# ACETIC / VOLATILE ACIDITY IN WINE, CIDER, VINEGARS AND JUICES

Acidity is a characteristic determined by the total sum of acids that a sample contains. We can quantify the set of all of them in an undifferentiated way (total acidity) or in a grouped way (fixed acidity and volatile acidity). Fixed acidity corresponds to the set of low volatility organic acids such as malic, lactic, tartaric or citric acids and is inherent to the characteristics of the sample; volatile acidity corresponds to the set of short chain organic acids that can be extracted from the sample by means of a distillation process: formic acid, acetic acid, propionic acid and butyric acid. Of all of them, the acid responsible for approximately 99% of the volatile acidity corresponds to acetic acid, so that its determination is often enough to reliably determine the total volatile acidity. Furthermore, volatile acidity appears as a consequence of the metabolic transformations of the fruit and is zero or very close to it for fresh fruit.

The presence of acetic acid in a fermentation product is a consequence of the metabolism of yeasts and bacteria, both in their anaerobic (fermentation) and aerobic (glycolysis) metabolism. These bacteria transform ethanol into acetaldehyde first and then, if there is sufficient oxygen (as occurs in partially filled tanks), into acetic and ethyl acetate by esterification with ethanol. The problem appears when, due to a growing population (especially Gluconobacter, Acetobacter and Bretanomyces bacteria, but also *Saccaromyces mycoderma* and cerevisiae), acetic levels increase to a level where its characteristic odor begins to be perceived clearly (from 0.8 g / L) and modifies the aromatic characteristics ('chopped' flavor, ethyl acetate, acetoin). The formation of a whitish superficial veil is a clear sign of the presence of these organisms. In the case of juices, the presence of acetic acid is indicative of a fermentation (deterioration) process started and therefore a good indicator of its quality. This phenomenon can occur immediately in juices, which is avoided by cold treatment of the juice and subsequent pasteurization.

Initially *G. oxydans*, the species with the highest presence in fruits including grapes, starts the acetification process by consuming the available sugars and converting them into ethanol (this genus of bacteria lacks enzymes of the Krebs cycle, so they cannot completely oxidize the sugar up to CO2), but it inhibits its growth at moderate concentrations of alcohol, which is why it tends to be replaced by *A. aceti* once fermentation has started. *A. aceti* is especially dominant in botiritis infected berries and is capable of surviving in wine, even under anaerobic conditions, and multiplying rapidly in the presence of minimal amounts of oxygen, which makes it necessary to establish additional protection mechanisms (sulphites, low temperature).

Acetic values above 1.2-1.5 g / L are no longer legal for wine (the specific limit depends on local legislation), with the exception of botirised wines, where it can reach up to 2.1 g / L. In cider, the legal limit is 2.2 g / L. The acetification process can be carried out intentionally in the production of vinegars by raising the acetic concentration to beyond 50 g / L (5%).

The determination of the acetic content is a process control requirement in the production of wine, cider, beer, or vinegar (and by extension, of any product in which a natural fermentation takes place

as part of its production process), and industrial juices, must or any other food in which fermentation should be avoided. The traditional procedure (included in many of the official standards) consists of a distillation or steam stripping of all the volatile components that are subsequently titrated with sodium hydroxide with phenolphthalein as an indicator of change (official AOAC method). These volatile components, in addition to acetic, formic, butyric and propionic, include free and total sulfites in the sample (which also contribute acidity), therefore, in the same procedure they are evaluated immediately afterwards. The main drawbacks of this method are the subjectivity associated with accurately determining the turning points (solvable through the use of potentiometers), the errors inherent in a manual method in the preparation and handling of the reagents and, very especially, the time required to carry out the distillation process itself (from 10 to 15 minutes, depending on the sample volume), which makes it especially tedious in case of having to process a significant number of samples, increasing the risk of unintentional errors.

Since a few years ago, the OIV has included the enzymatic method, it has been included among the official methods for the determination of acetic acid (OIV Resolution 621-2019). Enzymatic methods are based on the ability of enzymes to act specifically on a substrate, in this case acetic acid, and can be performed directly on the sample with little or no manipulation. The proposed method is based on the transformation of acetic acid to acetylphosphate by consuming ATP by means of acetate kinase; the ADP formed is regenerated to ATP by quantitatively transforming phosphoenolpyruvate to pyruvate by pyruvate kinase and, then, into lactate by lactate dehydrogenase with consumption of NADH, so that the reaction can be quantified by the spectrophotometric measurement of the disappearance of NADH.

The main advantage of this method is that it does not require any type of sample manipulation, it is highly specific for acetic acid, fast (results in five minutes) and can be automated, with minimal reagent consumption. By means of the enzymatic method, it is possible to determine acetic acid with a high sensitivity (from 0.03 g / L) and up to values of approximately 1.2-1.4 g / L of acetic acid, sufficient for the vast majority of samples, both juice (need for sensitivity) and wine (intermediate values); in the case of vinegars, a predilution of the sample is required (around 1/50) until it reaches values suitable for the measurement range.

Sinatech offers a range of highly reliable and precise enzymatic reagents for the specific and precise determination of sugars and acids in fruit juices and derivatives accepted among official methods of analysis. The Dionysos system is an optimal tool for the control of the production process, capable of guaranteeing the quality and food safety requirements demanded by the existing regulations.