

Vinegar

Raquel M. Callejón*, Rocío Ríos-Reina, M. Lourdes Morales, Ana M. Troncoso
Departamento de Nutrición y Bromatología, Toxicología y Medicina Legal
Universidad de Sevilla, Spain.

**E-mail corresponding author: rcallejon@us.es*

Freddy Thomas*
Eurofins Analytics France, Nantes, France
**E-mail corresponding author: FreddyThomas@eurofins.com*

Federica Camin*
Unità Tracciabilità -Dipartimento Qualità Alimentare e Nutrizione
Fondazione Edmund Mach, Italy
**E-mail corresponding author: federica.camin@fmach.it*

General overview of the product

Vinegar is one of the oldest fermented products in the world and its production dates back to around 2000 BC. Its acidic character (until the description of sulfuric acid, it was the strongest known acid) facilitated its use as a preservative due to its antimicrobial activity. Nowadays it is extensively used as a preservative, flavouring agent, and in some countries even as a healthy drink. Although, vinegar is mostly consumed in the food and beverage industry, it also finds applications in the healthcare and cleaning industry. The global vinegar market has reached values worth around USD 1.26 billion in 2017 growing at a rate of 2.1 % during 2010-2017 [1] and is further expected to reach a value of around USD 1.50 Billion by 2022.

As for fermented foods and beverages in general, the consumption of vinegar is a cultural trait. In Mediterranean countries, most vinegar is used directly or added to salads or to raw or cooked vegetables; thus, the appreciation of the organoleptic characteristics is straightforward. Therefore, “quality” vinegars are closely associated with these patterns of consumption. In contrast, in other countries, most vinegar is used for pickling or as part of sauces, and the impact of the organoleptic qualities, although possibly relevant for the final product, is less evident [2].

Types and major regions segment the global vinegar market. Different types of vinegar available are mostly balsamic vinegar, wine vinegar, cider vinegar, malt vinegar and rice vinegar. Geographically, Europe represents the biggest market for vinegar (more than half of the total global market share) followed by North America and the Asia Pacific region. In 2016, balsamic vinegar exhibited a clear dominance with the majority of market share. The use of vinegar is increasing in different cuisines, which results in increasing demand. Growing populations, rising disposable incomes, increasing health consciousness among consumers and the food and beverage

industry are the main driving factors of the vinegar market. It is expected that the global vinegar market will witness growth both in terms of revenue and volume during the following years. Growth will come from changing consumer lifestyles and preferences. The interest in cooking gourmet and ethnic foods have increased among many consumers, thus prompting the sales of various dressings, most of which use vinegar as one of the key ingredients.

Some premium vinegars are being commercialised worldwide. A typical example of this trend is the increased consumption and trade of Balsamic Vinegar of Modena (Aceto Balsamico di Modena). In fact, Italy is the country that exports the most vinegar, providing twice the quantities of the other main exporters, Germany, Spain and France. Moreover, in terms of revenues, Italian vinegars are exported at much higher values than Spanish or German vinegars. The situation in Germany is different, considering that most German vinegar is sold for the pickling or sauce industry, whereas Spanish exports include also some premium vinegars such as Sherry vinegars (Vinagre de Jerez).

Sherry vinegars that are included in the European Union's Protected Designation of Origin (PDO) framework derive from Sherry wines and are necessarily aged in wood barrels for at least six months. This aging can be performed by a dynamic system (i.e., passage through different barrels containing vinegar from different vintages or different ages) or a static system. A more complex example is Aceto Balsamico, which is either Aceto Balsamico Tradizionale (ABT), regulated by two different PDO labels (ABT di Modena or ABT di Reggio Emilia), and Aceto Balsamico di Modena, which has a Protected Geographical Indication (PGI) status. The production of ABT is a long process that starts with the cooking of the grape must, which increases the sugar concentration to 400-500 g/L. Next, a partial alcoholic fermentation, which is initiated by osmophilic yeasts, produces a "sweet wine" of approximately 7 % (v/v) ethanol concentration and over 200 g/L of residual sugars. Then, some mother of vinegar is added to this "sweet wine," and it is left to be acetified. Once is acetified, the vinegar is placed in a "bateria" formed by five to seven barrels of different woods (oak, mulberry, chestnut, cherry, juniper, ash and acacia) and decreasing volumes (from 60 to 15 L), which are filled up to 2/3 of their total volume. This "bateria" is kept for at least 12 years with a yearly refilling from the previous barrel in a dynamic aging process. During this aging process, two phenomena occur: the transfer of components from the wood to the ABT and, more importantly, the concentration of the product and the integration of its components. The final product can have up to 500 g of sugar per kg of product, 7 % acetic acid (v/v) and 20 g of gluconic acid per kg. The oxidation of glucose by acetic acid bacteria yields gluconic acid. The result is a dark, concentrated and thick product sold in 100 mL bottles and with a market value that can easily reach 100 euros [3,4]. In contrast, Aceto Balsamico di Modena (ABM) is a PGI (Protected Geographical Indication) salad dressing ingredient now renowned throughout the world, obtained from cooked and/or concentrated grape must (at least 20 % of the volume), with the addition of at least 10 % of wine vinegar and a maximum 2 % of caramel for colour stability that is aged at least two months, not necessarily in barrels [5]. The geographical origin of ABM ingredients is not specified. However, some of these ABM can be aged for more than three years and are labelled "Invecchiato" (Aged). Overall, ABM is a cheaper version of ABT that has been popularized all over the world.

Some Asian vinegars, such as black vinegars from China or "kurosu" from Japan, are produced from rice and other cereals (including sorghum, wheat, and others) with a very important aging process in which concentration and thickening occur in a similar manner to ABT.

1. Product Identity

1.1. Definition of the product and manufacturing process

In general, food regulations consider vinegar the result of a double fermentation (alcoholic and acetous or acetification) of any sugar substrate, usually agricultural raw materials of plant origin with the exception of those produced from whey or honey.

In the European Union, the established limits for acidity and residual ethanol content are strictly set. Thus, the acidity of wine vinegar (acetification obtained exclusively from wine) must be at least 6 % (w/v), and the maximum residual ethanol allowed is 1.5 % (v/v) [4]. However, the variety of raw materials used in the production of vinegar is important, ranging from by-products and agricultural surpluses to high-quality substrates for the most unique and prized vinegars, such as Sherry vinegar (Spain) and Aceto Balsamico Tradizionale (Italy). There are up to ten types of vinegars, which include wine, fruit, cider, alcoholic, cereal, malt, malt distillate, honey and whey vinegars. Undoubtedly, wine vinegar is the most common type in Mediterranean countries, although the latest gastronomic trends have led to a considerable expansion of the varieties available in recent years. However, worldwide most of the vinegar produced is “white” vinegar, that is, vinegar produced directly from diluted alcohol [3]. In Asia, rice vinegar is the most common type, although other types are also found, many of them following very traditional systems of production.

Table 1: Different vinegars of the world are classified according to substrate, name and region/country of production and distribution [6]

Substrate (Raw material)	Name	Region/Country (Production & distribution)
Grape	Wine vinegar	Global
	Balsamic vinegar	Global
	Red vinegar	Global
	White vinegar	Global
	Distilled white vinegar	Global
	Sherry vinegar	Global
	Traditional Balsamic vinegar	Global
Apple	Cider vinegar	US, Canada
Different fruits (mango, kaki, berries)	Fruit vinegar	East and Southeast Asia
Date	Date vinegar	Middle East
Coconut	Coconut vinegar	Tropical Africa
Rice	Rice vinegar	China, Japan, Korea
	Kurosu	China, Japan, Korea
Malt	Malt vinegar	USA, Northern Europe
	Distilled malt vinegar	USA, Northern Europe
Whey (dairy by-products)	Whey vinegar	Europe
Honey	Honey vinegar	Global

Therefore, vinegars can be classified by their substrates of origin or by their systems of production. It is necessary to differentiate between those products derived from the double fermentation of a single fruit (most often grapes or apples) and those that are “flavored” vinegars, that is, vinegars of various origin with added concentrates of different fruits, flowers, or spices. Although these “flavoured” vinegars are not considered vinegars in some countries, lax regulations in other countries allow these products or condiments to be sold as “vinegars”.

The first fermentation is an alcoholic fermentation and transforms sugars or processed starches into ethanol. This process is performed by yeast, mostly from the species *Saccharomyces cerevisiae*, although some other species can also perform the alcoholic fermentation, partially or totally. The final result is considered the substrate of the second transformation, to convert ethanol to acetic acid. Although this second process is often referred as “acetic” or “oxidative” fermentation, it is not biochemically a fermentation but an oxidation. The proper term is thus “acetification” and involves a two-step oxidation, from ethanol into acetaldehyde and further from acetaldehyde into acetic acid, whereby the end of this process requires an electron acceptor, with molecular oxygen being the most common [2]. The microorganisms responsible for this process are acetic acid bacteria. These bacteria are found on substrates containing sugars and/or alcohol, such as fruit juice, wine, cider and beer. On these substrates, the sugars and alcohols are incompletely oxidized, leading to the accumulation of organic acids, such as the production of acetic acid from ethanol. Although more than 60 species have been described as acetic acid bacteria, only a limited number of them are involved in the production of vinegar. The species most commonly found in the production of vinegar are *Acetobacter pasteurianus*, *Komagataei bacterioplanus* and *Komagataei bacterxylinus*. Several attempts have been done to have single, well-defined species of acetic acid bacteria for the production of vinegar, although it has been concluded that a mixture of at least two species (one of them as “starter” and the other as “finisher”, with different acetic acid sensitivities) is the most appropriate to be used as inoculum for the production of vinegar, especially those above 5 % (w/v) acetic acid [7,8].

Vinegars can also be differentiated by their production systems. In traditional vinegars, the transformation of ethanol into acetic acid is performed by a static culture of acetic acid bacteria at the interface between the liquid and air. Barrels are filled to 2/3 of their capacity, as to leave an air chamber, which is kept in contact with the outside air using one opening or various types of openings. This production system, which is considered to be the traditional method, is called “surface culture”. A more standardized version of this method, the “Orleans method,” includes side holes for air circulation and adds a funnel with an extension to the base of the barrel to allow wine to be added at the bottom of the barrel, preventing the alteration of the “mother of vinegar”. This mother of vinegar is the biofilm formed by the transforming microorganisms, i.e. the acetic acid bacteria, which develops on the surface due to the need for oxygen. The vinegars produced by this traditional system are generally considered of high quality because of their organoleptic complexity, which is mainly due to the metabolism of the acetic acid bacteria and the overlapping vinegar production with aging. However, this process is very slow, and the production of vinegar can take from months to years.

To reduce the acetification time, other methods, such as the Schutzenbach systems with submerged cultures, have been developed. Bacteria are immobilized on wood chips or charcoal, forming a solid bed on which the vinegar spreads. After passing through the bed of chips, the vinegar is collected in a container at the bottom and pumped back to the same fixed bed. The acidity successively increases, and it is possible to obtain vinegar of reasonable quality within a week.

Submerged culture systems provide a much faster alternative. These systems rely on suitable turbines to generate a flow of air bubbles into the wine or alcoholic solution. The oxidative process occurs in the air-liquid interfaces of the air bubbles. Improvements to this process generally involve engineering (improving the maintenance and persistence of the bubbles in the liquid, the uniformity of the bubble size, the recovery of lost aromas, etc.). Vinegar is then produced at a significantly lower cost, the bacteria act as bioreactors for the transformation of alcohol into acetic acid, the airflow contributes to a considerable loss of the volatile compounds present in the original alcoholic solution, resulting in a less complex product from a sensory point of view. Although early containers for submerged culture processing were made of wood, the usual containers are stainless steel, which is more hygienic and resistant to acidic conditions. The limitations can be compensated by subsequent aging in barrels or by submerging wood fragments or wood chips, which may contribute to the recovery of some of the missing organoleptic character. Despite the loss in product quality, this methodology has two important advantages: speed (the vinegar can be produced in cycles of 24 hours, or even shorter) and acidity (the product can reach concentrations of acetic acid of up to 23-25 %, compared to the 6-13 % achieved with other systems). Higher acidity helps to reduce transportation costs by reducing water transport.

An important aspect that contributes to the organoleptic quality of vinegars is aging, which enables the integration of the different compounds in vinegars. The increase in organoleptic quality during aging is remarkable; in addition to interactions with the wood, a series of chemical reactions, evaporation, the production of esters, reactions between acids and residual alcohols, and other processes result in better integration of aromas and metabolites and a reduction in the pungency of acetic acid.

1.2. Current standards of identity or related legislation

Vinegar is regulated by different standards, and even the legal definition itself varies from country to country [3]. The regional European Codex standard for vinegar dates back to 1987 [9], and it states that vinegar is as any liquid fit for human consumption, produced exclusively from suitable products containing starch and/or sugars by the process of double fermentation, first alcoholic and then acetous. Although several attempts have been made to convert the regional standard into a world-wide standard, this conversion has so far not been addressed, especially in view of trade patterns and significant regional differences. The standard describes different kinds of vinegar, essential composition and quality criteria together with optional ingredients, contaminants, hygiene, weights and measures as well as methods of analysis. This regional standard has not taken up by all national legislations of the Member States due to the fact that in two States the name 'vinegar' applies to the product obtained by dilution of synthetic acetic acid.

In the USA, the FDA (Food and Drug Administration) requires that vinegar products must contain at least 4 % acids. There are no FDA standards of identity for vinegar, however the "Compliance Policy Guides" establishes the labelling requirements for cider, wine, malt, sugar, sugar and vinegar blends.

In the EU, Regulation (EC) 1493/1999 [10], there are currently established thresholds for acidity and residual alcohol. Hence vinegars are those products having a minimum 5 % (w/v) acidity and a maximum of 0.5 % (v/v) ethanol, with the exception of wine vinegar which is exclusively obtained from wine and whose acidity is 6 % (w/v) at least and has a maximum ethanol concentration of 1.5 % (v/v). More recently the European Commission published Commission Regulation (EU) 2016/263 [11] amending Annex II to Regulation (EC) No 1333/2008 [12] of the European Parliament and

Council as regards the title of the food category 12.3 Vinegars. The new title of the food category 12.3 is now: Vinegars and diluted acetic acid (diluted with water to 4-30 % by volume). This category was renamed because *in some Member States only vinegars obtained from the fermentation of agricultural products are allowed to be named 'vinegars'. In other Member States, however, both products obtained from the dilution with water of acetic acid and vinegars obtained from the fermentation of agricultural products are marketed under the name 'vinegar'.*

Three EU schemes of geographical indications and traditional specialties, known as Protected Designation of Origin (PDO), Protected Geographical Indication (PGI), and Traditional Specialities Guaranteed (TSG), promote and protect names of quality agricultural products and foodstuffs. Products registered under one of the three schemes may be marked with the logo for that scheme to help identify those products. The schemes are based on the legal framework provided by EU Regulation No 1151/2012 [13] of the European Parliament and of the Council of 21 November 2012 on quality schemes for agricultural products and foodstuffs. This regulation (enforced within the EU and being gradually expanded internationally via bilateral agreements between the EU and non-EU countries) ensures that only products that originate from that particular region are allowed to be marketed as such. Regarding vinegars, there are currently five PDO registered categories and one PGI. Among PDOs: three from Spain (Vinagre de Jerez, Vinagre de Montilla-Moriles, Vinagre de El Condado de Huelva) and two from Italy (Aceto Balsamico Tradizionale di Modena, Aceto Balsamico Tradizionale di Reggio Emilia). Lastly Aceto Balsamico di Modena is registered as a PGI.

Currently there is no European trade association of vinegar producers. The Vinegar Institute is the international trade association representing the vast majority of vinegar manufacturers and bottlers, mainly those with activities in the USA.

2. Authenticity issues

2.1. Identification of current authenticity issues

2.1.1. Framework of national and international legislation

Due to the observed differences in the laws on vinegar from one country to another, it is clear that if a vinegar produced in one country is commercialized in another in which the definition of vinegar changes, it poses a problem and risk for consumers and can become an authenticity issue if its origin is not clearly declared. Thus, a number of examples exist where a vinegar from one country is commercialized in other country in which the legal definition of this kind of vinegar varies. For example, while in the European Union, the term vinegar describes 'a product of a double fermentation (alcoholic and acetic fermentation) from substances of agricultural origin', in the USA a 'synthetically-produced acetic acid diluted with water' can also be labelled as vinegar. Hence, if the latter is sold in Spain, it could be considered a fraud to the consumers. Other example of this problem occurs between Germany and Europe. The German legal definition of 'wine vinegar' permit the production of vinegar by acetic fermentation from natural ethanol, by diluting acetic acid with water or by blending fermentation vinegar with synthetic acetic acid, or with vinegar made from synthetic acetic acid [14]. However, European regulations indicate that wine vinegar can only be produced through the acetic fermentation of wine produced from fresh grapes. So commercialising some 'wine vinegars' from Germany produced with alcohol from different origins as genuine wine vinegar in a European country, could mislead the consumer.

2.1.2. Raw materials

One of the main problems in the vinegar industry lies in the difficult distinction between the use of low-quality and high-quality raw materials, between true vinegars rich in extracts from the raw materials or their blends, as well as to distinguish between highly valued, high quality wine vinegars or aged balsamic vinegars and their cheaper alternatives derived from other raw materials such as malt or alcohol and/or vinegar adulteration with diluted synthetic acid [15]. Within this section, the following issues are discussed.

2.1.2.1. Addition of chemical acetic acid

One of the first frauds in the vinegar industry, and one that has been occurring for more than eighty years, is the addition of chemical or non-biological acetic acid to different types of vinegar contrary to the vinegar industry regulations. The vinegar obtained by chemical acetic acid is called *wood vinegar* or *vinegar essence*, and it cannot be sold as *fermented vinegar* due to it contains more heavy metals per kg of pure acetic acid than the regulated permitted amount (maximum of 5 mg/kg pure acetic acid), which supposes a risk for the consumer. In this sense, European legislation indicates that authentic wine vinegar cannot contain acetic acid obtained from either petroleum derivatives or wood pyrolysis (synthetic acetic acid). These adulterated products constitute a fraud for consumers and are unfair practices to other vinegar producers. To detect the addition of chemical acetic acid to vinegar, the determination of formic acid, derived from the pyrolysis of wood, has demonstrated to be an indirect indicator of it [16], although the detection of synthetic acid added to spirit vinegar or to relevant products produced with the adulterated vinegar or synthetic acetic acid still remains difficult.

2.1.2.2. Addition of water to dried grapes or to must concentrate

The production of vinegar from dried grapes diluted with water is an unfair practice more related to the industry of wine vinegars. This so-called ‘raisin vinegar’ is commonly produced in some Mediterranean countries by fermenting dried grapes and rehydrating with tap water, but it cannot be regarded, or labelled, as ‘wine vinegar’. Due to the fact that this method reduces the price of production, it can be considered, in some Europe countries, as a fraudulent activity. Thus, it has been noticed that some Greek vinegars produced by the above water addition method have been improperly imported into Italy as ‘wine vinegar’ [17].

2.1.2.3. Use of alcohol or sugar not from wine

Commercialising vinegars produced with alcohol from different origins other than grapes, as genuine wine vinegar, is one of the most common fraudulent activities in the vinegar industry. This fraudulent practice aims to reduce manufacturing costs and constitutes a fraud to consumers. Another unfair practice that is currently happening, is the addition of different proportions of alcohol vinegar to wine vinegar samples, which makes the product cheaper. This unfair economic advantage poses an important threat for this sector. These adulterations are difficult to detect because the alcohol added to the base wine prior to the commencement of the fermentation process does not always have a well-known botanical origin [18]. The alcohol added to wine vinegars should come from the fermentation of skins of grapes, but sometimes its origin is fairly diverse: molasses, sugar beet, or sugar cane. Therefore, authenticity issues arise in the ability to detect if the source of the acetic acid and the grape sugars is truly grape (wine) ethanol or wine must, or other ethanol made from fermentation of some other cheaper agricultural products (cereal, potato starch, beetroot or sugarcane), that is called synthetic acetic acid. In the case of

balsamic vinegar as Aceto Balsamico di Modena IGP, there could also be the unfair practice of adding exogenous sugars to cooked and/or concentrated grape must.

2.1.2.4. *Blends of different type of vinegars*

Another common fraudulent practice in the elaboration and commercialisation of vinegar is the mixture of different proportions of wine vinegar and alcohol vinegar. The authenticity issue in this case occurs when this blend is sold under the denomination of wine vinegar, as if it was a pure product. Generally, a good method for a safe differentiation between them is the identification of specific fruit acids, although this can be manipulated easily with the addition of fruit-specific acids and amino acids.

2.1.3. Authentication of geographical indications

The existence of protected origin designations or quality labels in vinegars, which is very common in Southern Europe, provides a greater guarantee to the product although, at the same time, encourages the picaresque nature of unfair producers. The basic requirements for the product to receive such protection is that it must be closely associated with a particular geographical area and with a traditional production procedure which account for the specific quality and characteristics of the vinegar, and therefore, they have higher prices. Some of these characteristics that are defined and established under the PDO Regulations and are mandatory for these vinegars are for example, total acidity, total dry extract or total ash content. Although these PDOs strictly regulate these parameters - all regularly controlled by an inspection authority - some adulteration or frauds have occurred. All too often, however, they are condoned by leading manufacturers, mainly due to the powerful argument of extra profit. Examples include the well-known case of Traditional Balsamic Vinegar of Modena PDO (Protected Designation of Origin) and the Balsamic Vinegar of Modena PGI (Protected Geographical Indication). The former is produced by a traditional, time-consuming and expensive production method obeying very strict rules of raw material provenance and production methods, ensuring a high quality. The second one is produced industrially and is a much cheaper product made from cooked must, concentrated must and wine vinegar via a complicated process [19,20]. Due to their different prices, frauds and mislabelling are frequent, and many brands of these popular vinegars commercialised in the market are in fact merely a sweetened red wine vinegar with food colouring.

Also of considerable interest is the differentiation between Spanish PDO vinegars. Good and promising results in the characterisation and classification of these PDO vinegars have been achieved using different analytical procedures [21-24], but there is still a long way to go. The need to develop methods to distinguish vinegars with this recognised label from non-authentic product is obvious, as not only will the consumer be cheated, but he or she will lose confidence in PDO/IGP labels.

2.1.4. Production process and aging

Adulteration related to production processes occur mainly in vinegars produced by traditional systems such as Sherry vinegar or Traditional Balsamic Vinegar of Modena and Reggio-Emilia. There is an increased interest in differentiating vinegars that have been produced by a traditional method from those produced by a quick production method, due to the fact that the former is associated with a higher quality but also with a longer processing time and a higher cost of production. A further authenticity issue arises when there is a specified minimum aging time for a particular vinegar, as in the case of Sherry vinegars or Traditional Balsamic Vinegar of Modena, the latter being only sold after following an ageing process of at least 12 years in a set of wooden casks

of decreasing volume [19]. The organoleptic vinegar properties developed during ageing make the finished product very appealing. Nevertheless, the production time and costs are too excessive to permit a lucrative trade. Hence, an objective of the vinegar industry is to produce these aged vinegars with the same organic characteristics related to aging, but making it in the most economic and rapid way. For these reasons, the vinegar industry has a very real interest in speeding up ageing if this can be done in a way which does not produce an inferior product or result in the consumer being misled. In this context, the use of wood chips is being investigated. Moreover, there is an increasing necessity to develop simple methods able to detect specific metabolites in vinegars as possible indicators for the ageing process and traditional procedures, in order to protect the consumers and avoid unfair competitions.

2.1.5. Adulteration by addition of grape must caramel

The colour of the vinegars is an important quality parameter as it can, for example, indicate that a wine vinegar has undergone a process of aging in wood barrels. The wine vinegar colour changes during aging from amber to mahogany due to the changes that occur, in the content of polyphenols, tannins and anthocyanins as well as an oxidation process, which are responsible for the darkening of the vinegar. In this context, although the addition of grape-must caramel is allowed by the current legislation to correct and unify the final colour of the different batches, sometimes it could be added to simulate the effect of a greater aging of wine vinegar in wood, which would be considered as an unfair practice.

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

To assess the quality and authenticity of vinegars, several countries have established acceptable methods and ranges or guide values for some vinegar parameters, based on results obtained on the analysis of a large numbers of authentic samples. However, current national and international directives include more methods designed for vinegar identification and generally control than for authenticity issues. In this section officially recognised methods used on a regular basis for vinegars are described (cf. Table 2).

Table 2: Officially recognised methods to test for vinegar authenticity

Method	Reference	Technique	Objective
For wine vinegars			
Determination of total acidity content	OENO 52/2000	Neutralisation of acids in sample by alkali solution	To comply with legal requirements (definitions, PDO, PGI...)
Determination of the fixed acidity content	OENO 53/2000	Neutralisation of the (non-volatile) acids of the residue in an aqueous solution using an alkali solution	To comply with legal requirements (definitions, PDO, PGI...)
Determination of the volatile acid content	OENO 54/2000	Calculation of difference between total acidity and fixed acidity, expressed in grams of acetic acid per L	To comply with legal requirements (definitions, PDO, PGI...)
Detection and quantification of the presence of synthetic acetic acid	OENO 55/2000	After extracting the acetic acid using sodium hydroxide, complete by liquid scintillation the reactivity ^{14}C of the product converted into benzene	Authentication: Values less than the characteristic ^{14}C contents of the assumed year of production represent either a mixture with products from more recent years, or the addition of all or part of the synthetic acetic acid
Determination of the residual alcohol content	OENO 56/2000	Distillation of vinegar, oxidation of ethanol by potassium dichromate and determination of its content by titrating the excess potassium dichromate by a solution of iron sulphate and ammonium	To comply with law requirements (legal definitions, PDO, PGI...)
Determination of total dry extract content	OENO 57/2000	Evaporation of sample and drying in oven, then weighing	Detection of frauds: the addition of water or an aqueous solution of acetic acid (very low total dry extract value) or the addition of non-volatile substances (very high total dry extract value). Database for the type and origin of the vinegar is necessary.
Determination of ash content	OENO 58/2000	Incineration of the vinegar extract between 500°C and 550°C through to complete combustion of the carbon	Detection of frauds: the addition of water or an acetic acid aqueous solution (very low ash content) or the addition of non-volatile substances (very high ash content). Database for the type and origin of the vinegar is necessary.
Determination of the non-volatile reducing substances content	OENO 59/2000	Evaporation of volatile substances, hydrochloric hydrolysis, oxidation by a copper alkali solution in excess with titling by iodometry of copper ions	Detection of frauds: the addition of non-volatile substances.
Determination of the total sulphur dioxide content	OENO 60/2000 + OENO 13/2008	Iodometric titration direct (free SO_2) and after double alkaline hydrolysis (combined SO_2)	Control the level of SO_2 and check compliance with standards
Determination of the total ascorbic acid content	OENO 61/2000	Oxidation of ascorbic acid by iodine with transformation into dehydroascorbic acid, precipitation with 2,4 – dinitrophenylhydrazine. Separation by thin film chromatography, solubilisation in acetic medium and colorimetric determination at 500 nm.	Detection of a fraudulent technological use.

Method	Reference	Technique	Objective
Measurement of chloride content	OENO 62/2000	Potentiometric titration of Cl ions with a solution of silver nitrate, in an acidic environment, after prior measurement of the potential equivalent point of a standard chloride solution	Detection of the fraudulent increase in the dry extract by the addition of sodium chloride
Measurement of sulphate content	OENO 63/2000	Precipitation of sulphates with barium chloride, drying, calcination and weighing	Detection of frauds (aimed at increasing the total dry extract).
Measurement of copper content	OENO 64/2000	Direct measurement by atomic absorption spectrophotometry.	Contamination from contact materials during manufacture, and the iron of the wine itself. Excessive content could cause haze or serious alterations in colour.
Measurement of zinc content	OENO 65/2000	Direct measurement by atomic absorption spectrophotometry.	Contamination from contact materials during manufacture, and excessive content could cause hazes or serious alterations in colour.
Measurement of iron content	OENO 66/2000	Direct measurement by atomic absorption spectrophotometry.	Contaminations from contact materials during their manufacture, and of course the iron of the wine itself. Excessive content could cause haze or serious alterations in the colour.
Measurement of lead content	OENO 67/2000	Direct measurement of lead content in the vinegar by atomic absorption spectrometry without flame (electrothermal atomisation).	The presence of lead in vinegars mainly has its origin in contaminations from contact materials during their manufacture, and the lead of the wine itself from which the vinegar has been made
Measurement of mercury content	OENO 68/2000	Mineralisation. Reduction by permanganate Measurement by atomic absorption spectrometry (cold vapour).	Toxicologic issue
Measurement of the acetoin content	OENO 69/2000	Neutralisation of the sample at pH 7.00 with calcium hydroxide. Direct measurement of the acetoin via gas chromatography	Authentication: Determination of quality and origin by the analysis of acetoin content in the wine vinegars (between 100 mg/L and over 400 mg/L)
Measurement of the methanol, superior alcohols and ethyl acetate	OENO 70/2000	Neutralization of the sample at pH 7.00 with a sodium hydroxide solution. Measurement, via GC, of some volatile components: methanol, propan-1-ol, butan-2-ol, 2-methylpropan-1-ol, butan-1-ol and 2-methylbutan-1-ol + 3-methylbutan-1-ol	Organoleptic and possibly toxicologic issue
Authentication by SNIF-NMR® and other isotopic methods	OENO 71/2000	Extraction of the acetic acid from the vinegar with ether. Purification using a Cadiot column. Determination of the purity of acetic acid. Measurement of the site-specific deuterium/hydrogen ratio in the resulting acetic acid, via deuterium NMR.	Detection of frauds: detection of synthetic acetic acid in vinegars and any other downgrading of vinegars. Detection of possible addition of alcohol-vinegar coming from plants whose metabolism is C ₄ (sugar addition from cane) or C ₃ (beet)

Method	Reference	Technique	Objective
Detection of synthetic acetic acid in wine vinegars by the determination of beta radioactivity of ¹⁴ C of acetic acid by liquid scintillation	OENO 12/2006	Extraction of acetic acid from the vinegar. Acetic acid of mineral origin (Control) is counted in the same way. β emission value of the ¹⁴ C in the sample compared with the average value of the β emissions of ¹⁴ C found in the ethanol in genuine late harvest wines.	Detection of fraud: detection of the addition of synthetic acetic acid (levels lower than those for a given year) or the entire content of it. Control of the year of production of the raw wines.
Method for ¹³ C/ ¹² C isotope ratio determination of acetic acid in wine vinegar by isotopic mass spectrometry	OIV-OENO 510-2013	¹³ C/ ¹² C isotope ratio of acetic acid by Isotope ratio mass spectrometry (IRMS)	Detection of frauds related to the botanical origin of acetic acid and revelation of the addition of synthetic acetic acid. Determination of sugar addition (cane)
Method for ¹⁸ O/ ¹⁶ O isotope ratio determination of water in wine vinegar using isotopic mass spectrometry	OIV-OENO 511-2013	¹⁸ O/ ¹⁶ O isotopic ratio of water by Isotopic Ratio Mass Spectrometry (IRMS)	Detection of frauds related to the production of vinegars from fresh grapes or from dried grapes with water addition
Determination of the distribution of deuterium in the acetic acid of vinegar wine by Nuclear Magnetic Resonance (NMR)	OIV-OENO- 527-2015	Composite ¹ H-NMR and ² H-SNIF–NMR	Detection of frauds about botanical origin of acetic acid and revelation of the addition of synthetic acetic acid
For all vinegars			
Isotopic analysis of acetic acid and water Part 1: ² H-NMR analysis of acetic acid. Part 2: ¹³ C-IRMS analysis of acetic acid. Part 3: ¹⁸ O-IRMS analysis of water in wine vinegar	CEN, EN 16466-1,2,3 (2012)	SNIF-NMR (D/H), ¹³ C/ ¹² C IRMS, ¹⁸ O/ ¹⁶ O IRMS	Determination of frauds related to vinegar acetic acid, water and sugar addition (beet, cane)

3.2. Other commonly used methods

3.2.1. Sensory analysis

Sensory analysis has proven to be a simple and reliable tool for assessing the quality of vinegars [25]. The appropriate sensory methodology must be clearly defined and the attributes used in discriminant or descriptive analysis must be precise and well-recognised by the panel. The sensory characterisation of vinegars for monitoring vinegar quality has been widely performed in many studies over a number of years [26-31]. Moreover, in some vinegars, quality control is mainly based on their sensory properties, as is the case for Traditional Balsamic Vinegar of Modena. Sensory vinegar analysis can be performed by olfactive and gustative analyses, as well as by the determination of other parameters such as viscosity and colour.

3.2.1.1. Odour and taste

In order to analyse the taste and odour of the vinegars, there are different protocols such as preparing the vinegar in a way that most resembles how it is normally consumed (using lettuce suspended in the vinegar [27] or diluting with cold or hot water), or testing and smelling vinegar as is, using opaque cups to avoid colour influences, being it the usual sensory analysis for vinegar cellars [26].

Within the different types of sensory analysis, the most used are the descriptive test, that is useful for determining the sensory profile of the samples, and the discriminatory test, which include a wide range of tests such as triangular test (ISO, 2004, standard 4120) [32] and Paired Comparison tests (ISO, 1983b, standard 5495) [33], preference test, etc. These methods require a well-trained testing panel, and concrete and adequate attributes.

3.2.1.2. Viscosity

Viscosity is another important parameter in the sensorial quality of some vinegars such as in the case of the Traditional Balsamic Vinegar of Modena. Nevertheless, no procedure has yet been established to determine this objectively, as it is assessed in an empirical manner and wrongly expressed as physical density.

3.2.1.3. Colour

Colour is one of the most important parameters used by consumers to assess the quality of a food product. Some studies have described a relationship between some compounds and a darker colour such as melanoidin, and products from the degradation of sugars and Maillard reactions [3]. A darker colour is also related to a longer aging period in wine vinegars and Traditional Balsamic vinegar of Modena. Some techniques such as UV-Visible spectrophotometry or excitation-emission fluorescence or transmission colorimetric techniques are being used with promising results for this issue [34-36]. However, the colour could be easily modified with the use of grape must caramel or other additives and no methods have been officially established to assess and control this parameter.

3.2.2. Physicochemical analysis

Notwithstanding the fact that the quality of vinegars has been traditionally evaluated by using a trained sensory panel, more rapid and objective methodologies have been tested and performed by instrumental measurements.

3.2.2.1. Chromatographic techniques

Chromatographic techniques have been widely applied, for a long time, to determine certain vinegar compounds useful for characterising, classifying or detecting adulteration in vinegars.

High-performance liquid chromatography-mass spectrometry (HPLC)

HPLC has been widely used to analyse compounds such as phenols. Phenolic compounds are present in wine vinegars due to their natural content in grapes or as a result of contact with wood during the aging process, and they have demonstrated to be important in the determination of origin and the technology involved in the production of wine vinegars [37-39].

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS)

Gas chromatography (GC) is the official method for the determination of acetoin content, methanol, superior alcohols and ethyl acetate (OENO 69-70/2000) [40,41], and has also been applied to determine poly-alcohols in vinegars, all of them related to quality and origin. In addition to this method, gas chromatography coupled with mass spectrometry (GC-MS) has been the most efficient and widely employed technique to date to determine the volatile composition of vinegars which is also directly related to the quality of the vinegar. This technique normally requires a prior extraction step (such as dynamic and static headspace extraction, solid phase microextraction, stir bar sorptive extraction or liquid-liquid extraction methods). Examples of the efficiency of this

methodology are the determination of volatile aldehydes as discriminant parameters in quality vinegars or the determination of the volatile profile as a classification parameter of different vinegar types or geographical indicators [42-45]. However, regardless of the fact that these sampling methods have been widely employed in the volatile analysis of vinegars, the experimental sources of variability related to GC-MS (e.g. columns, stationary phase, temperature or experimental conditions and sample preparation) still cause some variations that directly affects the final results. These problems are being recently resolved by chemometric tools such as Multiple curve resolution (MCR) or Parallel factor analysis (PARAFAC) [44].

Gas chromatography coupled with olfactometry (GC-O)

The intensity and quality of the aroma constitutes the primary quality factor in vinegars. Although the aroma of vinegars is widely studied by sensory analysis and GC-MS methodologies, all volatile compounds determined in vinegar do not have the same contribution to the overall aroma of the product. Gas chromatography-olfactometry (GCO) is the most appropriate analytical technique to determine these compounds with real impact of the aroma of a vinegar, known as impact odorants, among the whole volatile fraction. This technique provides instrumental and sensory analysis simultaneously as the eluted analytes are perceived at the same time by the human nose and a conventional detector, such as the flame ionic detector (FID) or the mass spectra detector (MSD), which turns this technique into a powerful one in food aroma characterisation. However, little research can be found in the literature regarding the application of this technique in vinegars. Thus, only a few papers deal with a comprehensive characterisation of the aroma profile of red wine vinegars [31], some Chinese vinegars [46] or with the quality perception of Sherry vinegars [47].

3.2.2.2. Spectroscopic techniques

Near infrared spectroscopy

Near-infrared spectroscopy (in the range of 5000 - 15000 cm^{-1}) is a potential spectroscopic technique that has been applied to the analysis of vinegars. Near-infrared spectroscopy (NIR) has the advantages of high speed, accuracy, simplicity, and low cost. NIR spectra can record the multifrequency and co-frequency information of organic molecules, which involves the response of molecular bonds of C-H, N-H, C-O, and O-H, being useful for determining organic acids and pH in vinegars, as in the case of MIR, mentioned below [48]. The vinegar sample is either placed in a cuvette and the spectrum collected by absorption mode or the bottles can be directly scanned in transmission mode. A multivariate analysis of the data is usually employed to develop models able to classify the different classes of vinegars, different geographical origins [23,49] or even to predict or monitor the vinegar ageing process [50,51].

Mid-infrared spectroscopy

Mid-infrared spectroscopy (MIR) (in the range of 500 - 5000 cm^{-1}) has also been shown to be able to address a wide range of issues and provide solutions for rapid analysis and on-line control of vinegars. This technique combined with chemometrics has gained wide acceptance for authenticity and classification purposes in food, being informative at the molecular level and produces a single spectral fingerprint of each sample. Moreover, the use of an accessory of Attenuated Total Reflectance (ATR) allows the direct analysis of liquids in a simple, fast, only a few minutes, and non-destructive manner, involving minimal sample preparation. This method provides a greater amount of chemical information compared to NIR spectroscopy in terms of chemical assignment of observances and allows the interpretation of the spectra without the need of complex chemometrics. Thus, Fourier transform mid infrared spectroscopy (FT-IR) coupled with

ATR has been applied to investigate its potential as a tool for characterising different categories of high-quality vinegars by studying the differences in the spectra. FT-IR spectra have also been used to predict the sensory score of traditional balsamic vinegar of Modena by the performance of different partial least squares (PLS) regression models [52] as well as obtaining a full calibration model for organic acids in vinegars [53]. Finally, the technique can also be used to control certain steps and factors of the production processes in industry, making it possible to carry out necessary corrective actions without delay [54].

Fluorescence spectroscopy

Fluorescence spectroscopy is also being investigated as an alternative quality control tool for vinegars, with the same attributes as those mentioned above. Different methods of analysis are possible, the conventional one being the measurement of the excitation or emission spectra at a single emission or excitation wavelength, respectively. However, instead of measuring a single emission spectrum at a selected excitation wavelength, the emission spectra at different excitation wavelengths can be recorded, in a technique known as excitation-emission fluorescence. The latter results in a bi-dimensional Excitation- Emission Matrix (EEM), which contains unique information of each measured sample, having the advantage of containing more information about the fluorescent species than the conventional excitation and emission spectra separately. Moreover, the potential of the EEM technique can be improved by applying multivariate methods in the analysis of the fluorescence results such as Parallel Factor Analysis (PARAFAC) and its combination with PLS discriminant analysis. PARAFAC is used to decompose fluorescence EEMs into different independent groups of fluorescence components (fluorophores), as well as their relative concentration (scores) in each sample. This method extracts the most relevant information from the data in order to build further robust calibration and/or classification models. For this reason, this technique has been more widely applied in the study of wine vinegars than the simple excitation or emission analysis. Thus, Callejón et al. [48] and Ríos-Reina et al. [16] studied fluorescence excitation–emission spectroscopy combined with suitable multivariate methods. In these studies, the fluorescence Excitation-Emission Matrices (EEMs) were obtained by varying the excitation wavelength ranging between 250 and 700 nm (every 5 nm), and recording the emission spectra from 300 to 800 (every 2 nm). For these measurements, excitation and emission slits were both set at 5 nm, and the scan rate was fixed to 1200 nm min⁻¹. These studies demonstrated this method's ability to characterise and classify three Spanish PDO wine vinegars according to their protected designation of origin, as well as their categories (aged and sweet) [24; 55]. However, despite the promising results obtained, it is not yet widely in use in this field.

Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectroscopy, which has the advantage of being a rapid and non-selective analysis without any manipulation or derivatisation, has recently achieved general acceptance as a powerful tool for vinegar quality and authenticity determination. NMR can provide information on chemical composition, concentration of soluble metabolites and their structure in the samples such as sugars, acids and flavonoids, with the advantage of providing the best combination of fast data acquisition and predictive capability. However, the large amount of data needs to be treated by multivariate methods such as principal component and discriminant analysis with the final objective of making models able to discriminate authentic and non-authentic vinegars, origins, or vinegar types.

Different nuclei to which the spectrometer is tuned have been investigated for vinegar authentication. The most commonly applied NMR technique for origin authentication, and recently recognised as an official method, is deuterium SNIF-NMR (Site-specific Natural Isotopic Fractionation studied by nuclear magnetic resonance spectrometry). However, another very used

method with promising results is proton nuclear magnetic resonance (^1H -NMR) spectroscopy, which, combined with multivariate statistical data analysis, has demonstrated its usefulness in the characterisation of the ageing process and the discrimination of different vinegar types [19,56]. The application of ^{13}C NMR, two-dimensional ^1H - ^{13}C heteronuclear multiple-bond correlation (HMBC), and ^1H - ^{13}C heteronuclear single quantum coherence (HSQC) spectra for the characterisation and discrimination of Balsamic vinegars of Modena in order to obtain an indirect indicator of authenticity and a quality control tool have also been studied, although to a lesser extent [57]. It should be also considered that as vinegar samples contain a high amount of water, optimising water suppression methods is required, since it is one of the elements that most impacts the overall quality of the spectrum [58]. Moreover, as NMR generates a complex spectrum containing information on all proton/carbon bearing compounds, multivariate data analysis such as principal component analysis or discriminant analysis is employed to develop classification/authentication models.

3.2.2.3. *Other techniques*

Trace metal analysis

Trace metal analysis using inductively-coupled plasma optical-emission (ICP-OES), atomic absorption spectrometer spectroscopy (AAS), flame absorption (FAAS) and emission spectrometry (FES) has been applied to determine the mineral composition and the trace metal contents in vinegars to determine geographical origin, type of raw materials and different production processes [59,60]. Since the mineral composition of the plant reflects the mineral composition of the soil where it is growing, accordingly, soil differences and differences in grape varieties could be reflected in the mineral composition of the vinegars, providing information about the geographical origin. The main parameters found in the case of Spanish PDO wine vinegars were Ca, K, Mg, Na, P and S, that are natural components of grape juice, K being the pre- dominant cation.

Isotope analysis

The analysis of the isotope ratios of the bio-elements ($^2\text{H}/^1\text{H}$, $^{13}\text{C}/^{12}\text{C}$, $^{18}\text{O}/^{16}\text{O}$ or $^3\text{H}/^1\text{H}$, $^{14}\text{C}/^{12}\text{C}$) has also shown to be useful for providing proof of vinegar authentication and for detecting frauds such as the addition of synthetic acetic acid or water and the source of this acid [22]. In fact, isotopic methods have been recently recognised by the European Committee for Standardization (CEN) and in part by the OIV as a means of detecting the presence of exogenous acetic acid and tap water in wine vinegars.

Recently [61] it was found that the above listed OIV and CEN methods for the analysis of stable isotope ratios D/H and $^{13}\text{C}/^{12}\text{C}$ in ethanol and acetic acid and of $^{18}\text{O}/^{16}\text{O}$ in water can be applied to the ingredients of balsamic vinegar such as Aceto Balsamico di Modena IGP to evaluate their authenticity. The standard deviation of repeatability and reproducibility are indeed comparable in wine vinegar and balsamic vinegar and generally lower than those quoted in the official methods. Moreover, no changes in the isotopic values from wine to vinegar and to balsamic vinegar, and from the original must to the balsamic vinegar must were found. This provide experimental evidence that reference data from isotopic wine databanks [61] can also be used to evaluate the authenticity of the ingredients of vinegar and balsamic vinegar.

4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address.

Analytical Technique	Indicative data or Analyte	Authenticity issue or information
Colorimetric analysis	Total acidity content and fixed acidity content; total ascorbic acid	To comply with legal requirements
Gravimetric analysis	Residual alcohol content	To comply with legal requirements
	Total dry extract content; ash content; non-volatile reducing substances content; sulphate content	Detection of frauds
Iodometric analysis	Total sulphur dioxide content	To comply with legal requirements
Potentiometric analysis	Chloride content	Detection of frauds
Beta radioactivity ^{14}C	Synthetic acetic acid	Raw material and year of production
Sensory analysis	Odour and flavour attributes	Characterisation; ageing evaluation; quality certification (PGI, PDO); raw materials and production process
HPLC	Phenolic acids	Production process; Origin and technology involved
	Phenolic compounds	Ageing; production in different wood types
GC	Polyalcohol content	Origin
	Acetoin content, methanol, superior alcohols and ethyl acetate	Determination of quality and origin
GC-MS	Volatile aldehydes	Raw material and ageing
	Volatile compounds	Raw material and production process; quality certification (PGI, PDO); ageing
GC-O	Odour impact	Characterisation
NIR	Spectral profile	Raw material and production process; detection of frauds; origin; authentication (PGI, PDO)
MIR	Spectral profile	Ageing; raw material and production process; quality certification (PGI, PDO vinegars)
Fluorescence	Spectral profile	Ageing and authentication (PGI, PDO)
^1H -NMR	Spectral profile and vinegar metabolites	Authentication (PGI, PDO) and detection of frauds
	Organic components	Raw material and production process
^{13}C NMR, HMBC, and HSQC	Spectral profile and vinegar metabolites	Authentication (PDO, PGI...)
ICP-OES/ICP-MS	Mineral composition	Geographical origin
IRMS, SNIF-NMR	Site-specific D/H isotope ratio of acetic acid, $^{13}\text{C}/^{12}\text{C}$ ratio of Acetic acid and $^{18}\text{O}/^{16}\text{O}$ ratio of water	Detection of frauds: addition of synthetic acetic acid, water or sugar, from plants C_3 or C_4
IRMS	$^{13}\text{C}/^{12}\text{C}$ isotope ratio of acetic acid	Botanical origin, addition of sugar from C_4 sources
	$^{18}\text{O}/^{16}\text{O}$ isotopic ratio of water	Addition of water to dried grapes
SNIF-NMR	Site-specific D/H ratio of acetic acid	Botanical origin, addition of synthetic acetic acid
FES, FAAS, AAS	Metallic and trace element components	Production process
Colorimetric techniques	Volatile organic compounds	Production process
E-tongue, E-nose	Aroma and taste signals	Raw materials and ageing

5. Conclusion

The issues mentioned in the sections above are those that have already been identified and remain the most economically viable forms of adulteration at the present time. However, in the future, there could be more problems that should be kept in mind. These problems will most likely concern the growing range of new vinegar types, less common nowadays in the market or the emergence of other food ingredients that can create new, potential areas of deception when used improperly.

The diversity of vinegars in the market and the increase in demand makes it necessary to characterise them to establish quality control parameters. The characterisation of the vinegar covers different objectives including the authentication and classification of the product based on quality criteria. Consequently, there is an increasing need for investigating reliable analytical methods able to detect the possible adulterations and frauds as well as to assess the authenticity of the vinegar.

In recent years, there has been a growing need to develop fast, cheap, robust and effective analytical methods that do not require much sample manipulation such as sensors and spectroscopic techniques (e.g. MIR, NIR, Fluorescence, NMR and UV) coupled to chemometric tools. These techniques take into account both the individual contribution and the interactions of the different components presented in the vinegar, generating a global fingerprint of a food product. However, one of the main disadvantages is their ability to recognise just a limited number of molecules.

Finally, given the complexity of vinegars, and the fact that they are perceived by the consumer in a global way, they must be evaluated from a multivariate point of view. For this reason, a new trend in food authentication based on a combination of more than one of the aforementioned techniques has appeared. This promising methodology known as “data fusion” should be further studied for vinegar authentication.

6. Bibliographic references

- 1 Vinegar Market: Global Industry Trends, Share, Size, Growth, Opportunity and Forecast 2018-2023. Available at: <https://www.imarcgroup.com/vinegar-manufacturing-plant>.
- 2 Mas, A., Troncoso, A. M., García-Parrilla, M. C. & Torija, M. J. (2016). – Vinegar. *Encyclopedia of Food and Health*, 418–423. doi:10.1016/B978-0-12-384947-2.00726-1
- 3 Solieri, L. & Giudici, P. (2009). – *Vinegars of the World*. Springer-Verlag Italia.
- 4 Mas, A., Torija, M. J., García-parrilla, M. C. & Troncoso, A. M. (2014). – Acetic Acid Bacteria and the Production and Quality of Wine Vinegar. *The ScientificWorld Journal*, 1–7. doi:<https://doi.org/10.1155/2014/394671>.
- 5 Commission Regulation (2009). – Commission Regulation (EC) No 583/2009 of 3 July 2009 entering a name in the register of protected designations of origin and protected geographical indications Aceto Balsamico di Modena (PGI). Official Journal of the European Union. L 175/7-11.
- 6 Frias, J., Martínez-Villaluenga, C. & Peñas, E. (2017). – *Fermented Foods in Health and Disease Prevention*. Elsevier. Available at: <http://csu-cvmb.colostate.edu/Documents/erhs-fermentedfoods-HDprevention-2016.pdf%0A>
- 7 Gullo, M., De Vero, L. & Guidici, P. (2009) – Succession of selected strains of *Acetobacter pasteurianus* and other acetic acid bacteria in traditional balsamic vinegar. *Applied and Environmental Microbiology*, 75, 2585–2589.
- 8 Hidalgo, C. *et al.* (2010). – Effect of barrel design and the inoculation of *Acetobacter pasteurianus* in wine vinegar production. *International Journal of Food Microbiology*, **141**, 56–62. doi:10.1016/j.ijfoodmicro.2010.04.018.

- 9 Organización Mundial de la Salud (OMS) y la Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO) (1987) – Norma Codex para el Vinagre. **1**, 3–6.
- 10 Council Regulation (1999). – Council Regulation (EC) No 1493/1999 of 17 May 1999 on the common organisation of the market in wine. Official Journal of the European Communities. L 179/1 -83.
- 11 Commission Regulation (2016). – Commission Regulation (EU) 2016/263 of 25 February 2016 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council as regards the title of the food category 12.3 Vinegars. Official Journal of the European Union. L50/25 .
- 12 Regulation EC (2008). – Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. Official Journal of the European Union. L 354/16 -33.
- 13 Regulation EU (2012). – Regulation (EU) No 1151/2012 of the European Parliament and the Council of 21 November 2012 on quality schemes for agricultural products and foodstuffs. Official Journal of the European Union. L 343/1-29.
- 14 Werner, R. a. & Roßmann, A. (2015). – Multi element (C, H, O) stable isotope analysis for the authentication of balsamic vinegars. *Isotopes in Environmental and Health Studies*, **51**, 58–67. doi:10.1080/10256016.2015.1011154.
- 15 Grégrová, A., Čížková, H., Mazáč, J. & Voldřich, M. (2012). – Authenticity and quality of spirit vinegar: Methods for detection of synthetic acetic acid addition. *Journal of Food and Nutrition Research*, **51**, 123–131
- 16 Bourgeois, J. F., McColl, I. & Barja, F. (2006). – Formic acid, acetic acid and methanol: Their relevance to the verification of the authenticity of vinegar. *Archives Des Sciences*, **59**, 107–112.
- 17 Camin, F. *et al.* (2013). – Control of wine vinegar authenticity through δ 18O analysis. *Food Control* **29**, 107–111. doi:10.1016/j.foodcont.2012.05.055.
- 18 Sáiz-Abajo, M. J., González-Sáiz, J. M. & Pizarro, C. (2004). – Classification of wine and alcohol vinegar samples based on near-infrared spectroscopy. Feasibility study on the detection of adulterated vinegar samples. *Journal of Agricultural and Food Chemistry*, **52**, 7711–7719. doi:10.1021/jf049098h.
- 19 Consonni, R. *et al.* (2008). – NMR and Chemometric methods: A powerful combination for characterization of Balsamic and Traditional Balsamic Vinegar of Modena. *Analytica Chimica Acta*, **611**, 31–40. doi:10.1016/j.aca.2008.01.065.
- 20 Consonni, R., Cagliani, L. R., Rinaldini, S. & Incerti, A. (2008). – Analytical method for authentication of Traditional Balsamic Vinegar of Modena. *Talanta*, **75**, 765–769. doi:10.1016/j.talanta.2007.12.005.
- 21 Ríos-Reina, R., Callejón, R. M., Oliver-Pozo, C., Amigo, J. M. & García-González, D. L. (2017). – ATR-FTIR as a potential tool for controlling high quality vinegar categories. *Food Control*, **78**, 230–237. doi:10.1016/j.foodcont.2017.02.065.
- 22 Ortiz-Romero, C., Ríos-Reina, R., Morales, M. L., García-González, D. L. & Callejón, R. M. (2018). – A viability study of C–O isotope fingerprint for different geographical provenances of Spanish wine vinegars. *European Food Research and Technology*, **0**. doi:10.1007/s00217-017-3026-8.
- 23 Ríos-Reina, R., García-González, D. L., Callejón, R. M. & Amigo, J. M. (2018). – NIR spectroscopy and chemometrics for the typification of Spanish wine vinegars with a protected designation of origin. *Food Control* **89**, 108–116. doi:10.1016/j.foodcont.2018.01.031
- 24 Ríos-Reina, R. *et al.* (2017). – Characterization and authentication of Spanish PDO wine vinegars using multidimensional fluorescence and chemometrics. *Food Chemistry*, **230**, 108–116. doi:10.1016/j.foodchem.2017.02.118.
- 25 Tesfaye, W. *et al.* (2010). – Descriptive Sensory Analysis of Wine Vinegar: Tasting Procedure and Reliability of New Attributes, *Journal of Sensory Studies*, **25**, 216–230. doi:10.1111/j.1745-459X.2009.00253.x.
- 26 Tesfaye W., García-Parrilla M.C., T. A. M. (2002). – Sensory Evaluation of Sherry Wine Vinegar. *Journal of Sensory Studies*, **17**, 133–144. doi:10.1111/j.1745-459X.2002.tb00338.x.
- 27 González-Viñas, M. A., Salvador, M. D. & Cabezudo, M. D. (1996). – Taste group thresholds and sensory evaluation of spanish wine vinegars. *Journal of Sensory Studies*, **11**, 129–140. doi:10.1111/j.1745-459X.1996.tb00037.x.
- 28 Callejón, R. M., Morales, M. L., Silva Ferreira, A. C. & Troncoso, A. M. (2008). – Defining the typical aroma of Sherry vinegar: Sensory and chemical approach. *Journal of Agricultural and Food Chemistry*, **56**, 8086–8095. doi:10.1021/jf800903n.
- 29 Lalou, S. *et al.* (2015). – Beyond traditional balsamic vinegar: Compositional and sensorial characteristics of industrial balsamic vinegars and regulatory requirements. *Journal of Food Composition and Analysis*, **43**, 175–184. doi:10.1016/j.jfca.2015.07.001.
- 30 Hillmann, H. *et al.* (2012). – Sensomics analysis of taste compounds in balsamic vinegar and discovery of 5-acetoxymethyl-2-furaldehyde as a novel sweet taste modulator. *Journal of Agricultural and Food Chemistry*, **60**, 9974–9990. doi:10.1021/jf3033705.

- 31 Charles, M. *et al.* (2000). – Potent aroma compounds of two red wine vinegars. *Journal of Agricultural and Food Chemistry*, **48**, 70–77. doi:10.1021/jf9905424.
- 32 ISO (International Organization for Standardization) (2004) Methodology: Triangular Test. In: Sensory Analysis. ISO 4120.
- 33 ISO (International Organization for Standardization) (1983) Methodology: Pair Comparison Test. In: Sensory Analysis. ISO 5495.
- 34 Palacios, V., Valcarcel, M., Caro, I. & Perez, L. (2002). – Chemical and biochemical transformations during the industrial process of sherry vinegar aging. *Journal of Agricultural and Food Chemistry*, **50**, 4221–4225. doi:10.1021/jf020093z.
- 35 Zhu, D., Ji, B., Eum, H. L. & Zude, M. (2009). – Evaluation of the non-enzymatic browning in thermally processed apple juice by front-face fluorescence spectroscopy. *Food Chemistry*, **113**, 272–279. doi:10.1016/j.foodchem.2008.07.009.
- 36 De la Haba, M. J., Arias, M., Ramírez, P., López, M. I. & Sánchez, M. T. (2014). – Characterizing and authenticating montilla-moriles PDO vinegars using near infrared reflectance spectroscopy (nirs) technology. *Sensors (Switzerland)*, **14**, 3528–3542. doi:10.3390/s140203528.
- 37 García Parrilla, M. C., Heredia, F. J. & Troncoso, A. M. (1999). – Sherry wine vinegars: Phenolic composition changes during aging. *Food Research International*, **32**, 433–440. doi:10.1016/S0963-9969(99)00105-2.
- 38 García-Parrilla, M. C., Camacho, M. L., Heredia, F. J. & Troncoso, A. M. (1994). – Separation and identification of phenolic acids in wine vinegars by HPLC. *Food Chemistry*, **50**, 313–315. doi:10.1016/0308-8146(94)90140-6.
- 39 Cerezo, A. B. *et al.* (2008). –The phenolic composition of red wine vinegar produced in barrels made from different woods. *Food Chemistry*, **109**, 606–615. doi:10.1016/j.foodchem.2008.01.013.
- 40 Resolution OENO 69-2000. XVIII. Wine vinegar - Measurement of the acetoin content.
- 41 Resolution OENO 70-2000. XIX. Wine vinegar - Measurement of methanol, superior alcohols and Ethyl acetate.
- 42 Durán-Guerrero, E., Chinnici, F., Natali, N. & Riponi, C. (2015). – Evaluation of volatile aldehydes as discriminating parameters in quality vinegars with protected European geographical indication. *Journal of the Science of Food and Agriculture*, **95**, 2395–2403. doi:10.1002/jsfa.6958.
- 43 Cocchi, M. *et al.* (2004). – Application of a wavelet-based algorithm on HS-SPME/GC signals for the classification of balsamic vinegars. *Chemometrics and Intelligent Laboratory Systems*, **71**, 129–140. doi:10.1016/j.chemolab.2004.01.004.
- 44 Ríos-Reina, R., Morales, M. ., García-González, D. L., Amigo, J. M. & Callejón, R. M. (2018). – Sampling methods for the study of volatile profile of PDO wine vinegars. A comparison using multivariate data analysis. *Food Research International*, **105**, 880–896. doi:10.1016/j.foodres.2017.12.001.
- 45 Chinnici, F. *et al.* (2009). – Gas chromatography-mass spectrometry (GC-MS) characterization of volatile compounds in quality vinegars with protected European geographical indication. *Journal of Agricultural and Food Chemistry*, **57**, 4784–4792. doi:10.1021/jf804005w.
- 46 Zhou, Z. *et al.* (2017). – Elucidation of the aroma compositions of Zhenjiang aromatic vinegar using comprehensive two dimensional gas chromatography coupled to time-of-flight mass spectrometry and gas chromatography-olfactometry. *Journal of Chromatography A*, **1487**, 218–226. doi:10.1016/j.chroma.2017.01.014.
- 47 Callejón, R. M., Morales, M. L., Troncoso, A. M. & Silva Ferreira, A. C. (2008). – Targeting key aromatic substances on the typical aroma of sherry vinegar. *Journal of Agricultural and Food Chemistry*, **56**, 6631–9. doi:10.1021/jf703636e.
- 48 Liu, F., He, Y., Wang, L. & Sun, G. (2011). – Detection of Organic Acids and pH of Fruit Vinegars Using Near-Infrared Spectroscopy and Multivariate Calibration. *Food and Bioprocess Technology*, **4**, 1331–1340. doi:10.1007/s11947-009-0240-9.
- 49 Yu, H., Fu, X., Xie, L., Ying, Y. & Zhou, Y. (2007). – Discrimination between Chinese rice wines of different geographical origins by NIRS and AAS. *European Food Research and Technology*, **225**, 313–320. doi:10.1007/s00217-006-0416-8.
- 50 An, Z., Lu, H., Jiang, H. & Ying, Y. (2011). – Prediction of marked age of mature vinegar based on fourier transform near infrared spectroscopy. *IFIP Advances in Information and Communication Technology*, **344** AICT, 737–743. doi:10.1007/978-3-642-18333-1_89.
- 51 Casale, M., Sáiz Abajo, M. J., González Sáiz, J. M., Pizarro, C. & Forina, M. (2006). – Study of the aging and oxidation processes of vinegar samples from different origins during storage by near-infrared spectroscopy. *Analytica Chimica Acta* **557**, 360–366. doi:10.1016/j.aca.2005.10.063.

- 52 Versari, A., Parpinello, G. P., Chinnici, F. & Meglioli, G. (2011). – Prediction of sensory score of Italian traditional balsamic vinegars of Reggio-Emilia by mid-infrared spectroscopy. *Food Chemistry*, **125**, 1345–1350. doi:10.1016/j.foodchem.2010.10.003.
- 53 Regmi, U., Palma, M. & Barroso, C. G. (2012). – Direct determination of organic acids in wine and wine-derived products by Fourier transform infrared (FT-IR) spectroscopy and chemometric techniques. *Analytica Chimica Acta*, **732**, 137–144. doi:10.1016/j.aca.2011.11.009.
- 54 Durán, E., Palma, M., Natera, R., Castro, R. & Barroso, C. G. (2010). – New FT-IR method to control the evolution of the volatile constituents of vinegar during the acetic fermentation process. *Food Chemistry*, **121**, 575–579. doi:10.1016/j.foodchem.2009.12.050.
- 55 Callejón, R. M. *et al.* (2012). – Classification of Sherry vinegars by combining multidimensional fluorescence, parafac and different classification approaches. *Talanta*, **88**, 456–462. doi:10.1016/j.talanta.2011.11.014.
- 56 Boffo, E. F., Tavares, L. A., Ferreira, M. M. C. & Ferreira, A. G. (2009). – Classification of Brazilian vinegars according to their ¹H NMR spectra by pattern recognition analysis. *LWT - Food Science and Technology*, **42**, 1455–1460. doi:10.1016/j.lwt.2009.05.008.
- 57 Graziosi, R. *et al.* (2017). – Novel 2D-NMR Approach for the Classification of Balsamic Vinegars of Modena. *Journal of Agricultural and Food Chemistry*, **65**, 5421–5426. doi:10.1021/acs.jafc.7b01927
- 58 Giraudeau, P., Silvestre, V. & Akoka, S. (2015). – Optimizing water suppression for quantitative NMR-based metabolomics: a tutorial review. *Metabolomics*, **11**, 1041–1055. doi:10.1007/s11306-015-0794-7.
- 59 Paneque, P., Morales, M. L., Burgos, P., Ponce, L. & Callejón, R. M. (2017). – Elemental characterisation of Andalusian wine vinegars with protected designation of origin by ICP-OES and chemometric approach. *Food Control*, **75**, 203–210. doi:http://dx.doi.org/10.1016/j.foodcont.2016.12.006.
- 60 Guerrero, M. I., Herce-Pagliai, C., Cameán, A. M., Troncoso, A. M. & González, A. G. (1997). – Multivariate characterization of wine vinegars from the south of Spain according to their metallic content. *Talanta*, **45**, 379–386. doi:10.1016/S0039-9140(97)00139-2.
- 61 Perini, M. *et al.* (2014). – Stable Isotope Ratio Analysis for Verifying the Authenticity of Balsamic and Wine Vinegar. *Journal of Agricultural and Food Chemistry*, **62**, 8197–8203. doi:10.1021/jf5013538.
- 62 Commission Regulation (EC) (2008). – Commission Regulation (EC) No 555/2008 of 27 June 2008 laying down detailed rules for implementing Council Regulation (EC) No 479/2008 on the common organisation of the market in wine as regards support programmes, trade with third countries, production potential and on controls in the wine sector. Official Journal of the European Union. L 170/1-80.