CHAPTER 4

Technological and Microbiological Aspects of Traditional Balsamic Vinegar and Their Influence on Quality and Sensorial Properties

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Contents

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Abstract The term "balsamic" is widespread and popular all over the world of vinegar and fancy foods; it is used generally to refer to vinegars and sauces with a sweet and sour taste. However, the original is the European Protected Denomination, registered as "Aceto Balsamico Tradizionale of Modena, or of Reggio Emilia" that should not be confused with the "Aceto Balsamico di Modena" very similar in the name, but completely different for technology, raw material, quality, and sensorial properties. Traditional balsamic vinegar is made by a peculiar procedure, that starts with a thermal concentration of freshly squeezed grape juice, followed by alcoholic and acetic fermentations and, finally, long aging in a wooden barrel set, by a procedure which requires a partial transfer of vinegar from cask to cask with the consequential blending of vinegars of different ages. In addition, water transfer occurs across the wood of the barrels, the result being an increase of solute concentration of the vinegar. The chemical and physical transformations of the vinegar are mainly directed by the low water activity of the vinegar. High-molecular polymeric compounds are the main and characteristic constituents of original and old traditional balsamic vinegar, and the major cause of its rheological and sensorial properties.

I. INTRODUCTION

"Traditional balsamic vinegar of Modena" and "traditional balsamic vinegar of Reggio Emilia" (here collectively abbreviated as TBV) are two similar types of vinegar, both characterized by a strong local identity as well as chemical–physical and sensory properties, defined by Italian and European legislation. One of the main features of TBV is its aging period, fixed at a minimum of 12 years. During this time, chemical–physical changes take place and give to the vinegar its characteristic sensorial properties. In short, time plays the central role in the overall production process.

Control over TBV and its reputation is protected by the constitution of local associations of producers and experts in the field, usually called *Consortia*. The aim of these *Consortia* is to promote the culture of TBV and to survey their production and distribution. There are now four active *Consortia* in both of the interested cities—Modena and Reggio Emilia—with the same purpose and functions. For the TBV of Modena, these are *Consorzio Produttori Aceto Balsamico Tradizionale di Modena*, *Consorzio*

Tutela Aceto Balsamico Tradizionale di Modena, and *Consorteria dell'Aceto Balsamico Tradizionale di Modena*; for the TBV of Reggio Emilia, they are *Consorzio fra Produttori di Aceto Balsamico Tradizionale di Reggio Emilia*, *Confraternita dell'Aceto Balsamico Tradizionale di Reggio Emilia*, and *Sindacato Produttori Aceto Balsamico Tradizionale di Reggio Emilia*.

A. The "balsamic family"

TBV belongs to the wider group of vinegars made from grapes, known worldwide under the generic and legally dubious appellation: ''balsamic vinegars.'' The market for balsamic vinegars and related products has developed very quickly in a short space of time. It is, nowadays, composed of a wide range of products that at first glance can seem quite similar not only in appearance but also from the sensory point of view; however, all these products can actually be very different in respect of their ingredients, market claims, price, and legal status. It can be very difficult to understand the true differences among this heterogeneous group of products. For this reason, we attempt to present here a summary of what can be called the ''balsamic family.'' A first clarification can be achieved through observing the legal definitions, as a base on which we can identify three sets, and related protection levels:

1. *Condiments*: This set is composed of products that cannot be defined as vinegars because of their composition, low acidity level or intended use. There are no limitations as to their composition: they can contain thickeners, preservatives, colors, flavors, and any kind of additives, both artificial and natural. They can be liquid, solid, or semisolid. Sometimes they can resemble vinegars, even balsamic vinegars, in many aspects. Their price and their qualitative level are widely variable and depend on the cost of the raw materials. Balsamic sauces, glazes, jellies, flavored vinegars, dual oil and vinegar compositions, various fruit and vinegar compositions, vinaigrettes are members of this set. General food laws, according to national and international regulations, cover these products. The reasons for the production of condiments are manifold: (i) offering variants of extant products, with altered properties, for example, lower acidity, food colouring, flavor, higher viscosity, etc.; (ii) selling good quality products at a low price, for example, by avoiding the aging time; (iii) developing vinegar- or balsamic-like products containing new raw materials, for example, fruits, vegetables, honey, etc.
2. *Vinegars*: These are a subset of the larger condiments group. According to a generally accepted definition, vinegars are liquids obtained by the acetic fermentation of any suitable foodstuff. Vinegars are legally defined in many states of the world and sometimes are subject to specific legislation. They usually have a minimum acidity level. To cite just a few

examples in Europe, vinegars must have a minimum of between 5 and 12 g of acetic acid per 100 ml of product, except wine vinegar which has a minimum of 6 g; however, in the United States, the minimum is 4 g/100 ml (FDA ORA quality manual) and in Australia, it is 4 g/100 g (FSANZ Standard 2.10.1). Vinegars are usually cheap everyday condiments, but their price can rise if they require complex production steps, long aging or highly priced raw materials (e.g., PGI wines). This group includes vinegars such as wine vinegar, rice vinegar, apple cider vinegar, malt vinegar, and honey vinegar that are usually obtained from a single foodstuff through alcoholic and subsequent acetic fermentation. For some countries, flavored vinegars are included in this category.

3. *Specialty vinegars*: This subset includes vinegars that are legally recognized as peculiar and different from the products of the "normal" vinegar group, for historical, cultural, or other plausible reasons. These types of vinegar are subject to special and dedicated regulations or under special protection such as PGI or PDO. The vinegars belonging to this group are often expensive and produced on a reduced or small scale, like TBV. However, they can sometimes reach huge production numbers, like the Jerez Vinegar or the balsamic vinegar of Modena (not to be confused with TBV), which are actually industrial products consumed worldwide like wine vinegar. Balsamic family products can belong either to the condiments set or to the specialty vinegars set. Figure 4.1 illustrates the three legal levels of vinegar and the balsamic family, while Table 4.1 shows a summary of the balsamic family features. The technological and microbiological aspects described in this review are not specifically mentioned, except in regard to TBV.

B. Historical note

TBV is generally described as vinegar of ancient origin, possibly dating back to the Middle Ages and deeply embedded in the gastronomic history of the Italian Provinces of Modena and Reggio Emilia. TBV has achieved worldwide fame in recent times, boosted by several marketing promotions. Notwithstanding their famed time-honored traditions, the culture, the history, and the complexity behind this product are far from being correctly reconstructed and properly understood. It seems that serious comprehensive research into the original production process, the aging system, the sensory profile, and the analytical parameters that properly describe TBV has not yet been accomplished. The piecing together of reliable information from various sources, and the consequent reconstruction of the true history of the TBV of Modena or Reggio Emilia, is a very challenging task because documents and testimonies about them are few

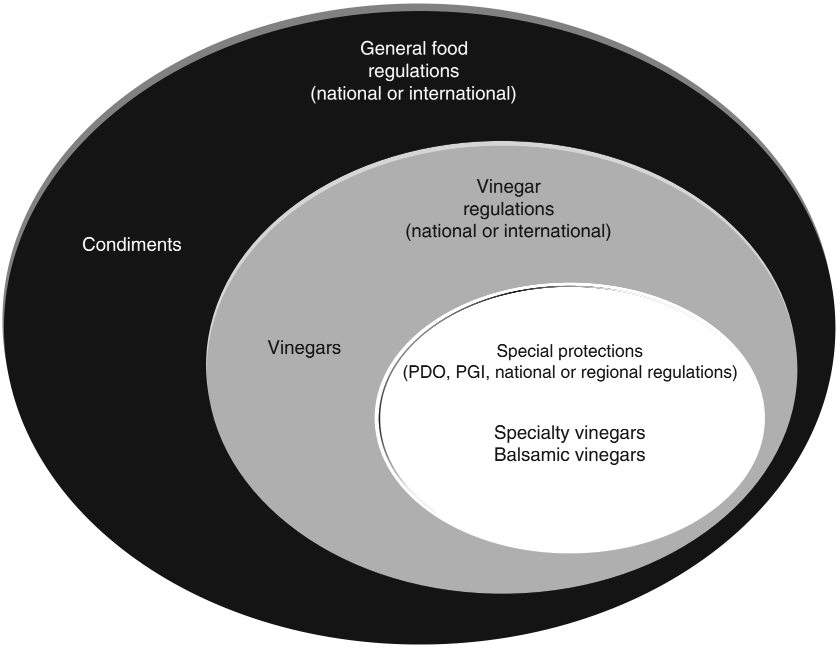

FIGURE 4.1 The three legal levels of the balsamic family: condiments, vinegars, and specialty protected vinegars.

and often confusing. Secondly, very few of the publications on TBV and related products can be considered as a valuable source of information, because producers or commercial associates to create a cultural background to use for marketing and promotional purposes. The claimed antiquity of this product and its culture is affected by too many expectations, and derives more from *ex nihilo* publications than from serious research, whether historical or scientific. However, we can report that according to popular folklore, the world famous condiment now known as TBV originated in the Italian region of Emilia. As far as we can deduce from recent research, it has been steadily produced in its present form in the provinces of Modena and Reggio Emilia; however, little is known about the original recipes and related production practices (Benedetti, 2004; Giudici *et al.*, 2008).

As an example of a common misunderstanding of the early documents on vinegar, one of the first generally accepted written testimonies about balsamic vinegar is the allusion to a precious and highly prized condiment made in the province of Reggio Emilia in the poem *Acta Comitissae Mathildis*, also known as *Vita Mathildis* or *De Principibus Canusinis*, written in the 12th century by the monk Donizo of Canossa. In the first book of

TABLE 4.1 The main features of vinegars and "balsamic family"

Product type	Vinegar	Grape must (%)	Added sugars	Caramel color (E150a–d)	Thickeners	Flavors	Aging	Market protections	Legal acidity limit	State
Vinegar (wine, apple, honey, malt, whey, . . .)		No	No	No	No	Natural flavors only	Optional not mandatory	No	EU 5 –12% USA > 4% AUS > 4%	Liquid
Special vinegars										
Jerez vinegar (Spain)	Yes	0–50% (also used as color)	No	No	No	No	Yes	PGI	>7%	Light to high-bodied liquid
Orléan vinegar (France)	Yes	No	No	No	No	Natural flavors only	Yes	PGI	>5%	Liquid
Balsamic vinegars										
Balsamic vinegar	Yes	5–60%	No	Yes	No	–	Usually no	No	–	Light to high-bodied liquid
Balsamic vinegar of Modena	Yes > 10%	5–60% (*should be >20%*)	No	Yes 2% max	No	No	60 days min (*usually not observed*)	PGI	>6%	Light to high-bodied liquid
Traditional balsamic vinegar of Modena	Yes (*should NOT be used*)	50–100 %	No	No	No	No	12 years min (not declarable)	PDO	>4.5%	High-bodied liquid
Traditional balsamic vinegar of Reggio Emilia	Yes (*should NOT be used*)	50–100%	No	No	No	No	12 years min (not declarable)	PDO	>5%	High-bodied liquid

Condiments										
Liquid condiments not qualifiable as ''vinegar'' nor ''balsamic''	Depends on recipe	Depends on recipe	Permitted	Depends on recipes	Permitted (usually not used)	Permitted	No	No	No	Liquid
Balsamic sauces, glazes or jellies (condiments)	Permitted—depends on recipe			Depends on recipe	• Modified or native starch (maize, potato, wheat, . . .) • Glucose/ fructose syrup (fluid or dehydrated) • Pectins • Dextrose • Gums (xanthan, guar, . . .) • Carob seed flour • Lactose	Permitted	No	No	No	Thick liquid—semisolid

the poem, there is an account of a luxurious gift given to the Emperor Henry III of Franconia by the Marquis Bonifacio, Lord of Canossa (Reggio Emilia), who in order to display his wealth and power, gave as a present a silver barrel filled with the famous, precious, exquisite vinegar made in the Castle of Canossa (Donizo, *Acta Comitissae Mathildis*). An important detail not mentioned by previous authors is that in the Italian poem the adjective ''balsamic'' is never used, even though the words ''balsam'' and ''balsamic'' were already present in Italian dialects. What this ancient document tells us is only that a famous vinegar was made in this area; nothing is said about its properties and composition. Similarly, other early references to vinegar are always cited in order to demonstrate the ancient tradition of ''balsamic'' vinegar making; however, the documents from the area of Modena and Reggio Emilia always cite only the word ''vinegar.'' It seems that the first use of the adjective *balsamic*, referring to a particular kind of vinegar, is not documented before the 18th century, where it is found mainly in records of donations or nuptial gifts. From the 19th century, however, we can see a number of testimonies clearly speaking of ''balsamic vinegar'' and the first recipes or suggestions on how to produce it from the grape must (Benedetti, 2004).

Even if the conclusion that the balsamic vinegar cited in those records is directly related to the one we know now is in some way inviting, it should be kept in mind that we have no further evidence to back up this statement. According to other authors, it is also possible that the adjective ''balsamic'' has been used since those early years to designate any kind of generically aromatic vinegar or mixed vinegar—not just the product obtained from the fermentation of grape must alone (Saccani and Ferrari Amorotti, 1999), as it now must be according to the legal definitions of ''traditional balsamic vinegar of Modena'' and ''traditional balsamic vinegar of Reggio Emilia.'' These considerations go to show that we cannot be sure when in history the TBV of Modena and Reggio Emilia began to be produced in the way we are accustomed to today. In addition, no conclusive research has been done to understand historical aspects of the production such as the types of grapes (e.g., by pollen analysis), cooking and fermentation methods, and possible aging systems. Regarding the aging practice, we have to note that the technique used today and which is expressly prescribed by law is the so-called *rincalzo* (''refilling''), which is carried out by fractional blending in a barrel set, so that the finished product is a mixture of ages, with the average age gradually increasing as the process continues over many years. The vinegar refines in a barrel set of at least five casks of different sizes and woods. Every year, a small quantity of the aged vinegar is withdrawn from the smallest barrel. This barrel is then refilled from the contents of the preceding barrel and this operation is repeated, up to the first and largest cask, which receives the new cooked grape must (Giudici and Rinaldi, 2007). This method of aging is not exclusive to TBV, even though it is an essential part of the TBV culture and mandatory in production regulations. It is also possible that the *rincalzo* method did not originate in Emilia, nor even in Italy: it is clearly a system invented to save time from year to year in the aging of each barrel (see “Refilling procedure” chapter) and is virtually identical to the Spanish *solera* system. According to extant documents, the *solera* system was used in Spain after the Napoleonic Wars and began to spread abroad after the second half of the 19th century (Simpson, 2003). It is thus possible that this aging practice spread from Spain to Italy during the Cisalpine Republic (a French client republic founded by Napoleon Bonaparte in Northern Italy that lasted from 1797 to 1802) mediated by French oenological culture. Further historical research in this direction would be highly useful to understand whether the *rincalzo* and *solera* systems are in some way related, or developed independently in Spain and Italy.

C. “Balsamic”: From Semitic languages to Italian legislation

The word *balsamic* in the English language is attested as having been in use since the Middle Ages as an adjective from the noun *balsam*, meaning (i) any agency that soothes, restores, or comforts or (ii) certain officinal plants and/or products thereof.

The words *balsamic* and *balsam* have gone through different language families, over a very long period of time. In the modern languages of Europe and the Americas, the linguistic form of the name and derived adjectives are still well preserved, because of the common Latin and Greek origin, as we can see from Table 4.2. The various forms of the word balsam all come from Latin *Balsamum*, which itself came from Ancient Greek *Bálsamon* and has been recorded since the 4th century BC. The form *Bálsamon* has actually been borrowed from the Hebrew *baśam*, which is related to Aramaic *busma* and the Arabic *baśam*, meaning “balsam, spice, perfume, incense.” The root on which the words *balsam*/*balsamic* has formed is thus clearly of Semitic origin (Table 4.3) and is usually represented as *bśm* (Köbler, 2006; Murtonen 1986; Nielsen, 1986; The American Heritage Dictionary of the English Language, 2004; Vocabolario degli Accademici della Crusca, 2008). Regarding the ancestral origin of this ancient root, very little is known and we can only hypothesize that the Semitic *bśm* may be akin to an even older root recognizable in a larger number of languages. So, the adjective “balsamic” is associated not only with TBV but also with an entire class of products far less expensive and “traditional,” well represented in the vinegar and condiment market, with much higher selling volumes than TBV (as shown below).

This alternative class of products belongs to the “balsamic vinegar of Modena” (BVM) group and has been legally recognized since 1965. Whereas TBV production is small scale and expensive, BVM is cheaper

TABLE 4.2 Comparison of the word "balsam" in different European languages

Language	Form	Attestation time
Modern English	Balsam, balm	After 1500 AD
Old Spanish (Old Castilian)	Balsamo (?)	900–1500 AD
Middle English	Balsamum/balsaum	1175–1225 AD
	Basme	1220 AD
Old French	Basme/balsme/ balme	1000–1300 AD
Old Norse	Balsam	1000–1300 AD
Old English	Balsam/balsame	Before 1000 AD
Old High German	Balsamo	Around 1000 AD
Vulgar Latin (*Toscano* Italian)	Balsamo/balsimo	Since 900 AD

The American Heritage Dictionary of the English Language 2004; The Merriam-Webster Online Dictionary; Köbler, 2006; Vocabolario degli Accademici della Crusca (2008).

TABLE 4.3 The borrowing of the *balsam* forms by Latin and Ancient Greece from Semitic languages

Language	Form	Attestation time
Latin	Balsamum	4th century BC
Ancient Greek	Bálsamon	4th century BC
Hebrew	Bośem/baśam	
Aramaic	Besma/busma	
Arabic	Baśam	

The Merriam-Webster Online Dictionary (2004); The American Heritage Dictionary of the English Language (2004).

and intended for a very wide market; thus it is a fully industrial product. To distinguish these two classes of balsamic vinegar, the ''traditional'' and the ''industrial,'' in 1983, a group of producers proposed and obtained legal recognition of the denomination ''traditional balsamic vinegar of Modena,'' which still exists today in the PDO regime granted by the European Union. The difference between the two denominations relies solely on the adjective ''traditional''; this is misleading, as it means that the cheaper product receives a sort of marketing benefit from TBV, just through the presence of the adjective ''balsamic'' in its commercial name. Here it is worth mentioning that, through the centuries, the names associated with balsamic vinegar have never been clear to the general public, and that the existence of these two differing ''classes'' of balsamic vinegars has been recorded at least since the 19th century (Saccani and Ferrari Amorotti, 1999), more than 100 years before true industrialization began. This situation is further confused by the fact that before the legal distinction was made between TBV and BVM, balsamic vinegars were recorded in historical documents under a variety of names such as *aceto alla modenese* (''Modena-style vinegar''), *aceto del duca* (Duke's vinegar), and *aceto balsamico naturale* (natural balsamic vinegar).

D. Legal aspects

TBV was granted PDO status by the European Community on April 17, 2000 by EC Council Regulation No. 813/2000, in which the Production Regulations proposed by the Consortia were accepted, both for Modena and for Reggio Emilia TBV; they were published on May 15, 2000. The two products must be retailed in a distinctive bottle, one for each TBV, with a legally defined shape and design, sealed and numbered. The grape used for the TBV must come from the Provinces of Modena or Reggio Emilia, following the specified local tradition, and without additives. Each TBV has its specific Production Regulation, though there are no substantial differences between the two recipes. Basically, there are only two significant aspects: the minimum total acidity, which is 4.5% for the TBV of Modena and 5% for the TBV of Reggio Emilia; and the minimum density which is 20 °C: 1.24 g/ml for the TBV of Modena and 1.20 g/ml for the TBV of Reggio Emilia. The reason for these discriminations is possibly due to a desire to impose a parametric differentiation between the two types of TBV, which would otherwise remain indistinguishable.

According to product regulations in force, TBV is made exclusively from a single ingredient: grape must, cooked in open vats, and matured with a long acetification process through natural fermentation, followed by progressive aging in a series of casks made of different woods (oak, chestnut, mulberry, cherry, and juniper being the most common) even though it is still unclear whether the type of wood has any real influence on the final product (Giudici *et al.*, 2008).

Concerning the single-ingredient composition, it is necessary to state that, in spite of such strict legal statements, basically derived from historical practice, many producers, from the second half of the 20th century onward, have begun to produce TBV by mixing cooked grape must and wine vinegar, thereby avoiding the difficult fermentation phase. This ''mixture preparation'' for TBV has probably been borrowed from industrial balsamic vinegar production, which eschews fermentation after blending, thereby gaining faster results, and more control of acidity and viscosity. However, over a long time scale, this system can easily produce negative effects, mostly in the sensorial features, because it does not produce the overall complexity of the fermented vinegar, causing in many cases excessive bitterness, loss of heady flavors, flat aromas, and other sensory degradations. Many chemical–physical defects are also associated with the two-ingredient system, such as liquid-to-solid transitions and the progressive loss of total acidity (Giudici *et al.*, 2008).

E. Sensorial aspects

A commission of expert tasters trained by the Consortia performs the sensory analysis of TBV. According to production regulations and published PDOs, the sensory profile of TBV should be evaluated by hedonic judgment expressed in all cases through a numeric score by a panel that evaluates the following sensory attributes: (1) visual-related aspects such as free flowing, color and clearness; (2) olfactory-related aspects such as flavors (fragrance) and aroma in terms of their intensity, persistence, and pungency; and (3) taste-related aspects of vinegar body in terms of its intensity, harmony, and acidity. Ideally, the vinegar color is defined as dark brown, nearly black, but full of ''warm light''; good texture requires the vinegar to be dense, with a fluid- and syrup-like consistency; good fragrance requires the vinegar to be sharp and unmistakably but pleasantly acid; good aroma requires the vinegar to show the traditional, inimitable sweetness, and sourness in perfect proportion: vivid, full-bodied, velvety, intense, and lingering.

The score achieved is used by the Consortia to certify the two possible levels of aging that according to product regulations should be specified as (1) ''*Affinato*'' (''fine'') or (2) ''*Extravecchio*'' (''fine old''). No reference to the year of production or the presumed age of the product may be stated on packaging; ''Extravecchio'' is the only age-related statement permitted on packaging for this aging level.

It is remarkable that, in existing regulations, no methods are specified to evaluate in an objective way the effective aging of the products; it is evaluated only through a panel-tasting test, whose effectiveness for this purpose is clearly inadequate. Among the attempts that have been made for age evaluation, a simple calculation model has been proposed recently to estimate the TBV age (see TBV age paragraph) but, at present, no official Consortia in Modena or in Reggio Emilia have adopted it, or any analogous procedure, as a control system.

II. BASIC TECHNOLOGY

The process of TBV production can be divided into four main steps as shown in Fig. 4.2: (i) cooking of grape juice; (ii) cooked must fermentation; (iii) acetic oxidation; and (iv) slow aging of vinegar. The cooking of the grape juice takes between 12 and 24 h and produces chemical and physical

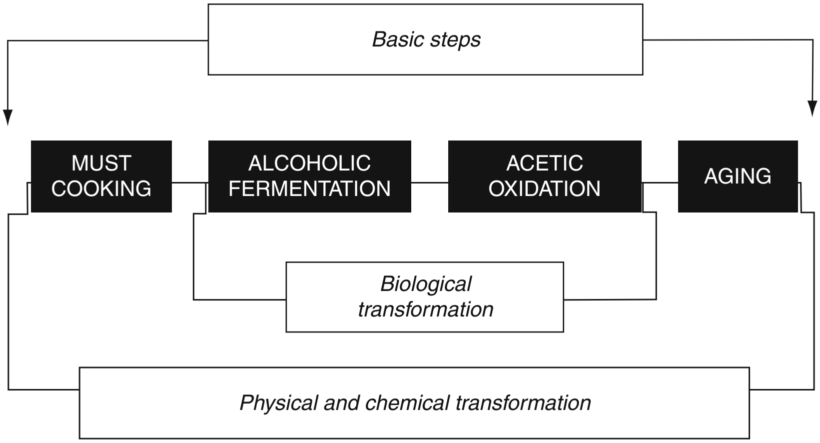

FIGURE 4.2 Basic technology of the traditional balsamic vinegar.

modifications that strongly affect the successive stages of TBV production. After cooking, the must undergoes alcoholic fermentation of sugars by yeasts, followed by acetic oxidation of the ethanol by acetic acid bacteria (AAB), both biological processes taking place in a cask, the ''*badessa*.'' These two biological processes take more or less 1 year to be fully completed. The alcoholic conversion is easier to control than that of the acetic acid, which is a serious problem for TBV production because incomplete oxidation of the ethanol produces vinegars with low titratable acidity, affecting negatively the sensory perception of the end quality. Finally, such vinegar undergoes slow aging in the barrel set to concentrate flavors. Aromatic compounds accumulate and intensify over decades, with the vinegar kept in fine wooden casks becoming sweet, brown, viscous, and concentrated. The aging of vinegar is the longest step and it occurs inside a set of barrels of different volumes, made of different types of wood.

A. Raw material

1. The grape

In oenology, it is a general opinion that the quality of wines is born in the vineyard. It is widely accepted that a large variability in grape composition is expected as a function of the grape cultivar, climate, and agricultural conditions (Ribéreau-Gayon *et al.*, 1980). Grape variety and cultivar, agronomical operations, climate, and degree of grape ripeness exert a strong influence on wine composition and sensorial properties. The literature on these topics is extensive, consistent, and widely accepted. However, to our knowledge, there are no significant research data related to grape composition and TBV quality. Literature on this topic is poor and vague, suggesting simply that the grape needs to be "well-ripened." This is a really weak point, because TBV is a sweet/sour vinegar and the balance between acidity and sweetness has been proved to be a very important trait, one which, as everyone concerned realizes, may also be influenced by the berry composition. The major grape constituents are sugars and organic acids, the former consisting mainly of glucose and fructose in a ratio close to 1:1, their precise quantity being strongly related to grape variety, climate, and time of harvest. The other constituents, organic acids, are present in salt form: among them are tartrate, malate and, in smaller amounts, citrate. The balance between sugars and acids changes with grape ripening: in particular, the sugar increases with time whereas malate decreases. Grapes permitted in TBV production come from vineyards of the Emilia Romagna region and have a minimum sugar content of 15% (w/w). According to DOP rules, numerous grape varieties are allowed, with white or red berries; among the most important are Lambrusco (including all varieties and clones), Ancellotta, Trebbiano (all varieties and clones), Sauvignon, Sgavetta, Berzemino, and Occhio di Gatta. However, although the DOP rules on grape varieties and their sugar content are very stringent, to date, there has been no scientific study of the quantitative influence of grape composition on TBV quality. In addition, the length of aging and the refilling procedure required in making TBV complicate the picture. Actually, the balance between sugars and acids is easily modified by collecting grapes with different degrees of ripening. Our recent data show that the ratio between sugars (expressed in g/l) and organic acids (expressed as g/l of tartaric acid equivalents) ranges from 13 to 30, when the sugar content of the berries is at least 16% (w/v). But the proper sugar/acid balance for TBV is still an intriguing unsolved question. For example, in the past, it was usual practice to add common ash obtained from burned woods to decrease the acidity of the grape must before cooking (Sacchetti, 1970). Nowadays, this practice is in total disuse, and a contrary approach is standard: grape musts with high-fixed acidity are required to increase the acidity of TBV. In wine science, other grape constituents such as anthocyanins, polyphenols, and tannins have inspired scientific studies due to their central role in sensorial properties, shelf-life, and wine stability (Ribéreau-Gayon *et al.*, 1980). During the cooking of grape juice in an open pan, a portion of these minor compounds separate from liquid bulk due to their interaction with proteins and other colloidal material; while, another portion can be involved in polymerization reactions with sugars during the aging process (see Section II.D.3).

The grape berry composition is *per se* not uniform: the pulp is richer in sugar and organic acids; the skin in anthocyanin (red grape) and flavonol (white grape); the seed in polyphenols and tannins (Ribéreau-Gayon *et al.*, 1980). The grapes are crushed with specialized wine making equipment, the juice being separated from the pomace by soft pressure for up to a yield of 70%. Juice composition is expected to be strongly influenced by the pressing conditions of grapes: the earlier juice is rich in sugars and acids, the latter in tannins and salts.

B. Cooking technology

Fresh grape juice is boiled down to approximately 30% of the original volume to produce a concentrated must. Cooking is carried out in batch-type stainless steel pans operating at atmospheric pressure, heated by a direct flame burner for 12/24 h at a temperature close to boiling point. The heating causes the formation of foams and natural colloids, mainly coagulated proteins, all needing to be removed mechanically from the surface of the must.

Cooking is presumed to exert a key role in the TBV quality. The transfer of mass and energy takes place, and many chemical reactions and physical transformations are activated. Cooking induces the formation of compounds that will act as precursors in the formation of particular sensory-related constituents of TBV; at the same time, it induces the formation of compounds potentially toxic for microbial activity and/or human consumption.

This is a long-term practice for some traditional Italian foods and beverages, including traditional balsamic vinegars, and for some special liquor known as ‘‘vino cotto’’ (‘‘cooked wine’’). Cooked grape must is also produced in Spain for sweet wines (Riviero-Pérez *et al.*, 2002). The general use of cooked must in European countries is regulated by International laws (Regulation CE 1493/99, 1999).

1. Heat-induced changes during cooking

a. Solute concentration The most evident effects of cooking are water vaporization and color change. The process of vaporization involves simultaneous heat and mass transfer, and depends on the heat supply (Fig. 4.3). The extent of vaporization is strictly related to the effectiveness of both the mass and energy transfer. Both, in turn, depend on the rheological properties of grape juice as well as on the evaporator size and on fouling resistances increasing during cooking. Mass and energy fluxes are coded and presented in Fig. 4.3. In a simplified model where the juice is brought instantaneously to the boiling point (Tb), the quantitative analysis of fluxes is based on the conservative mass balance equation:

$$G_2 = L_\mathrm{i} - L_\mathrm{f} \tag{1}$$

while, for water it is

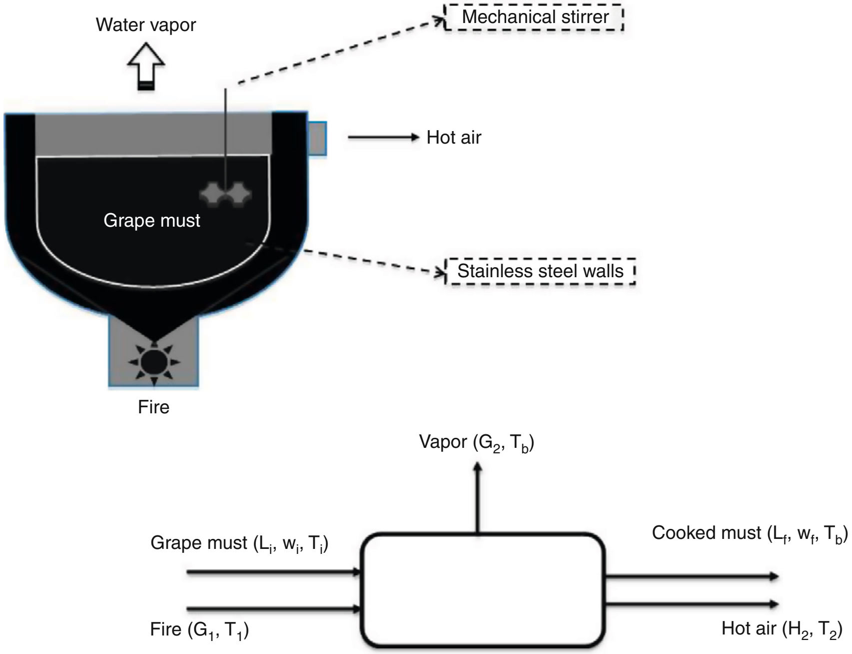

FIGURE 4.3 Schematic representation of process streams during must cooking (upper side). Symbols indicate the steady-state condition (bottom side): L_i (kg/batch) is the fresh grape must entering the open pan; G_2 (kg/batch) is the water vapor leaving the open pan; G_1 and H_2 (kg/batch) are the hot-dried air streams; w_i (kg/kg) is the solid concentration in L_i; T is temperature (°C).

$$L_{\mathrm{i}}(1 - w_{\mathrm{i}}) = G_2 + L_{\mathrm{f}}(1 - w_{\mathrm{f}}) \tag{2}$$

Therefore, the amount of water lost by vaporization at boiling temperature per batch can be calculated by

$$G_2 = L_{\mathrm{i}}\left(1 - \frac{w_{\mathrm{i}}}{w_{\mathrm{f}}}\right) \tag{3}$$

A mass balance can be analyzed for each solute of interest: in the case where it takes part in a chemical reaction, so that its specific extensive properties (such as mass or moles) can be measured over time, the generation term must be accounted for in the balance equation:

$$\text{inflow} - \text{outflow} + \frac{\mathrm{d}s}{\mathrm{d}t} = 0 \tag{4}$$

where "*s*" is the solute of interest, which can act as reactant, intermediate or product of a reaction.

The size of the pan is equally important and deserves consideration: it determines the rate of juice-heating. The heat transfer rate, expressed as kg/batch, can be calculated by

$$q = UA(Tb - Ti) \tag{5}$$

where U is overall heat transfer coefficient (W/m^2/°C), A is the area (m^2) of the pan walls, and q is the heat flux (kJ/s). It should be noted that the coefficient U is a function of the sum of the resistances to the heat transfer. The most important resistances are the increasing viscosity of the juice and the semisolid layers on juice surfaces.

b. Chemical changes It is well documented that heat up-take induces some complex transformations in sugar-rich foods. Some of them lead to the formation of brown compounds, the so-called ''melanoidins.'' These compounds are expected to have significant effects on the end quality and in consumer acceptance of widely consumed dietary goods (e.g., coffee, cocoa, bread, malt, and honey) thanks to their antioxidant properties (Delgado-Andrade and Morales, 2005; Rufian-Henares and Morales, 2007; Verzelloni *et al.*, 2007), antimicrobial activity (Rufian-Henares and Morales, 2007), antihypertensive properties (Rufian-Henares and Morales, 2007), prebiotic activities (Borrelli and Fogliano, 2005), browning properties (Gogus *et al.*, 2007; Hofmann, 1998), and foam stability (D'Agostina *et al.*, 2004). Sometimes, melanoidins are considered to be potentially undesirable compounds playing a strong role in the binding of nutritionally important metals (O'Brian and Morrisey, 1989) and flavored compounds (Hofmann *et al.*, 2001). All of these functionalities are presumably derived from the fact that the melanoidin structures are sufficiently diverse to have complex functional behavior. The heat supply leads to the formation of some potential toxicants: methylglyoxal, furfuryl, and furan derivates including 5-hydroxymethylfurfural (HMF). HMF is a cytotoxic, genotoxic, and tumorigenic agent (Janzowski *et al.*, 2000; Zhang *et al.*, 1993). However, HMF and methylglyoxal act as intermediate chemicals and their concentration and lifetime are related to the initial reducing sugar concentration and to the extent of sugar degradation. In model systems, the accumulation of 5-hydroxymethyl furfural is a function of the kind of sugar and amino acids (Gogus *et al.*, 2007); in grape juice, fructose is more reactive than glucose. Masino *et al.* (2005) observed that cooking promotes the formation of some furanic congeners in grape must: at the end of cooking they found from about 3.4 to 6.8 ppm of HMF; from 3.8 to 2.3 g/kg of furoic acid; and from 7.8 to 4.8 ppm of furfural as a function of the starting grape must. Water activity plays a central role on the kinetics of HMF formation upon grape must cooking (Muratore *et al.*, 2006).

Sugars undergo other degradation reactions leading to the formation of acetic and lactic acids, the last one in both the D and L isomers (Fig. 4.4). In particular, the extent of these degradations is strictly related to the grape juice composition. The time-dependent accumulation of solutes follows different kinetics leading to high-chemical potentials that are responsible for the accumulation of newly formed compounds with high-molecular size, over 500 kDa (Fig. 4.10).

c. Physical changes During cooking, the grape juice undergoes profound changes related to the solute interactions which generally affect juice density, refraction index, viscosity, boiling point, specific heat, and coefficient of thermal expansion (Rao *et al.*, 1984; Saravacos, 1970; Schwartz and Costell, 1986). Cooking stops all enzymatic browning reactions that rapidly occur inside fresh grape musts by polyphenol oxidase, and progressively promotes grape must discoloration (due to the heat-induced deactivation of proteins including browning enzymes). In addition, it promotes nonenzymatic browning reactions.

The most evident effect of water vaporization is the increase of solute concentration and viscosity; clarified juice concentrates show Newtonian behavior (Ibarz and Ortiz, 1993; Rao *et al.*, 1984; Saenz and Costell, 1986; Saravacos, 1970) although some authors have found a small pseudoplasticity in the flow of grape juice for concentrations above 55°Bx (Brix degree). However, other authors have observed that juice concentrates behave as Newtonian fluids even at high-soluble solid concentrations of 60–70°Bx (Rao *et al.*, 1984; Schwartz and Costell, 1986). It has been postulated that pectins and tartrates affect the rheology of grape juice in a significant way during cooking (Moressi and Spinosi, 1984; Saravacos, 1970), but others assert that high-molecular size biopolymers induced by thermal treatment play the most important role (Falcone and Giudici, 2008).

C. Fermentation

1. The scalar fermentation

Fermentation is the name conventionally attributed to any industrial transformation that involves microorganisms. In TBV production, there are two distinct fermentations: alcoholic and acetic; the first is carried out by yeasts of different genera and species, the second by AAB. Usually, the two fermentations occur in the same vessel (*badessa*) and are performed by wild strains without any control. The results are unpredictable: sometimes the alcoholic fermentation is inhibited by acetic acid produced by AAB; often the acetic acid fermentation, for various reasons, does not occur. To solve the problem, a two-stage fermentation procedure has been suggested: the oxidation of ethanol should always follow alcoholic

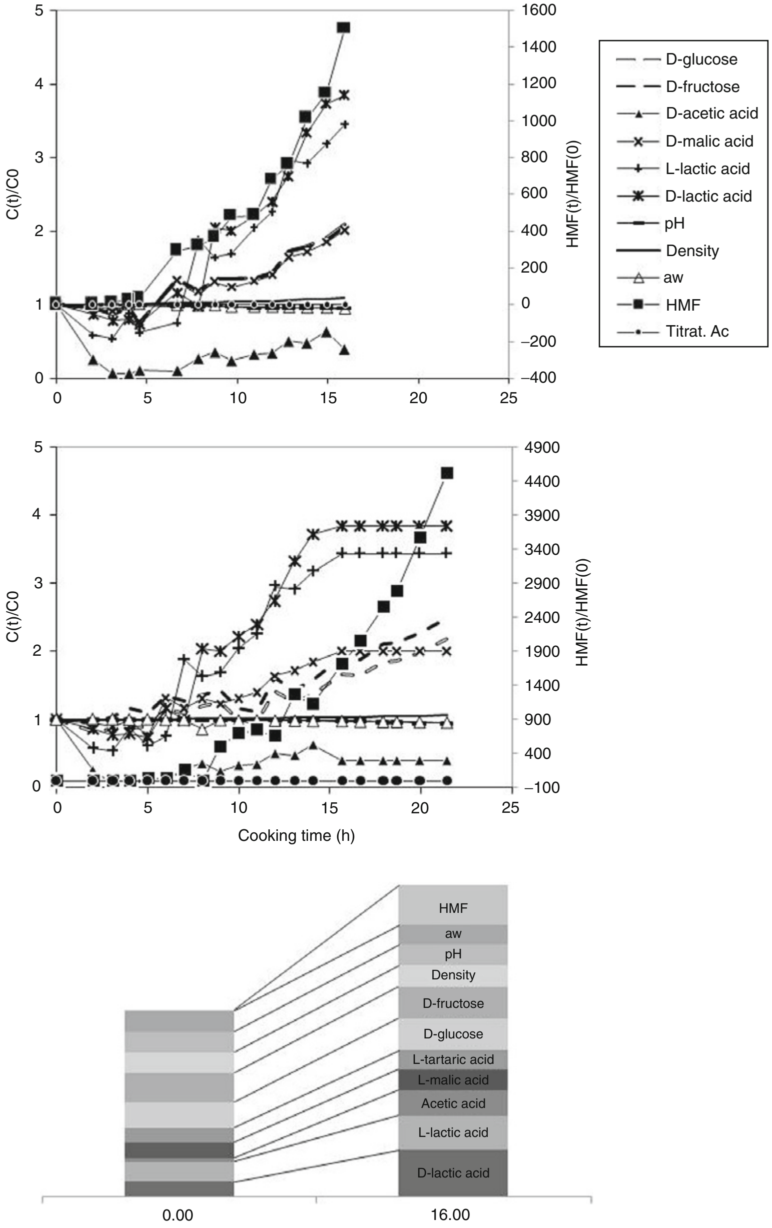

FIGURE 4.4 Effect of the cooking time on the composition of grape juice from the Trebbiano grape variety. "A-type" grape must was harvested in mid-October 2009; while "B-type" grape must was harvested 2 weeks later. All investigated properties have been normalized in respect to their initial value to the fresh juice; corresponding differences before and after 16 h of heating are reported at the bottom of the table.

fermentation, in a scalar way and in two different vessels (Giudici *et al.*, 1992). In the past, it was accepted that fermentation of cooked must was a commensalistic interaction between yeasts and AAB (Sacchetti, 1932, 1970). The idea of commensalisms has not completely gone away; and some practices, such as the use of one single vessel for both fermentations, are still in use.

2. Yeast and alcoholic fermentation

a. The yeast involved The alcoholic fermentation of grape juice has been an ecologically well-studied process since the time of Pasteur. During the last 100 years, papers have focused on the dynamics of yeasts during wine fermentation, elucidating the role of *Saccharomyces* and non*Saccharomyces* yeasts (Amerine and Kunkee, 1968; Davenport, 1974; Fleet and Heard, 1993; Kunkee and Amerine, 1977; Kunkee and Bisson, 1993). Only recently has the importance of yeasts for TBV production and quality become clear that their metabolism is responsible for major physical–chemical changes of cooked must (Landi *et al.*, 2005; Solieri *et al.*, 2006, 2007).

The first study of TBV yeast dates back to the 1930s. In a paper published by Sacchetti (1932), later summarized in a book (Sacchetti, 1932, 1970), the author recognized strains belonging to the genus *Zygosaccharomyces* (very similar to those recognized as *Z. rouxii*, according to the latest nomenclature) as the predominant TBV yeasts, and proposed the idea of a commensalistic interaction between yeasts and AAB. In the 1980s, Turtura and coworkers investigated the main TBV-related species (Turtura, 1984, 1986; Turtura and Benfenati, 1988). They reported the presence of *Z. bailii* and *Z. rouxii*, identified on the basis of morphophysiological features, such as the ability to grow at 1% acetic acid concentration. Afterward, the occurrence of *Saccharomycodes ludwigii* strains, together with *Z. rouxii* and *Z. bailii*, was demonstrated. Recently, a complex yeast microflora, including *Z. bailii*, *Z. rouxii*, and *S. ludwigii*, *Z. mellis*, *Z. pseudorouxii*, *Z. bisporus*, and *Z. lentus*, two species belonging to *Hanseniaspora* genus (*H. osmophila* and *H. valbyensis*), two *Candida* species (*C. stellata* and *C. lactis-condensi*) and an *S. cerevisiae* species have been found by Solieri *et al.* (2005, 2006).

All *Zygosaccharomyces* species recovered from TBV are osmophilic yeasts growing in media with a high sugar concentration (50–60%) and are responsible for the spoilage of sugary beverages and food (Fleet, 1992; Loureiro and Malfeito-Ferreira, 2003; Pitt, 1975). Among TBV yeasts, it is remarkable that many strains belong to *Z. lentus*, a new osmotolerant species first described by Steels *et al.* (1998, 1999) in spoiled beverages, such as orange juice and tomato ketchup. TBV has also been the isolation source of a new putative species, provisionally named *Z. pseudorouxii* (Solieri *et al.*, 2006).

Some other TBV-associated species, mainly *Candida* and *Hanseniaspora* spp., are rarely detected in spoiled foods and beverages, but are prevalently associated with the early stages of wine fermentation, even though they can also occur during middle and late phases. In spite of being a nonosmophilic species, *S. cerevisiae* has been frequently detected in the lowest sugary, cooked musts (Solieri *et al.*, 2006); this agrees with Deak and Beuchat (1996) who found that some strains of *S. cerevisiae* are able to grow in food or beverages with high sugar content.

b. The product of fermentation The influence of *S. cerevisiae* and non*Saccharomyces* yeasts on the flavor of wine and wine vinegar is well characterized (Ciani, 1998; Fleet, 2003). *C. stellata* strains have been found to produce high glycerol, succinic acid, ethyl acetate, and acetoin concentrations that influence positively the aromatic profile of wine vinegar (Ciani, 1998). Two other TBV-associated yeasts, *H. osmophila* and *S. ludwigii*, produce high amounts of ethyl acetate, acetoin, acetic acid, and acetaldehyde and are considered detrimental yeasts in wine fermentation (Ciani and Maccarelli, 1998; Granchi *et al.*, 2002). *S. ludwigii* has been proposed for continuous production of vinegar by Saeki (1990). The role of yeast secondary metabolites in TBV sensorial quality has not yet been studied; deeper knowledge of this topic is required.

3. Acetic acid bacteria and oxidation

The generic name ''acetic acid bacteria'' indicates a heterogeneous group of strictly aerobic bacteria. Nowadays, the AAB group includes spoilage bacteria of fermented beverages, bacteria exploited in biosynthesis with highly economically relevant molecules, vinegar bacteria, and recently pathogenic bacteria. However, historically, AAB were recognized as ''vinegar bacteria,'' showing the important traits for bioconversion of ethanol into acetic acid (Gullo and Giudici, 2008). The most relevant phenotypic feature of AAB is related to their ability to carry incomplete oxidation of broad ranges of carbohydrates (aldehydes, ketones, and organic acids) that are secreted almost completely into the medium.

In TBV, the oxidative fermentation is carried out by spontaneous acetification due to the natural occurrence of AAB in the environment. Recently, the application of selected AAB strains in TBV production has been proposed, and a procedure has been developed for the scale-up of the fermentation process at the vinegar factory scale (Fig. 4.5) (Gullo *et al.*, 2009). However, the actual basic technology used for cooked must fermentation is without any physical–chemical control; therefore, fermentation breakdowns cannot be predicted. Ecological studies on AAB of TBV were first conducted by culture dependent methods. The oldest studies date back to 1970 and 1988 (Sacchetti, 1970; Turtura and Benfenati, 1988) and deal with the ecological aspects of AAB in TBV.

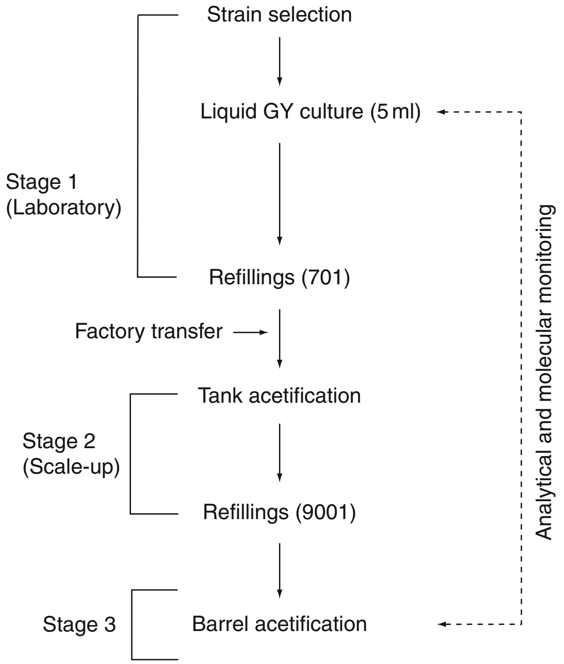

FIGURE 4.5 Critical steps in the scale-up of the fermentation by selected starters from laboratory to industrial scale (adapted from Gullo *et al.*, 2009).

More recently, intermediate products of TBV have been investigated using both culture-dependent and -independent methods. In particular, strains belonging to the following species were detected: *Gluconacetobacter europaeus*, *Gluconacetobacter hansenii*, *Gluconacetobacter xylinus*, *Acetobacter pasteurianus*, *Acetobacter aceti*, and *Acetobacter malorum*. All of these species were previously detected in different kinds of vinegars, except for *A. malorum* (De Vero *et al.*, 2006; Gullo and Giudici, 2006, 2008). Among the species recovered until now, the *Ga. europaeus* strains seem to be the most widespread.

a. The products of oxidation Many studies have been carried out on AAB metabolism, mainly focusing on the physiological behavior of strains in defined media as well as an understanding of the enzymatic system of AAB. The majority of studies focused primarily on the *Gluconobacter oxydans* species because of its relevance in biotechnological applications, such as the synthesis of Vitamin C, gluconic acid, and several biopolymers (Adachi *et al.*, 2003; De Vero *et al.*, 2006; Deppenmeir and Ehrenreich, 2009; Macauley *et al.*, 2001). In general, sugars, alcohols, and polyols are oxidized via two alternative pathways by two types of enzyme systems, differing in location and function within the cell, that are capable of dehydrogenation reactions.

However, the chemical composition of TBV, relating to the AAB oxidation products, is highly variable and depends on several factors such as the type of grape must, cooking modality, oxidation temperature, and others. A lack of information about the compounds originating from AAB is due to the fact that importance has been given to substances detected in the final product while the dynamics of these compounds over the course of the production time has been ignored. Some volatile compounds (acetoin, diacetyl and ethyl acetate) have proved to be useful in characterizing the bacterial strains involved in the acetification process (Gerbi *et al.*, 1995). Interest has been shown in TBV as well as in other vinegars produced by traditional technology, with the aim of differentiating them from vinegars produced by quick processes (Tesfaye *et al.*, 2002).

Among organic acids, acetic acid is the most studied compound, due to the fact that it is strictly related to both the safety and the sensorial quality of TBV. High amounts of this organic acid can mask other sensorial properties and potentially adulterate vinegars. Among sugar acids, several authors have studied gluconic acid. The availability of glucose together with very low nitrogen and phosphorus sources are the optimal conditions for the gluconic acid production in vinegar: it has been found at up to 3.0% in TBV (Giudici, 1993; Plessi *et al.*, 1989) and at lower concentrations (0.37% and 0.28%) in wine vinegar, cider vinegar, and balsamic vinegar (the last obtained with a short-time acetification of a blend of cooked grape must and wine vinegar). The occurrence of gluconic acid has been proposed as an indicator of TBV quality, a way of differentiating it from other balsamic vinegars (Giudici and Masini, 1995).

D. The barrel set

The TBV is aged in a barrel set usually composed of at least five casks of different sizes and woods, mainly chestnut, acacia, cherry, oak, mulberry, ash, and, in the past, juniper. It is generally agreed that the type of wood used plays an important role in the aging process and sensorial properties of the TBV. The casks are arranged in decreasing scalar volume, generally from 75 to 16 l (Fig. 4.6). The smallest one contains the oldest vinegar of the set and is conventionally numbered ''1.''

The barrel set behaves essentially as a device for vinegar concentration. Two types of process streams take place: (i) the mass transfer from vinegar bulk toward the ambient throughout the wooden casks (water lost by evaporation, vinegar leakage throughout the staves, and solutes lost by precipitation) and (ii) the mass transfer from cask to cask spanning the barrel set (see ''refilling'' paragraph). Evaporation varies as a function

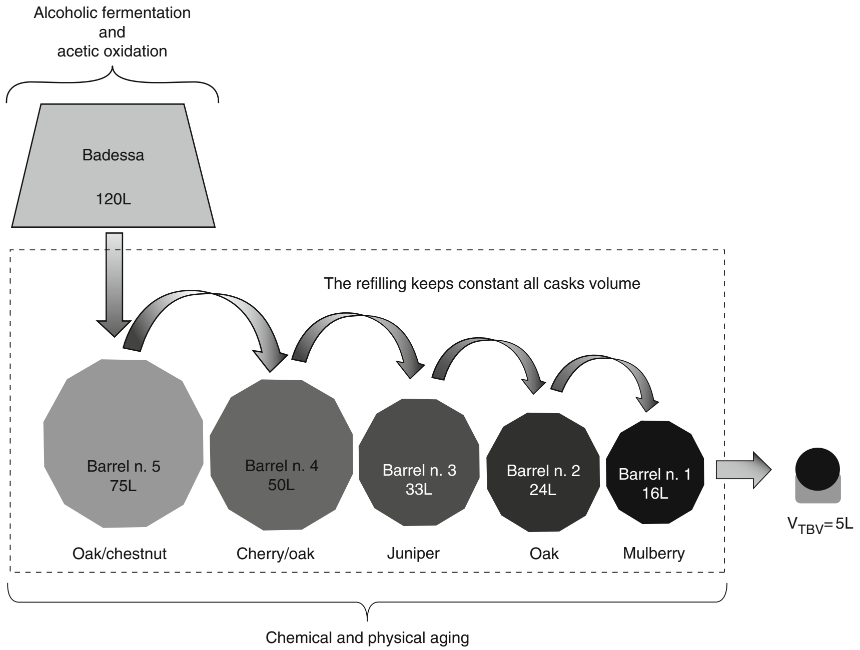

FIGURE 4.6 A possible configuration of the barrel set. The withdrawn of TBV is made once per year. The refilling procedure is aimed at keeping constant the volume of vinegar in all the casks. The casks have a decreasing volume. Conventionally the smallest cask is referred to as "barrel no. 1."

of cask features and ambient conditions. Leakage may occur in the presence of holes and/or defects in the wood, often caused by rapid changes of hygroscopic conditions, and may result in buckling and cracking of the stave. Solute precipitation may occur depending on the precise amount of insoluble matter and their degree of solubility. In particular, the wooden cask works as a molecular separation device: low-steric dimension molecules pass selectively throughout the wood pores and the higher ones are retained (Siau, 1984), resulting in a general increase of the solute inside the vinegar bulk. The residence time (RT) of the vinegar inside the barrel set depends on both the rate of water lost by evaporation and the mass flux to volume ratio. The rate of water transfer through the wooden casks is influenced by several factors including the hygroscopicity (water activity) of the vinegar, the relative humidity of the surrounding air, the type of wood, its thickness, and the size and shape of the cask. Another factor is the way the barrels are closed on the topside: historically, each cask had either a large rectangular opening, covered by a cloth, or a round hole covered by a round rock from local rivers.

Recent evidence shows that the use of hermetically sealed barrels improves the TBV quality because it serves to preserve the vinegar's aromatic compounds.

1. Yield

The *yield* can be calculated easily as the mass fraction ratio between the cooked must and TBV withdrawn from the smallest cask of the barrel set:

$$\text{yield} = \frac{m_{\text{TBV}}}{m_{\text{FCM}}} 100 \qquad (6)$$

Yield indicates the ability of a barrel set to concentrate cooked must at given operating conditions. It is worthwhile noting that neither the volume of casks nor their number affects *yield*, which is affected only by the solid concentration in the cooked must coming from the ''*badessa*.'' For example, a cooked must containing 35–40% (in weight) of glucose and fructose is fermented by yeasts to have ethanol at around 5% and residual sugars between 20% and 25%, and at the end of aging between 40% and 50% in weight. Using Eq. (6), it is easy to calculate that a yield of TBV ranging between 40% and 65%—in other words, 3 kg of cooked and acidified must—can provide between 1.2 and 1.8 kg of TBV. The yield of TBV production does not depend on the size of the barrel set; rather, it depends on the flux of masses across the barrel set.

2. Refilling procedure

a. Process streams in wooden barrels The barrel set is managed with a traditional procedure that resembles the *Solera* method used for making Sherry wine. In particular, a coded procedure is followed for the annual cask refilling, consisting of withdrawing only a part of the vinegar from the smallest cask, which is then refilled with the vinegar coming from the next barrel, and so on. The biggest one receives new cooked and oxidized must (see Fig. 4.6). The purpose of refilling is to keep the volume of the vinegar constant inside every cask of the barrel set, reintegrating the product withdrawn and/or lost by evaporation and/or transferred from cask to cask.

The mass transfer of vinegar throughout the wooden staves involves the following aspects: (i) a transition time is required to reach the outside of the stave; (ii) the loss by evaporation consists mainly of water; (iii) the mass flux throughout the staves is a function of wood type; (iv) the mass flux is more pronounced when the wood is in direct contact with the vinegar; and (v) the mass flux changes with ambient conditions. The most common model used to describe the mass transfer across the wooden barrels assumes three mechanisms in series: absorption, diffusion, and evaporation. A Fickian model for calculating water losses from oak casks depending on conditions in aging facilities has been recently proposed by

Ruiz de Adanaa *et al.* (2005). These authors proved that the pressure potential drives the water flow across the staves within free domains according to Darcy's law; while, the diffusion mechanism drives the water transfer within hygroscopic domains inside the staves according to the Fick's law. The lower the relative humidity, the higher the transfer of water. However, mass diffusion through the wooden staves represents a rate-limiting stage of the vinegar transfer throughout a barrel, and Fick's second law describes quantitatively the mass transfer under isothermal conditions:

$$\frac{\partial M}{\partial t} = \frac{\partial}{\partial x}\left(D\frac{\partial M}{\partial x}\right) \tag{7}$$

where x is the thickness of the stave, and D the diffusion coefficient (m^2/s) of the wood. In a simplified model, diffusion occurs under a gradient of moisture in the wood, and the mass flux is proportional to the driving force; that is, the difference between the average water content within the wood and the water content at the saturation equilibrium point of wood. Conversely, water may be transferred also from the ambient into the wood. Of course, mass transfer occurs if driving forces can prevail over the opposing resistances such as the concentration of the hygroscopic solutes in vinegar and the thickness of the stave. The aging causes the water activity of vinegar to decrease due to the increase of solute interaction and their ability to link water molecules so that the water activity will become progressively the limiting driving factor for the evaporation.

It is useful to define an overall mass transfer coefficient as a function of the ambient conditions so that a surface emission coefficient gradient can be defined by

$$\mathrm{d}S = \frac{\partial S}{\partial T}\mathrm{d}T + \frac{\partial S}{\partial RH}\mathrm{d}RH + \frac{\partial S}{\partial V}\mathrm{d}V \tag{8}$$

where temperature (T), relative humidity (RH), and air velocity (V) contribute differently to the mass transfer. In general, higher temperatures and lower relative humidity make vinegar concentration faster.

b. Vectorial concentration model There is experimental evidence that the concentration of solutes increases from left to right along the barrel set (Fig. 4.6) (Sanarico *et al.*, 2002). This vectorial product concentration (VPC) is due to two additive factors: the annual refilling of casks and the water lost by evaporation. In particular, the refilling procedure is responsible for solute dislocation: the same amount of solutes (dry matter) is transferred from cask to cask (Table 4.4) but in a decreasing volume, as required by the water evaporation. The theoretical basis of the VPC model is explained with the analysis of the degree of freedom for the process

TABLE 4.4 Analysis of the degree of freedom (DOF) of the process streams across a barrel set consisting of five of casks, see Fig. 4.9

	Set of equations (E)
Technological constraints	$V_{i+1} = kV_i$
Physical constraints	$s_i + w_i = 1$
Overall volume transferred across the barrel set	$R_{\mathrm{TBV}} = R_{\mathrm{FCM}} - \sum_{i=1}^{5}(W_i)$
Dry matter transferred from cask to cask	$m_{\mathrm{FCM}}s_{\mathrm{FCM}} = m_i s_i = m_{\mathrm{TBV}}s_{\mathrm{TBV}}$
Mass of water leaving the barrel set	$\sum_{i=1}^{5}(m_w)_i = [m_{\mathrm{FCM}}w_{\mathrm{FCM}}] - [m_{\mathrm{TBV}}w_{\mathrm{TBV}}]$
Mass of water transferred from cask to cask	$m_{\mathrm{FCM}}(1 - s_{\mathrm{FCM}}) = m_i(1 - s_i) = m_{\mathrm{TBV}}(1 - s_{\mathrm{TBV}})$

Assuming null all vinegar lost (L_i), we have 17 unknown variables (i.e., six streams with two components, that is, water and solutes; five streams with one component, i.e., water), 12 mass balance equations, and constraints: DOF = 22-E-11-5. The mass transfer becomes a solvable problem if we have 11 independent equation (E).
w_i and s_i are the mass fraction of water and dry solutes within the streams, respectively; $R_{i+\,1,i}$ refers to the vinegar volume withdrawn from the barrel $i + 1$ and used to refill the barrel i; W_i are the water volume lost by evaporation; m_i are the mass fluxes; i is the number of the barrel (with $1 \leq i \leq 5$ and "1" is the smaller barrel).

streams involved in any cask as reported in Fig. 4.7. All sets of equations necessary to evaluate mass transfer are reported in Table 4.4.

A practical way to save time in TBV production is to extend the vectorial concentration in the barrel set for example by increasing the number of casks.

3. The aging

a. Definition of the age and its descriptors The meaning of the age as related to a special food product such as TBV calls for two distinct concepts. Firstly, irrespective of how much or how little the vinegar properties change, we can define the TBV age as the time that it spends in the barrel set. In this case, the TBV age corresponds to the turnover time; that is, the time it takes to completely replace the product in the cask (RT). Secondly, accounting for time-dependent changes in chemical, physical, and sensorial properties occurring at a given RT, we can define an appropriate physical age of TBV. In both cases, the variable chosen to describe the TBV age must have additive property. Consequently, as is true for every extensive property, the descriptors of TBV age must depend on the size of the barrel set and will accumulate during the storage time.

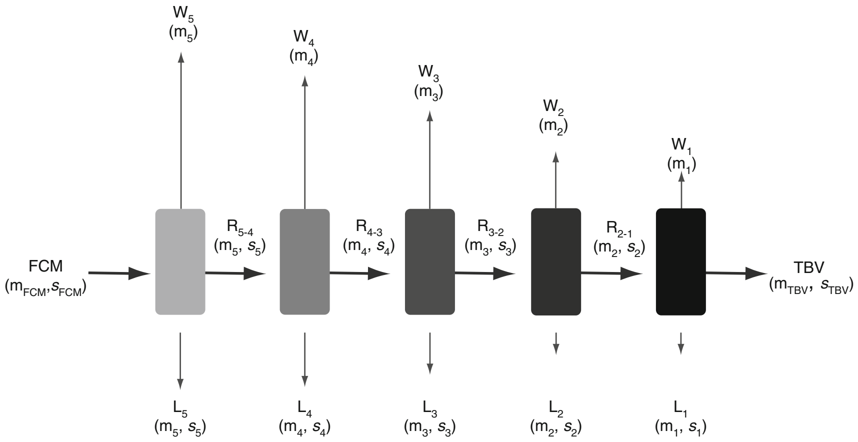

FIGURE 4.7 Vinegar streams across the barrel set during aging. FCM refers to the new cooked must from the "badessa"; TBV refers to the vinegar volume ready to be bottled; W_i refers to the water volume lost in evaporation; $R_{i+1,i}$ refers to the vinegar volume withdrawn from the barrel $i + 1$ and used to refill the barrel i; L_i refers to the vinegar volume lost from staves; m_i are the mass fluxes; s_i is the mass fraction of dry solutes within vinegar streams; i is the number of the barrel (with $1 \leq i \leq 4$ and "1" is the smaller barrel). The vinegar volume inside the casks (i) is kept constant by the annual refilling: the solute translocation caused by refilling practice and water lost for evaporation determines the vectorial product concentration across the barrel set.

b. Residence time Due to the refilling procedure, each barrel contains a blend of vinegars of different composition and age: intuitively, one would expect the age to increase according to the VPC model and, therefore, from the largest barrel to the smallest. What complicates the assessment of RT is the discontinuous refilling practice: each barrel, every year, contains a new blend of vinegars of different ages. Casks behave as continuous evaporators and as splitter devices at the end of each year during refilling (Fig. 4.8). Giudici and Rinaldi (2007) proposed a simple model to calculate the age of the vinegar blend as the pondered value of the RT of vinegar inside the cask (i) before refilling plus the RT that the vinegar used for refilling has spent inside the previous cask ($i + 1$):

$$RT_{\text{vinegar blend}}n = V_iRT_in + R_iRT_{i+1}n \tag{9}$$

where n is the number of years (and the number of refillings performed).

In particular, the authors formulated $RT(n)$ as a way of defining the following extensive quantity that accumulates over the years:

$$RT_in = RT_i(n-1) + 1 \tag{10}$$

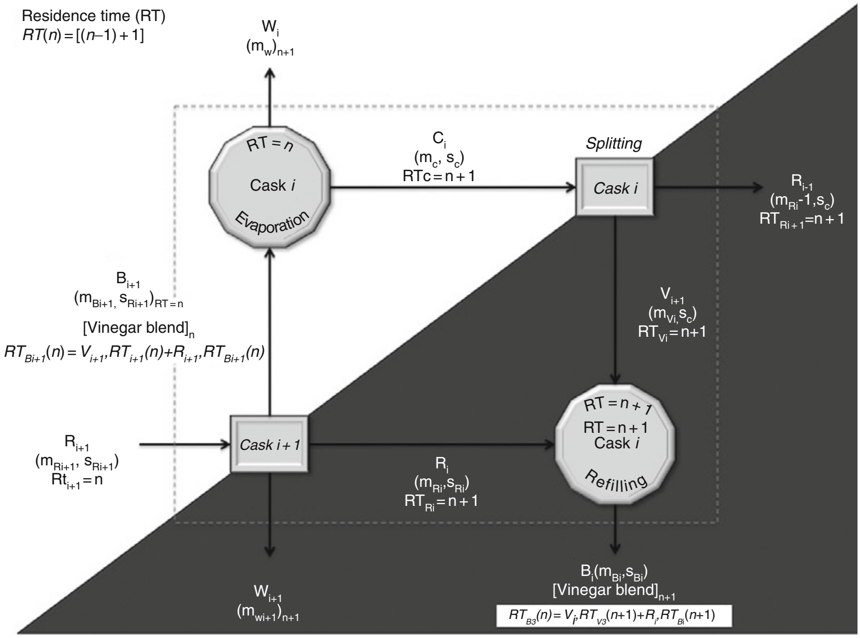

FIGURE 4.8 Virtual splitting of the vinegar streams involved in the cask (*i*) of the barrel set during aging. Casks behave as continuous evaporators and, as splitter devices at the end of each year, during refilling. The age of the vinegar in the cask (*i*) is the pondered value between the RT of the vinegar coming from the cask ($i + 1$) and that of the vinegar present into the cask (*i*).

This definition of the age is valid for $1 \leq i \leq 4$ because the age of the cooked and acidified must is assumed to be nil.

The study carried out by Giudici and Rinaldi (2007) demonstrated that: (i) the RT of vinegar in a barrel set reaches an upper finite limit at the steady-state; (ii) limit increases from the biggest to the smallest cask according to the ratio between refilled volume and volume of cask; and (iii) the upper aging limit is a decreasing function of the volume leaving the barrel set, that is, the quantity of TBV withdrawn. It is worth noting that the above-mentioned limits are reached with n; that is, the number of years theoretically goes to infinity, but in reality, each RT limit is more or less reached after very few years of storage (Fig. 4.9A and B).

The mathematical model can be used to find how much TBV can be withdrawn to satisfy the legal limit of 12 years or, conversely, to verify whether the minimum legal age required has been reached at a given productivity. According to the proposed model, an increase in the production rate leads to a decrease of RT: in fact, the solids concentration inside the process streams decreases, even though more feed (cooked

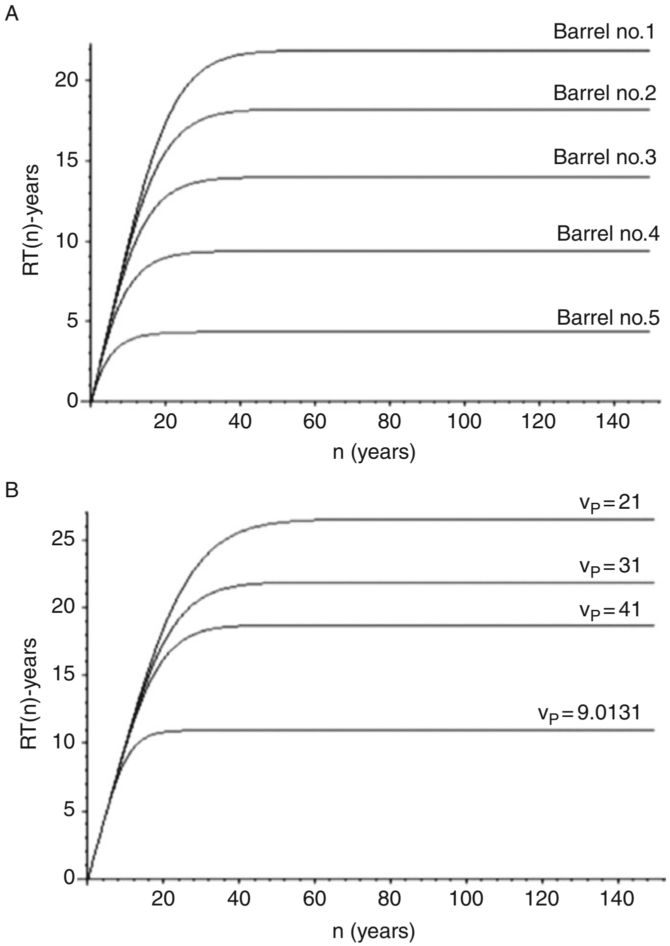

FIGURE 4.9 Numerical simulation of the effects of the refilling on the upper limit of the RT for each barrel of the set with a withdrawal of 3 l (V_p), and on the hypothesis of constant water evaporation (5% of the volume per year) (A); upper limit of the RT for the smallest cask with different volumes of vinegar withdrawn (B). The increase of the vinegar withdrawn leads the vinegar age to decrease (adapted from Giudici and Rinaldi, 2007).

must from *badessa*) is being processed, because the time of residence is reduced.

c. Physical ripening time At a given RT, each vinegar is characterized by thermodynamic properties that are the results of specific microstates, the actual interaction among all the constituents. Refilling practices, of course, periodically perturb all chemical potentials among vinegar constituents as

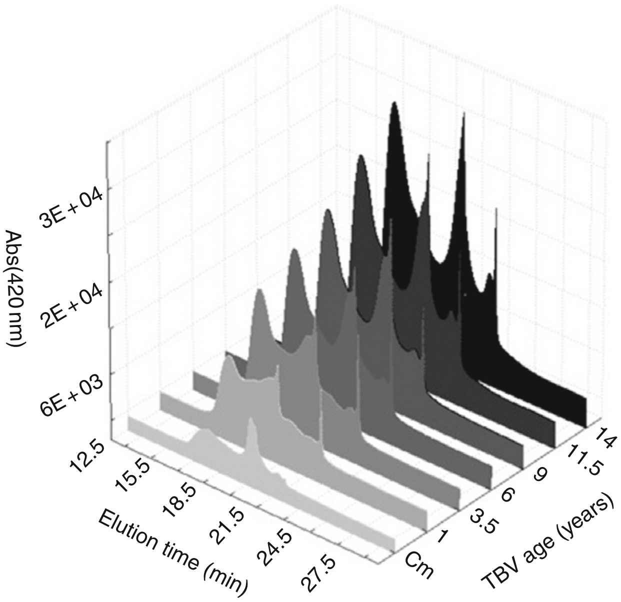

FIGURE 4.10 Distribution of the brown-labeled chromophores of melanoidins accumulated in the vinegar during aging. The vinegar age was calculated as RT; elution time is the HPLC-column elution time; and Cm represent the cooked must for which RT is considered to be nil. Signal is the 420 nm-radiation absorption of melanoidin (adapted from Falcone and Giudici, 2008).

well as the balance of their interactions. The thermodynamic state of TBV is described by intensive and extensive quantities. The former (temperature, volume, pressure, internal energy, chemical potential) are independent of the amount of material in the vinegar at a given time and are therefore not sufficient to provide unbiased information on TBV constituents' microstate upon ripening. On the other hand, the extensive quantities (entropy, total volume, total surface area, mole of solutes, mass of solutes, number of charges or electrons) may be easily linked to the vinegar age. Vinegar behaved as an ''out of equilibrium'' system in the early years, with the extensive properties continuously changing towards a ''partial equilibrium'' after a long RT (Falcone and Giudici, 2008). The products of polymerization reactions are good descriptors of TBV aging since they are related both to RT and PRT. The evolution of the molecular size as a function of RT of TBV is reported in Fig. 4.10. These polymers possess a distribution character in a variety of physical properties simultaneously, such as molar mass, density, chemical composition, viscosity, and thermal properties. The distribution characteristics are good descriptors of the physical age (PRT, Fig. 4.11).

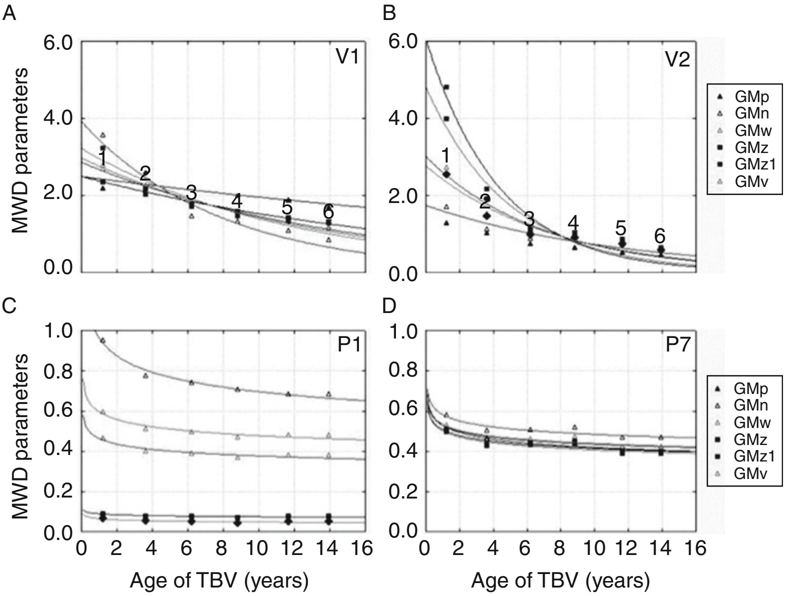

FIGURE 4.11 Biopolymers' absorbance at 280 nm (A, B) and 420 nm (C, D); GMp, GMn; GMw, GMz1, and GMv are the characteristics of the molecular size distribution, having physical meaning as described by Falcone and Giudici (2008), all describing quantitatively the physical age of the traditional balsamic vinegar. All values have been normalized with respect to their initial value in the fresh cooked must. The vinegar age corresponds to the RT (adapted from Falcone and Giudici, 2008).

Attempts to identify descriptors of TBV age have been made by other authors (Cocchi *et al.*, 2002, 2006; Consonni *et al.*, 2008; Masino *et al.*, 2005) but with unsatisfactory results, mainly due to the uncertainty of the RT of the samples investigated.

III. CHEMICAL COMPOSITION

The full picture of TBV composition is actually difficult to complete for at least four reasons: (i) the empirical approach that is used to manage the barrel sets; (ii) the technology for TBV production is roughly the same everywhere but there are differences among the producers; (iii) the analytical techniques used to determine vinegar composition are frequently transferred directly from wine science without any specific validation for TBV; and (iv) the data available in the literature are mainly focused on the macro constituents (i.e., all those present with a concentration of g/l of TBV such as reducing sugars and organic acids): little attention has been paid to minor but not less important constituents including polyphenols, volatile compounds, and the end-products of nonenzymatic sugar degradation reactions.

A. Major compounds

1. Sugars

The first studies on monosaccharides in TBV were based on enzymatic methods (Plessi *et al.*, 1987): the authors proposed glucose, fructose, together with glycerol, ethanol, and xylitol as vinegar quality criteria. D-Glucose and D-fructose have been studied in TBV in relation to its degree of crystallization (Giudici *et al.*, 2004). Other sugars such as xylose, ribose, rhamnose, galactose, arabinose, mannose, and sucrose were detected in very small amounts (g/kg) (Cocchi *et al.*, 2006).

Glucose and fructose are the main components of TBV, their average mass fraction being 23.60 (± 3.45) and 21.14 (± 3.57), respectively; the average and standard deviations (SDs) were calculated on the data of 100 samples randomly chosen during the TBV exhibition held in Modena, 2005 (Table 4.5). It is consolidated practice to use the °Bx to indicate the overall content of sugars in TBV, but this assumption is conceptually wrong and creates confusion as to the real solute composition. In addition, sugar and °Bx are uncorrelated (Fig. 4.12), which makes it impossible to compare old data with new. Each soluble solid affects TBV's optical properties and the °Bx value is the additive and/or synergic result of the contribution of the individual solute to the overall refractive response of

TABLE 4.5 Chemical characteristics of traditional balsamic vinegar, expressed as mean of 104 samples (adapted from Falcone *et al.*, 2008)

Parameter	Mean[a]	Standard deviation (SD)
Soluble solids	73.86	±1.73
Titratable acidity	6.67	±0.88
"*R*" ratio	11.27	±1.53
Glucose	23.60	±3.45
Fructose	21.14	±3.37
Tartaric acid	0.78	±0.25
Succinic acid	0.50	±0.70
Acetic acid	1.88	±0.45
Malic acid	1.04	±0.32
Gluconic acid	1.87	±1.27
Lactic acid	0.12	±1.07

[a] Amount expressed in g/100 g of TBV, as mean value of 104 samples presented at the annual competition held at Modena in 2005; titratable acidity is expressed as gram of acetic acid per 100 g of TBV.

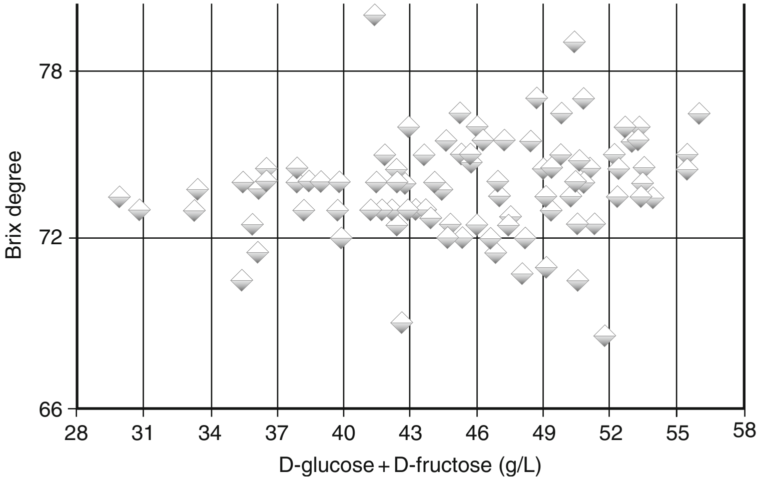

FIGURE 4.12 Relationship between sugars and Brix.

vinegar. Moreover, the solute-specific refractive index depends on several factors including temperature and concentration, as well as molecular weight and polydispersity of the solute. It is easy to infer that the higher the vinegar concentration, the higher the overestimation of sugar content: the solute contribution to the refractive response of the vinegar has been proved to be higher than that of the cooked must, most probably because of the accumulation in vinegar of high-molecular-weight biopolymers (Falcone and Giudici, 2008).

The sugars in solution are present in different isoforms, each of them having different solubility and reactivity, which is an important concern for TBV's physical stability. Both α and β pyranosidic and furanosidic forms are present in sugar solution, whereas, in TBV, glucose is present in the pyranosidic forms and fructose in both pyranosidic and furanosidic forms (Consonni *et al.*, 2008).

2. Organic acids

The ratio between °Bx and titratable acidity (''*R*'') was first described by Gambigliani Zoccoli (unpublished data) and it is still used as a quality criterion for TBV (Giudici *et al.*, 2006). Tritatable acidity is usually expressed as grams of acetic acid per 100 g of vinegar, but this does not describe either the type or the amount of the individual organic acids. In particular, the sum of the individual acids, expressed as equivalents, is always less than the corresponding titratable acidity. The degree of vinegar sourness is related to the titratable acidity, the pH, the relative amount of dissociated and undissociated acid, buffer capacity and the relative quantities of individual acids. The acids are all more or less sour and some have a characteristic flavor (Ough and Amerine 1988): succinic acid is bitter, acetic acid is pungent, and so on. Recently, the concentration of single organic acid has been determined by selective methods such as enzymatic-based techniques (Giudici *et al.*, 2004, Plessi *et al.*, 1987), chromatographic techniques (Cocchi *et al.*, 2002, 2006), and NMR (Consonni *et al.*, 2008). Unfortunately, the data obtained by different methods cannot be compared, due to the different sensitivity and specificity involved. We believe that the most reliable composition data are obtained by enzymatic methods because they allow the separation of D/L isomers. Table 4.5 shows the average and SD of the main individual organic acids and titratable acidity of a large number of TBV samples. The samples have roughly the same titratable acidity as each other, but they show different compositions for the individual acids (with the exception of tartaric acid because the main part of it precipitates as potassium and calcium salts). In general, TBV contains, in descending order: acetic, gluconic, tartaric, succinic, malic, and citric acids, plus a small amount of lactic acid.

The origin of the individual acids varies: some arise from grapes, some from yeasts and bacteria metabolism, and others have more than one origin (Table 4.6). Acetic acid is the result of ethanol oxidation by AAB: a very small amount is formed during must cooking due to sugar degradation. Tartaric acid comes only from grapes. Gluconic acid is produced by AAB but can also be a natural constituent of grapes undergoing mold deterioration, that is, by *Botrytis cinerea*. It has been proposed as an indicator of TBV authenticity (Giudici, 1993). Succinic acid comes from yeast metabolism and D-/L-lactic acid, which is generally present in a low concentration, comes mainly from AAB and the thermal treatment of grape must (Fig. 4.4).

B. Minor compounds

1. Volatile compounds

The volatile and aromatic fraction profile of TBV varies strongly in relation to the method used in their determination. Recent studies have shown the presence of several aldehydes, ketones, alcohols, and esters in TBV (Del Signore, 2001; Natera *et al.*, 2003; Zeppa *et al.*, 2002). Quantitative determinations of volatile compounds have been used to group and discriminate vinegars from different origins. Among alcohols, ethanol and 2-propanol are present in a relatively high concentration in common vinegars; while 1-propanol, isobutyl alcohol, isoamyl alcohol, and 1-hexanol are present in BV. Acetaldehyde is present in a relatively high concentration of common vinegars; while diacetyl, hexanal, and heptanal were three and five times higher in TBV, compared to BV and common vinegars. Esters were found in BV and common vinegars in concentrations higher than that found in TBVs, apart from 1,3-butanediol diacetate (Del Signore, 2001).

TABLE 4.6 Organic acids: origin and microorganism involved in their formation

Organic acid	Origin	Metabolism	Microorganism
Tartaric acid	Grapes	Degradation	Lactic acid bacteria
		Oxidation with high oxygen concentration	Acetic acid bacteria
Acetic acid[a]	Acetic acid bacteria Yeasts Lactic acid bacteria	Carbon dioxide and water	Acetic acid bacteria
Succinic acid	Yeasts	Oxidation with high oxygen concentration	Acetic acid bacteria
Citric acid	Grapes	Acetic acid	Lactic acid bacteria
Gluconic acid	Botrytized grapes Acetic acid bacteria		*Schyzosacchaomyces*
Malic acid	Grapes	L(+)-lactic acid + carbon dioxide	Acetic acid bacteria
	Yeasts	Ethanol + carbon dioxide	Acetic acid bacteria
L-Lactic acid and D-Lactic acid[a]	Lactic acid bacteria yeasts	Oxidation with high oxygen concentration	
Piruvic acid	Yeasts and bacteria	Oxidation with high oxygen concentration	Acetic acid bacteria

[a] Small amounts can be produced by sugars thermal degradation.

2. Phenolic compounds

Phenols include several hundred compounds that are broadly grouped as flavonoids and nonflavonoids. The former include anthocyanins, catechins, and tannins; the latter include stilbenes and other compounds derived from benzoic, caffeic, and cinnamic acids.

In red wine, up to 90% of the phenolic content consists of flavonoids. These compounds derive in a decreasing order from grape stems, seeds, and skins. Tannins refer to a wide range of high-molecular-weight compounds including the two classes of compounds generally known as ''natural tannins'' and ''pigmented tannins.'' The natural tannins (proanthocyanins) derive, in descending order of volume, from grape skins, stems, and seeds. The reaction between natural tannins and/or anthocyanins with other phenols, mainly catechins, results in the formation of pigmented tannins. Finally, the barrel wood may itself contribute to the formation of the pigmented tannins, in which case they are known as ''hydrolyzable tannins'' since they contain ellagic and gallic acids that are usually determined by chemical hydrolysis. However, it is reasonable to suppose that the older barrels contribute to the tannin formation less than the new ones.

To date, both qualitative and quantitative judgments of the individual phenolic compounds in TBV are still unsatisfactory. The phenols in TBV were first studied in terms of their overall content by colorimetric methods and GC–MS techniques by Plessi *et al.* (2006); and by means of colorimetry alone by Verzelloni *et al.* (2007). The latter showed that the overall amount of the phenolic compounds is strictly related to the antioxidant properties of TBV. Phenols take part in the polymerization reactions during the TBV aging (Tagliazucchi *et al.*, 2008; Verzelloni *et al.*, 2007) and probably the reactions start during the cooking of the must.

Discrimination of TBV based on phenolic compositions, aroma compounds, and organic acids has been investigated by Natera (2003): data from 83 vinegars of different origins and raw materials were studied by linear discriminating analysis which allowed differentiation between the 88% of samples investigated, according to their raw materials, and the 100% investigated according to the presence or absence of the aging period in wood.

3. Furanic compounds

The most studied product of sugar thermal degradation is HMF, but other furanic congeners (furoic acid, formaldehyde, and acetoxymethylfurfural) were quantified in a barrel set (Chinnici *et al.*, 2003; Masino *et al.*, 2005). HMF is a molecule of public concern for its potential toxicological activity; several vinegars were grouped on the basis of the content of this compound. In particular, BVM showed a concentration ranging between 300 and 3300 mg/l, and higher concentrations—up to 5500 mg/kg—were found in TBV samples (Theobald *et al.*, 1998). Other authors reported similar results for BVM (Bononi and Tateo, 2009; Giordano *et al.*, 2003). It is a general opinion that HMF and related congeners are formed during the cooking of the must, and that these compounds are a highly reactive intermediate of sugar degradation, including Maillard reactions (Berg and Van Boekel, 1994). HMF formation and Maillard reactions occur also at room temperature, especially in sugary food with low water activity. TBV is a typical beverage in which these reactions occur, because of its peculiar chemical composition, low water activity, and long aging time.

C. Melanoidins and other biopolymers

Melanoidins are high-molecular biopolymers, which arise from sugar degradation reactions. They are important for the rheological properties of different vinegars including TBV. Other functionalities arise from their complex molecular structure and size, depending on the extent of the polymerization reactions leading to their formation. Falcone and Giudici (2008) have recently used size-exclusion chromatography with a dual detection system (UV–Vis and DRI) to characterize the distribution properties of the molecular size of these biopolymers; the data obtained suggest that TBV behaves as a heterogeneous mixture of four classes of copolymers at least, all ranging from 0.2 kDa to beyond 2000 kDa, and highly polydispersed in respect to their molecular size and composition. All classes of melanoidins of TBV are labeled by ultraviolet- and/or visible-radiation absorbing chromophores. Since the relative concentration of melanoidins evolves according to their initial value into the cooked must, the size-exclusion chromatographic profiles can be safely used as a fingerprint of the overall composition of the TBV throughout the manufacturing process.

D. TBV composition during the last three decades

TBV composition and characteristics have changed over the years. In particular, during the last three decades, the most evident changes have related to the °Bx and the titratable acidity levels: from ~62 to ~ 73°Bx, and from ~ 9% (sometimes even as low as 11%) to ~ 6%. Nowadays, it is unusual to find a TBV with a titratable acidity greater than 6% (w/w) (Table 4.7). The composition and sensorial properties of TBV are now very different from those of the past: in general, more acid, more pungent, and less sweet. The new taste seems to meet consumer preferences as well as those of the professional panel testers, who assign higher sensory scores to the sweeter vinegars. The relationship between composition and sensorial properties in TBV has been investigated by several authors (Chinnici *et al.*, 2003; Cocchi *et al.*, 2002, 2006; Giordano *et al.*, 2003; Plessi *et al.*, 2006; Sanarico *et al.*, 2002; Zeppa *et al.*, 2002). Unfortunately, different and often opposite conclusions have also been reported. This is due to the lack of preliminary validation studies with reliable procedures aiming to investigate the TBV sample with respect to the age claimed by producers.

TABLE 4.7 Composition related-changes of the traditional baslamic vinegar during the latest three decades (adapted from Giudici *et al.* 2008)

	1982			1996			2004		
Sample	°Br	TA	“*R*”	°Br	TA	“*R*”	°Br	TA	“*R*”
1	61.00	11.40	5.35	73.80	7.32	10.08	74.50	6.52	11.43
2	61.50	7.26	8.47	75.80	8.37	10.06	77.00	6.93	11.11
3	64.40	9.72	6.62	76.30	8.99	8.49	71.50	6.99	10.23
4	61.40	9.90	6.21	70.80	8.25	8.58	72.50	7.17	10.11
5	63.60	8.76	7.26	73.80	7.32	10.08	72.25	8.87	8.15
6	63.60	7.92	8.03	73.80	7.69	9.60	74.50	7.18	10.38
7	65.70	9.12	7.20	71.30	9.30	7.67	75.00	6.31	11.89
8	70.00	8.70	8.04	75.80	8.68	8.73	73.00	5.91	12.35
9	63.60	8.40	7.57	71.80	8.31	8.64	73.00	7.06	10.34
10	57.30	9.00	6.36	72.30	6.82	10.60	74.00	6.49	11.40
11	61.00	8.70	7.01	71.80	6.20	11.58	74.00	6.25	11.84
12	59.20	11.58	5.11	73.80	8.80	8.39	74.00	8.18	9.05
Mean	62.69	9.21	6.93	73.43	9.18	9.18	73.77	6.99	10.69

“*R*” is the ratio between Brix and titratable acidity.

IV. PHYSICAL PROPERTIES

A. Rheological properties

Aqueous solutions containing glucose, fructose, and acetic acid in a range of concentrations simulating a typical TBV exhibit some Newtonian viscosity; while samples of genuine TBV exhibit Newtonian viscosity of two or three times greater (Falcone *et al.*, 2006). Glucose and fructose act as structure-promoting compounds, but other constituents exert a greater structuring ability (Falcone *et al.*, 2006). In particular, it was recognized that such biopolymers are responsible for the high viscosity level in TBV. As mentioned, these compounds are formed during the cooking of grape juice and accumulate and evolve during aging (Falcone and Giudici, 2008). These polymers contribute to other physical properties including colligative ones, the refractive index, density, specific heat capacity melt, and viscoelastic properties.

B. Color and spectrum absorbance

The color of vinegar during aging changes from yellow/brown to brown/black, due to the accumulation of chromophore-labeled melanoidins (Falcone and Giudici, 2008). At least four classes of melanoidins contribute to this coloration. Falcone and Giudici (2008) propose the ratio between the absorbance at 420 nm of TBV and the absorbance of the cooked must (brown index, BI) as a descriptor of the vinegar color and physical age (PRT):

$$\mathrm{BI} = \left\{ \frac{A_{\mathrm{RT_V}}}{A_{\mathrm{CM}}} \right\}_{420\,\mathrm{nm}}$$

where $A_{\mathrm{RT_V}}$ is the average residence time of the vinegar (V) calculated according to the Giudici and Rinaldi (2007) model.

The BI follows a two-rate kinetic with the second stage starting at about the 6th year of aging with a high rate; the relative melanoidins content follows a similar trend (Fig. 4.13). The UV–Vis spectrum (from 200 to 700 nm) of TBV samples during aging exhibits a well-defined absorption peak at 280 nm and featureless absorptions as the wavelength increases. Spectra of this kind were observed for other naturally occurring melanoidins (Hofmann, 1998; Riviero-Pérez *et al.*, 2002; Rizzi, 1993) and in synthetic solution, where the interaction between lysine, glucose, and fructose results in chromophore and molecular size distributions resembling those which occur in the cooked must (Falcone and Giudici, 2008).

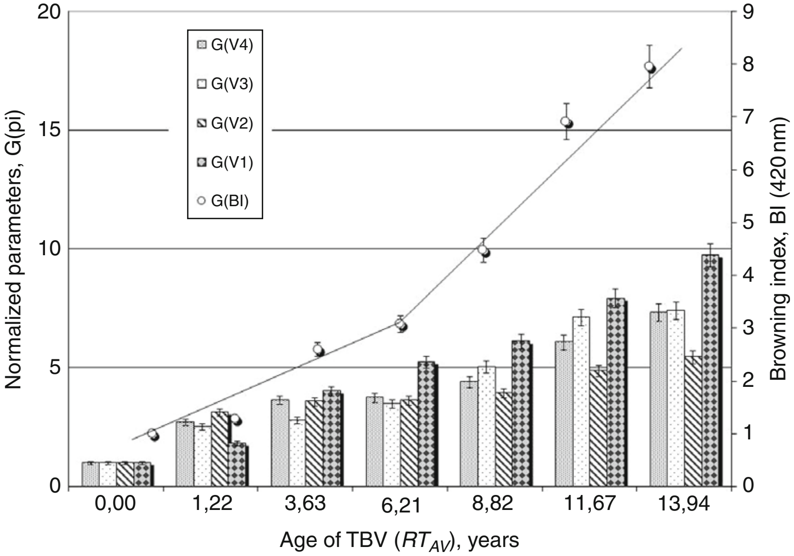

FIGURE 4.13 Time evolution vinegar color during aging. *G*(BI) is the brown index as defined in the text; *G*(V1), *G*(V2), *G*(V3), and *G*(V4) are the relative concentration of high-molecular size in vinegar melanoidins having brown-labeled (420 nm-radiation absorbing) chromophores. All data are normalized with respect to their initial value (in the cooked must). The age of the vinegar was assessed according to the Giudici and Rinaldi (2006) model (adapted from Falcone and Giudici, 2008).

V. CONCLUSION

Several different products are known worldwide with the generic and legally dubious appellation ''balsamic vinegars.'' It is very difficult to understand the true differences among these products, which include condiments, vinegars, and specialty vinegars legally recognized and protected by special regulations such as PGI and PDO. Most ''balsamic products'' are simply a blend of ingredients, in different amounts and ratios, such as vinegar, concentrated must, sugars, food colouring, and thickeners. Also among the balsamic vinegars produced in Italy, wide differences exist between the PDOs traditional balsamic vinegar of Modena and the PGI balsamic vinegar of Modena, the first is the only one produced by fermentation, acetification, and aging as described in this review; the second is a blend of wine vinegar and cooked must with the addition of caramel to improve the color. From a scientific point of view, TBV is an important model to understand as it answers important questions in food sciences: fermentation of selective media, sugar degradation, Maillard reactions, mass transfer, physical aging, polymerization reaction in complex and long preserved media, and the role of high-molecular-weight compounds on rheological properties.

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