Chapter 12. Post-fermentation treatments of vinegars

(An overview of existing technologies/methods for vinegar decolourisation, clarification and pasteurisation systems, and current trends with results and potential for industrial application)

Note: *if these topics are away from your field of expertise, you may alternatively consider contributing a chapter* ***only on industrial vinegar decolourization & clarification***.

12.1 Introduction.

The vinegar produced by any fermentation process described in the previous chapters may present cloudiness since it contains acetic bacteria and suspended matter coming from the original raw material, such as unstable phenolic compounds, pectins and traces of proteins that can form mists or deposits. Thus, vinegars usually need to be clarified and/or stabilized, and the traditional method consists of adding an adjuvant, such as bentonite, to facilitate the process and remove the unstable compounds from the product. Vinegars made from surface methods usually have few bacteria in suspension, while vinegars obtained by the submerged method have large amounts of bacteria. The quality of the vinegar may also be affected by chemical phenomena, as well as by the actions of microorganisms, yeasts and vinegar eels. Therefore, before consumption, it is advisable to treat the vinegar to achieve the best possible color, cleanliness and presentation conditions before it reaches the consumer.

12.2 Alterations of vinegar.

Once vinegar is elaborated, it can present chemical defects or biological diseases. Among chemical defects, the so-called iron or blue casse, white or persistent casse and darkening or brown casse should be highlighted. Furthermore, microbiological diseases (such as the mouldiness, weakening and the presence of mucilage) must be distinguished by those produced by other organisms such as eels, flies and mites. Next, the vinegar alterations are described in more detail.

The iron or blue *casse* is produced by an excessive presence of iron, which reacts with the tannins forming a precipitate. The presence of excessive iron can also affect the taste of vinegar. White or powdered *casse* occurs when phosphate contents exceed tannins' ones; then, iron reacts with phosphate forming white precipitates. The darkening or brown *casse* is characterized by a brown color caused by vinegar in contact with air or insufficiently sulphited (Casale et al, 2006). The enzymes responsible of brown *casse* are the so-called "enoxidases" or "oxidases" (polyphenol oxidases, PPOs), which oxidize the polyphenols to quinones with the presence of oxygen and, subsequently, the flavonols are responsible of turbidity and browning (Waterhouse and Nikolantonaki, 2015).

Regarding diseases of biological origin due to microorganisms, the so-called weakening should be highlighted. Weakening is due to a phenomenon of over-oxidation (acetic acid step to carbon dioxide and water) caused by the same acetic bacteria and / or other bacteria such as *Acetobacter xylinus* (LeFevre 1924, Mas et al 2014). Weakened or reduced vinegar can be also produced due to the action of molds and mucilages. In addition, the preservative action will decrease while losing acetic acid, obtaining a less stable product. Furthermore, the molds occur on the surface, spreading later to the whole of vinegar. Deuteromycetes, pelleting yeasts and lactic acid bacteria destroy acetic acid when it is diluted by oxidation. The destruction of acetic acid is the consequence of a low alcohol content or an excess of ventilation. Other alterations are caused by microorganisms such as *Lactobacillus* which transforms malic acid into lactic acid and CO_2, reducing vinegar acidity (Bartowsky, 2009). Fortunately, they are almost never pathogenic. Butyric bacteria (genus *Clostridium*, sporogenous and aerobic) transform the soluble carbohydrates into acetic acid, butyric acid, CO_2 and H_2 (Murali et al. 2017).

Regarding other organisms that can cause alterations in vinegar, vinegar eels (*Anguillula acetiglutinis*) should be highlighted. Vinegar eels are ubiquitous nematodes, inhibiting damaged fruit such as grapes and apples, and consequently they can often be seen swimming on the surface of vinegars. Not much is known about the role and the effect of vinegar eels on vinegar production and not many studies have been carried out to clarify this issue (Rainieri and Zambonelli, 2009). These small worms (*Anguillula* aceti) are viviparous and harmless to man, but that causes turbidity (they are perfectly visible with backlight) and alterations in taste and smell of vinegar. They usually appear in vinegars produced by surface acetification methods (Orleans and Shützenbach), near mothers. Vinegar eels can even destroy the film when they become large enough and, consequently, causing it to sink and interfering with the acetification process. Even though, their putrid decomposition when die give bad smells to the vinegar, making it not suitable for consumption. On the other hand, vinegar flies (*Droftophila* spp.) breed in the juices of decaying fruits and also around the openings of vinegar containers or wherever they find vinegar exposed to the air. These are known as fruit or vinegar flies. In the case of high population, the larvae of these flies can enter in the reactors and destroy the mother of the vinegar. They may also be responsible for the introduction of *Bacterium xylinum*, an undesirable acetic bacterium. Finally, in vinegar factories that do not maintain proper cleaning and disinfection**,** mites can appear which are small and prolific. Mites breed in the woods of the acetifiers, looking for a temperate and humid environment, and can be readily destroyed by the use of hot water or steam. (LeFevre 1924)

12.3 Vinegar treatments.

Firstly, it is convenient to differentiate fining from clarification. Clarification can be defined as the treatment used to diminish the turbidity of a product removing the solid particles from the liquid, while fining is associated with the treatment used to maintain the vinegar stable along time from a colloidal point of view. Nevertheless, in Europe the treatments applied to the vinegar during its elaboration can be regulated. For example, Spanish legislation allows all practices as far as the post-fermentative treatments indicated in the article 4 of the Real Decreto 661/2012.

12.3.1 Storage and maturation.

Maturation of vinegar is required for the development of a pleasant aroma to achieve a high quality product. Traditionally, the rough stock vinegar (that is, the resulting liquid after acetification) could be stored for up to 1–2 years in wooden barrels, whereas today vinegar is stored, at the most, for 1–2 months in barrels or in stainless steel tanks before bottling (Lea, 1989, Heikefelt, 2011). At 1-2 months of maturation, vinegar has high level of acetic acid and low level of alcohols (which produces esters). The concentration of these compounds has a significant impact on the unique flavor and bouquet for the case of malt vinegars. The pH of the vinegars has by this time dropped below 3 and various polyphenols, etc. will slowly drop out of solution, giving the final vinegar a much more stable character (Grierson, 2009). In industry, this diminution of maturation time of vinegar is due to higher production costs and the expense of storage. In some plants, producers may undergo rough filtrations at first stages. In the productions of cider vinegar, many changes occur during storage. Its harsh flavor changes to more pleasant aroma and bouquet, probably due to the oxidation of vinegar caused by air entering though he pores of the wood. Acetic acid may also react with residual alcohol to form ethyl acetate, which has a fruity flavor, and the color of cider vinegar varies during aging (Joshi and Sharma, 2009).

For quality products, the aging of vinegar is usually performed in wooden containers. The production of traditional vinegars such as vinegars in Jerez or Modena, aging procedures are well established and regulated. However, in recent years there have been some studies to accelerate the maturation and aging of vinegars. Wang et al 2017 applied ultrasounds to accelerate Zhenjiang vinegar maturation. In their study, the ultrasonic treatment (optimal conditions) was determined to be equivalent to 2–3 years of natural aged Zhenjiang vinegar. This study has showed that ultrasound is promising not only in shortening the aging time and lowering costs for the vinegar-making industry, but also in producing fine vinegar.

12.3.2 Vinegar clarification.

One of the most important treatments in the elaboration of vinegars is the clarification to improve the appearance and stability of the product. Turbidity is due to larger particles as plant debris, yeast and bacterial cells, and smaller material as carbohydrates, polyphenols and proteins (García-García et al, 2009, Heikefelt, 2011). The clarification can be accomplished spontaneously, called spontaneous clarification or self-clarification. This methodology consists in allowing the vinegar to rest, in order to precipitate the suspended particles in the bottom of the tank by gravity. For an optimal performance, it is convenient to avoid cloudiness. The process is slow and needs recipients with large capacity to be carried out. In addition, self-clarification by sedimentations depends upon acidity and maturation time (Ormaechea-Landa, 1991). Cider vinegar with low acidity normally does not clear even after long storage, whereas high-strength vinegars usually do so within a couple of months (Joshi and Sharma, 2009). The risk of uncontrolled self-clarification is that vinegar may integrate unpleasant odors and tastes. Sometimes it is convenient to help this clarification by sedimentation using adjuvants that favor and accelerate the clarification.

Another way to clarify the vinegar is mechanically by using the centrifugation technique, which consists of accelerating the deposition of particles through the effect of centrifugal force. This can be done by different equipment: hydrocyclones, centrifugal decanters or clarifiers (plate separators).

Hydrocyclones are based on the principle of free fall, according to which the liquid flows freely following a spiral along the trunk-conical body. The solid particles are separated from the liquid and adhere to the wall. They then fall by gravity and are collected in a thick chamber, while the clarified liquid rises to the top.

Centrifugal decanters are useful equipment to recover liquids and separate solids, being effective to separate coarse particles, representing a handicap to obtain a fully clarified product, however they can be useful as pre-filtration treatments, if this is the limiting stage.

Vertical centrifugal clarifiers also called plate centrifuges are the most suitable equipment to obtain a good clarification of the vinegar, since they allow a rapid elimination of suspended solids. The product losses are low as well as the generation of waste, allowing to regulate the turbidity of the vinegar with the speed of rotation. The process can be carried out continuously and automated. It has the advantage of not needing adjuvants and can act quickly on the vinegar to be clarified. It is important to note that vertical centrifugal clarifiers are very versatile since they can be used before the acetic fermentation in the prior clarification of the wine if necessary, as well as in the treatment of waste, either to concentrate or recover vinegars from low deposits. This equipment is compact, occupying little space in the vinegar factory, as well as a relatively low operating cost. However, it has a high investment cost. Ultra-high-speed centrifuges are available with which to remove any particulate matter and produce bright, straw colored liquid, which is fairly stable (Grierson, 2009).

Finally the vinegar clarification can be done by filtration. Plate and frame filtration is an alternative method of clarifying the rough vinegar. This process consists of filter mixing filter powder, usually diatomaceous earth, with the vinegar, which is then pumped through the filter, with the powder building up a fine filter bed on the plates and with the frames filling with powder, giving a depth filter bed to enhance the clarity of the vinegar. Membrane filters may also be employed to produce the required clarity (Grierson, 2009).

The use of membrane technology in tangential flow filtrations improve the vinegar productions process, reduce the environmental impact and makes cold sterilization possible, thus reducing the vinegar treatment to a minimum, preserving the vinegar's own qualities and minimizing production cost (López, 2012). Nevertheless very few studies have been made of the application of membrane technology to vinegar. The application of MF of vinegar has been reported by main membrane manufacturers. In industrial production, the filter modules used in cross-flow MF are hollow-fiber membranes or spiral-wound membranes manufactured with organic materials, such as polysulphone or regenerated cellulose, and ceramic tubular membranes manufactured with materials, such as zirconia or alumina. The pore sizes that are most commonly used are of 0.2, 0.45 and 0.65 μm, but the current trend is to use 0.2 μm. Nevertheless, ultrafiltration (with a typical cut-off point of 50,000 molecular weight) were used to replace normal filtration and sterilization procedures in cider vinegars. To minimize the risk of bacterial contamination, the vinegar is filtered immediately before bottling. Ultrafiltration does not prevent the formation of non-microbiological post-bottling haze, since haze precursor molecules (procyanidins) have molecular weights ranging from 500 to 2500 and can, therefore, easily pass through even the smallest of ultrafiltration membranes (Joshi and Sharma, 2009).

López *et al* . (2005) made a study of the clarification of vinegars (white, rosé and red) by cross-flow MF on an industrial scale, and the main conclusions were as follows.

- The turbidity for the three kinds of vinegars was reduced considerably; for filtered vinegars, it was less than 0.5 NTU.
- The reduction of total solids in suspension was complete.
- MF could be used to simultaneously clarify and cold sterilize the vinegar.
- The effect of MF on color and polyphenol content was acceptable. The reduction in color, expressed as a decrease in modified color intensity, was practically negligible for white vinegars, 11% for rosé vinegar and 37% for red vinegar.
- The reduction in polyphenol content was less than 15% in all vinegars. The polyphenolic profile of the filtered vinegars is quite similar to that of the initial vinegar.

12.3.3 Vinegar fining.
The fining is an alternative or complement to the clarification, that is used both in small and larger scale. This treatment improves the clarity even more, and decreases the risk of developing turbidity during storage (Lea, 1989, Heikefelt, 2011).

Chemical-physical stabilization is based on the use of clarifying additives, which usually require a subsequent treatment by filtration (Joshi and Sharma, 2009). In this treatment, the first stage consists in the dispersion of the clarifier, followed by coagulation, which causes an increase in turbidity, and finally flocculation, which consists of an increase in the size of the particles that facilitates sedimentation at the bottom of the tank. The temperature of the treatment must be low, since it favors the coagulation and the flocculation of the particles. For the realization of this treatment the tanks have to be suitable for the operation, in particular with smooth walls.

The clarifiers have to be inert from an organoleptic point of view, since they do not have to transmit strange smells and flavors to the vinegar. Another important feature is that the flocs formed are of high density. Another aspect to consider is the acidity of the vinegar, since it affects the clarification. As a guide, in vinegars with a pH of 4, around 1 hour is necessary to clarification, while for pH of 3 about 12 hours are necessary, and at a pH of 2 the clarification does not run correctly. However, each clarifier has its optimal pH range. For a pH of 4 gelatin works well; however for pH of 3, clarifiers such as albumin, casein and potassium caseinate are more suitable.

Among the clarifiers, two types can be distinguished: organic and inorganic. The organics are usually effective but can modify vinegar composition, so it is advisable to monitor the dosage (over treatment). These include traditional clarifiers such as gelatin, albumin, casein, potassium caseinate, complex clarifiers, antioxidant clarifiers (which have a double, clarifying and stabilizing effect), enzymatic clarifiers, tannins, PVPP (Guzmán-Chozas, 1998). Joshi and Sharma (2009) proposed for cider vinegar a fining procedure such as the addition of gelatin, bentonite and/or liquid silicon dioxide, following the two main protocols described below: (i) 260 g gelatin and 400 g bentonite are added to each 1000 L of cider vinegar, and the suspension is stirred and left to settle for at least 1 week before racking, or (ii) liquid silicon dioxide (5 L at 30 % solution for 5000 L of cider vinegar) followed by gelatin (1 kg per 5000 L) are added to cider vinegar and left to settle. A final filtration could be necessary to completely remove suspended materials and bacterial cells.

The inorganic clarifiers base their effect on the formation of a gel that traps the particles in suspension that favors their decantation. They do not usually modify vinegar composition. Bentonite and silica gel are the most notable inorganic clarifiers, which are often used together for greater efficacy of the treatment. Normally the choice and dosage of clarifiers should be determined in laboratory tests (Ormaechea-Landa, 1991).

12.3.4 Microbiological stabilization

Vinegar is a product that normally does not need to indicate legally the expiration date due to its characteristics. However, it can be associated with a shelf life and restricted stability, even if stored cold. In addition, an unprocessed product may contain potentially dangerous microorganisms that may be present without damaging it. Various methods can be used to eliminate unwanted microorganisms, including pasteurization, sterile filtration and different additives (Heikefelt, 2011).

Pasteurization is an operation that aims to destroy bacteria and inactivate enzymes that may cause later alterations in vinegar. This treatment can also be used to kill vinegar eels, followed by filtration to eliminate them. The treatment has to avoid modifying the organoleptic characteristics of the treated vinegar, however heat might decrease the organoleptic quality by affecting its color and flavor (Choi and Nielsen, 2005). Within the pasteurization, we can distinguish different steps, heating the vinegar at temperatures that oscillates between 50-85ºC and the treatment time varies according to the selected temperature (Ormaechea-Landa, 1991). The standard procedure consists on heating the vinegar to temperatures between 65-70ºC and successively bottling, sealing and a slow cooling. Alternatively, vinegar can be bottled and sealed first, and heated to 65-70ºC consequently. Furthermore, sterilize vinegars produced in submerged cultures with high bacterial concentrations may require pasteurization temperatures of 77-80ºC. (Dinsmoor Webb, 2007).

Joshi and Sharma, 2009 describe a particular chemical pasteurization, called the 'silver process', which is based on flowing cider vinegar through silver-bearing sand, to reach a silver ion concentration (about 2 ppm) sufficient to sterilize it.

Membrane technologies, such as the cold sterilization method, have also been applied to food products. Cross-flow microfiltration is a useful technique because clarification and sterilization can be carried out simultaneously (Lopez, 2012). Cold sterile filtration through a membrane with pore size less than 0.2 µm, is an alternative to pasteurization. This method can only be applied to clear products; otherwise the fine membranes that are used tend to clog (Heikefelt, 2011). Membrane application in the vinegar industry makes it possible to avoid a pasteurization stage, which is still useful in this production area (Ormaechea-Landa, 1991).

Sulphiting is an effective method for inactivation of microorganisms, and this chemical stabilization is usually carried out by adding SO_2 up to a maximum permitted dose of 170 mg/L (Regulation (EC) No 1333/2008). Usually it is introduced in the form of gas (E220) or as potassium metabisulfite (E224), its efficiency is reduced to half and it is difficult to dissolve it cold as potassium metabisulfite. It is a self-limiting additive in its use (above a certain dose alters the taste characteristics of the product) (Ormaechea-Landa, 1991). It is especially effective in an acid medium, inhibiting bacteria and molds, and to a lesser degree, yeasts (bactericidal action up to values of 5-10 mg/L).

However, in some Denomination of Origin other antioxidant additives such as ascorbic acid (E-300), which prevents oxidative browning, potassium sorbate (E-202), which is applied as an inhibitor of secondary fermentation in the wine industry, allowing to reduce the dose of SO_2. Ascorbic acid is an antioxidant which is less effective than SO_2 unless used in very high amounts (>250 ppm). However, the breakdown products of ascorbic acid (dehydroascorbic acid and diketogulonic acid) are carbonyls which have been found to be potent pro-oxidants, encouraging browning reactions and haze formation unless excess ascorbic acid is present. The addition of small amounts of ascorbic acid (<100 ppm) to cider vinegar are probably worse than useless (Joshi and Sharma, 2009). Sorbates are low in toxicity, among the lowest among all preservatives, which is why their use is authorized all over the world. Sorbic acid (E-200), is an unsaturated fatty acid, naturally present in some vegetables, but manufactured for use as a food additive by chemical synthesis. It is used in the preservation of acidic foods and beverages, since its action at neutral pH is almost nil. They have the technological advantages of being active in low acid media and of practically lack flavor. Their main drawback is that they are comparatively expensive and that they are partly lost when the product is boiled. They are especially effective against molds and yeasts, and less against bacteria. It is commonly used in oenology associated with sulfur dioxide or another antiseptic. Currently it is not authorized in vinegars, but in condiments.

12.3.5 Other treatments

Vinegar coloring. The color of vinegar normally comes from the natural color of the raw material. The caramel (E-150) is the only coloring authorized, and in general is used to give a balsamic aspect to the final product. The authorized dose is usually *quantum satis*. Malt vinegar is a pale straw-colored liquid, with a strong acetous flavor, whereas dark malt vinegar has an ark brown color. The dark color is derived from the addition of barley extract or caramel to the malt vinegar (Grierson, 2009). Balsamic vinegar of Modena is a flavored wine vinegar obtained by blending cooked must and wine vinegar and, in some cases, by adding a small amount of caramel (Giudici et al, 2009).

Vinegar discoloration. It is common practice in the vinegar industry to decolor a fraction of vinegar and blend it with colored vinegar to obtain a standard final product of the same characteristics and quality. The total or partial discoloration of the coloring matter of the vinegar is usually carried out using powdered oenological activated carbon (vegetable origin), which is added to the vinegar, allowed to settle and then filtered. This type of treatment usually lasts 48-72 hours, since it takes a long time for the sedimentation of the carbon particles. Normally, the consumption of activated carbon is high, and the decoloring process can uses doses of 10–20 g/L of activated carbon, which increases production costs, vinegar losses and an important generation of residues (Achaerandio et al 2002 a,b, López et al. 2003).

Other alternatives to the discoloration of vinegar by using powdered activated carbon is the use of activated carbons in pellets, which can be packed in columns, allowing the process to be carried out continuously, minimizing product losses and generation of waste (Achaerandio et al 2002a, López et al.2003).

In cider vinegars, the color is modified during the ageing step. Specifically, the color, which it is intensified by polyphenol oxidase activity (a constitutive enzyme of apples) during milling and pressing processes, fades during the fermentation and maturation phases, probably due to the polymeration of procyanidins and other polyphenols. It is a general practice to use polyvinylpolypyrrolidone (PVPP) and carbon to reduce the color by removing oxidized and polymerized procyanidins (Joshi and Sharma, 2009).

Another alternative is the use of exchange resins (not currently authorized) (Achaerandio et al 2003, 2007), or using tangential nanofiltration (NF). In vinegar decolorization, NF can be an alternative technique of processing rather than the traditional system using activated carbon. Güell and López (2001) decolored vinegar with a small pilot-plant that operates with a titanium dioxide membrane fused on a porous ceramic tube with a nominal molecular cut-off of 1 kDa. The decolorization efficiency was 88.2% and 35.5% for the red and white vinegars, respectively. It has also been observed that the membrane treatment affects the fixed acidity and the dry matter content of the product, but leaves the other parameters almost unaffected. This study suggests that NF can be used successfully to reduce color in vinegar.

Vinegar deodorization. Deodoration is the total or partial elimination of substances that give an unpleasant aroma to vinegar using deodorant charcoal. The treatment procedure is similar to that used for the decolorization of vinegars by activated carbon powder.

Citric acid (E-330) forms a stable complex with metals, such as iron and copper, which catalyze the oxidative polymerization of polyphenols and, it acts preserving the color, the aroma and the content of the vinegar vitamins (Joshi and Sharma, 2009).

Pectin and Arabic gum can be added to stabilize cider vinegar against haze formation (Joshi and Sharma, 2009).

Potassium ferrocyanide is used as a fining agent (blue fining) to remove iron from vinegars formation (Joshi and Sharma, 2009).

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