12.1 Introduction

Apple vinegar or cider vinegar (CV) is made with apple juice or concentrated apple juice (CAJ) through a double fermentation: alcoholic and acetic. Cider vinegar is extensively used in several countries including Austria, the UK, the USA and Switzerland (Ebner, 1982; Lea, 1988).

The minimum legal strength for cider vinegar varies from country-to-country and in USA it is 4% acetic acid (w/v). Cider vinegar is classified into low-strength and high-strength depending on the chemical composition of the cider used. Low-strength cider vinegar refers to vinegar that is produced from cider with a solute concentration (acid % w/v plus alcohol % v/v) of less than 8-9%. High-strength cider vinegar is made from cider with more than 9% and up to 13% solute concentration.

Natural apple cider vinegar is made from fresh, crushed, organically grown apples and is allowed to mature in wooden barrels.

In the traditional method, both alcoholic and acetic fermentation of apple juice is carried out in the same barrel by naturally occurring yeasts and acetic acid bacteria (AAB). The barrel is placed in a warm, damp place and the bung-hole of the barrel is covered with a piece of cloth to keep out the dust and flies. It takes about 5-6 months to complete the whole alcoholic and acetous fermentation to form CV from apple juice. The main drawbacks of this process are that alcoholic fermentation is often incomplete and slow, and also that acetic fermentation has low yields, resulting in a CV of poor quality.

Nowadays, cider vinegar is made mainly by submerged culture, as for wine vinegar; a complete description of submerged culture is reported in Chapter 6.

Cider vinegar is popular in folk medicine and is suggested as a remedy to various diseases, from obesity and overweight to arthritis, but also for asthma, coughs, diarrhoea, colitis, eczema, hair loss, and many other conditions.

More conventional uses of cider vinegar are as a flavouring agent and as a food preservative.
12.2 Microbiology of Cider Vinegar

Different microorganisms play an important role during CV production. Like that of grapes, the microflora of apples consists of yeasts (*Kloeckera apiculata*, *Metschnikowia pulcherrima*, *Candida* spp. and *Pichia* spp.), lactic acid bacteria (*Lactobacillus*, *Pediococcus*, *Leuconostoc* and *Oenococcus* spp.), and acetic acid bacteria (*Acetobacter* and *Gluconobacter*) (Fleet, 1998). Yeasts carry out the glycolytic conversion of sugars to alcohol by the EMP pathway, which gives a practical yield of alcohol of around 50% of the sugar weight initially present. The wild yeast microflora, which are present on fruit or come from the surfaces of the processing equipment, perform the first phase of alcoholic fermentation, but *Saccharomyces cerevisiae* and *Saccharomyces uvarum* (or *Saccharomyces bayanus*) are the most important species because of their special traits of great ethanol tolerance and high fermentation rate.

Acetic acid bacteria (AAB) are responsible for the oxidation of ethanol to acetic acid. Nowadays, they are classified into the family *Acetobacteraceae* as a branch of the acidophilic bacteria in the α-subdivision of the Proteobacteria (De Ley et al., 1984; Sievers et al., 1994). Genotyping relationships among AAB, based on 16S rRNA gene sequences and ubiquinone systems, have revealed that the currently recognized AAB are classified into ten genera; more details on current classification are reported in Chapter 3. AAB species are widely distributed in nature in plant material rich in sugar, and include *Acetobacter pasteurianus*, *Acetobacter oboediens*, *Acetobacter pomorum*, *Gluconacetobacter hansenii*, *Gluconacetobacter europaeus* and *Gluconacetobacter xylinus* (Kittelmann et al., 1989; Sievers et al., 1992; Swings, 1992; Sokollek et al., 1998). In particular, *Ga. europaeus* has been described as the dominant species in industrial submerged-culture vinegar manufacturing in central Europe, whereas *Ga. xylinus* has been frequently recovered from traditional processes.

Many vinegar fermentations are carried out by mixed and wild AAB cultures, not microbiologically defined, that are generally called ‘seed vinegar’. Experimental methods for starter preparation have been described and applied to the cultivation and preservation of isolates from vinegar fermentation at laboratory scale (Sokollek and Hammes, 1997; Sokollek et al., 1998). Although species belonging to the genera *Acetobacter*, *Gluconacetobacter* and *Gluconobacter* are resistant to acetic acid concentrations, they differ in several metabolic aspects. For instance, *Acetobacter* and *Gluconacetobacter* spp. are able to carry out the overoxidation of acetic acid to CO$_2$ and water from ethanol, whereas *Gluconobacter* spp. are not able to do this, due to their non-functional α-ketoglutarate dehydrogenase and succinate dehydrogenase of the tricarboxylic acid cycle.

12.3 Cider Vinegar Production

Processing from apples to CV can be summarized in three steps: (i) raw material preparation; (ii) alcoholic fermentation of apple juice to produce cider; (iii) acetous