identified in beer. Most of the aromatic compounds have a major effect on the beverage flavour even when present in low quantities due to their low flavour threshold. An overview of the important metabolites formed during beer fermentation is shown in figure 1.

Yeasts obtain their supply of amino acid nutrients from malt. These amino acids are firstly transferred into the cell and are utilized through transamination reactions. Through this process yeasts remove the amino group from amino acids that are transferred at a faster rate and utilize their amino groups to synthesize essential amino acids (e.g. amino acids that are absorbed slowly) by attaching the corresponding organic acids to them. The remaining organic acids are converted into aldehydes through loss of a CO_2 residue (decarboxylation) and are ultimately converted to higher alcohols (also called fusel alcohols). This is known as the catabolic (Ehrlich) route to higher alcohol formation. They can also be synthesized from carbohydrates through similar decarboxylation and reduction reactions on the anabolic route of higher alcohol formation. Some higher alcohols are also obtained by reduction of aldehydes and ketones in wort [16].

Production of higher alcohols following transamination and reduction reactions imparts various flavours to beer. The fusel alcohols namely propanol, isobutanol, 2-methylbutanol, 3-methylbutanol are significant in imparting distinct flavours to beer. However, among

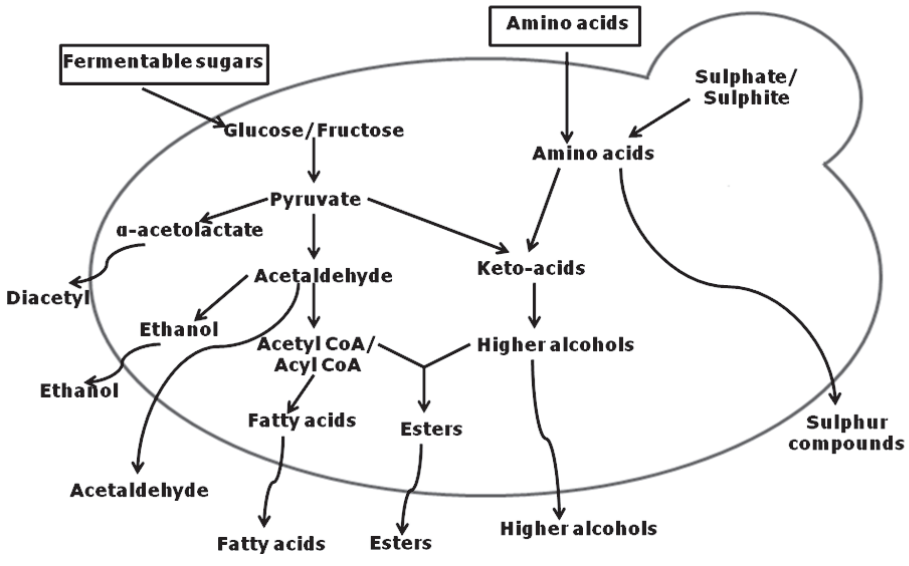

Fig. 1 Metabolic pathways in brewers' yeast leading to flavour production

Table 3 Higher alcohols and their corresponding amino acids involved in flavour compound production

Amino acids	Higher alcohols	Flavour/Aroma
Leucine	Isoamyl alcohol	Bitter
Isoleucine	Amyl alcohol	Alcohol,vinous
Valine	2-Methylpropanal	Alcohol
Phenylalanine	2-Phenylethanol	Rose, perfume
Tryptophan	Tryptophol	Almonds
Tyrosine	Tyrosol	Bitter
Metheonine	Methionol	Cooked vegetable
Isoleucine	2-methylbutanol	Alcohol
Leucine	3-methylbutanol	Fusel, pungent
Threonine	Propanol	Alcohol

these compounds, 3-methylbutanol is the largest contributor to the flavour while n-propanol is the smallest [32]. Optimal amino acid concentrations are highly essential for appropriate higher alcohol concentrations in beer. Surplus or shortage of a particular amino acid could lead to inhibition or over-production of their corresponding alcohols respectively. Also excess supply of amino acids (FAN) leads to increased levels of higher alcohols in beer.

Amino acids are involved in the formation of fusel acids and fusel alcohols including aromatic amino acids (phenylalanine, tyrosine, and trytophan), branched-chain amino acids (leucine, valine, and isoleucine), and the sulphur-containing amino acid (methionine). Fusel alcohols produce esters by their reaction with Acetyl-CoA producing fruity flavour in beer. They also have significant influences on alcoholic and solvent-like aroma. The relation of the higher alcohol and the amino acid from which it is formed and the resultant flavour produced in beer is listed in table 3.

4 Esters, organic acids, ketones and aldehydes in the formation of flavour-active compounds

Several by-products of amino acid metabolism in yeast yield different flavours in beer. These flavour-active esters are formed by the condensation reaction between either acetyl/acyl-CoA and higher alcohols or ethanol. The formation of these esters in yeast confers fruity-flowery flavour to beer. Some examples of different flavours produced by ester compounds include ethyl-acetate with acetone (solvent-like) flavour, iso-amyl-acetate with banana (fruity) flavour, phenylethyl acetate with roses, honey, apple flavours, ethyl caproate and ethyl caprylate with apple flavour.

Organic acids are mainly obtained from wort while the rest is synthesised by yeast. These organic acids confer a sour taste to beer.

Table 4 Flavour compounds produced by yeasts during beer fermentation

Flavour	Compounds
Body	Polysaccharides (Dextrins)
Sour, acidic	Lactic acid, Acetic acid
Bitter	Iso-alpha acids
Sulphury	Dimethyl Sulfide (DMS)
Sulphudic	Hydrogen sulfide (rotten or boiled egg like)
Cooked vegetable	Dialkyl sulfides, DMS, Butyl mercapton, Ethyl mercapton
Metallic	Ferrous iron and some organic compounds
Salty	Sodium chloride, Magnesium sulphate, other mineral salts
Papery (cardboard)	Aldehyde, 2-trans-nonenal
Buttery	Diacetyl
Phenolic	Chlorophenols
Caramel,burnt	Melanoidins
Resinous,grassy	Aldehyde (hexanal)
Solvent-like	Ethyl acetate and other esters/fusel alcohols
Estery,fruity	Ethyl acetate, Ethyl caprylate, Ethyl caproate
Floral,hoppy	Phenethanol
Husky, grainy	aromatic aldehyde cyclopentyl methanol (husky) Melanoidins such as 2-acetylpyridine and trimethylpyrazine (grainy)
Sweet	Dextrins

Among several organic acids in beer, those which are important for beer flavour include isovaleric acid (old hops flavour), caprylic and caproic (goat-like odour), phenylacetic acid (astringent flavour).

Among all ketones produced, vicinal diketones are of major importance to beer flavour. Diacetyl due to its low flavour threshold (0.1–0.15 ppm) offers an unpleasant buttery flavour to beer. Diacetyl is synthesized by the spontaneous decarboxylation reaction of α-acetolactate, a by-product in the valine bioynthesis pathway.

Acetaldehyde, another significant flavour compound in beer is an intermediate during alcohol formation and amino acid metabolism. Aldehydes in beer are largely derived from wort and are also produced by yeast from oxo-acid pools both via anabolic process (carbon source) and the Ehrlich pathway (amino acids). The presence of acetaldehyde produces fruity flavours (green apples, pumpkin) in beer. Typical flavour producing compounds in beer are listed in table 4 [26].

5 Influence of amino acid permeases on beer flavour

Different nitrogen sources including ammonium, amino acids, and di- and tripeptides play an important role in influencing beer flavour production. However amino acids represent the major source of the assimilable nitrogen in wort. The final concentrations of higher alcohols and ethyl ester or acetate ester derivatives are therefore dependent on the uptake efficiency of the corresponding amino acid and the sugar utilization rate. As seen previously, yeasts have a well regulated amino acid transport system involved in the uptake of various nitrogen sources required for its growth. The uptake of amino acids is highly regulated through mechanisms like nitrogen catabolite repression (NCR) and SPS systems. Different specific and general amino acid permeases involved in the uptake of amino acids are listed in table 2.

Amino acids are taken up into yeast by the Ehrlich pathway. After the initial transamination reaction, the excess α-keto acids are converted into fusel alcohols or fusel acids and excreted into the medium. The accumulation of a particular by-product in the beer is dependent on the uptake order and rate of the corresponding amino acid. For example, the ready assimilation of glutamate in yeast results in a fifth flavour, Umami. Also amino acids like valine, leucine, isoleucine, methionine, and phenylalanine are continuously assimilated via the Ehrlich pathway throughout the course of the fermentation resulting in fusel oil accumulation in beer.

Another means of influencing beer flavour is through addition of particular amino acids to the wort. For example, supplementation of the medium with valine and isoleucine results in reduced levels of VDK (vicinal diketone) production during fermentation [22]. Higher availability of the amino acids results in enhanced uptake of the particular amino acid leading to feedback inhibition in the amino acid biosynthesis pathway of valine and isoleucine, thereby reducing VDK levels. But increased assimilation could also lead to enhanced utilization of the particular amino acid thereby influencing the levels of flavour compound production. For example, addition of certain amino acids like alanine, proline, valine, leucine and isoleucine resulted in higher concentrations aliphatic alcohols and esters (ethyl acetate, isoamyl acetate, n-propanol, isobutanol and amyl alcohols) [9].

5.1 Amino acid transporters involved in major flavour producing compounds during beer fermentation

In yeast, various amino acid permeases are involved in the transport of amino acids across the plasma membrane with different affinities, specificities, capacities and regulations. Those amino acid permeases that are involved in the transport of various amino acids that contribute to important flavour in beer are described below.

5.1.1 Branched-chain amino acid permeases (Bap2p, Bap3p) influences vicinal diketone and fusel alcohol levels

Bap2p, Bap3p are amino acid permeases involved in the uptake of leucine, isoleucine and valine (branched-chain amino acids). The expression of these permeases is under the control of the plasma membrane Ssy1-Ptr3-Ssy5 (SPS) sensor. Previous studies showed that the transcription of *BAP2* is greatly induced in the presence of leucine leading to the increase in branched-chain amino acid uptake. However this induction by leucine was only effective in the case of cer-*BAP2* (*Saccharomyces cerevisiae BAP2* gene) and not for Lg-*BAP2* (lager part in the brewing yeast).

The increased levels of uptake of the branched chain amino acids will have positive effects on flavour production. When cells have sufficient valine uptake, diacetyl levels are reduced due to feedback inhibition in valine biosynthesis pathway. Similarly reduced levels of 2,3-pentanedione was achieved upon increased uptake of isoleucine. Overexpression of this leucine transporter also increased isoamyl alcohol production leading to increased bitterness flavour in beer. Similarly, the production of 2-methylbutanoate esters (fruity flavour) was increased upon enhanced uptake of isoleucine [30]. Also addition of valine significantly increased production of the expected corresponding alcohol and ester (2-methylpropanol and 2- methylpropanoic acid ethyl ester) [34].

5.1.2 Control of higher alcohol and ester production using tyrosine and tryptophan amino acid transporters (Tat1p and Tat2p)

In addition to ethanol, several higher alcohols are synthesized during beer fermentation and contribute most significantly to alcoholic flavour and warm mouth-feel. The formation of these higher alcohols is maximized during amino acid starvation (low FAN levels), unfavourable to beer flavour. Control of higher alcohol formation can therefore be controlled by uptake efficiency of the corresponding amino acid and the sugar utilization rate.

The tryptophan amino acid transporters, Tat1p and Tat2p mediate high affinity uptake of aromatic amino acids tyrosine, tryptophan and phenylalanine. They are also involved in the low affinity transport of valine, leucine, isoleucine and histidine. The expression of these permeases is under the control of the amino acid sensor Ssy1p-mediated regulation [21]. Increase in the uptake of these aromatic amino acids should have its effect on the production of the corresponding fusel alcohols. For example, increased uptake of tryptophan can lead to increased production of tryptophol (almond flavour). Similar effect could be observed upon increase in theronine and phenylalanine uptake, producing higher alcohol and flowery flavours respectively.

5.1.3 Sulphur compounds production and methionine and cysteine transporters (Mup1p and Yct1p)

Sulphur is essential for yeasts in the formation of amino acids, proteins and Coenzyme A. The presence of sulphur compounds in beer produces dramatic effects on its flavour. Sulphur compounds are produced from sulphate, sulphite and sulphide ions present in the wort. During fermentation the yeasts produce hydrogen sulfide (H_2S), which when present in lower levels gives the desirable flavour of pale lager beers. However at higher concentrations, H_2S gives rise to the rotten egg smell responsible for the skunky odor in bad beer.

Another compound responsible for off-flavour in beer is Dimethyl sulfide (DMS) which when present in high concentrations (>100

µg/L) imparts a cooked sweet corn flavour to beer [19]. Trans-2-nonenal is another compound associated with unfavourable papery and cardboard-like flavour in beer. However when sulphur dioxide present in beer reversibly reacts with trans-2-nonenal, it produces other flavour-inactive compounds thereby reducing its adverse flavour impact. Hence increased SO_2 production is desirable during fermentation [39].

Sulphur containing amino acids like cysteine and methionine are very important in beverages as they are responsible for aromatic structure of beer and wine. Yeasts transport cysteine and methionine using high affinity permeases like Mup1p (methionine and cysteine transporter) and Yct1p (cysteine transporter). The expression of these nitrogen permease genes is subjected to SPS-regulation mechanism. The sulfate compounds are taken up into yeast and used in the biosynthesis of methionine, and cysteine resulting in the release of off-flavour by-products like H_2S in the process. Defective uptake of cysteine and methionine by the respective amino acid permeases (also due to lack of sufficient nutrients in the wort), may affect yeast growth and also result in excess production of sulfur compounds.

5.1.4 Nitrogen assimilation through ammonia, glutamate and glutamine transporters

Nitrogen utilization in yeast involves assimilation of the three key compounds: ammonia, glutamate and glutamine. Glutamate and glutamine provide nitrogen to the cells for the synthesis of amino acids and proteins. Previous research shows that glutamate and glutamine are the major donors of nitrogen in both yeasts and bacteria [33].

The assimilation of various nitrogen compounds gives rise to higher glutamate and glutamine levels in the cells. The nitrogen compounds that are transported into yeast via permeases are utilized for the biosynthesis of various amino acids and/or converted into ammonium and glutamate. Glutamine is synthesized from glutamate and ammonium condensation reactions using glutamine synthetase (*GNL1*). The ammonium, glutamate, and glutamine together form the hub of nitrogen metabolism, yielding several flavour compounds in beer. Accumulation of glutamate results in the umami flavour in beer. Likewise, ammonia produces caramelization in beer as it reacts with wort sugars (Maillard reaction) giving rise to a burnt flavour in the beverage.

The specific permeases that are involved in the transport of these nitrogen compounds are therefore responsible for the various flavour compounds produced. Since glutamate and glutamine are considerd as good nitrogen sources, their accumulation in the cell will result in the down regulation of NCR-sensitive genes. *GNP1* is the high-affinity glutamine permease which also transports Leu, Ser, Thr, Cys, Met and Asn. The expression of these permeases is modulated by the Ssy1p-Ptr3p-Ssy5p (SPS) sensor of extracellular amino acids.

DIP5 (Dicarboxylic amino acid permease) mediates high-affinity and high-capacity transport of L-glutamate and L-aspartate. This permease is also involved in the transport of Gln, Asn, Ser, Ala, and Gly. *MEP1* and *MEP2* are the ammonium permeases involved in the transport only ammonium ($NH4^+$). The expression of this permease is under the nitrogen catabolite repression regulation. Due to this reason, the high level transcription of *MEP2* leading to ammonium assimilation during the early hours of fermentation takes place only upon low concentration of glutamine, a key component of NCR regulation.

5.1.5 Beer flavour during nitrogen limitation conditions

The quality of the flavour compounds produced can be greatly influenced by the availability of nitrogen sources in the wort, which in turn activates the transporter genes to take up amino acids that support good growth. However, the permeases of amino acids subjected to NCR will be derepressed when the good sources (glutamate, ammonium) are depleted and only poor nitrogen sources are available. These permeases include general amino acid permease Gap1p (transporting all naturally occurring amino acids) and Put4p (transporting proline) [38,20].

During limited availability of good nitrogen sources, the poor nitrogen sources are utilized for obtaining the required amino acids which could in turn lead to stress–related off flavours in the beverage [11]. For example, low concentrations of valine and leucine inhibit formation of isoamyl acetate. Also the uptake of amino acids is related to the formation of hydrogen sulfide (H_2S) and sulfur dioxide (SO_2) formation.

The general amino acid permease (Gap1p), which is said to be the major transporter of arginine, senses amino acid substrates to transport all available nitrogen sources into the cell during conditions of nitrogen starvation. GAP1 is transcriptionally regulated by the available nitrogen source and is under the control of nitrogen catabolite repression mechanism. Gap1p regulation is complex taking place both transcriptionally and post-translationally. In the presence of good nitrogen sources (glutamate or glutamine) the amino acid transport activity of Gap1p is low whereas their activity in the presence of poor nitrogen sources (proline or arginine) is high.

Similarly proline permease (PUT4) is required for high-affinity transport of proline. Although proline is the least-preferred nitrogen source for yeast and is normally not taken up during fermentation, it is the most abundant source of nitrogen wort and must [18]. During the unavailability of good nitrogen source, yeasts degrade proline into glutamate through the proline utilization pathway.

Other permeases that are constitutively expressed in the presence of the particular amino acids include Hip1p (histidine transporter), Can1p (arginine transporter), Lyp1p (lysine transporter) and Tat2p (tryptophan transporter) [12, 28, 37, 35].

6 Conclusion

Sufficient FAN amounts in the wort are necessary to promote adequate growth and a good flavour profile during alcoholic fermentations. Previous studies have revealed that, different nitrogen combinations can produce variations in aroma outcomes which are strain dependent. Another important factor that alters flavour production is the timing of nitrogen addition [2]. Certain flavour compounds

are dependent on the addition of nitrogen source while certain others are independent of their addition. For example, higher FAN concentrations in the wort produced higher amounts of isoamyl acetate [15]. The uptake of various amino acids through the regulations of their amino acid permeases can therefore be said to have a strong influence on flavour compound production.

The availability of certain amino groups (e.g. branched chain amino acids) significantly alters the production of higher alcohols or aroma compounds like diethyl succinate (fruity/sweet ester). Furthermore, the timing of nitrogen source addition seems to favour different pathways of aroma compound formation. During initial fermentation the anabolic formation of aroma compounds is favoured leading to the uptake of the preferred nitrogen sources resulting in higher concentrations of related esters and fatty acids.

The order of assimilation of nitrogen substrates depends on the availability of nitrogen compounds and on the strain used. The utilization of the nitrogen sources are also dependent on the amino acid permeases whose expression levels may vary between different strains. For example, the lager brewing strains are aneuploid/polyploid in nature and are known to show interspecies differences in their phenotype (flavour production) due to copy number variations, single nucleotide polymorphism, variations in gene activation in response to environmental stress etc. As a result, the extent to which different strains are able to activate amino acid uptake and catabolism could largely vary, which leads to variations in their flavour profile production.

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