

# **Oral Session I**

## **Traditional Vinegar Fermentation**

## I-1: Traditional Rice Vinegars That Are Considered to Have Health Benefit

Yoshikatsu Murooka

Dept. Health Science, Hiroshima Institute of Technology, Hiroshima 731-5193, Japan  
E-mail: murooka@bio.eng.osaka-u.ac.jp

According to Chinese literature rice wine, *sake*, and vinegar were produced in the Xia-Shang (Yin) period (ca. 2000 B.C. - 1027 B.C.). Rice vinegar brewing techniques traveled from China to Korea and Japan around A.D. 300. Black rice vinegar was probably the first vinegar variety brewed in these countries. We produced three types of rice vinegars; amber (clear/pale amber), used mainly in sweet-and-sour dishes and 'sushi' rice; red, a popular accompaniment for boiled crab in China; and black, used mainly as a table condiment and health drink. In Japan, fermented vinegars are further classified into polished rice vinegar (*komesu*), unpolished rice vinegar (*kurosu*), *sake-lee* vinegar (*kasuzu*), and other grain vinegars.

*Komesu* and *kurosu* are produced from rice by the same process, saccharification, alcohol fermentation, and oxidation of ethanol to acetic acid. Although some vinegar companies produce *kurosu* using a modern submerged fermentation process, most *kurosu* is produced by static fermentation method. Traditionally, ceramic pots have been used for the *sake* to vinegar production in China and Japan. The alcohol liquid media, *sake*, is produced from rice by *koji* mold, *Aspergillus oryzae* for saccharification of rice starch and then fermented by *sake* yeast, *Saccharomyces cerevisiae*. In some cases *Asp. awamori*, *Asp. usami*, or *Rhizopus* spp. are used in stead of *Asp. oryzae*. In production of red rice vinegar, red *koji*, *Monascus purpureus* is used in China.

We investigated acetic acid bacterial strains from *komesu* and *kurosu* productions in Japan. By DNA fingerprinting analysis and 16S rDNA sequence, one group of *Acetobacter pasteurianus* overwhelmingly dominated all stages of acetic acid fermentation. Since no purified strain was inoculated appropriate strains of acetic acid bacteria spontaneously established pure cultures in one century of these traditional rice vinegar fermentations. In *sake* lee vinegar, *kasuzu*, fermentation, *Gluconobacter intermedius* subsp. nov. *tamanoi* was found from the late phase fermentation in addition to *A. pasteurianus*.

Rice vinegar, especially black vinegar, *kurosu*, is now experiencing a boom in popularity as a health drink in Japan. *Kurosu* extract was recently shown to suppress lipid peroxidation, express stronger antioxidative activity, lower blood pressure, and promote anti-tumor activity. With increasing public interest in health, effective health-related elements of traditional vinegars are being carefully researched.

## I-2: Basic Technology and microbiology of Traditional Balsamic Vinegar: influence on sensorial properties and quality

P. Giudici<sup>1</sup>, M. Gullo<sup>1</sup>, L. Solieri<sup>1</sup>, P.M. Falcone<sup>2</sup>

<sup>1</sup>*Dept of Food Sciences and Agriculture, University of Modena and Reggio Emilia, Via Amendola 2 Padiglione Besta, 42100 Reggio Emilia, Italy*

<sup>2</sup>*Centro Ricerche Aceti di Fermentazione CRACEF, Via Amendola 2 Padiglione Besta, 42100 Reggio Emilia, Italy*

E-mail: paolo.giudici@unimore.it

Historically, Traditional Balsamic Vinegar (TBV) was not traded and it was mostly consumed by the family of producers or at least offered as a present. The making process was different from one producer to another one and only recently a standard procedure has been coded and reported on the disciplinary of production. The basic technology could be grouped in four steps: 1) grape must preparation; 2) alcoholic fermentation; 3) acetification; 4) ageing.

1) The grape must is heated in an open pan to a final sugar concentration ranging from 30 to 50%. During the heating several transformations occur, in particular Maillard reactions<sup>1</sup> involving sugars, amino acids and polyphenols. Tritable acidity and pH change due to the effect of the solute concentration and salts precipitation. The ratio between sugar and tritable acidity is the main parameter for evaluating the quality of cooked must for TBV.

2) Alcoholic fermentation of cooked must is carried out by indigenous yeasts belonging to different species, such as *Zygosaccharomyces rouxii*, *Zygosaccharomyces bailii* and other osmotolerant species, but also *Saccharomyces cerevisiae* occurs in samples with low soluble solids concentration<sup>2</sup>. The high number of species reflects the wide variability of TBV samples, differently produced from one farm to another. The composition of cooked must (sugar amount, pH values and acetic acid concentration) seriously affects the yeast species, the growth rate and the species ratio. On the other hand many evidences suggest that yeast metabolism influences the TBV quality.

3) Acetic acid bacteria (AAB) that occur in TBV belong to *Acetobacter* and *Gluconacetobacter* genera and the main species detected until now is *Gluconacetobacter europaeus*, followed by *Acetobacter pasteurianus*, *Acetobacter aceti* and *Acetobacter malorum*. Sugar and alcohol content strongly affect the AAB growth. In general, few AAB species are able to oxidise must with sugar concentration higher than 25% (w/v), therefore AAB layer has been observed only in the largest barrels with the lowest sugar content<sup>3,4</sup>.

4) TBV is aged for a long time in set of barrels arranged in decreasing scalar volume. New cooked must is added and aliquots of product are transferred from barrel to barrel every year; the refilling procedure provides a blend of vinegars of different age. The age of the product in the single barrel has been described by sequence of real numbers depending upon the year number of the barrel and volume of vinegar transferred<sup>5</sup>. Moreover, during the ageing several chemical and physical transformations occur. In particular, polymers with high molecular weight are formed, affecting the structure-related properties of TBV, including viscosity, colligative properties, refractive index, density, specific heat, glass transition temperature, and others<sup>6</sup>.

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3. Gullo and Giudici, *Int. J. Food Microbiol.*, **125**, 46-53 (2008)

4. Gullo, M. et al., *Int. J. Food Microbiol.*, **106**, 209-212 (2006)

5. Giudici, P. and Rinaldi, G. *J. Food Eng.*, **82**, 121-127 (2007)

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### I-3: Biochemical and Morphological Characterization of a Fungal Strain as a New Contaminant of Balsamic and Cider Vinegar

S. Schindhelm<sup>1</sup>, A. Weber<sup>1</sup>, C. Schelling<sup>2</sup>, A.M. Stchigel<sup>3</sup>, J. Cano<sup>3</sup>, J.-L. Veuthey<sup>2</sup> and F. Barja<sup>1</sup>

<sup>1</sup>Department of Plant Biology, Bioenergetics and Microbiology Laboratory, University of Geneva, Chemin des Embrouchis 10, CH-1254 Jussy-Geneva, Switzerland

<sup>2</sup>Laboratory of Pharmaceutical Analysis Chemistry, University of Geneva, Bvd d'Yvoy 20, CH-1211 Geneva 4, Switzerland

<sup>3</sup>Unitat de Microbiologia, Facultat de Medicina, Universitat Rovira i Virgili, Reus, Spain

E-mail: Francois.Barja@bioveg.unige.ch

Vinegar fermentation is an oxidative process in which diluted ethanol is oxidized to acetic acid and water by acetic acid bacteria (AAB) under aerobic conditions. Healthy and active AAB are prerequisites for trouble free vinegar production. Biological factors such as contamination by fungi, yeasts, nematodes and chemical products, such as sulphur dioxide or chlorine, can disturb the optimum performance of vinegar production.

The aim of this study is to determine morphological, biochemical and some kinetic characteristics of a fungal strain as a new contaminant of balsamic and cider vinegars.

The examination of fungal contamination of 100 balsamic and cider vinegar samples showed that 90% of the samples contained more than  $10^4$  colony forming units (CFU/mL), in most of the samples (65%) more than  $10^5$  CFU/mL were recovered. A variety of molds were isolated, especially belonging to genera *Monascus*, *Moniliella* and *Epicoccum*. The most frequent mold was *Monascus*; 80% of the samples were contaminated with the species of this genus and the number of CFUs varied between  $2 \times 10^3$ /mL to  $6 \times 10^5$ /mL. This mold can produce secondary metabolites such as citrinin<sup>1,2</sup> and monacolin K<sub>L</sub> (lactone form) and monacolin K<sub>A</sub> (acid form)<sup>3,4</sup>. Using high-performance liquid chromatography-fluorescence detection, citrinin was found in 68% of analyzed samples. The concentration varied between 0.016 and 0.072 ppm. Monacolin was not detected in all samples analyzed. The concentrations of citrinin were very low and we can anticipate that this compound has nontoxic effects on renal cells at these levels.

In conclusion, the balsamic and cider vinegar samples examined are frequently contaminated with fungi, some of them belonging to the genus *Monascus*, which can produce pigments and metabolites such as citrinin and monacolin. The low concentration of citrinin detected in the vinegar samples probably would not be toxic for the cells in human kidneys and liver.

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## **I-4: Characterization of Wine Vinegar from the New Protected Designation of Origin (PDO) “Vinagre Montilla-Moriles”**

I. López-Infante<sup>1</sup>, V. González-Caballero<sup>1</sup>, J. Morales-Ordóñez<sup>1</sup>, P. Ramírez-Pérez<sup>1</sup>, I. García-García<sup>2</sup>

<sup>1</sup>*IFAPA Centro de Cabra. Ctra. Cabra-Doña Mencía, Km. 2,5. 14.940 Cabra (Córdoba) Spain.*

<sup>2</sup>*Chemical Engineering Department, University of Cordoba, 14071 Cordoba, Spain*

E-mail: marii.lopez.infante@juntadeandalucia.es ; isidoro.garcia@uco.es

From ancient times, the high quality wine vinegars produced in the province of Cordoba (Spain) have been appreciated. In 2008 an official first step has been done<sup>1</sup> in order to approve new regulations<sup>1</sup>, by the system of The Protected Designation of Origin (PDO), which will recognise and confer judicial protection as well as assuring the quality and the origin of these vinegars. So, “Vinagres de Montilla-Moriles” will hopefully be the third PDO mark in Spain. At present, “Vinagre de Jerez”<sup>2</sup>, “Vinagre de Condado de Huelva”<sup>3</sup> (both in Spain) and Traditional Balsamic Vinegar of Modena (ABTM)<sup>4</sup> Traditional Balsamic Vinegar of Reggio Emilia<sup>4</sup> and Balsamic Vinegar of Modena (ABM)<sup>5</sup> (in Italy) enjoy the recognition of the PDO mark in Europe.

The “Montilla-Moriles” vinegars<sup>1</sup> are exclusively produced from “Montilla-Moriles” wines (PDO): “Finos”, “Olorosos”, “Amontillados” and “Pedro Ximénez”. Traditional methods, similar to those Sherry wine vinegars, are used. Basically, two methods are followed: dynamic and static one. The first method, also known as “Solera”, consist in an ageing process in oak butts by which some volume transfers are carried out between butts containing vinegar of different age. Contrarily, in the static method, the vinegar is kept in the same butt during the ageing period. Two main types of vinegar are regulated: aged vinegars (“Crianza”, “Reserva” and “Gran Reserva”) and sweet vinegars (Muscatel and “Pedro Ximénez”).

In order to prepare the new regulations, products from 23 vinegarmakers of the Region were studied. Samples were analysed for: dry extract, ash, total acidity, volatile acidity, pH, final alcoholic content, sodium, potassium, total sulphur anhydride, colour intensity and tonality, total polyphenols, acetoin, methanol and sensorial analysis.

The results show that from both a physical-chemical basis as well as organoleptic point of view the uniqueness and authenticity of these products could be assured.

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4. *Council Regulation (EC)* No 813/2000 of 17 April (2000)
5. 2007/C 152/08 Publication of an application pursuant to Article 6(2) of Council Regulation (EC) No 510/2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs.. *Official Journal of the European Union* C 152, Volume 50, 6 July (2007).
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## **I-5: Microbiological Profile of Fig Vinegar Produced Traditionally in Turkey**

I. Yucel Sengun and S. Karabiyikli

*Ege University, Engineering Faculty, Food Engineering Department, 35100, Bornova-İzmir/TURKEY*

E-mail: ilkinyucel@yahoo.com

Fig vinegar plays an important role in the development of vinegar. Although, it is not produced industrial scale, the limited production of this product is going on as a family art. The objective of this research was to determine the microbiological profile of fig vinegar. Two different homemade vinegar samples were analysed by standard procedures for total viable count (TVC), mould and yeast count, Lactic Acid Bacteria (LAB) count and Acetic Acid Bacteria (AAB) count. TVC of samples were found as  $1.4 \times 10^3$  cfu/ml and  $4.4 \times 10^2$  cfu/ml. Three different media were used for mould and yeast count. For one vinegar sample, mould and yeast counts on Potato Dextrose Agar (PDA), Malt Extract Agar (MEA) and Dichloran Rose Bengal Chloramphenicol Agar (DRBC) were found as  $1.8 \times 10^2$  cfu/ml,  $2.1 \times 10^2$  cfu/ml and  $<1$ , respectively. For the second sample, no mould and yeast growth were observed on all of the media. LAB counts for two vinegar samples were found as  $1.2 \times 10^3$  cfu/ml and  $2.0 \times 10^2$  cfu/ml on M17 agar while the counts on Man-Rogosa and Sharp Agar were  $3.0 \times 10^0$  cfu/ml and  $4.0 \times 10^2$  cfu/ml. GYC (10% glucose, 1.0% yeast extract, 2.0% calcium carbonate, 1.5% agar) and YPM (0.5% yeast extract, 0.3% peptone, 2.5% mannitol, 1.2% agar) were used for AAB counts of the samples<sup>1</sup>. For both samples AAB counts were ranged as  $8.8 \times 10^2$  cfu/ml and  $1.6 \times 10^2$  cfu/ml on GYC medium and  $9.1 \times 10^2$  cfu/ml and  $5.5 \times 10^2$  cfu/ml on YPM medium. These two media were also used for the isolation of AAB and 72 purified isolates were placed at  $-20$  °C in GYC and YPM broths containing 15% glycerol for the future identification. pH and the total acidity of samples were also determined in the study.

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## I-6: The role of acetic acid bacteria in traditional fermentations

L. De Vuyst, T. Lefeber, Z. Papalexandratou and N. Camu\*

*Research Group of Industrial Microbiology and Food Biotechnology, Department of Applied Biological Sciences and Engineering, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium (\*Present address: Barry Callebaut NV, Wieze, Belgium)*

E-mail: ldvuyst@vub.ac.be

Acetic acid bacteria (AAB) play an important role in the production of fermented foods and beverages, due to their capacity to incompletely oxidize different kinds of alcohols, sugars, sugar alcohols, and organic acids. Typical products are vinegar, nata, palm wine, kombucha, kefir and koumiss, and cocoa-based products. Also, this property involves them in the biosynthesis of *L*-sorbose (part of the chemical synthesis of vitamin C) and of 6-amino-6-deoxy-*L*-sorbose (part of the synthesis of the type II diabetes drug miglitol), in the production of polysaccharides, such as bacterial cellulose and acetan, and in the production of plant growth promotors (nitrogen fixation). Conversely, AAB cause spoilage of beverages, such as beer, wine, and cider.

Vinegar is an aqueous solution of acetic acid that is produced by AAB from a dilute solution of ethanol (acetification step). The primary alcoholic fermentation step, necessary for the production of ethanol, makes use of sugar-containing juices or mashes such as wine, rice, and cider. Today, three different methods are known for the production of vinegar. In the traditional, surface cultivation methods, *i.e.*, field processes (weeks to months) and open vats or Orleans/French procedure (back-slopping with two to three weeks per cycle), the ethanol solution is placed in vats exposed to air. AAB develop as a surface film on top of the liquid where the oxygen concentration is high. In the trickling generator process, referred to as quick vinegar or German process, ethanol is trickled from the top through a tank filled with beech-wood shavings on which the bacteria grow. Air is introduced through holes in the bottom and passes upward to improve oxygen uptake. Submerged fermentation employs Frings acetators, fermentors equipped with an aeration and agitation device allowing improved oxygen transfer from the medium to the bacterial cells.

Bulk vinegar production is mainly carried out by strains of *Acetobacter aceti*, *A. malorum*, *A. pasteurianus*, *A. pomorum*, *Gluconacetobacter entanii*, *Ga. europaeus*, *Ga. hansenii*, *Ga. intermedius*, and *Ga. oboediens*, which have been isolated from different production facilities during the last decades. To inoculate the fermentor, a seed vinegar is used, *i.e.*, a microbiologically undefined starter culture usually obtained from a previous fermentation. The lack of defined starter cultures is mainly due to problems with isolation, cultivation, and preservation of vinegar AAB. Specialty vinegars include, for instance, traditional Balsamic vinegar produced by surface culture fermentation in Italy. A back-slopped mother culture of yeasts and AAB is used.

During fermentation of cocoa bean pulp, which is manually scooped out of the cocoa pods and piled into heaps or placed into boxes or baskets or on platforms, successive microbial activities by naturally occurring yeasts, lactic acid bacteria, and AAB take place. In particular, AAB convert ethanol, formed by the yeasts out of sucrose under conditions of high carbohydrate concentrations (characteristic for fresh cocoa pulp), limited oxygen availability (due to tight packing of the beans), and a pH of below 4.0 (due to the relatively high content of citric acid in cocoa pulp), into acetic acid under conditions of high ethanol concentrations (due to yeast activity) and satisfactory oxygen availability (due to air penetration into the fermenting cocoa bean mass, which is caused by reduction of viscosity and drainage of the cocoa pulp, in turn due to the pectinolytic activity of the yeasts that break down the walls of cells in the pulp). Mainly *Acetobacter* species (*A. fabarum*, *A. ghanensis*, *A. pasteurianus*, *A. senegalensis*) play an important role in cocoa bean fermentation, among which *A. pasteurianus* is predominant.

Alternatively, AAB play an important role in texture formation of nata. Further, associations of yeast and AAB occur in the production of palm wine, kefir, koumiss, and kombucha, giving these products a sparkling appearance and/or a slightly sour taste.