MICROBIAL PRODUCTION OF VINEGAR

The word Vinegar is derived from the French Vin (wine) and Aigre (sour). Vinegar is a sour and sharp liquid used as a condiment and food preservative (Cruess 1958). The available definition from the Codex Alimentarius (1987) states that vinegar is “a liquid, fit for human consumption, produced from a suitable raw material of agricultural origin, containing starch, sugars, or starch and sugars, by the process of double fermentation, first alcoholic and then acetous”.

The raw materials used for vinegar production include rice, grapes, malt, apple, honey, potatoes, whey or any other sugary food (Bamforth 2005). In human history, vinegar appears at the beginning of agriculture with the discovery of alcoholic fermentation from fruits, cereals and vegetables. The genesis of vinegar can hardly be distinguished from the origin of wine. Although vinegar has always been considered among the lowest quality products of fermented foods, it has also been used as a food condiment, as a preservative agent and, in some countries as a healthy drink (Solieri and Giudici, 2009).

Vinegar contains about 5% acetic acid in water, varying amounts of fixed fruit acids, colouring matter, salts and a few other fermentation products which impart a characteristic flavour and aroma to the product.

Vinegar production

Vinegar is the product made from the conversion of ethyl alcohol to acetic acid by a genus of bacteria, Acetobacter. Therefore, vinegar can be produced from any alcoholic material from alcohol-water mixtures to various fruit wines (Pepppler and Beaman 1967). Vinegar bacteria, also called acetic acid bacteria (AAB), are members of the genus Acetobacter and characterised by their ability to convert ethyl alcohol (C\textsubscript{2}H\textsubscript{5}OH) into acetic acid (CH\textsubscript{3}COOH) by oxidation as shown below:

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\begin{align*}
\text{Glucose} & \xrightarrow{\text{Yeast}} 2\text{C}_2\text{H}_5\text{OH} \\
\text{Ethanol} & \xrightarrow{\text{AAB}} 2\text{CH}_3\text{COOH} + 2\text{H}_2\text{O} \\
\end{align*}
\]

Vinegar is a solution of acetic acid produced by a two-step bioprocess. In the first step, fermentable sugars are transformed into ethanol by the action of yeast. In the second step, AAB oxidize the ethanol into acetic acid in an aerobic process. AAB are well known for their ability to spoil wines because they can produce large amounts of acetic acid from ethanol and other compounds present in wines (Joyeux et al., 1984).

Substrates used in vinegar production

One of the critical steps in vinegar production is the preparation of the raw material (Solieri and Giudici 2009). This step is required to obtain the fermentable sugar and juice solution to be acetified. The processing differs depending on the raw material used. In general, fruits require less preparation
than seeds; however, seeds are more easily stored and preserved after harvest. Fruits are highly perishable, rich in water, and need to be processed very quickly. Therefore, basic safe food handling practices, storage, and processing are essential to prevent the growth of pathogenic microorganisms.

These microorganisms could alter the quality of the final product or even produce dangerous toxins such as aflatoxin. Processing has expanded the market of both fruit and vegetable products (Singh and Verma 1995). The seasonal gluts are avoided by the utilization of fruits/vegetables in processing industries for the preparation of various value added products. In advanced countries, 70-75% of perishables are processed before reaching the consumer’s table, whereas in India only 1-2% of the total produce is processed utilizing only 40% of the installed processing capacity.

**Microorganisms involved in the vinegar production**

After raw material preparation, the alcoholic fermentation and acetylation processes play a key role in vinegar production. Depending on the environmental factors (temperature, pH, water activity) or the nutrients (carbon sources) and the microbial diversity present in the raw material, different biotransformations could take place. Microbial species involved in fermentations may range from yeast and lactic acid bacteria (LAB) to molds and AAB. The microorganisms involved in the elaboration of vinegars are mainly yeasts and AAB. The former being responsible for the alcoholic fermentation, and the latter needed for the acetylation (Nanda *et al.* 2001; Haruta *et al.* 2006; Wu *et al.* 2010).

**Acetic Acid Bacteria (AAB)**

The ninth edition of Bergey’s *Manual of Systematic Bacteriology* classified the acetic acid bacteria (AAB) in the family Acetobacteriaceae and Gluconobacter. AAB are Gram-negative or Gram-variable bacteria, with ellipsoidal to rod-shaped morphologies, being motile due to the presence of flagella, which could be either peritrichous or polar. Their size varies between 0.4-1 μm wide and 0.8-4.5 μm long. They are observed as individual cells, in pairs or in chains. They show a strict aerobic metabolism with oxygen as the terminal electron acceptor, and are catalase positive and oxidase negative (Gonzalez *et al.*, 2004).

Gullo and Giudici (2008) reported that the AAB were present in the environment and in the raw material, but they cannot grow during alcoholic fermentation because of the anaerobic conditions. When the alcoholic liquid was exposed to oxygen, the AAB started their growth on the surface. Du Toit and Pretorius (2002) reported that most AAB growth was observed between pH 5.4 - 6.3, but they also could grow at pH values lower than 4. They also reported that AAB could also be isolated at pH values of 2.0-2.3 in media containing acetate, if they were aerated. The optimal temperature for their growth was 25-30°C, but their growth was also observed between 38-40°C and weakly at temperatures as low as 10°C.
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\text{CH}_3\text{CH}_2\text{OH} + \text{O}_2 \rightarrow \text{CH}_3\text{COOH} + \text{H}_2\text{O} + 493 \text{ kJ}
\]

**Uses of vinegar in food industry**

- The use of vinegar to flavour food is centuries old. Whether naturally produced during fermentation or intentionally added, vinegar retards microbial growth and contributes sensory properties to a number of foods. It has been used as a medicine, corrosive agent, pickling agent and can be directly consumed in diluted form as a beverage. In the food industry, vinegar is used mainly as an acidulant, but it has also many other food processing applications. It is found in hundreds of different processed foods, including salad dressings, mayonnaise, mustard, ketchup, bread and bakery products, canned foods, marinades and the current falling wine consumption have favoured an increase in vinegar production (De Ory et al., 2002).

- Vinegar is the world’s oldest cooking ingredient and food preservation method. Vinegar’s use can be traced back over 10,000 years. In fact, flavoured vinegars have been manufactured and sold for almost 5,000 years. The wide variety of vinegars available today is nothing new. Until the six century BC, the Babylonians were making and selling vinegars flavoured with fruit, honey, malt, etc. to gourmets of the time. In addition, the Old Testament and Hippocrates recorded the use of vinegar for medicinal purposes (Kehrer 1921; Conner 1976).

- The use of vinegar to flavour food is centuries old. It has also been used as a medicine, a corrosive agent, and as a preservative. In the middle Ages, Alchemists poured vinegar onto lead in order to create lead acetate, called “sugar of lead”, it was added to sour cider until it became clear that ingesting the sweetened cider proved deadly. Production of vinegar was also burgeoning in Great Britain and it became so profitable that a 1673 Act of Parliament established a tax on so called vinegar-beer. In the early days of the United States, the production of cider vinegar was a cornerstone of farm and domestic economy, bringing three times the price of traditional hard cider.