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Review

Nitrogen compounds in brewing wort and beer: A review

Thiago Rocha dos Santos Mathias[1]*, Pedro Paulo Moretzsohn de Mello[2] and Eliana Flavia Camporese Sérvulo[1]

[1]Department of Biochemical Engineering, School of Chemistry, Federal University of Rio de Janeiro. Athos da Silveira Ramos, 149, ZIP CODE 21941-909, Rio de Janeiro, RJ, Brazil.
[2]Technology Center of Food and Beverage - SENAI, Nilo Peçanha Street, 85, ZIP CODE 27700-000, Vassouras, Rio de Janeiro, RJ, Brazil.

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Traditionally, beer is obtained from the treatment and processing of three raw materials (barley malt, hops and water). From it, brewing wort fermented by the action of yeasts is obtained. Wort composition depends on the quality and type of raw materials used, as well as the control of the various processing steps. Wort composition also depends on the concentration and profile of nitrogen compounds, such as proteins, polypeptides and amino acids. In general, it has significant influence on the entire process and on the quality of the beer produced, especially color, texture, turbidity, foam formation, CO_2 retention and microbial nutrition. This paper presents a review of nitrogen composition in brewing wort, its influence on brewing and the quality of the final product during its storage period.

Key words: Nitrogen compounds, protein, beer, beer quality, wort composition.

INTRODUCTION

Beer is the product of alcoholic fermentation process, by the action of yeast; it is also the product of wort obtained from malted cereal (barley), other cereals or sugar sources (adjuncts) and hops (Tschope, 2001; Rehm and Reed, 1983; Prescott and Dunn, 1949). The composition of wort is extremely important for microbial activity and the quality of the final product. It must contain organic sources of carbon (carbohydrates) and nitrogen (mainly proteins, peptides and amino acids), as well as phosphorus, sulfur, minerals and vitamins (Bamforth, 2003).

Also present are hop compounds such as bitter resins, essential oils and polyphenols (Haunold and Nickerson, 1993). Nitrogen contents in beer and wort are considerably lower than carbohydrate contents. They also play an important role in beer quality. The presence of proteins and their derivatives in wort can be associated with several factors influencing the nutritional value of the

*Corresponding author. E-mail: thiago.rsmathias@gmail.com.

drink, the turbidity effects and colloidal stability, microbial nutrition, by-products formation during fermentation, and foam stability.

Generally, nitrogen concentration and the profile of nitrogen compounds in beer, and wort beer are highly influenced by raw materials, as well as by the management of each step of the process.

BARLEY MALT AS NITROGEN SOURCE

Traditionally, the introduction of nitrogen sources in wort beer occurs solely by using barley malt as raw material. The addition of hops can lead to a slight increase of nitrogen compounds. However, it can be considered insignificant as compared to the content provided by the main raw material.

Specifically in malted barley, proteins represent the largest percentage of nitrogen sources (8 to 16% w/w), although it may also contain small amounts of amino acids and nucleic acids. The largest amount of protein is located in the inner part of the grain; it is split into two groups based on the solubility of the compounds in water, totaling four different types: (i) soluble ones, which are proteins with enzymatic action, or those proteins that represent reserved material, comprising albumin and globulin (equivalent to 4-11%) and 15-30% protein fraction, respectively; and (ii) insoluble ones, the structural proteins found in the cell wall of starch granules, denominated hordein (36%) and glutelin (30%) (Hornsey, 1990).

The quality and protein contents of barley and malt depend on several factors, among which are the seeding rate, soil fertilization with nitrogen and the variety of the plant. Edney et al., (2012) observed that barley from high seeding rate has lower protein content; however, high germination indicates greater enzymatic power. Also it generates a wort with lower levels of beta-glucans, indicating better modification of the endosperm. The barleys produced under high fertilization have higher nitrogen content; however, less germination and minor modification of the endosperm result in worts with high content of beta-glucans.

NITROGEN COMPOUNDS' PROFILE

Nitrogen compounds present in wort may have different molar mass, and this profile influences the brewing process and the quality of the final product. Its influence is detailed throughout the work. In brief, proteins with high molar mass ($\geq 10^6$ Da) contribute to beer texture and the formation of foam, although those proteins might be related to haze formation in the product during its storage time (Bamforth, 2003). Generally, majority of insoluble proteins with very high molar mass are removed with the used grain (O'Rourke, 2002).

On the other hand, proteins with medium molar mass and polypeptides derived from malt lead to freshness sensation, CO_2 retention and stability of the foam when they are hydrophobic compounds (Schonberger and Kostelecky, 2011; Onishi and Proudlove, 1994). Proteins with low molar mass as well as peptides and amino acids found in the wort (molar mass $\leq 10^3$ Da) are fundamental to yeast metabolism during the fermentation stage. Therefore, they exert influence on quality and quantity of the by-products (such as vicinal diketones), changing the composition of the final product. They may influence the beer's color and flavor, due to the formation of Maillard compounds, which is a result of the interaction between low molar mass proteins and reducing sugars, when wort is boiled (Bamforth, 2003; O'Rourke, 2002; Kunze, 1999). They also contribute to the stabilization of foam (Dale et al., 1989).

According to Dale et al. (1989), not only the molecular mass of proteins, but also their amino acids compositions affect various characteristics such as isoelectric point, surface charge, hydrophobicity and tendency to react with other molecules such as polyphenols. According to Pomilio et al. (2010), amino acid composition determines the type of malts and beers. The role of amino acids and their influence on the brewing process will be discussed later.

Wort contains approximately 19 amino acids that are consumed (or not) neatly in different stages of fermentation. According to the sequence of absorption by yeast, the amino acids can be divided into different groups. In the first group, they are rapidly absorbed at the beginning of the process, including glutamic acid, glutamine, aspartic acid, asparagine, serine, threonine and lysine and arginine, which are the last two amino acids of great importance for fermentation. The second group includes those that are absorbed only after a lag phase of growth (glycine, phenylalanine, tyrosine, and alanine).

Finally, the amino acids with the slowest absorption and of great importance to the brewing process are valine, leucine, isoleucine and histidine. There is also a single amino acid (proline) that is not utilized by the yeast; it remains in the wort during the entire process (Stanbury et al., 1995; Fix, 1993).

THE BREWING PROCESS AND NITROGEN SOURCES

Nitrogen compounds may represent about 5% of wort, and generally available in the form of amino acids, peptides and proteins, derived from malt during its hydrolysis (Bamforth, 2002; Kunze, 1999).

However, the content and profile of nitrogen compounds in wort beer (high, medium and low molar mass) depend on raw materials; that is the type of barley malt, the adjuncts utilized, the ratio of the cereal and water used.

Furthermore, it depends on several other factors, such as the management of each process steps, including malting, milling, mashing and wort boiling (Dragone and Silva, 2010; Celus et al., 2006; Priest and Stewart, 2006; Briggs et al., 2004; Anibaba and Osagie, 1997).

Generally, nitrogen content tends to decrease in the process. This is because it is coagulated during wort boiling, utilized during metabolic activity of yeast in fermentation and precipitated or removed when beer matures to avoid turbidity. According to Cortacero-Ramirez et al. (2003), the final product, beer, contains between 2 and 6 g/L of protein or substances derived from this.

Gorinstein et al. (1999) evaluated the protein and amino acid content of more than 15 different types of commercial beers at different stages of the production process. They determined the concentration of nitrogen compounds as follows: wort, 9.16 g/L; fermented wort, 8.55 g/L; green beer, 8.50 g/L; and aged beer, 6.37 g/L, confirming this reduction.

The main steps and key factors that influence the nitrogen composition of the wort and beer or that are influenced by the same are discussed as follows.

Malting

Due to the inability of the brewing yeast to produce extracellular enzymes to metabolize macromolecules available from barley and/or adjuncts, a previous step called malting is required (O'Rourke, 2002). The enzymatic capacity of the malt is known as diastatic power and is closely related to the nitrogen content in the grain (Lima et al., 2001).

According to Dragone and Silva (2010), during the malting process, the barley grain undergoes significant changes due to increased soluble nitrogen fraction (10-12 to 35-50%), development of proteolytic activity (from undetectable to 15-30 units of activity) and significant increase in its diastatic power (50-60 to 100-250° Lintner).

MacWilliam (1971) observed slight increase in the total nitrogen content of barley during malting (0.71 to 1.18% of dry matter, in average data). Crabb and Hudson (1975) noted that the addition of exogenous gibberellic acid (hormone responsible for the germination of cereal) promoted an increase of 10% in the fraction of soluble nitrogen in malt. These changes have important influence in the mashing stage, as seen subsequently. Chandra et al. (1999) concluded in their studies that the variety of barley changes the protein content.

It is observed that during the malting process, for all varieties studied, the protein content undergoes modification due to the breaking down of its high molar mass. During the drying step of the malt, another problem may Be generated, since part of the proteolytic enzymes can be denatured by being more sensitive to temperature. Thus, the hydrolysis of proteins may be inappropriate during mashing, which results in worts with a high concentration of high molecular mass proteins (Lewis and Young, 2001).

Adjuncts utilization

Introducing starchy or sugary adjuncts in large proportions promotes the dilution of wort nutrient; for example, nitrogen sources, as the main function of adjuncts substitute the carbohydrate provided by barley malt (Dragone and Silva, 2010). Bvochora and Zvauya (2001) evaluated the production process of beer by increasing the carbohydrate concentration through the addition of adjuncts. They observed no increase in the concentration of free amino nitrogen in the wort of any beers produced. This dilution promotes slow and prolonged fermentation, characterized by the release of high levels of microbial metabolism by-products, besides reducing formation of beer foam and stability (Briggs et al., 2004; Bradee, 1977).

The most common adjuncts utilized, the grits of rice and corn have lower protein content (about 6, 5 and 8%, respectively) than wheat and barley that contain acceptable amounts of soluble proteins (Hough, 1990). Sorghum is commonly used in Africa, but it contains very low enzymatic power (Palmer, 1992); however, Agu and Palmer (1998) argue that this is no problem because the protein content of the grain is smaller than that of barley.This leads to proteolysis and releases the starchy fraction more easily. According to Campbell (2003), the highest protein content of barley malt compared to that of corn or rice is strongly associated with its higher enzyme activity, which plays an important role in the preparation of wort.

Currently, it has been studied, the substitution of malted barley for alternatives cereals or pseudocereals that lack gluten. This enables people with celiac disease to consume the drink. It contains sorghum, rice, maize, oats, quinoa and amaranth. However, in general, these cereals or pseudocereals have low enzymatic power and reduced protein content.

As a result, the addition of exogenous enzymes for the preparation of wort is required (Hager et al., 2014; Phiarais and Arendt, 2008).

High gravity for wort production

The production of worts with high concentrations of fermentable carbohydrates is common nowadays in large breweries that use high or very high gravity process to obtain fermented worts that have high alcohol content and which are diluted subsequently before being filled.

There can be observed a significant increase in productivity and reduction in the generation of wastewater (Puligundla et al., 2011). However, the increased carbohydrate content is as a result of adding sugary adjuncts and lack of nitrogen, which lead to the problems seen above.

Several authors note the need for the enrichment of wort with nitrogen sources for this type of process to be successful and the problems minimized (Gibson, 2011; Jones and Ingledew, 1994a). Dragone et al. (2004) observed a significant increase in productivity of ethanol (0.374 to 0.694 g / Lh) through the enrichment of worts, whose high gravity fermentation temperature was set at 15°C.

Jones and Ingledew (1994b), on the other hand, studied the effect of adding commercial proteolytic enzymes for mash assessment. It resulted in increased concentration of free amino nitrogen released in the wort (up to 83 mg/L), slight increased production of ethanol (up to 2%) and significant reduction in fermentation times (up to ⅓ of control fermentations).

Mashing and boiling

Mashing is the breaking down (hydrolysis) of macromolecules from barley by the activity of inherent enzymes of the malted cereal. The main objective of this step is to hydrolyze starch, protein and β-glucans, to release simple sugars (glucose and maltose) as substrate, and other nutrients that can be assimilated by the yeast.

Traditionally, the preparation of wort is done by heating in order to establish adequate temperatures in accordance with different enzymatic groups: proteolytic enzymes and mainly amylolytic enzymes (Kunze, 1999; Hough, 1990).

According to O'Rourke (2002), about 35 to 40% of the malt protein is solubilized during mashing by the action of endo, exo or aminopeptidases from malt, whose optimum pH and temperature are about 5 and 50ºC, respectively. Several problems might arise through inadequate management of the mashing, or even if the proteolytic step is suppressed with the purpose of saving energy and time.

Crabb and Hudson (1975) evaluated three different processes of mashing: two of them by infusion (with proteolytic and without proteolytic step at 48°C) and one by decoction (with proteolytic step and ramp heating promoted by boiling a portion of the wort). For the three commercial malts used, there was an increase in total nitrogen in the wort by allowing the action of proteases (increases of 11.1, 9.4 and 6.2%, respectively). The increase was lower (8.1, 8.9 and 5.2%, respectively) in the decoction process, possibly due to coagulation of protein during the boiling of the wort to increase temperature.

The addition of malt adjunct also has effect on mash. Curi (2006) evaluated the production of beer using different proportions of unmalted barley and maltose powder from corn as adjuncts. As a result, it was observed that the mash yield reduced from 80 to 70%.This was due to the high amount of barley added (50%), with low diastatic power. It resulted in loss of substrate with bagasse cereals. To avoid this problem, exogenous proteinases can be added during the mashing step (Bamforth, 2009).

Without the ideal hydrolysis of the protein fraction, there will be greater concentration of high molecular mass proteins that will denature and coagulate with polyphenols (compounds derived from the malt husk and hops) during the boiling of wort. Such complexes are insoluble and precipitate together with other components to form the hot trub (Barchet, 1993), leading to considerable reduction in the protein content of the wort (Miedaner, 1986), with losses up to 6%.

These precipitates must be removed to prevent the occurrence of problems in subsequent steps, especially an eventual slow fermentation and the formation of by-products with unpleasant flavor and aroma, as well as beer colloidal stability throughout its storage (Priest and Stewart, 2006).

Mello (2008) determined the protein content in the hot trub and found values ranging between 50 and 60%, which depends on the quality of the malt, its protein content, the type of beer produced and the mashing and boiling steps (Nathan, 1930a).

Still during cooking, many other changes occur in the composition of the wort. They include the formation of various reducing compounds, mainly melanoidins and volatile heterocyclic compounds, derived from the Maillard reaction between amino acids and carbonyl groups (especially reducing sugars), which have significant effects on color, flavor and aroma of the beverage (Miedaner, 1986).

Once again, the addition of adjuncts to the wort has an effect on the color of the product. Curi (2006) observed a significant reduction in color intensity of wort (21 to 11 EBC units) and beer (from 11.4 to 7.4 EBC units). This was caused by reduction in the content of low molecular mass nitrogen compounds when high proportion of unmalted adjuncts or maltose was added.

Fermentation

During fermentation, there is reduction in the nitrogen content of wort, due to its consumption by the brewing yeast. The work of Bvochora and Zvauya (2001) showed a significant reduction, between 40 and 75% (calculated data from their results) in the content of free amino nitrogen before and after fermentation, for all the beers produced.

Moreover, when the protein hydrolyzed is not sufficient to generate adequate amounts of essential amino acids for metabolic activity of yeast during fermentation, an alternative metabolic pathway can be utilized to synthesize them using other compounds. This metabolic deviation is dependent on the microbial strain, the number of reutilization of cells, the fermentation temperature, the inoculum concentration, the oxygen content (Briggs et al., 2004; Bamforth, 2003; Kunze, 1999), and mainly the content of nitrogenous compounds of low molar mass available in the wort (Iersel et al., 1999).

During this process of synthesis, significant sensory changes may be seen in the beer, due to the formation of by-products that exert greater influence on it, such as vicinal diketones, comprising diacetyl (butane-2,3-dione) and pentane-2,3-dione. This leaves the beer with buttery flavor. Once it is present, the vicinal diketones are posteriorly absorbed by the yeast and are reduced to promote the reoxidation of metabolic factors. However, this process requires long maturation time and yeasts with high vitality (Fix, 1993).

According to Brites et al. (2000), the concentration of diacetyl present in the fermented wort increases from 0.2 to about 1 ppm due to the addition of adjuncts for substituting 50% of barley malt, which causes reduction of the content of amino acids or low molar mass proteins.

Besides diacetyl, other by-products of importance are released during the metabolic activity of brewing yeast and can be related with the metabolism of nitrogen sources, among other factors. Examples of such compounds are aldehydes (especially acetaldehyde), higher alcohols (Campbell, 2003; Brown and Hammond, 2003), aromatic esters (Broderick et al., 1977), organic acids (Araujo et al., 2003) and glycerol.

Kitagawa et al. (2008) observed a significant increase in production (up to 2% concentration) and productivity (up to half of the time) of ethanol during fermentation of the wort enriched with soy peptides or free amino nitrogen mixture.

The metabolism of the nitrogen compounds and the generation of by-products is dependent on the type of yeast used (top or bottom fermentation). This topic has been of interest for long due to its significant effect on the sensory characteristics of the beverage. Barton-Wright (1949) studied the consumption of different nitrogen components of wort.

According to their study, the total soluble nitrogen has higher absorption rate in the first 48h of fermentation. They also observed that amino acids are the main sources of nitrogen for the yeast metabolism, reducing their levels between 42 and 62%. This is because the protein content is lower and these compounds are scarcely hydrolyzed during fermentation.

At the end of fermentation, the scarcity of nitrogen compounds in the wort plays an important role in reducing microbial activity as well as in yeast flocculation and its consequent sedimentation (Vidgren and Londesborough, 2011).

Colloidal stability

After fermentation, beer presents significant turbidity. Low temperatures promote the precipitation of turbidity compounds, called cold trub. Several authors indicate that the protein fraction of wort beer has significant impact on the turbidity of the beverage, since this precipitate consists mainly of complexes formed by proteins of high molecular weight (specifically the molecules that contain amino acids proline at the end of the sequence), and oxiized polyphenols derived from malt and hops (Priest and Stewart, 2006; Siebert, 2006; Rehmanji et al., 2005; Markovic et al., 2003; Kunze, 1999).Moreover, if there are still high molecular mass proteins, they may precipitate during the pasteurization step of beer, increasing its turbidity even before reaching the consumer (Nathan, 1930b).

The amount of cold trub formed depends on factors such as: the type of malt utilized, the amount of hops added, the temperature controlling mashing and fermentation (Lewis and Young, 2001; Barchet, 1994), the decreased pH of beer (which promotes protein coagulation) and the alcohol content in final product (Reinold, 2007). Gorinstein et al. (1990) evaluated the turbidity of beers prepared with different amounts of proteins and polyphenols, and observed that the beers with the highest levels of these compounds showed the highest levels of turbidity.

Again, if the mash is badly handled, especially on the degree of proteolytic activity, there will be greater concentration of high molecular mass proteins. These tend to be complexed, damaging the colloidal stability of the beverage, which generally requires stabilization for removal of these compounds.

The cold trub can be removed by sedimentation, which occurs naturally throughout the maturation at low temperatures. However, this is a slow process and it is not able to eliminate all turbidity (Lima et al., 2001). In order to speed up the deposition and increase the clarity and colloidal stability of beer, major breweries frequently promote the introduction of adsorbents agents (clarifiers) as additives for removing the excess of haze compounds. At this stage, the protein content of the beverage undergoes some losses, with the purpose of increasing its colloidal stability during the storage. These agents are destined primarily for adsorption of precipitated protein-phenol compounds, or phenol and proteins with high molecular weight, separately.

Currently, gel polyvinyl-polypyrrolidone (PVPP), polyamides (nylon 66), silica (hydrogel or xerogel), clays (bentonite), collagen and isinglass are utilized. Such agents have net positive charge and, thus, they interact forming aggregates (which make precipitation faster) with substances that have a negative charge, such as proteins (Priest and Stewart, 2006; Bamforth, 2003). This causes loss of nutritional quality, texture and foam stability of the final product.

An alternative technique is the addition of proteolytic enzymes in order to reduce the molecular weight of proteins responsible for turbidity, consequently reducing its impact on haze formation in beer (Bamforth, 2009). In this case, the beer must undergo heat treatment to inactivate these enzyme complexes before it is ready for distribution and marketing (Dragone and Silva, 2010; Nguyven et al., 2007; Carvalho et al., 2007). In the latter procedure, beer has its colloidal stability increased, without changing the content of nitrogen compounds.

Foam stability

Proteins are components which have significant effect on the formation and stability of the foam, during the fermentation or in the final product. Generally, albumins (including the so-called protein Z) and hordeins (including lipid transfer protein - LTP) are found in beer foam (Bamforth, 2011). The concentration of these components in the foam is two times greater than its concentration in the wort, and hence the use of worts with low levels of these components promotes considerable reduction in the quantity of foam (Kordialik-Bogacka and Ambroziak, 2004). Some studies suggest that the presence of their hydrolysates (low molecular weight) promotes an increase in the foam stability, and that the presence of each of these types of proteins isolated has greater effect on foaming than when present together (Kapp and Bamforth, 2002; Bamforth and Milani, 2004).

BEER NITROGEN COMPOUNDS AND HEALTH

Throughout its history, beer has been linked to several purposes, among them, the daily feeding and therapeutic uses (Mataix, 2004; Kondo, 2004; Saura et al., 2003). For at least 20 years numerous biochemical investigations have shown that moderate consumption of beer has many health benefits to its consumers (Sánchez et al., 2010), since it is a highly nutritional drink, rich in carbohydrates, proteins and amino acids, vitamins, minerals, phenolics, essential oils, etc. Compared with wine, its consumption can also contribute substantially to diet (Wright et al., 2008; Denke, 2000).

In relation to the content of nitrogen compounds, including proteins, peptides and amino acids, several authors indicate a significant concentration in beer, ranging between 3 and 5 g/L (Bamforth, 2002; Gonzalez-Gross et al., 2000), value greater than that found in many other beverages, including wine (Bamforth, 2011; Wright et al., 2008; Cortacero-Ramirez et al., 2003). According to Sanchez et al. (2010), beer has in its composition 20 essential amino acids, and other non-essentials. It also has amino acid tryptophan, which leads to the production of the melatonin hormone in humans, that has positive effects on sleep and reduces anxiety of consumers.

Gorinstein et al. (2002) reported an increase in total antioxidant activity and a significant reduction in levels of total cholesterol, low density lipoproteins (LDL) and triglycerides in the blood of rats whose diet included lyophilized proteins from commercial beers.

FINAL CONSIDERATIONS

The composition of wort beer has significant influence on the composition and quality of the beverage obtained. The contents of nitrogen compounds, as well as the molecular profile of these compounds are two important characteristics of the wort which is influenced due to changes in the composition of raw materials and their processing during the production of the beer. They also have influence on the beverage quality. They have great effect on turbidity, color, foam quality and stability, aroma, flavor, CO_2 retention, and generation and composition of wastes, such as used grain and hot trub. Furthermore, beer has significant protein content, contributing substantially to the diet of the moderate consumers.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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