HYGIENIC ASPECTS AT THE PRODUCTION OF GLUCOSE SYRUPS THROUGH ACID HYDROLYSIS

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Abstract: Glucose syrup is a valuable product, obtained through acid hydrolysis at the factory "Amylon" in Sibiu Romania. The technology is complex and the risks of contamination during the processing steps or in the final product are high. The microbiological analysis in different places from the glucose syrup compartment revealed the presence of bacteria, as coliforms, lactic acid bacteria and *Bacillus*. Moulds spores and even mycelia from *Aspergillus niger, Penicillium sp, Fusarium sp.* were found too. A specie of *Aureobasidium pullulans* resistant to very high glucose concentrations was isolated from the glucose syrup tanks. Two contamination sources in the glucose syrup factory were identified: the deficiency at the separation of cereals storages (rich in vegetative microorganisms and spores) from the glucose syrup compartments and the deficiency in maintain the hygiene in the storage recipients.

Keywords: Hygiene, glucose syrup, acid hydrolysis, *Lactobacillus, Leuconostoc*, coliform bacteria, *Aureobasidium*, *Aspergillus niger*

INTRODUCTION

After cellulose, starch is the second polysaccharide found in nature, produced by plants as reserve material. Starch is a biopolymer consisting of α-D-glucose bound α-(1→4) and α-(1→6) forming two types of molecules, amylose and amylopectin (Smith, 2001). The break-off of these bounds is called hydrolysis. Hydrolysis can be realised using enzymes or acids. Many factories utilise the acid conversion to obtain glucose syrup (especially for the sugar confectionery industry), because of some advantages of this process: short hydrolysis time, simplicity of installations, low prices for the materials.

The raw material at the obtaining glucose syrup is starch slurry. The industrial process of acid hydrolysis of starch is conducted using

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hydrochloric acid as catalyst, at high pressures. After hydrolysis, the starch hydrolysate is neutralised to pH 4.6 – 4.8 with sodium carbonate; the lipids and the proteins are so precipitated (Howling, 1992). Precipitated impurities may be removed by centrifugation or by skimming operations; solid impurities can be removed by passing the liquor through deep tanks with a weir that separate the fat and protein flocks from the liquor. The remaining impurities are colour precursors, flavour and odour contaminants, proteins or protein hydrolyse products, peptides, aminoacids and are refined using powdered or granular activated carbon techniques or ion-exchange resins (Cotillon, 1992). After refining, the syrup liquor is adjusted to ~80% dry solids and cooled. The final product called glucose syrup is packed in containers.

Although the theory discuss very few the safety aspects in the compartments for obtaining acid-conversed starch syrups (Adams and Moss, 2000), they are many possibilities for microbiological contamination. This contamination is due to the existence of glucose, maltose and other sugars with small molecule in the composition of starch hydrolysate, which are used by microorganisms as carbohydrate sources. A practical study, made at the "Amylon" factory in Sibiu Romania reveals the necessity to analyse seriously this aspect. So, after the study of all the steps in the factory, a block diagram was constructed. The diagram gave an overview of the production of glucose syrup by acid hydrolysis from starch suspension and is presented in detail in (Mironescu and Mironescu, 2001), together with the process flow diagram. The process flow diagram showed in detail the various steps of the process (inclusively the entry of ingredients, all processing's steps, packaging, storage, distribution).

The theoretical study of both diagrams and the practical tests demonstrated the importance of the microbiological analysis in the starch factories. The aim of this paper is to realise a systematically study on the microorganisms which inhabits the glucose factories, adapted to the technological schema of glucose syrup obtaining at "Amylon" and to identify the potentially action of microorganisms on the glucose syrup quality.

MATERIAL AND METHODS

For the microbiological analysis, assays from the starch suspensions, glucose syrup, machinery's, recipients and from various places in the factory were sampled. The samples were diluted (1 ml from each solution and 1 g from

each solid product was diluted with sterile distilled water, dilution 10^2) and 1ml from the last dilution was poured into Petri dishes. The assays were incubated at 37°C for 72 hours.
The contaminant moulds were identified by colonial studies and by optical microscopy (Dan et al., 1999) (Larone, 1995) at the cultivation on malt agar.
Coliforms were identified on solid media according to (Feng et al., 1998) using violet red bile agar (VRBA) as cultivation substrate. Invert solidified plates were incubated 24 h at 35°C. The coliformic bacteria appear as purple-red colonies that are 0.5 mm or larger in diameter and surrounded by zone of precipitated bile acids. To confirm that the colonies are coliforms, each colony was transferred to a tube of Brilliant green lactose bile (BGLB) broth 2% with Graham tube and incubated at 35°C. In the tubes having gas bubbles, Gram stain was performed. The Gram-positive bacteria were considered to be lactic bacteria from the genera *Lactobacillus, Leuconostoc, Pediococcus* and *Streptococcus* (Axelsson, 1998). The Gram-negative bacteria were assigned as coliformic bacteria.
From the lactic bacteria group, *Leuconostoc* was identified based on its ability to produce dextran from sucrose (Sutherland, 1996). The tubes with lactic bacteria were poured on plates containing a synthetic growth medium, containing sucrose 110mM, yeast extract 20g/l, K_2HPO_4 20 g/l, $MgSO_4 \cdot 7H_2O$ 0.01 g/l, $MgSO_4 \cdot 7H_2O$ 0.01 g/l, NaCl 0.01 g/l, $CaCl_2$ 0.02 g/l, $FeSO_4 \cdot 7H_2O$ 0.01 g/l, buffered at pH 6.9 (Dols et al., 1997). 5 g/l agar was added. The colonies having mucilage were considered to be *Leuconostoc*.
For the identification of *Lactobacillus* species, samples were mixed with de Man, Rogosa and Sharpe (MRS)-agar medium (Oxoid) and incubated at 37°C for 48 h under anaerobiosis (Giraud et al., 1991). The Gram-positive bacteria were analysed microscopically and the rod-shaped bacteria were analysed following the protocol presented in (Batt, 1999): the identification of fermentation, motility test and catalase test. The bacteria fermentative, non-motile and catalase-negatives were considered to be *Lactobacillus*.
The identification of *Bacillus* species was based on four characteristics: *Bacillus* species are rod-shaped, endospore-forming, Gram-positive, catalase-positive (Turnbull et al., 1990). For its identification, samples from the factory were pasteurised at 80°C for 15 minutes, then plated onto nutrient agar and incubate at 37°C for 24 hours up to several days. The plates were examined after 24 hours for typical *Bacillus* colonies identified as catalase-positive, Gram-positive, endospore-forming rods (***).
All analyses were made at the “Amylon” factory.

RESULTS AND DISCUSIONS

The results of microbiological tests are presented in Table 1. The microorganisms found in different places of the factory are bacteria and moulds. From these two categories, bacteria are very demanding, requiring complex media for growth and warm, moist conditions for maximum survival. The conditions in the factory are very different from their requirements; for example, the proteins and aminoacids are absent in all places analysed, water is absent on the plastic containers and less than 20% in the final product (Mironescu and Mironescu, 2001). Their presence could be due on the hygiene problems caused by human actions or on the accidentally arrival with wind.

Table 1: Microorganisms found in the factory before cleaning and disinfecting

Place	**Microorganisms identified**	
Storage tanks of starch slurry	Moulds spores	*Fusarium sp., Absidia sp.* *Penicillium glaucum* *Aspergillus niger*
	Bacteria	*Lactobacillus*
Storage tanks of carbon-kieselgur suspension	Bacteria	*Coliformic bacteria*
	Moulds spores	*Aspergillus niger*
Plastic containers	Moulds	*Penicillium glaucum* *Aspergillus niger* *Aureobasidium pullulans*
	Bacteria	*Leuconostoc, Bacillus* *Coliformic bacteria*
Walls	Moulds	*Aspergillus niger*
Final product (glucose syrup)	Moulds spores and colonies	*Penicillium glaucum* *Aspergillus niger* *Aureobasidium pullulans*
	Bacteria	*Leuconostoc, Bacillus* *Lactobacillus* *Coliformic bacteria*

Although in the factory the coliform bacteria begin to die, they are most likely to survive in these conditions. Coliform bacteria are an indicator of the

contamination and possible presence of intestinal parasites and pathogens. The coliform bacteria are present in much larger numbers than the more dangerous pathogens, and react to the natural environment and treatment processes in a manner and degree similar to pathogens. Thus by observing coliform bacteria, the increase or decrease of many pathogenic bacteria can be estimated. Their presence in the storage tanks of carbon-kieselgur suspension and in the plastic containers indicates very probably the absence of human hygiene at the manipulation of recipients. However, the number of coliformic bacteria was in the limits admitted by the Romanian standards, as in the case of final product.

The lactic acid bacteria derive almost all of their energy from the conversion of glucose to lactate during homolactic fermentation, when 85-90% of the sugar utilized is converted to lactic acid (Salminen et al, 2004). In the glucose factories, an infection with lactobacilli is not desired and the presence of these microorganisms must be monitored. As Table 1 shows, they are quite everywhere in the factory. *Lactobacillus* can grow in the storage tanks of starch slurry, where the conditions allows its multiplication (pH is neutral or weak acid, the oxygen is present at the surface, dry mass is around 40%). Many species of *Lactobacillus* are able to synthesise α-amylases (Calderon et al., 2003), making possible the consumption of starch. For this reason, the slurry maintained in the storage tanks will be light acidified, depending on the storage time.

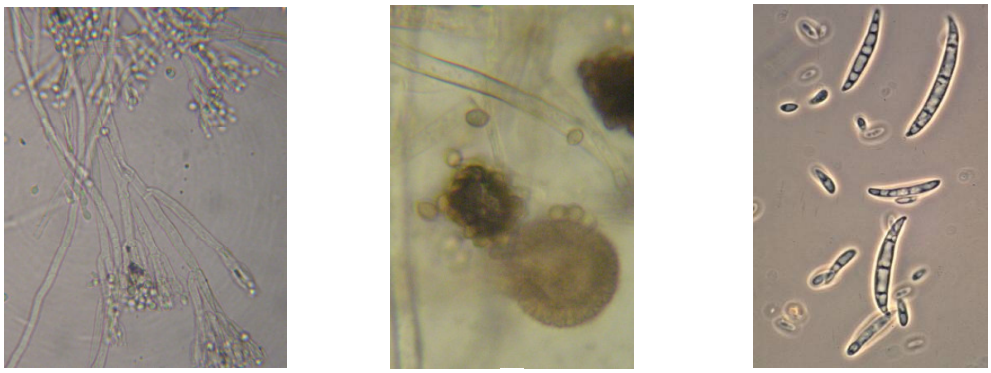
The hydrolysis conditions are very aggressive (pH = 2.5, temperatures of 185°C, high pressure), the glucose syrup obtained after hydrolysis is neutralised and cooled to 70°C (Mironescu and Mironescu, 2001). These conditions are not favourable for the multiplication of bacteria identified in different places, including coliforms; most of them are mesophylic, although thermophilic strains exists (Akelsson, 1998). They survive in the glucose syrups during various treatments, but they are not active.

Bacteria can grow in the plastic containers when the temperature at storage is around 35°C (in summer) and the containers are wet or in the containers with glucose syrup stored at high temperature in wet places. In these conditions, the spores of *Bacillus*, which survived in the product, germinate and become vegetative cells.

There is great diversity in physiology among members of the *Bacillus* genus, whose collective features include degradation of most all substrates derived from plant and animal sources, including cellulose, starch, pectin, proteins, agar, hydrocarbons, and others (***). Aerial distribution of the dormant

spores probably explains the occurrence of *Bacillus* species in the storage places in the factory. The main cause of the presence of these spores could be the deficient separation of the two main sections, for starch slurry and glucose production. A large number of microorganisms, including sporulated bacteria from the genus *Bacillus*, arrive in the factory with the cereals (Dan, 2000), the raw material for starch obtaining. The presence of cereals storages near the compartments for glucose syrup production can cause the contamination with bacteria, especially Bacillus spores, but also coliforms.
Beside degradation, the specie *Bacillus cereus* can produce toxins, causing two types of food-borne intoxications (***). For these two reasons (degradation and toxin production), the presence of the *Bacillus* species must be monitored in all the storage places in the factory.

Moulds are not so sensible to nutrients or moisture, they growing in poor media with less humidity as bacteria. This is the explanation why moulds were found quite everywhere in the factory. Only superior moulds were found.
In Figures 1 a, b,c are presented some microscopic images of common moulds found in the factory.



a b c

Figure 1. Reproductive hyphae and spores of *Penicillium* (a), *Aspergillus* (b) and *Fusarium* (c) species seen by optical microscopy.

All these three identified genera (*Penicillium*, *Aspergillus* and *Fusarium*) can produce micotoxines.
The most common dispersal way for moulds is by spores. Once an infection is produced and the fungal mycelium is formed, the microorganism produces

spores which are spread by any currents or by wind. This is the explanation why *A. niger* was found in all factory, the main cause being the presence of this mould on the walls. According to this, a serious disinfection of walls is recommended.
As in the case of bacteria, one possible cause of the strong contamination could be the faulty design of the factory.

Beside the three fungal genera, a specialised *Aureobasidium pullulans* was found in the factory in the plastic containers and in the final product, growing in the concentrated glucose syrup. *Aureobasidium pullulans* is commonly considered as a contaminant (Collier et al., 1998); it produces a black pigment, melanin and a polysaccharide, pullulan, in great quantities. This polysaccharide is not toxic, but it modifies the rheology of the glucose syrup and his composition (Rau, 2004).

Macroscopic characteristics of *A. pullulans* found in the factory

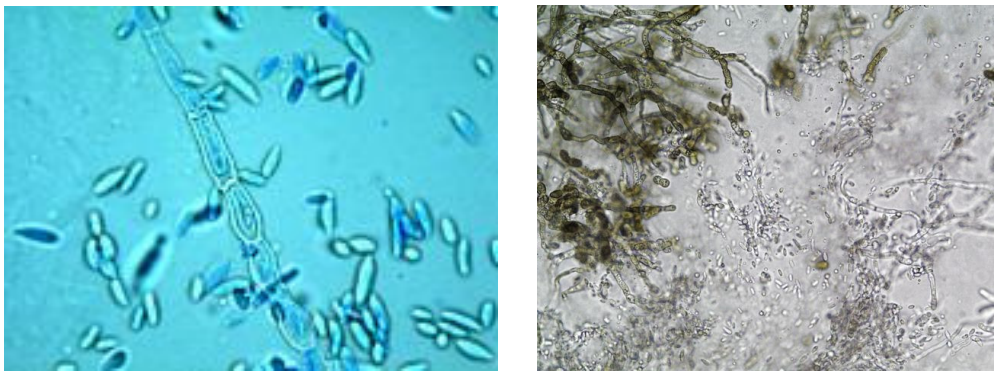
The colony diameter is 1 to 3 cm following incubation at 25°C for 5 days on malt agar. The colonies are flat, smooth, moist, yeast-like, mucoid to pasty, shiny and leathery in appearance. The surface is white, pale pink or yellow at the beginning and becomes brown to black and velvety with a grayish fringe by aging. Reverse is pale or black.

Microscopic characteristics of *A. pullulans* found in the factory

In Figure 2 two images of *A. pullulans* hyphae and conidia are presented. Unicellular budding yeast cells (blastoconidia) were observed microscopically, especially in the young colonies (Figure 2a). By aging, while the colony gets black and velvety, hyphae became visible. Blastoconidia were pale in colour. hyphae appears hyaline at the beginning and gets dark brown by aging (Figure 2b) due to the formation of melanin.

CONCLUSIONS

From a microbiological point of view, the technological process at the obtaining of glucose syrup through acid hydrolysis is aggressive and unfavourable to the microorganisms multiplication. Still, the risks are possible and are due to the external contamination at mixing, packaging and storage or to the unhygienic manipulation and/or maintenance of equipments.



a b

Figure 2. Hyphae and blastoconidia of *A. pullulans* observed by optical microscopy using objectives of 90x (a) and 40x (b). In (a), methylene blue was used as colorant. In (b) the black colour is given by the pigment melanin.

The bacteria that can inhabit the starch factories are coliforms, lactic bacilli and *Bacillus* species.
The moulds are more frequent, *Aspergillus niger* causing a general infection in the factory. A specie of *Aureobasidium pullulans* resistant to high glucose concentrations was isolated

Two reasons for the contamination in the glucose syrup factory are identified:

- Deficiency at the separation of cereals storages (rich in vegetative microorganisms and spores) from other compartments;
- Deficiency in maintain the hygiene in the storage recipients. Adequate codes of practice and good manufacturing practices can eliminate these dangers.

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