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(nitrosamines and biogenic amines)

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Content:
1. Guide on the application of the good practices in small and medium size malt-houses and beer breweries: the case of nitrosamines and the biogenic amines

Summary:
This document is worked out on the base of the experience of all the scientists involved in the research unit. Therefore, following the HACCP procedure described in the Codex Alimentarius for the management of any hazard, all the hypothetic measures for the control of the nitrosamines and biogenic amines in beer production are discussed.
Over the last years, a number of investigators have demonstrated that nitrosamines and biogenic amines are present along the beer chain, and are a risk for human health.
Current scientific literatures and the experience of all the scientists involved in it were used to study the possibility to control these risks, and interviews to small-size brew masters were used too for the applicability and efficacy of the proposed control system.
The preliminary scope of this is to explain to the operators of the small and medium size industries how to implement a specific HACCP system for nitrosamines and biogenic amines, and the prerequisite programmes are part of the information added to describe the area of discussion (as for the scientific literature).
As the result of this study, for the successful development and implementation of the HACCP system, it is necessary that operators take into account and implement the general application of GMP, GHP and the pre-requisite programmes for specific hazards such as nitrosamines and biogenic amines. These requirements (GMP, GHP and pre-requisite programmes) are activities that reduce or eliminate certain food safety hazards and reduce the number of CCP in the HACCP plan.
The scheme of the CCP and CP view in the table 1 & 2 was elaborated, in according with the results that will be reported in the deliverable D2B.2.4, with the purpose to simplify the comprehension and further application of the guide from the operators in small and medium size industries. This document should be translated in specific operative manuals that will be adopted for specific producing units.
Guide to good practice in small and medium size malt-houses and beer breweries: the Nitrosamines and the Biogenic Amines case

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“*”: prerequisites related with the management of nitrosamines and biogenic amines hazards.
1. Introduction
Food safety is a result of several factors: legislation (should lay down minimum hygiene requirements); official controls (should be in place to check food business operators’ compliance) and food business operators (should establish and operate food safety programmes and procedures based on the HACCP principles). The HACCP system is a tool to help food business operators to attain a higher standard of food safety and allows identifying, evaluating, and controlling hazards which are significant to ensure the safety of food (Lee and Hathaway, 1998; Ropkins and Beck, 2000; Efstratiadis and Arvanitoyannis, 2000; Mortimore, 2001; FAO/WHO, 2001; Reg. 852/2004; Senires and Alegado, 2005; Sperber, 2005; Rush, 2006).
HACCP is a tool to assess hazards and establish control systems focusing on prevention rather than relying mainly on end-product inspection. Any HACCP system is capable of accommodating change, such as advances in equipment design, processing procedures or technological developments (Codex Alimentarius, 1997).
Food safety management systems such as HACCP, and the pre-requisite systems of Good Manufacturing Practices (GMP), Good Hygiene Practices (GHP), Good Agricultural Practices (GAP), and Good Storage Practices (GSP) provide the operators in the food supply chain with excellent tools, and are used for design and implement a specific food manufacturing process in a proper and diligent way (FAO/IAEA, 2001; Gorris, 2005).
The HACCP principles are incorporated in food safety legislation, as well as a liable component of the standardization of international food quality control and assurance practices (Ropkins and Beck, 2000).
The pursuit of a high level of protection for human life and health is one of the fundamental objectives of European food law, as defined in Regulation (EC) N. 178/2002. Council Directive 93/43/EEC of the 14th of June 1993 on the hygiene of foodstuffs has defined the general rules of hygiene for foodstuffs, and the procedures for verification of compliance with these rules.
The Regulation (EC) N. 852/2004 requires to food business operators to put in place, to implement and to maintain a permanent procedures based on the HACCP principles.
An integrated approach is necessary to ensure food safety from the site of primary production of raw material and ingredients up to and including distribution on the market and selling. Every food business operator along the food chain should ensure that food safety is not compromised.
Beer may be contaminated by biological, chemical and physical hazards at various stages within the process. Adopting the HACCP approach, the operators can control this possibility; the HACCP program is recognized worldwide as a systematic and preventive approach to beer safety that addresses risk through prevention rather than finished product inspection (EBC, 2006). Over the last
years, a number of investigators have demonstrated nitrosamines and biogenic amines are present and represent risks for the beer chain (Smith, 1994; Longo et al., 1995; Izquierdo-Pulido et al., 1996a; Izquierdo-Pulido et al., 1996b; Sen et al., 1996; Shalaby, 1996; Beatriz et al., 1997; Gloria and Izquierdo-Pulido, 1999; Halász et al., 1999; Izquierdo-Pulido et al., 2000; Kalač et al., 2002; Kalač and Křížek, 2003; Loreto et al., 2005; Yurchenko and Molder, 2005; VLB, 2006; Cortacero-Ramirez et al., 2007; Marconi et al., 2007). Therefore, taking into account the Regulation (EC) N. 852/2004 is important to set up specific procedures to assure food safety in the beer production chain for stated and emerging risks too.

Nitrosamines are formed by reaction of secondary or tertiary amines with a nitrosating agent (Mangino and Scanlan, 1981; Poocharoen et al., 1992; Lijinsky, 1999; Kalač and Křížek, 2003; Yurchenko and Molder, 2005). The same reactions can develop in several natural matrices. Biogenic amines are commonly found in foods such as cheese, meat and fish products, wine, beer, and other fermented food (Stratton et al., 1991; Halász et al., 1994). These amines can have aliphatic, aromatic or heterocyclic structures, and most of them can generate by microbial decarboxylation of free amino acids (Brink et al., 1990). The presence of biogenic amines in foods and alcoholic beverages is important for toxicological point of view (Kalač and Křížek, 2003; Yurchenko and Molder, 2005).

This deliverable presents a HACCP based approach for evaluating the specific risk of nitrosamines and biogenic amines in the malting and brewing industries. The aim of this research was to identity the Critical Control Points (CCPs) for N–nitrosamines and biogenic amines that could occur in malting and brewing processes which may cause the end-product to be unsafe for human consumption. More specific concerns regard the application of this HACCP study to Small and Medium-sized Enterprises (SMEs) because of particular needs in terms of raw material management, processing and logistics.

2. **Scope and application**

This manual should be applied to the malt-houses, microbreweries and medium size beer industries from the intake and storage of barley to the final product, beer.

The purpose is to help the chain operators specifically for the identification of nitrosamines and biogenic amines hazard that could occur in barley and other cereals grain management, malting and beer processes.

All industries have to provide evidence that the prerequisite requirements and critical control points are under control by a management system, assuring to prevent, eliminate or reduce hazards to acceptable levels.
This specific manual implemented for the nitrosamines and biogenic amines should be integrated with the other hazards of malting and beer process.

3. Definition
The following list of definitions helps the operators to use of the Good Practices guide:

**Cleaning in Place (CIP):** systems which allow food processing plants to be cleaned and sanitized automatically.

**Contamination:** the presence of hazard in food.

**Corrective action:** the action to be taken when the results of monitoring indicate that a control has exceeded its critical limit.

**Control Point (CP):** if a control could easily be implemented and a hazard was just as easily prevented, then a process step easily merely identified as a control point.

**Critical Control Point (CCP):** any point or procedure in a food process where loss of control may result in an unacceptable health risk.

**Critical limit:** a criterion that defines a safe process step from an unsafe process.

**European Union: EU**

**Good Agricultural Practices (GAP); Good Hygienic Practices (GHP); Good Manufacturing Practices (GMP); Good Storage Practices (GSP):** a series of rules put in practice by the industries to help operators to product safe foodstuffs, according to the Codex General Principles. Good practices are ongoing measures designed to ensure an effective ongoing approach to product quality control and risk management.

**Green barley:** undried barley.

**Hazard:** an agent which, when present in food, renders it unsafe.

**Likelihood:** term used to describe whether something is probable: the probability if a hazard occurring.

**Monitoring:** the act of conducting a planned sequence of observation or measurement of control parameters in order to assess whether a CCP is under control.

**Water:** water used in the production must comply with the appropriate European and National water quality legislation.

**Preventive action:** action taken to prevent a process deviation before a critical limit is exceeded.

**Prevention or control measures:** an action or activity that eliminates a hazard or reduces it to an acceptable level.

**Risk:** a measure of the impact of a hazard and the likelihood that it will occur.
4. Prerequisites
Malt houses and Brewing Industries must comply with the EU and National legislation and regulations.

For the successful development and implementation of a HACCP system there are a number of requirements and systems that must be in place. These requirements and systems are usually activities that result in reduction/elimination of certain food safety hazards, thus reducing the number of Critical Control Points in the HACCP plan or they are processes that are required to operate the HACCP system efficiently.

Prerequisites programmes must be developed, documented and implemented in order to control factors that may not be directly related to manufacturing controls, but which support the HACCP plan.

The prerequisites signed with the “*” are specifically related with the management of nitrosamines and biogenic amines hazards.

4.1 Additives & Allergens
All processing additives used must be suitable for food use.

European Union legislation requires foods, including alcoholic beverages, to be labeled if they contain recognized allergens.

4.2 Fabrication/Equipment *
Buildings should be fit for their purpose, adequately maintained and cleaned.

Equipment should be designed for purpose intended and easily cleaned.

Planned maintenance programs should be in place.

4.3 Glass
Glass or brittle plastics should not be used where possible in construction or operation in production areas. Protection should be in place in case of breakage where this is unavoidable. The possibility of contamination should be reduced by the use of safety bulbs or tight covers wherever possible. Procedures for action on the discovery of dirty or broken glass should be documented, and staff trained to be aware of these procedures.

4.4 Housekeeping & hygiene *
Proper cleaning and sanitation procedures are important in the production of safe food and beverages. Pathogens can grow and reach contamination levels in a food processing facility because of improper cleaning and sanitation, poor handling procedures, and inadequate equipment.

Procedures for cleaning the building, to schedule defined by risk assessment, have to be put in place.
The effectiveness of cleaning, and the removal of cleaning agents from plant and packaging materials shall be verified.

The risk related to the cleaning materials used shall be documented, and procedures be put in place to deal with accidental spillage which would result in contamination of product with these materials.

A policy and good standards of housekeeping and cleanliness must be set and communicated to all the staff.

Schedules have to be laid down for routine housekeeping.

Cleaning and disinfection products must conform to any National legislation. They have to be clearly labeled and stored appropriately in a place where there are no dangerous contaminating raw materials, process stream or finished product.

4.5 Location

The site should be located, or sufficiently protected, in order to prevent and to minimize the chances of contamination.

The access to the site should be controlled.

Procedures for site protection should be periodically reviewed.

A risk assessment should be carried out in order to establish the extent of control required in each area of site to protect product against contamination. High-risk areas will require more stringent controls than low-risk areas.

4.6 Management system *

The following processes have to be put in place:
- document control and management;
- appropriate records of relevant testing;
- training;
- instruments management;
- auditing;
- traceability (both forwards to the customer or backwards to each of the raw materials, additives or processing aids used in production);
- corrective actions.

Standard operating procedures have to be written. They are detailed procedures indicating step by step how to do a job, including sanitation and HACCP related job.
4.7 Product recall *
Procedures shall be put in place to assure a rapid recall of any unsafe lot of food product from the market.

The recall procedure should include:

- identification of products, thought date or code identifying each lot;
- finished product distribution records should be maintained for a period of time exceeding the shelf life of product, and is at least the length of time specified by regulations. Records should be designed and maintained to facilitate the location of product in the event of a recall;
- records documenting all health and safety related complaints should be maintained, and the action taken must be filed;
- roles and responsibilities of the recall team should be clearly defined;
- procedures should be described in the event of a recall including extent and depth of recall;
- communication channels should be clearly defined to notify the affect customers in a manner appropriate to the type of hazard defined;
- control measures for the returned product.

4.8 Pest control
The risk of pest infestation on site and consequent, potential product contamination must be minimized. Any materials used in pest control should be used to prevent the materials from a risk of product contamination.

Pest control should either be subcontracted to a competent pest control company or conducted by suitably trained internal personnel. The procedures have to be documented and records of finding maintained.

All storages, process, packaging and dispatch areas should be protected against animal incoming. Doors and hatches should be kept closed when in use.

Waste materials, that might encourage pest should be regularly cleared away and disposed of.

All personal Have to be appropriately trained about the use of insecticides, in accordance with any National legislation.

The amount applied must be controlled, and conform to National and industry limits.

All agrochemicals have to be clearly labeled and should not be transferred to alternative packages. They have to be stored in a secure place away from the production areas.

Empty packages should be reused for other materials.
4.9 Silos & Stores
Empty silos and stores have to be swept or vacuumed to remove residues, and may be fumigated or treated with suitable insecticides according to company policy.
Only chemicals that are conform to legislation and are approved by the malting and brewing industry have to be used.
Records of chemical applications must be kept.

4.10 Supplier quality assurance *
Products should be bought from reputable supplier and from an agreed specification, described in the contract that should cover all relevant food legislation. Supply contract should include suppliers have a HACCP system and the possibility to carry out regularly audit.

4.11 Staff facilities & hygiene
Toilets and hand washing facilities should be available, but not open directly into production areas.
Staff should be trained to wash their hands before entering production areas after eating, drinking and visiting the toilet.
Do not eat or drink into production areas, but in designed areas.

4.12 Transport
Transport is a potential source of contamination. To prevent this problem, all vehicles used for the transport of raw materials and finished products have to be suitable for the purpose, and have to be maintained in good repair and hygienic condition. Vehicles, trailers and load carrying areas must be inspected before loading and, if necessary, cleaned/dried internally to remove any dust accumulations.
Loading and unloading of the vehicles should be conducted to prevent raw materials or products contamination or deterioration.
Exteriors of all vehicles must be presented in a clean condition for the carriage.
Vehicles must not be loaded until cleanliness inspection has been made at the collection point by driver, and the person appointed at the loading point.

4.13 Training *
All the staff, including temporary staff, have to be adequately trained.
Records of training must be kept.

4.14 Traceability *
Food business operators have to set up traceability system and procedures for ingredients and foodstuffs.
All food businesses within EU will be required to be able to identify the suppliers of food and ingredients to their businesses and the businesses, to which products have been sold. Operators will need to identify “one step forwards, one step back”.

Such information must be made available to enforcement authorities on demand.

Traceability system within food businesses are considered to be a good practice because they can assist in the management of business risk and bring business and consumer benefit.

5. Product description

5.1 Malt

Malt is the product coming from processed barley that has been steeped in water, germinated on malting floors or in germination boxes or drums, and later dried in kilns, under carefully controlled conditions.

Malt is used predominantly as the basic raw material for beer and spirit, with a much smaller quantity used in the food industry (e.g. bread, biscuits, etc.).

Barley can be distinct in: summer barley (spring sowing) and winter barley (late fall sowing), and between two-row and multiple-row barleys, according to the number of blossoms on the stalk. Multiple-row barleys produce malts richer in husks, protein, and enzymes, which may prove advantageous when using unmalted grain adjuncts. Two-row barley is divided into two main groups: the straight barley, and the “nodding” barley, whose ear hangs down during maturation. Two-row spring barley has the best malting and brewing properties for beer production.

5.2 Beer

Beer is a fermented beverage made in several steps from cereal grains such as malted barley. Other grains such as maize and rice are used to lower the production costs, with particular taste and flavor of the product. The primary ingredients are: malted barley, water, hops, and yeast. Part of the malt can be replaced by unmalted cereal (adjuncts): maize grits (moisture 10-14%), rice (moisture 12-13%), sorghum and wheat.

Water generally constitutes more then 90% of the finished product. The final alcohol content may vary from 0.5% to 15%, and the pH is typically 4.5 or less.

Malt is milled (ground) and mixed with water. During the subsequent procedure, called mashing, high molecular mass components (starch, polymerized carbohydrates) of malt are degraded by enzymes at specific temperatures. The suspension obtained is then filtered to separate the liquid (wort) from the spent grains. This process is called lautering. The wort is subsequently boiled with the addition of hops; the high temperatures cause a coagulation of constituents, which are then called hot trub. They are separated together with the solids from the hops (hot trub removal). The
clarified wort is subsequently cooled to the pitching temperature required by the fermentation method and the yeast strain used. The fermentation process is initiated by pitching: this consists of saturating the cold wort with air, and adding cultured yeast.

The fermentable low molecular mass components of the wort (glucose, maltose, etc.) are converted to ethanol and numerous aroma compounds according to the metabolism of the yeast. After maturing the taste and enriching with carbon dioxide produced by the fermentation, the beer is filtered to clearness, and bottled.
6. Flow Diagrams
The flow sheets of malt and beer production are here reported.

6.1 Malting

Coarse impurities; foreign deeds; metallic objects; sand; stones; dust; foreign and broken kernels.
6.2 Beer production

Malt Intake → Malt Storage → Water → Milling → Water → Mashing → Spent grain ← Lautering → Hop → Wort boiling → Steam ← Whirlpool → Yeast → Fermentation → Spent yeast ← Filtration → Maturation → Packaging → Pasteurisation → Beer

Adjuncts Intake → Adjuncts Storage

Fermentation in bottle ← Yeast
7. Hazard analysis: identify hazard and assess the risks associated with them at each step in the commodity system

Hazard analysis involves identifying and assessing the risks associated with each step in the commodity system. Hazards fall into three categories:

a) Biological contamination: by organic materials present (e.g. animal, bird, insect remains), or from toxins produced from moulds and bacteria.

b) Chemical and biochemical contamination: by chemicals deliberately introduced (e.g. pesticides), by accident (e.g. fuel), cleaning chemicals, or actually produced by the malt process (e.g. NDMA) and from biochemicals such as toxins produced by moulds and fungi.

c) Physical contamination: by physical objects present in the raw materials (e.g. stones, glass, metallic parts), or picked up from malting and brewing plants (e.g. metallic components, glass), or accidentally dropped in by process operators/contractors (e.g. pens, tools).

The hazard identification is based on the experience and qualitative reasoning (McMeekin et al., 2006). The first step of hazard analysis is the description of the product, and the identification of hazards associated with the raw materials. For the hazard analysis it is very important to define and to describe the process flow diagram and the product specifications (Lee and Hathaway, 1998; Mortimore, 2000; EBC, 2006).

The Italian legislation defines beer as the product made by the alcoholic fermentation of a wort obtained by malt (barley or wheat), water, hops and adjuncts (maximum 40%) as maize, rice, sorghum, wheat using the *Saccharomyces cerevisiae* and/or *Saccharomyces carlsbergensis* yeast (D.P.R., 1998).

The malting and beer production flow diagram are reported in paragraph 6.1 and 6.2 including the parameters for a generic lager beer production with use of adjuncts (Sunier, 1988; Hardwick and Dekker, 1995; Kunze, 2004). The example of lager beer produced with the use of adjuncts is typical for Italian industrial production. The flow diagram was used to manage the hazards identified, that in this study are nitrosamines and biogenic amines.

### 7.1 Nitrosamines

Nitrosamines are formed from the interaction of nitrites, nitrate or secondary and tertiary amines. They are present in almost all food and beverages. The typical source of nitrosamines is nitrite, which is added to meat to retard bacterial growth, but which can react with secondary and tertiary amines present in the food to form nitrosamines. The key role of nitrite and nitrogen oxides in forming N-nitroso compounds by interaction with secondary and tertiary amino compounds has led to the examination of foods for the presence of N-nitroso compounds, which have been found almost exclusively in those foods containing nitrite or which have become exposed to nitrogen oxides. Beer is a source of NDMA, in which as much as 70 μg/l has been reported in some German beers, although usual levels are 5-10 μg/l; this could be a
considerable intake for a heavy beer drinker. Nitrosamines levels have been declining during the past three decades, concurrent with a greater control of exposure of malt to nitrogen oxides in beer making and a generally lowering of the nitrite used in food (Lijinsky, 1999; Marconi et al., 2007).

The highest concentrations of NDMA were found in dark beers, high in malt content. The origin of the NDMA depends on alkaloids, hordenine and gramine and perhaps others present in barley and hence, in the malt. These alkaloids were derivatives of dimethylamine and were easily nitrosated, in this case, by nitrogen oxides as the nitrosating agent. The nitrogen oxides were in the flue gases from the burning of the fuel used to dry (heating) the malt and allowed to come in contact with the latter (Sen et al., 1996).

Microbiological nitrate reduction is the principal pathway of N-nitrosamine formation in beer brewing (Smith, 1994).

7.2 Biogenic Amines

Biogenic amines are natural antinutritional factors, and are important from a hygienic point of view as they have been implicated as the causative agents in a number of food poisoning episodes. Amine production results from the presence of bacteria (Enterobacteriaceae, Clostridium, and Lactobacillus species among others) that are capable of decarboxylating aminoacids (Shalaby, 1996).

Beer has been reported as a possible health risk for some consumers due to biogenic amines intake (Izquiedro-Pulido et al., 1994; Shalaby, 1996; Halász et al., 1999).

The high biogenic amines intake levels are not usually caused by high amine content in individual beers, but rather by very high beer consumption. These compounds arise from the bacterial decarbolxylation of the corresponding amino acids: histamine (HI) from histidine, tyramine (TY) from tyrosine, tryptamine (TR) from tryptophan, 2-phenylethylamine (PEA) from phenylalanine, agmatine (AGM) from arginine, cadaverine (CAD) from lysine, putrescine (PUT) from ornithine and agmatine, spermidine (SPD) and spermine (SPM) from the biochemical reaction of putrescine by the addition of aminopropyl moieties catalyzed by spermidine and spermine synthase. PUT, SPD, SPM and AGM can be considered as natural beer constituents primarily originating from the malt, and HI, TY and CAD usually indicate the activities of contaminating lactic bacteria during brewing. TY has been the main biogenic amine identified in beer that cause the adverse effects on human health (Kalač and Křížek, 2003; Marconi et al. 2007).

The biogenic amines in beer originate from the raw materials and the fermentation phases of beer production (Jurková et al., 2005)
8. Determination of the Critical Control Points (CCPs)

A CCP is a step or a procedure in the brewing process where control is essential to prevent, eliminate or reduce a hazard to an acceptable level. The World Health Organisation (WHO) recommends that CCPs should be determined using the HACCP decision tree established by FAO/WHO (2001). If a control could easily be implemented and a hazard is just an easily prevented, then a process step is merely identified as a control point (CP). Process steps that need close monitoring so that a hazard could be prevented, eliminated, or minimized is identified as CCPs (Senires and Alegado, 2005). It is very important to introduce a step where only a control or a control point (CP), help to take under control the process and prevent the formation of hazards. When multiple steps address a hazard, the CCP should be the step nearest the end of the process, if this step provides adequate control (Rush, 2006).

The results of the hazard analysis and the study of CCP, CP and the overall management of nitrosamines and biogenic amines in malting and brewing processes are reported in table 1 and 2. In these tables, the control measures, critical limits and corrective actions are reported for nitrosamines and biogenic amines for different processing steps in malting and brewing.

8.1 CCPs for Nitrosamines

8.1.1 Malting process

Kilning: malted barley is the primary source of NDMA and only malt dried by direct-fired kilning, where nitrogen oxides reacts with certain alkaloids, could account for the levels of NDMA found in final beer (Mangino and Scanlan, 1985; Izquierdo-Pulido et al., 1996a; Yurchenko and Molder, 2005).

The NDMA is formed at high temperatures and since the conversion from direct to indirect heating of kilns the hot nitrogen oxide-rich (NOX) gas used for killing is no longer passed through the malt. In the production of smoked malt, smoke is fed through the malt before kilning, which gives it its scratchy flavour. This does not incur the formation of NDMA (VLB, 2004).

The NDMA concentration in malt depends on the type of the drying technique: high combustion temperatures (usually from 1500 to 1800 °C) yield high NDMA concentration, but green malt is usually dried with hot air at the temperature of lower then 100 °C, and the malt germination is very short; the germ and root of the malt are usually very small, and this reduce the quantities of alkaloids in malt (Yurchenko and Molder, 2005).
8.1.2 Brewing process
Ingredients and Adjuncts: Study on beer ingredients showed that NDMA is not normally present in significant quantities in any processing aids, additives or ingredients other than malted barley although small amounts have been found in hops (in accordance with the results that will be reported in the deliverable D2B.2.4). Because the NDMA in hops is diluted during brewing, its contribution is negligible. The NDMA content of water is quite variable and charcoal filters should adsorb it. Experiences suggest that NDMA in supply water is a common source of NDMA in beer. The most convincing evidence is that NDMA is not normally formed during beer fermentation or during mashing or wort boiling (Wainwright, 1986a) (in accordance with the results that will be reported in the deliverable D2B.2.4).

The occurrence of Apparent Total N-Nitroso Compounds (ATNC) in beer is due to the metabolism of certain groups of bacteria and is thus indicative of hygiene problems at some stages in the brewing process (Smith, 1994).

A possible source of dimethylamine (DMA) is hordenine and gramine, which is formed in the barley kernels during malting. NDMA could be formed during the brewing process, if traces of nitrosating agents were available at that stage; nitrite could be formed from nitrate during processing or nitrogen oxides might be present. The concentration of NDMA depends on the percent of alcohol, since ethanol is an inhibitor of nitrosation (Yurchenko and Molder, 2005). But NDMA is not usually formed during production or storage of malt, except when gaseous N_2O_3 or N_2O_4 is present, or beer because the concentration of NO_2 and nitrite ions are too low at the temperature and/or pHs involved (Wainwright, 1986b).

8.2 CCPs for Biogenic Amines

Data on biogenic amines levels in malt, hops or hop derivatives as well as pitching yeast is very limited (Kalač and Křížek, 2003). Malt is a source of agmatine, putrescine, spermidine and spermine; while tyramine, histamine and cadaverine have been formed during the main fermentation by contaminating lactic acid bacteria (Kalač et al., 2002).

8.2.1 Malting
Process water: no amines should be present in water used during malting (Kalač and Křížek, 2003). Malting process: malt is the main source of the PUT, SPD, SPM and AGM in beer and their increase were observed during five days barley germination; malting condition such as germinating intensity, kiln temperature and barley variety will affect the final amine levels in malt (Kalač and Křížek, 2003). Decarboxylating enzyme activity of micro-flora is considered causing amine formation during barley malting (Kalač et al., 1997).
8.2.2 Brewing

Process water: no amines should be present in water utilized for the production of beer too (Kalač and Křížek, 2003).

Brewing process: in brewing, the types of amines are dependent on the raw materials (in according with the results that will be reported in the deliverable D2B.2.4), as well the method of brewing, and any microbial contamination (lactic acid bacteria activity) occurred during the brewing process or during the storage. Amines can be also formed by the activity of lactic acid bacteria during the storage of beer in bottles (Gloria and Izquiedro-Pulido, 1999; Kalač and Křížek, 2003).

9. Establish the critical limits

One (or more) prescribed tolerances (critical limits; maximum or minimum values; applicable targets) must be met to ensure that the CCP effectively controls the hazard (Codex Alimentarius, 1997). Critical limits must be set for each CCP identified. The critical limits define the difference between a safe and unsafe process, and it is not necessarily the legal limit of the contaminant in the product, but for nitrosamines and biogenic amines, the critical limits are connected with physical and chemical parameters affecting the occurrence of the hazard. In particular, the nitrogen along the production chain or the bacterial activity can be respectively exploited in different manners to define specific critical limits for nitrosamines and biogenic amines. The limit applies to the control measure and not the hazard, and must be able to be measured quickly and simply to enable prompt corrective actions (FAO/IAEA, 2001; Rush, 2006). Critical limits must be stated in terms of the safety requirements of the process, not overall process requirements (Rush, 2006).

9.1 Critical limits for Nitrosamines

It is generally “accepted” a level ≤ 5 ppb in beer (Izquierdo-Pulido et al., 1996a; Lijinsky, 1999). Industry’s voluntary limit is 0.5 μg/kg (Smith, 1994). Moreover, in Germany there exist a technical reference concentration of 2.5 ppb for malt and 0.5 ppb for beer (VLB, 2006).

9.2 Critical limits for Biogenic Amines

Safety limit of 20 mg/L for the sum of the TY+HI+CAD+PEA contents is proposed by Loret et al. (1999).

10. Establish a monitoring system

Monitoring is the scheduled measurement or observation at a CCP to assess whether the step is under control, i.e. within the critical limit(s) specified as above reported for the step 3 (FAO/IAEA, 2001).
Monitoring procedures specify how a critical limit has to be measured. Monitoring procedures should give the specific method (or include a reference to the method, the responsibility, and the frequency of monitoring) (Rush, 2006). Monitoring procedure could be in-line, on-line or off-line. Process parameters such as temperature, time and moisture should be controlled within the limits required (Codex Alimentarius, 1997).

10.1 Monitoring system for Nitrosamines

10.1.1 Malting process

Good Agriculture Practices (GAP) are request to malt suppliers. The GMP (e.g.: not use direct-fired in kilning; not use oil but natural gas as the kiln fuel source; use indirect malt drying techniques are needed) (Izquierdo-Pulido et al., 1996a; Sen et al., 1996) have to be implemented. The use of sulphur dioxide in the kiln air stream seems to reduce or prevent the formation of undesirable nitrosamines in malt; high application of bromates at the beginning of malting, or spraying or steeping green malt with dilute nitric acid before kilning is proposed too (Briggs et al., 1981).

Moreover, the concentration of NDMA can be reduced through the control of the exposure of malt to nitrogen oxides, efficaciously stopping nitrosation of the alkaloids in the malt (Lijinsky, 1999).

The use of formalized management system, GHP and GMP are requested for nitrosamines too.

10.1.2 Beer production

The use of formalized management system is here useful too. Cleaning-In-Place (CIP) procedures should be used to reduce potential contamination from bacteria.

10.2 Monitoring system for Biogenic Amines

The histamine content in beer is a good indicator for hygienic condition of barley storage, malting and brewing, as histamine content of the product does not originate from barley or from the malt (Halász et al., 1999).

10.2.1 Malting process

Malt coming from barley grown in soils with low potassium or sodium concentration, or with high ammonia level should not be used for beer brewing (Izquierdo-Pulido et al., 1994).

GMP for malting process: barley variety, storage, germinating intensity and kiln temperature will affect the final amine levels in malt (Halász et al., 1999; Kalač and Křížek, 2003).

10.2.2 Brewing process

The use of formalized management system (e.g.: application of GMP and GHP) is request for monitoring biogenic amines formation. The TY, HI and CAD formation was observed during the main fermentation by the presence of lactic acid bacteria (i.e. Pediococcus spp., mainly P.
*damnosus*, and species of *Lactobacillus*, *L. frigidus*, *L. brevissimilis* and *L. brevis* (Izquiedro-Pulido et al., 2000; Kalač and Křížek, 2003). The presence of these bacteria has to be controlled.

11. **Establish procedures for corrective action**

The establishment of a procedure for corrective actions is necessary to be adopted when monitoring a CCP it indicates a deviation from an established critical limit (FAO/IAEA, 2001). The corrective action must provide assurance that the CCP had been brought under control; it must also describe the proper actions to be taken in disposing of the affected product (Senires and Alegado, 2005) if needed. A corrective action must be established for each CCP: for the purpose of HACCP, the corrective action should address the actions to take to deal with the product produced while critical limit was begin violated and what steps are necessary to return the process to a state of control so that the operation may continue (Rush, 2006). Corrective action procedures and responsibilities need to be specified (Efstratiadis and Arvanitoyannis, 2000).

11.1 **Corrective actions for Nitrosamines**

11.1.1 Malting process

Do not use contaminated water.

Apply the GMP:

- Use indirect malt drying techniques (use indirect firing to dry malt);
- Check to control risk caused by exhaust gases of heating system and polluted drying air by NOx;
- High application of bromates at the beginning of malting, or spraying or steeping green malt with dilute nitric acid before kilning is proposed.
- Do not use oil but natural gas as the fuel source.
- Use sulphur dioxide in the kiln air stream.

11.1.2 Brewing process


11.2 **Corrective actions for Biogenic Amines**

11.2.1 Brewing process

Washing the pitching yeast with phosphoric acid reduce the presence of *Pediococci* and consequently reduce the TY content of beer (Kalač and Křížek, 2003).

Special attention is required in controlling the presence of lactic acid bacteria contamination (Izquiedro-Pulido et al., 2000). GHP (CIP) should be in place if contamination of bacteria is found.
Table 1: Hazard analysis and study of CCP, CP and the overall management of nitrosamines and biogenic amines in malting.

<table>
<thead>
<tr>
<th>Process Step</th>
<th>Hazard to be Controlled</th>
<th>Critical Limits</th>
<th>Control Measures</th>
<th>Corrective Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley intake</td>
<td>Biogenic Amines</td>
<td></td>
<td>CP: Document suppliers control: delivery will be accompanied with document.</td>
<td>Reject the delivery or ask suppliers the delivery documents</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Request to the farmer the implementation of the GAP and encourage growers to use</td>
<td>Request to the farmer the implementation of the GAP and encourage growers to use</td>
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<tr>
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<td>the GAP (not use barley grown in soils with low potassium or sodium or with high</td>
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<td></td>
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<td>ammonia).</td>
<td>ammonia).</td>
</tr>
<tr>
<td>Kilning</td>
<td>NDMA</td>
<td>≤ 5 ppb</td>
<td>CCP: malt analytical control</td>
<td>Application of the GMP:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Check to control risk caused by exhaust gases of heating system and polluted</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>drying air by NOx;</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>- Use indirect firing to dry malt</td>
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<td></td>
<td>- High application of bromates at the beginning of malting, or spraying or</td>
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<td></td>
<td>steeping green malt with dilute nitric acid before kilning is proposed</td>
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<td></td>
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<td></td>
<td>- Do not use oil but natural gas as the fuel source</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Use sulphur dioxide in the kiln air stream</td>
</tr>
<tr>
<td>Malting process</td>
<td>Biogenic Amines</td>
<td></td>
<td>CP: process water: periodic analytical control.</td>
<td>Add water from different incoming</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CP: GMP: have to be maintained during the malting production:</td>
<td>Once a contaminated product has been identified, clean-up, and separation of the</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- good hygienic condition during barley storage</td>
<td>batch are the first action to put in place.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- barley variety</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>- germinating intensity</td>
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<td></td>
<td></td>
<td>- kiln temperature</td>
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</tr>
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<td></td>
<td></td>
<td></td>
<td>GHP have to be in place to reduce bacteria contamination</td>
<td></td>
</tr>
</tbody>
</table>
### Table 2: Hazard analysis and the study of CCP, CP and the overall management of nitrosamines and biogenic amines in brewing.

<table>
<thead>
<tr>
<th>Process Step</th>
<th>Hazard to be Controlled</th>
<th>Critical Limits</th>
<th>Control Measures</th>
<th>Corrective Actions</th>
</tr>
</thead>
</table>
| Raw material intake (malt) | NDMA | ≤ 5 ppb | CCP: Qualified Supplier  
- deliveries should conform to brewers’ specifications  
- documents of analytical control must accompany every delivery to brewers | Reject the delivery or ask suppliers the delivery documents  
Use wet grinding |
| Beer Production | NDMA | ≤ 0.5 ppb | CP: GHP have to be in place:  
- CIP procedures should be used to reduce potential contamination from bacteria | Once a contaminated product has been identified, clean-up, and separation of the batch are the first action to put in place |
| Beer Production | Biogenic amines | Add water from different incoming | CP: process water: periodic analytical control | |
| Beer Production | Biogenic amines (lactic acid bacteria contamination) | ≤ 20 ppm (Sum of the TY+HI+CAD+PEA) | CP: GHP have to be in place:  
- CIP procedures should be used to reduce potential contamination from bacteria  
- Washing the pitching yeast with phosphoric acid | Once a contaminated product has been identified, clean-up, and separation of the batch are the first action to put in place |
12. Establish procedures for the verification
Each industry has to establish procedures to verify and to confirm the effectiveness of the HACCP plan. Such procedures include auditing of the HACCP plan to review deviations and product disposition, and random sampling and checking to validate the whole plan (FAO/IAEA, 2001). Accurately and efficiently documenting all activities related to the HACCP system is very important. This would include creating records of the hazards and their control methods, the monitoring of safety requirements, and the action taken to correct problems. All actions must be properly recorded also for the traceability needs (Senires and Alegado, 2005). The results of analytical control are part of the verification system for the contaminants here studied.

The surface malt pH level during the kilning is the indicator of the correct use of sulphuring to control the development of NDMA. Surface malt pH, and not the ground pH, below 4 give low NDMA values (Wainwright, 1986a).

The high total acidity in beer is an indicator of the activity of lactic acid bacteria (Kalač and Križek, 2003) for the presence of Biogenic Amines.

13. Establish document concerning all procedures and records appropriate to these principles and their application
Verifying whether HACCP works effectively is extremely important to determinate if there is a need to modify or alter the HACCP plans adopted and in operations, and the relevant procedures associated with it. Validation, on-going verification, reassessment, and auditing are the tools implemented in verifying the effectiveness of the HACCP system. These tools would detect if the preventive measures, corrective actions, critical limits, and risk assessments are sufficient to address the problems (Senires and Alegado, 2005). Records are the results of monitoring activities during the execution of the HACCP plan (Rush, 2006).

14. Concluding remarks
HACCP is an effective precautionary control system that, if applied correctly and systematically, offers the means for the identification and assessment of any possible physical, chemical and microbiological hazard, the detection and control of critical points in all food production steps. The evolution of an HACCP plan has become an important part of food safety for food and beverage industry.

This work hopefully provides a guide for operators in the development of their specific factory plans with regard to HACCP.
Food safety is assured by process control, and not only by finishing product testing. Post-harvest storage, transport and processing are all important stages in the food chain which need to be monitored and be managed by the application of the GAP and GMP.

Considering all this aspects and the results of the safety concerns reported in this document, it is possible to develop HACCP programmes for nitrosamines and biogenic amines, but this is not a simple issue. One possible approach to manage the risk associated with contaminants prevention is the use of an integrated system. The HACCP system should involve strategies for prevention, control, GMP and quality control at all stages of production, from raw material production (field) up to the final consumer.

It is evident that the need of managing the risks associated with the presence of these contaminants in the beer food chain can be taken under control by the use of proper HACCP principles. Prevention measures are available and should be exploited for any specific concern ensuring a safe final product. The risks associated with these hazards could be minimized at every phase of production.

The results here reported and described should be exploited for the implementation of the current HACCP factory documents already adopted. This is possible by the integration of these hazards management with the other biological, chemical and physical hazards in barley, malting and brewing food chain.

Specific needs for small and medium size industries are connected with the use of non-standardized barley or malt lots, it means that the particular attention of potentially dangerous factors affecting the use of unknown content of nitrogen in water, malt or other ingredients (adjuncts, etc.), and the proper hygienic control: all of them are examples of the specific and peculiar needs to avoid the production of the specific hazard.

Finally, to make a HACCP work in practice it does need to become a part of the company’s operating practices, and depend on the prerequisite programmes which support it, and on the people who operate it. Therefore, the proper training program should be adopted too.

15. Documents
Here are reported the most important references used.

15.1 References


- Jurková M. Kellner V. Horáček T. Čejka P. & Čulik J. 2005. The HPLC determination of biogenic amines in beer by solid phase extraction (SPE) and automatic precolumn derivatization. Proceeding of the 30th EBC Congress, Prague.


