

Paolo Giudici · Federico Lemmetti  
Stefano Mazza

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Tradition, Technology, Trade

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Paolo Giudici  
Department of Life Sciences  
University of Modena and  
Reggio Emilia  
Reggio Emilia, Italy

Federico Lemmetti  
Department of Life Sciences  
University of Modena and  
Reggio Emilia  
Reggio Emilia, Italy

Stefano Mazza  
Modena, Italy

ISBN 978-3-319-13757-5      ISBN 978-3-319-13758-2 (eBook)  
DOI 10.1007/978-3-319-13758-2

Library of Congress Control Number: 2015931542

Springer Cham Heidelberg New York Dordrecht London  
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# List of Abbreviations

AAB	Acetic acid bacteria
AGE	Advanced glycation end products
BC	Balsamic condiment
BI	Browning Index
BPF	Brown pigment formation
BV	Generic balsamic vinegar
BVM	Balsamic Vinegar of Modena
BVR	Base vinegar
BW	Base wine
CGM	Concentrated grape must
CI	Colour intensity
CKM	Cooked must
DOP	Denominazione di Origine Protetta
FA	Fixed acidity
GI	Geographical indication
GM	Grape must
GRAS	Generally recognised as safe
HMF	Hydroxymethylfurfural
HMW	High molecular weight
HORECA	Hotellerie Restaurant Cafè
IGP	Indicazione Geografica Protetta
IR	Infrared
LAB	Lactic acid bacteria
MEI	Methyl imidazole
PDO	Protected Denomination of Origin
PGI	Protected Geographical Indication
RD	Relative density
RT	Residence time
SEC	Size exclusion chromatography
TA	Titratable acidity
TBV	Traditional balsamic vinegar

TBVMO	Traditional Balsamic Vinegar of Modena
TBVRE	Traditional Balsamic Vinegar of Reggio Emilia
TSG	Traditional Speciality Guaranteed
VA	Volatile acidity

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# Chapter 1

## Vinegar: Definition, Diffusion, and Uses

The history of microbial biotransformation is closely associated with vinegar production, which dates back to around 2000 years BC. However, among fermented foods, vinegar has always been considered a poor commodity because it is not a “food”, lacks significant nutritional value, and it is produced from the transformation of richer and more nutritive fermented foods, such as wine and honey. Vinegar is a flavouring agent but also a preservative, and in some countries (Japan, China, and Korea) it is considered a healthy drink.

Vinegar production is a two step fermentation process, first by yeast and then by acetic acid bacteria, starting from almost any fermentable carbohydrate source: apples, pears, grapes, honey, syrups, cereals, hydrolysed starches, beer, and wine. With a few exceptions only, vinegar is an inexpensive commodity since low-cost raw materials are generally used for its production, like, for example, sub-standard fruits, seasonal agricultural surpluses, by-products from food processing, and fruit wastes.

Spirit vinegars are obtained directly through acetic oxidation of the ethanol from the distillation of fermented pulps and in some countries from petrochemical ethanol. Pyroligneous liquor, collected during wood carbonisation, is also called “vinegar” and it is used as an agricultural raw material, animal health product, cosmetic ingredient, and traditional medicine in Japan and East Asia (Mu et al. 2003, 2006).

Another subset includes flavoured vinegars, also known as herbal or fruit vinegars. Herbal vinegars consist of wine vinegars or white distilled vinegars, flavoured with garlic, basil, tarragon, cinnamon, cloves, nutmeg, or other herbs. Fruit-flavoured vinegars are wine and white vinegars sweetened with fruit or fruit juice to produce a characteristic sweet and sour taste. In these cases the fruit name does not indicate the raw material used for vinegar fermentation but rather the flavour added to achieve a specific taste and other characteristic features.

Some vinegars are very expensive and they are produced from regional and local foodstuffs according to longstanding traditions, such as Traditional Balsamic

Vinegar of Modena from Italy, Sherry vinegar from Spain, Oxos from Greece, and Shanxi mature vinegar from China.

## 1.1 Legal Definition and Composition

Vinegar production is regulated by an extensive set of statutes and the definition of vinegar varies from a country to another. The FAO/WHO defines vinegar 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. The residual ethanol content must be less than 0.5 % in wine vinegar and less than 1 % in other vinegars. (Joint FAO/WHO Food Standards Programme). In the USA, the Food and Drug Administration (FDA) requires that vinegar products must have a minimum acidity of 4 g per 100 ml, while in Australia the lower limit is 4 g per 100 g (FSANZ 2.10.1). This qualification ensures a minimum strength for vinegars sold in retail. There are currently no standards that identify vinegar; however, the FDA has established “Compliance Policy Guides” that the Agency applies for the labelling of vinegars, such as cider, wine, malt, sugar, spirit, and vinegar blends (FDA/ORA CPG 7109.22).

European countries have regional standards for vinegar produced or sold in specific areas. Unlike US law, the EU has established a threshold both for acidity and ethanol content. “Vinegar of X” is a general definition used for products with a minimum of 5 % (w/v) of acidity and a maximum of 0.5 % (v/v) of ethanol. Wine vinegar is obtained exclusively by acetous fermentation of wine, and it has at least 6 % of acidity (w/v) and 1.5 % (v/v) of ethanol (Regulation EC 479/2008). Table 1.1 shows the ranges of acidity and ethanol in some common vinegars.

**Table 1.1** Acidity and residual ethanol content in various types of vinegars

Vinegar	Acidity (% w/v)	Ethanol (% v/v)
Malt vinegar	4.3–5.9	–
Cider vinegar	3.9–9.0	0.03
Wine vinegar (semi-continuous process)	4.4–7.4 (8–14)	0.05–0.3 –
Rice vinegar	4.2–4.5	0.68
Chinese rice vinegar	6.8–10.9	–
Cashew vinegar	4.62	0.13
Coconut water vinegar	8.28	0.42
Mango vinegar	4.92	0.35
Sherry vinegar	7.0	–
Pineapple vinegar	5.34	0.67
Balsamic vinegar of Modena	>6.00	<1.5
Spirit vinegar		<20

In Europe vinegar is considered a flavouring or preservative food ingredient and, with few exceptions, its taste is generally sharp and sour. On the other hand, in Asia and Africa, vinegar is also a drink with a less sour taste. A lot of sweetened fruit vinegars characterised by low acidity and aromatic flavours are very popular in China and East-Southeast Asia. In Africa some fermented beverages can spontaneously acidify to produce alcoholic-acetous products, which are very difficult to classify as either alcoholic beverages or vinegars. Similarly, in Japan, black rice vinegar is usually diluted with fruit juice and consumed daily as a healthy tonic drink, representing 20 % of the Japanese vinegar market with a value of 21.46 billion yen in the year 2004. In some western countries, mainly the USA and Canada, apple cider vinegar is an old folk remedy claimed to be beneficial for treating a long list of diseases and it is consumed mixed with fruit juice. In the traditional wine-producing countries of Europe, it is very easy to differentiate between wine and vinegar, since the respective names are well established both historically and legally, with a precise definition for each. Wine can have a maximum of 1.2 g/l of acetic acid and, in all cases, the acetic acid content must be less than 1 % of the ethanol content. Vinegar must have a minimum of 6 % of titratable acidity and less than 1.5 % (v/v) of residual ethanol. In China, the word “vinegar” indicates products obtained from both fermentation and artificial processes, according to the Chinese National Standard definitions (CNS 14834, N5239 2004). In the previous National Industrial Standard of Vinegar, vinegar was classified in three grades, depending on its acetic acid concentration (3.5–4.5 %, 4.5–6 %, and >6 %, respectively). More recently, the Chinese State Administration Bureau for Quality and Technology issued a New National Standard Code of Condiments, introducing a definition of vinegar as either brewed or artificial (acetic acid blended with other ingredients, such as flavours). Each major vinegar also has its own local quality criteria and grading system. In the EU, some vinegars have a specific geographical indication (GI), a sort of intellectual property to protect regional food varieties and specialties, implemented by the European Union (EU) in a legal framework with three distinctive classes: Protected Designation of Origin (PDO), Protected Geographical Indication (PGI), and Traditional Specialty Guaranteed (TSG). See Chap. 8 for more details. Only a few vinegars bear a GI, an example being the Chinese Zhenjiang Xiang Cu (Table 1.2).

In general, vinegars are properly ascribed to the wider category of condiments, and they fall into two different sub-categories: with and without GI. Regarding the subset of balsamic vinegars, the main topic of this book, the grouping is more complex for at least two reasons: firstly, there is no precise and legally recognised definition of “balsamic vinegar”; and secondly, products bearing the “balsamic” tag can be very different one from each other (see Chap. 3).

Despite the fact that at first glance balsamic products can appear similar in appearance and sensory traits, they can actually differ markedly in ingredients, market claims, price, and legal status.

There are balsamic vinegars with more than 6 % of titratable acidity (TA, expressed as grams of acetic acid in 100 ml) and others with TA less than 4 %. TA is an important characteristic of vinegars. In oenology, TA is conventionally referred to the amount of strong basic molar solution necessary to neutralise (at pH

**Table 1.2** Vinegars with geographical indications (GI)

Dossier number	Country	Name	App type	Date	Status
IT/PDO/0017/1565	Italy	Aceto Balsamico Tradizionale di Modena (ABTM)	PDO	20/04/2000	Registered
IT/PDO/0017/1566	Italy	Aceto Balsamico Tradizionale di Reggio Emilia (ABTRE)	PDO	20/04/2000	Registered
IT/PGI/0005/0430	Italy	Aceto Balsamico di Modena (ABM)	PGI	04/07/2009	Registered
ES/PDO/0005/0723	Spain	Vinagre de Jerez	PDO	05/10/2011	Registered
ES/PDO/0005/0724	Spain	Vinagre del Condado de Huelva	PDO	05/10/2011	Registered
ES/PDO/0005/0726	Spain	Vinagre de Montilla-Moriles	PDO	09/10/2012	Published
CN/PGI/0005/0630	China	镇江香醋 Zhenjiang Xiang Cu	PGI	14/06/2012	Registered

Source: <http://ec.europa.eu/agriculture/quality/door/list.html>

8.2) a specific amount of wine, and it is expressed in grams of tartaric acid per litre of wine.

The major contribution to wine TA arises from non-volatile organic acids, a fraction conventionally defined as *fixed acidity* that includes tartaric, malic, succinic, and lactic acids. The *volatile acidity* fraction consists almost entirely of acetic acid, and it should be less than 1 g/l. As for wine, the TA of vinegars is determined by titration, and it is conventionally expressed as acetic acid, since this acid is the principal organic component of most vinegars. The exception to this rule is the balsamic category, in which sugars are the major constituents, and fixed organic acids can be present in large amounts (see Chap. 3).

From a sensory point of view, fixed and volatile acids play important but different roles. Olfactory pungency is due to acetic acid, while fixed acids are detected only by taste (see Chap. 7). With regard to the acidity fraction, some balsamic products are very unusual because they do not have significant amounts of acetic acid or even TA. In the authors' opinion, and also in the legislation of several countries, this class of balsamic products cannot be defined as vinegar, belonging instead to the wider and more general category of condiments (see Chap. 3). This applies to the two PDO Traditional Balsamic Vinegars of Modena and Reggio Emilia (TBVs), for which the lower limit of TA is 4 % (w/w) and 4.5 % (w/v), respectively. In Italy, the minimum legal TA content for wine vinegar is 6 % (w/v), and furthermore the TA of the finest TBV consists mostly of fixed acids (Lemmetti and Giudici 2011).

From a commercial point of view, the official annual production of TBV is so small that it can be considered a food curiosity, at less than 300,000 bottles of 100 ml capacity, a total of just 30,000 litres! In reality, the overall production

including the artisanal producers outside the consortium is larger, but a comprehensive survey does not exist.

In contrast, the other balsamic condiments without any sort of GI are commercialised in huge volumes around the world. These non-GI balsamic condiments differ from vinegars for their low acidity and their intended use. Balsamic condiments often imitate the characteristics of other expensive balsamic products, in some cases with good results although the price and quality vary widely and depend on the kind of raw materials used. Since balsamic condiments can contain thickeners, preservatives, colourants, flavours, and any other type of additive (both artificial and natural), these products are often called sauce, glaze, jelly, or dressing (see Chap. 3).

On the basis of certain particular characteristics and the technology applied, Shanxi mature vinegar could be grouped with balsamic vinegars, since it is cooked, concentrated, and its dark colour results from heat treatment and ageing. This product is the only balsamic vinegar not produced from grapes. Strictly speaking, there are also apple based balsamic vinegars, but the commercial quantities are practically zero).

## 1.2 Raw Materials and Critical Production Point

The majority of vinegars are of vegetable origin with only two exceptions: whey and honey. Whey is the residual milk serum from cheese making. It is rich in lactose and/or in the corresponding hydrolysed sugars, galactose and glucose, depending on the cheese making technology used. Honey is very rich in sugars (70–80 % w/w), mostly sucrose, fructose, and glucose, the ratio of which is influenced by the botanic origin of the nectar collected by the bees.

Several botanical species can be used for vinegar production on the condition they satisfy two main basic requirements. Firstly they must be safe for human and animal consumption, and secondly they must provide a direct or indirect source of fermentable sugar. General grouping can be made on the basis of the chemical composition of the edible parts of the plant and their proneness to fermentation:

- High acidity and ease of fermentation. pH below 3.5, glucose, fructose, and sucrose as main constituents; e.g. grapes, apples, plums, etc.
- Moderate acidity and ease of fermentation. pH between 3.5 and 4.5; e.g. figs, dates.
- Low acidity and ease of fermentation. pH higher than 4.5; e.g. palm sap.
- Non-fermentable. Hydrolysis is required before fermentation; e.g. seed crops and bananas.

The chemical composition of the raw materials exerts a strong selective pressure, effectively determining the dominant species of microorganisms involved in acetification.

The two fundamental steps in vinegar production are the preparation of the raw materials and fermentation. The first stage embraces all the necessary operations to ensure the availability of fermentable sugar and protein in solution, including slicing and/or crushing to obtain the fruit juice, enzymatic digestion of starch (for cereals), and in some cases cooking and steaming. In general, fruits require less processing than seed crops. Conversely, seed crops are more easily stored and preserved, making their use independent of harvesting time.

Fruits are highly perishable due to their high water content, and they need to be processed very quickly, in some circumstances (high temperatures, use of damaged fruits, etc.) even on the same day as harvesting. The logical outcome of these differences is that seed crops are typically transported and stored at large factories for transformation into vinegar. Instead, fruits tend to be processed at smaller, less technological factories, close to the production area.

### 1.3 Market Trends

From an economic point of view, vinegar production is a small industry in the overall economies of industrialised countries (Adams 1998). Global vinegar market shares in 2005 were balsamic vinegar (34 %), red wine vinegar (17 %), cider vinegar (7 %), rice vinegar (4 %), white vinegar (2 %), and finally other vinegars (36 %).

On the US market, white distilled vinegar has a 68 % unit share, cider vinegar 20 %, and specialty vinegar 12 % (data for 2007, [www.versatilevinegar.org](http://www.versatilevinegar.org)). In the specialty vinegar category, 39 % is from red wine, 30 % balsamic, 13 % all other wines, 12 % rice vinegar, and 6 % all other specialties.

The quantity of balsamic vinegar with GI, mostly Balsamic Vinegar of Modena (BVM), is in the range of 86–90 million litres (Anonymous 2014).

The main vinegar-producing countries are France, Italy, and Spain. In China the annual production for 2001 was  $2.0 \times 10^9$  kg of brewed vinegar and  $8.0 \times 10^9$  kg of distilled spirit vinegar (Wei 2001). In addition to white vinegar and fruit vinegars, brewed vinegar is also quite popular on the Chinese market. There are at least 14 types of traditional brewed vinegars, the six most widespread being Zhenjiang aromatic vinegar, Sichuan Bran vinegar, Shanghai rice vinegar, Jiangzhe rose vinegar, and Fujian red rice vinegar (Liu et al. 2004).

### 1.4 Use as a Preservative

Acetic acid is relatively weak and is well known as a very effective food preservative at low pH, and for this reason it is frequently associated with the strongest lactic acid. The bactericidal effect of acetic acid is related to the concentration of undissociated molecules in the medium. The non-ionic form of the acid is the

only species able to cross the cellular membrane and enter the cytoplasm, where the pH is close to seven. Here the molecules release  $H^+$  which dissipates the proton motive force of the cell (Eklund 1983, 1985; Salmond et al. 1984; Cherrington et al. 1990, 1991; Davidson 2001).

Vinegars with high TA are particularly effective against microorganisms and are used for the preparation of pickles, marinades, and sauces. In developing countries where food preservation technologies are scarce, vinegars can represent a very important option for preserving fresh fruit and vegetables from rapid deterioration, especially in the tropics where the prevailing environmental conditions accelerate food spoilage. Developing and improving small-scale vinegar production and food fermentation technologies in general was a goal of some FAO initiatives (Anonymous 1995).

Although the recipes for the preparation of pickles are numerous and often very elaborate, they all involve acidification, cooking, or pasteurisation, and then packaging which may follow or precede the heat treatment. The final pH value of the pickles must always be lower than 4.6, with at least 2 % of acetic acid in the brine. To achieve the required pH, a considerable amount of vinegar must be added to the vegetable raw materials, with a consequent marked modification of the sensory properties, and for this reason acidification of the brine is often achieved by addition of lactic and citric acid, which have a milder taste.

It is important to note that TBV's are not suitable for the preparation of pickles because of their low level of acetic acid, but they can be used as flavouring agents. Worldwide, the percentage of vinegar used as a preservative is close to 64 % of total vinegar production. It is used as an ingredient of dressings and sauces (16.8 %), for pickles (14.8 %), mustard (11.5 %), other processed foods (10.5 %), tomato products (8.5 %), and others (4.2 %). Source: <http://www.versatilevinegar.org/marketrends.html>.

## 1.5 Fermentation

Vinegar production involves different species of microorganisms during the various stages of the fermentation process, including lactic acid bacteria (LAB), yeasts, moulds, and acetic acid bacteria (AAB), whose habitats are often vegetable, fruit, and in general the raw materials used for vinegar production.

Two stages are common to all vinegars: alcoholic fermentation followed by acetic acid fermentation, respectively produced by yeasts and AAB. Other microorganisms are involved only in specific vinegars. Among yeasts, *Saccharomyces cerevisiae* is the most widespread species in fruit and vegetable vinegar, while the lactose fermenting yeast, *Kluyveromyces marxianus*, is the species responsible for whey fermentation. AAB are aerobic whole cell bio-catalysts involved in conversion of ethanol into acetic acid. AAB are common in fruits and in many sugar and acid environments, and their growth is promoted by procedures that increase

oxygen availability after yeast fermentation. Finally, a physical association of yeasts, LAB, and AAB is involved in kombucha tea.

There are ten AAB genera (Yuzo and Pattaraporn 2008), but the majority of species detected in vinegars belong only to the *Acetobacter* and *Gluconacetobacter* genera (Gullo et al. 2006). However, this outline is not definitive because other species and genera involved in vinegar production are probably yet to be described. Furthermore, the AAB taxonomy is under extensive revision and the species and genera will soon be reorganised.

Fermentation can be induced either as spontaneous fermentation, by back-slopping, or by addition of starter cultures.

In spontaneous fermentation the raw materials are processed and the environmental condition selects the indigenous micro-flora. The more stringent the growth conditions, the higher the selective pressure exerted on the indigenous microorganisms. In very acidic and sugary environment, for example certain fruit juices, only yeast and AAB can grow.

Spontaneous fermentation is only suitable for small-scale production and very selective juices, because the method is difficult to control and there are risks of associated spoilage microorganisms. In the majority of spontaneous fermentations, microbial succession occurs, often with LAB and yeasts initially dominating. These microorganisms produce, respectively, lactic acid and ethanol, which inhibit the growth of many bacteria species, in addition to reducing sugar content and so prolonging the shelf life of the goods. Moulds mainly grow aerobically, and so their occurrence is limited to specific production stages or on crops before and after harvesting. Moulds are a major safety concern, since some genera and species produce aflatoxins. Therefore, the moulds used for starch hydrolysis of seed crops should be generally recognised as safe (GRAS) microorganisms.

Back-slopping involves the use of part of a previously fermented batch to inoculate a new batch. This procedure increases the initial number of helpful microorganisms and ensures more reliable and faster fermentation than would occur spontaneously. Back-slopping represents a crude form of starter culture, with the best adapted species seeded over the indigenous population (De Vuyst 2000). Nevertheless, the finished products are still exposed to the risk of fermentation failure, and moulds or harmful spoilage bacteria can develop.

In general, back-slopping is considered the most practical because it encourages the growth of helpful yeasts, inhibits the growth of spoilage and pathogenic microorganisms, and avoids the laborious and time consuming starter selection process.

Back-slopping is particularly useful to inoculate AAB, which are very fastidious microorganisms and it is very difficult to establish a true starter culture. In semi-continuous submerged acetification, at least one-third of vinegar volume is left in the fermenter to inoculate the new wine (Gullo and Giudici 2008; Gullo et al. 2009), whereas surface layer fermentation requires the physical transplantation of an AAB biofilm.

A starter culture can be defined as a microbial preparation comprising large numbers of cells of a microorganism (in some cases more than one), which is added