
VINEGARS 2005

8-12th MAY, 2005

VINEGARS AND
ACETIC ACID BACTERIA

INTERNATIONAL
SYMPOSIUM

BOOK OF ABSTRACTS

REGGIO EMILIA - ITALY
2005

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ISBN 88-901732-0-3

208108



Dear Colleagues,

“.....the most desired ingredient of Western comfort: a clear signal of progress measured as some form of steadily increasing complexity for life as a whole thorough time. The basic evidence cannot support this view, for simple forms will predominate in most environments, as they always have.”

(Stephen Jay Gould from “The full house: The spread of Excellence from Plato to Darwin”, Harmony, New York 1996)

Simple living forms have prevailed on Earth since the beginning of Life. This scientific evidence is hard to accept since some of its philosophical implications clearly undermine the concept of Man as the center of the Universe. It is not my intention to go deeper into such kinds of speculation both because I do not have the competence and because it would be out of the scope of this Conference. However, reading Gould’s words in a minor key, it is beyond doubt that a higher consideration of microorganisms and of their scientific and industrial potential would bring innumerable advantages to the quality of our life. It has been largely demonstrated that microorganisms represent the most extensive combination of metabolic resources and that most of them have not yet been fully explored. Acetic acid bacteria are no exception. For a long time they have been associated simply with the production of vinegar and to the spoilage of fermented beverages, nevertheless, their role has recently been strongly re-evaluated. This is confirmed by the titles of the lectures and posters presented at this Conference covering a wide range of fields from Ecology to Technology and from Genetics to Metabolomics.

A large part of this Conference is dedicated to the vinegars of the world; the title of the Conference itself recalls the Session that is dedicated to this topic. This choice was not accidental; our aim was in fact to emphasize the great variety of vinegars and vinegar-based products that are produced and consumed worldwide, all products rich in tradition and culture, all so special that they are often considered

unique. We are certain that by putting together all these products contributes in some way to improve a common awareness between peoples.

It is far from my intention to flatter, but indeed the number of participants and their scientific reputation as well as the high scientific standard of the presentations, represent for us the most important reward for the effort made by the organization of this Conference. I hope that the expectations that have brought you to this small town will be totally satisfied during the Conference. We will do our best to make your stay here as fruitful as possible, and I am certain that my hometown, with the tradition of its history and culture will make your visit pleasant and relaxing.

It is not very important if this Conference is or is not the first, it is far more important to make sure that it will not be the last. Therefore we should all work to make sure this appointment becomes periodic.

We have issued the challenge, anyone taking up the baton?

Paolo Giudici

Reggio Emilia, May 2005

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Acknowledgements

The Organising Committee is deeply appreciative of the sponsorship generously provided by the following companies:



Consorzio Aceto Balsamico di Reggio Emilia
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Contributions

VINEGAR: MYTH, HISTORY AND CULTURE

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Aims: In Antiquity viticulture and wine-making represented common denominators of the Mediterranean culture. They became symbols of the civilization process and their origin was placed in the mythical times, deriving from divine generosity. When did vinegar start to be distinguished from wine? Which degree of acidity characterized each of them? How was vinegar produced and used in Antiquity? These are some of the questions my paper intends to answer.

Methods and Results: Methodology implies three levels of analysis.

- 1) Linguistic analysis: through the ancient sources, we aim at determinig the exact meaning and use of words connected with vinegar.
- 2) Description of the fermentation and acetification process of wine, with a specific accent on the typologies of fermented beverages widespread in Antiquity: some examples from myth and ancient history.
- 3) Use of vinegar and similar by-products: some recipes in order to clarify tastes and habits of the ancient peoples.

Conclusions: Life of the Mediterranean peoples was related to wine in each aspect: customs, traditions and religious practices. In spite of the changes in taste and customs, such a continuity may be defined as “culture of vinegar”.

Significance and impact of the study: A sort of short history of vinegar is composed, which proves a long-term acquisition of vinegar in nutrition.

Keywords: Mediterranean culture, fermentation, myth, Antiquity

VINEGAR IN THE ESTE DUCHY. A STORY OF CUSTOMS, TRADITIONS AND POLITICS

Ugo Rangone

Presidente “Confraternita dell’Aceto Balsamico Reggiano”

Aims: The image of the Este Duchy, which governed the two provinces of Reggio Emilia and Modena between 1300 and 1860, was linked to a particular grape derivate, produced by the local people for centuries with great passion: Balsamic Vinegar.

Methods and results: Traditional Balsamic Vinegar from Reggio Emilia is produced from the alcoholic and acetic fermentation of cooked and slightly concentrated, locally grown grape must.

It is dark brown in colour, with a characteristic bouquet and a sweet-sour flavour.

Protected by a DOP designation, it can only be produced in the provinces of Reggio Emilia and Modena.

It has been known throughout the world since 1046, when Boniface, father of Matilde and Lord of Canossa gave some as a gift to the future Emperor Henry II of Franconia.

From that time onwards until the end of the 19th century, it appears in notarial deeds recording weddings, inheritances, donations and archive documents concerning all of the vinegar barrels owned by the Este family, lords of Modena and Reggio.

From 1700 it was to be found on the dining tables at the European courts presided by the ambassadors of the Este Duchy.

It has been discussed and studied by the great agronomists of the past: Mitterparker in Buda, Filippo Re in Reggio, Fausto Sestini Ernesto Parisi and Mario Sacchetti in Bologna.

But was the vinegar of 1046, 1500, 1700 or that studied by these great agronomists similar to the one we produce today?

Significance and impact of the study: The tastes of our ancestors, the analytical studies and the information available in the archives tell us that there are indeed differences.

Keywords: Este Duchy, DOP designation, alcoholic and acetic fermentation

THE ROLE OF SENSORY SCIENCE IN DESCRIBING THE “IDENTITY” OF TYPICAL PRODUCT: THE CASE OF VINEGAR OF MODENA

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Aims: To exploit, diffuse and protect typical food products, analytical protocols are being developed in order to define and evaluate their unique characteristics. It is not surprising that the most interesting products from this point of view are those which associate a raw material of agricultural origin with traditional processing procedures. We can say that a typical food product is one which brings together various environmental factors and various people. When a consumer buys a typical food product he is actually choosing to buy a small piece of the territory of origin and the unique history of the place. The consumer is looking for a memory, the atmosphere of a particular region, and the experiences of all the other people who have tasted the product throughout time: a unique and one-of-a-kind product that unifies culture, environment and personal experience. In recent years, studies on the optimization of the perceptible characteristics of products have led to the realization of experimental protocols aimed at protecting their peculiar characteristics.

The objective of this presentation is to present a strategy which, starting from an available descriptive sensory profile of a particular type of product (Vinegars of Modena), permits identification of some attributes able to identify the typicity and their role in the consumer choices.

Methods and results: The experimental approach can be divided into three distinct phases: i) acquisition of a descriptive sensory profile of product by a panel of trained experts; ii) acquisition of an overall quality score by a group experts with specific knowledge regarding the product; iii) acquisition of preference data from regular product type consumers (but without specific knowledge regarding one specific product).

Through the study of the relationships among the three sets of data it has been possible to identify the sensory attributes that characterise the product (relationships between the profile and quality score provided by experts) and the sensory attributes that explain preference (relationship between the profile and preference score provided by consumers). Finally, the study of the relationship between the two groups of sensory attributes has led to insight about how much the identifying characteristics of product can influence the preferences of consumers.

Conclusion: The developed sensory strategy assure standards of production, processing and distribution of typical food product. Moreover it is possible to follow the evolution over time of a typical food product and to emphasise the role of the consumer in the optimization of the sensory profile of a typical food product.

Significance and impact of the study: The range of the intensity of the sensory attributes related to the product acceptability and its distinctiveness should be viewed as reference data for the optimization of productive process.

Key word: Vinegar of Modena, sensory analysis, trained judges, experts, consumer, overall quality

THE MANUFACTURE OF TRADITIONAL MALT AND DISTILLED MALT VINEGARS

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Aims: To illustrate the process for the production of Malt and Distilled Malt Vinegars.

Methods: to discuss the various processes involved in the production of Malted Barley, ie steeping,germinating ,kilning and packaging. To describe the process of production of these vinegars from the brewing and fermentation stages through the acetification, storage and packaging of the finished vinegars.

Key words: Brewing fermentation, maturation and distillation

ACETIC ACID BACTERIA IN TRADITIONAL JAPANESE RICE VINEGARS

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Aims: Japanese traditional rice vinegars, *komesu*, a polished rice vinegar and *kurosu*, an unpolished rice vinegar, are both produced using the traditional statistic fermentation process. This process is not costly in terms of factory investment, however considerable time is required to achieve fermentation. These vinegars are well-known for their health benefits; in the prevention of inflammation and hypertension to date. For the statistic fermentation process, an ethyl alcohol/acetic acid mixture, known as *moromi* is fermented in large containers fitted with covers. After a few days, crepe pellicles of acetic acid bacteria cover the moromi surface, at which point the fermentation process begins and is achieved in about one month. During this process, no strict sterilization measures are employed. No purified strain is inoculated either. Our bacteria cultures were taken from a fermentation plant which has carried out these processes for more than 100 years without a single inoculation of a pure culture. Another vinegar *kasuzu*, is produced by a fermentation process which uses sake lees, the waste materials from the production of Japanese sake. We analyzed bacterial flora at varying intervals during these two traditional fermentation processes for the above three Japanese rice vinegars.

Methods and results: Acetic acid bacteria isolated from samples during the commercial production of rice vinegars were analyzed by the ERIC-PCR and RAPID methods, and then classified into four groups, A, B, C, and D. Groups A and B, representing acetic acid bacteria from the kasuzu fermentation process showed PCR band patterns identical with those isolated from the komesu and kurosu statistic fermentation process. Strains in these groups were identified as *Acetobacter pasteurianus*. Strains in groups C and D were found only in samples from the late phase of the kasuzu fermentation process. Based on the 16S rDNA sequences, physiological characteristics, and ERIC-PCR patterns, we classified the strains in groups C and D under a novel subspecies, for which we proposed the name *Gluconobacter intermedius* subsp. nov. *tamanoi*. We also developed expression vectors and an efficient transformation system, which can be used for various species of acetic acid bacteria.

Conclusion: Optimum strains of acetic acid bacteria naturally established nearly pure cultures in one century in both the komesu and kurosu fermentation process. Also, we discovered a novel subspecies of acetic acid bacteria in the kasuzu fermentation process.

Significance and impact of the study: We conducted the first analysis ever of acetic acid bacteria taken from the statistic fermentation process. (used for the production of traditional rice vinegars)

Key words: rice vinegar, komesu, kurosu, kasuzu, *Acetobacter pasteurianus*, *Gluconobacter intermedius*

THE SENSORY ANALYSIS OF BALSAMIC VINEGAR OF MODENA: FROM THE QUALITY MODELS INDIVIDUATION TO THE CERTIFICATION

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Some years ago we started a path of sensory analysis of the Balsamic Vinegar of Modena which developed following the next five phases:

- Consumer tests to identify the quality models;
- laboratory tests to describe the quality models;
- correlations between objective and affective characters to determine the hedonic indexes and obtain detailed indications to effectively improve the product;
- creation of conformity profiles and four corresponding classes;
- creation of a consumer-oriented graphic system for the indication of the quality classes
- product certification with the “leaf system”.

During the first phase we submitted tens of samples to hundreds of consumer tests using the Stratus Testing Methodology that requires the random choice of the judges and the following profiling by means of a suitable form and particular statistic techniques. Thanks to this phase we identified which characteristics of Balsamic Vinegar of Modena were preferred by consumers and which were not, adopting the philosophy that the true quality comes from the product ability to satisfy its consumers.

A part of the samples, chosen among the preferred and not-preferred samples, was submitted to the evaluation of a laboratory panel, composed by qualified judges, that outlined the profile, firstly establishing the descriptors by means of a free description, and then choosing the descriptors that today we find on the official form of the Italian Balsamic Tasters, after a thorough check with a selected panel of manufacturers.

Using the Trialtest, method of highly formative utility, during the following phase the descriptive-quantitative profiles were determined in order to verify, in the quality area defined by consumers, the existence of four qualities of Balsamic Vinegar of Modena.

By this way, it was possible to create conformity profiles which have been then certified through an external and independent institute, profiles that have originated the present, so-called “leaf system” (after the symbols of one to four vine leaves which are adopted to indicate the quality class on labels) which allows consumers to easily individuate a Balsamic Vinegar of Modena with specific sensory characteristics, as well as to orientate their choices in view of their cooking needs. With the availability of a first producer to run a pilot test, the system feasibility and its response to the market needs were extensively checked both on domestic and international grounds. The success of the pilot test brought several new companies to adhere to the system developed by AIB.

Today the sensory experimentation is going on with a new group of tests – the Big Sensory Test – that allows to detail better the physiognomy of the product creating a portrait from the profile, picking up all the emotional aspects that represent the biggest input for the purchase and the usage of the product.

ACETO BALSAMICO DI MODENA BETWEEN TRADITION AND MARKET

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The developments occurred over an exceptionally long traditional process (almost twenty centuries) brought to the formation of two different versions of Balsamic Vinegars in the manufacturing areas: Aceto Balsamico Tradizionale, di Modena or di Reggio Emilia (*Traditional Balsamic Vinegar of Modena, or of Reggio Emilia*), and Aceto Balsamico di Modena (*Balsamic Vinegar of Modena*).

It is interesting to see how these two products interacted on the market during the said historical process: both deriving from grape must, which is reduced by heating, and with no other addition for the Tradizionale, many signs are that the noblest version was known and very much appreciated even in the beginning of the second Millennium (Emperor Henry IV required some liters of this product to Bonifacio di Canossa during his Italian journey, year 1049).

Made by local families with an addition of wine vinegar, to achieve a larger availability and a possibility to use it all year long, the other and cheaper product, Aceto Balsamico di Modena, was naturally the best candidate to conquer a wider consumption in the modern markets.

Already at the end of 1800 some small local operators (i.e. Giusti, owner of a food shop at the very heart of Modena) started exporting, and promoting their product at domestic and International Fairs, like in Florence and Paris. The price was extremely high if compared to regular vinegars, but the superior quality of the balsamic vinegar coming from Modena became the most efficient tool to gain success and visibility for its manufacturers.

Laws started recognizing Balsamic Vinegar of Modena much in advance than Traditional Balsamics: in 1933 the Italian Government - considering that a newly introduced text seemingly forgave the manufacturing of Balsamic Vinegar of Modena - guaranteed such production, declaring it a small and typical one for the area of Modena. In 1965 a revision of the whole wine, grape must and vinegars Laws in Italy gave birth to a first standard of Identity, which was in force till end of year 2004, and which represents one of the very first Standards of Identity for a food product in Italy (insofar partly excusing some lack of ruling details). A request for PGI (Protected Geographic Indication) is under way in Europe for Balsamic Vinegar of Modena.

The market of this product expanded very steadily, and several Countries (starting from the US, still today the largest market for Balsamic Vinegar of Modena) imported it for a spreading consumption.

Market figures for several markets tend to demonstrate that the product is partly substituting consumption of other vinegars, even much cheaper than balsamic itself, and that this vinegar tends to be inelastic to price. A good explanation also for the growing success of Traditional Balsamics, which, on a much smaller scale, are increasing their notoriety and exports especially after the EEC granted the PDO protection to their names and standards of identity.

This, finally, underlines the future issues, which will be probably mainly focused on the needs for controls over production, and protection from an increasing trend of counterfeiting.

THE SOLERA SYSTEM FOR THE VINAGRE DE JEREZ

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“The “Vinagre de Jerez” (Sherry Vinegar) Denomination of Origin was created in 1994 in order to control, defend and promote a very special vinegar produced for many years in the demarcated region of Jerez-Xeres-Sherry and aged in american oak barrels in an unique ageing system called “Solera and criadera”. Sherry Vinegar is a perfect combination between nature, tradition and technology. The special microclimate of the region, the architecture of the ageing cellars, the wooden butts and the unique ageing process confere to this vinegar a great intensity and richness of flavours, making it completely different to any other vinegar. Very well known in the world of “haut cuisine”, it is becoming increasingly popular for its use at home and therefore sales of Sherry Vinegar are growing every year.”

THEORETICAL APPROACH TO AGE DETERMINATION OF TRADITIONAL BALSAMIC VINEGAR

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Aims: The production of Traditional Balsamic Vinegar of Reggio Emilia (ABT) is simple for its basic steps, but indeed it is very complex for the chemical, physical and biological transformations which are involved. The process is also extended and it requires at least 12 years of aging. The vinegar is transfer from a barrel to another resulting a mix of products with different ages. Therefore it is very difficult to assess the age and the precise yield of ABT from cooked must. In this work we have developed a theoretical model which allows to calculate the age and the maximum admissible production of ABT per barrel.

Methods and Results: The model developed in this work is based on the volume of the barrels and the flow in entry of the cooked must necessary to feed the same barrels. The model is valid for barrels at the equilibrium and which are at least 12 years old. The age of the ABT in exit is a function of the mass of solutes in the barrels and the flow of solutes in entry.

Conclusions: The real age of the ABT, as well as the maximum admissible productivity for barrels, are important quality parameters. The development of a mathematical model to age determination of ABT is an important and objective tool for quality definition of balsamic vinegars.

Significance and impact of the study: The theoretical approach pointed out in this work is a required step for ABT authenticity protection.

Key word: Traditional Balsamic Vinegar, age of ABT, quality parameter, theoretical model

THE YEASTS OF TRADITIONAL BALSAMIC VINEGAR

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Aims: Production of Traditional Balsamic Vinegar (TBV) follows two biological process: alcoholic fermentation of cooked must sugars to ethanol by yeasts and oxidative fermentation of ethanol to acetic acid by Acid Acetic Bacteria. The TBV yeasts are not much known. In the present study, determination of most frequent yeast species isolated from Traditional Balsamic Vinegar and responsible of spontaneous alcoholic fermentation of cooked must was performed.

Methods and Results: In this work, 98 yeast strains were isolated from 39 different TBV or cooked must samples on Sabouraud medium and identified both by enzymatic digestion of amplified 5,8S-ITS regions and sequence analysis of D1/D2 domain of the large subunit 26S ribosomal DNA. On the basis of our data, 12 different species were detected, most of which were never found in TBV and cooked must samples. Among these species, *Zygosaccharomyces bailii* was the most frequent isolated species, being present in 18 different samples, but also *Zygosaccharomyces rouxii*, *Saccharomycodes ludwigii* and *Zygosaccharomyces mellis* were frequently recognized. *Saccharomyces cerevisiae* was detected too, although it is not a typical osmophilic species.

Conclusions: Although the cooked must and TBV are no-permissive media because of the pH, acetic acid and sugar concentration values, our results demonstrated that the indigenous TBV yeast population is complex and composed by yeast species number higher than that reported in literature.

Significance and impact of the study: On our knowledge, the first monitoring study of a high number of yeasts fermenting the cooked musts used for TBV preparation was carried out and, on the basis of results by two different molecular techniques, *Z. bailii*, *Z. rouxii*, *S. ludwigii*, *Z. mellis* and *S. cerevisiae* are the most frequent species detected.

Keywords: Traditional Balsamic Vinegars, D1/D2 domain, ITS regions, *Zygosaccharomyces bailii*

POPULATION DYNAMICS OF ACETIC ACID BACTERIA DURING COCOA FERMENTATION

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Aims: To study the population dynamics of acetic acid bacteria during cocoa fermentation.

Methods and results: During fermentation of cocoa beans the total count of microorganisms increases in the first 24-36 h, and then stabilizes or gradually reduces. The fermentation of cocoa can be considered as being divided into three phases: phase 1 or the anaerobic development of yeasts; phase 2 or the development of lactic acid bacteria (LAB); and phase 3 or the development of acetic acid bacteria (AAB). As a first step, the sugar of the plant juice is fermented to ethanol by yeasts, thus creating a medium highly suitable for the development of AAB. The yeast activity becomes inhibited by the alcohol concentration, increasing pH, and greater aeration, conditions that are more favorable to LAB. Also, AAB occur very early in the fermentation and persist until the end. As aeration increases, AAB become more important. Their main reaction is the conversion of ethanol to acetic acid. Acetic acid mainly causes the death of the beans, whereby the biological barriers (membranes) between the cells break down, and hence various enzymes and substrates are free to mix, and the subsequent reactions produce the necessary flavour precursors for cocoa processing. The AAB are also responsible for the oxidation of acetic acid to carbon dioxide and water. This strongly exothermic reaction is mainly responsible for the rise in temperature, which can reach 50°C in some fermentations.

Conclusion: In practice, there is considerable overlap between the phases, and the relative importance of each phase varies between regions and fermentation techniques. Also, the population dynamics of each group of microorganisms may vary, in particular that of the AAB, influenced by factors such as the evolution of the acidity and temperature during fermentation and drying.

Significance and impact of the study: Studying the population dynamics of cocoa bean fermentation will unravel the contribution of acetic acid bacteria in cocoa processing.

Key words: acetic acid bacteria, cocoa fermentation, acetic acid

ACETIC ACID BACTERIA OF TRADITIONAL BALSAMIC VINEGAR

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Aims: Traditional Balsamic Vinegar has been made by the ageing of cooked grape must into wooden barrels arranged in set of 5-7 casks each of varying capacity. Every year a small amount of vinegar is transferred from a bigger to a smaller barrel and new cooked must is added to the first barrel of the set. In this way the sugary concentration is increased from the biggest to the smallest barrel due to water evaporation across the wood.

Methods and results: Different molecular techniques were applied to study acetic acid bacteria in Traditional Balsamic Vinegar. In particular 16S-23S-5S rDNA PCR/RFLP analysis, DGGE and sequencing techniques were performed on both acetic acid bacteria type strains and isolated strains from vinegars.

Conclusion: *Gluconacetobacter xylinus* is the main species of Traditional Balsamic Vinegar and the greatest hurdle to the growth of acetic acid bacteria is high sugar concentration.

Significance and impact of the study: The results suggest new technological approach to vinegar production.

Key words: acetic acid bacteria, molecular techniques, glucose tolerance, vinegar.

ENVIRONMENTAL TECHNOLOGIES AND ACETIC ACID BACTERIA: YEAST BIOMASS RECOVERY AND ACETIC ACID PRODUCTIONS FROM CHEESE WHEY

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Aims: To set up in advanced microbial biotechnologies for the acetic acid and biomass production from cheese whey; to valorize the suitable products to destine as animal feedings and to reduce the environmental pollution.

Methods and Results: Selected yeast (*Kluyveromyces marxianus* 2A2 strain) and acetic acid bacteria (*Acetobacter aceti*, DSM G3508 strain) were used as inocula for alcoholic and acetic fermentations, respectively, on cheese whey, at laboratory scale and fermenting pilot plants (20 l - 1,000 l).

Data showed increase of ethanol production when operated with biomass recycling (18.6 gl⁻¹), acetic acid yield and great stability of fermentative trials (working period 28 days). Batch and feed-batch fermentation tests conducted increased and standardized alcoholic fermentation and acetic acid recovery (average lactose consumption 56%, ethanol 6.7 gl⁻¹day and acetic acid production 4.35 gl⁻¹day).

Conclusions: The experimentations furnished important data suggesting transferability in full scale plants with possibility to valorize elevated daily quantities of cheese whey by-products.

Significance and Impact of Study: Results clear evidenced that microbial biotechnologies, when well conducted, could be of significant importance towards both contributing the reduction on the polluting load and to develop an energy recovery system (protein and energy) for animal feedings.

Keywords: acetic acid bacteria, acetic acid, cheese whey, biomass

FRUIT VINEGARS BASED ON CITRUS WINES

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Aims: To evaluate the feasibility of new fruit vinegars based on citrus wines.

Methods and results: Natural or concentrated citrus juices - lemon, orange, or bergamot - and must from natural or dried grapes were differently mixed to prepare substrata for winemaking. The alcoholic fermentation trials were carried out using selected strains of *Saccharomyces cerevisiae* or *Hanseniaspora guilliermondii*. The obtained citrus wines and other control wines were acetified with selected strains of *Acetobacter aceti*, *A. pasteurianus* or *Gluconacetobacter hansenii*. The obtained vinegars were examined for the principal analytical parameters. Remarkable differences among the vinegars were observed.

Conclusion: To obtain citrus wines fit to produce vinegars it is advisable: a) the choice of an appropriate mixing ratio among the fruit juices, to attain sufficient sugar content; b) the utilization for the winemaking of suitable non-*Saccharomyces* yeasts. To obtain fruit vinegars based on citrus wines it is advisable to select bacterial strains showing high acid-resistance and ability to metabolize methylic alcohol.

Significance and impact of the study: The trials carried out with the present work constitute a basic step, with the ultimate goal to produce new fruit vinegars based on citrus wines.

Key words: citrus juices, citrus wines, fruit vinegars, mixing ratio, starter selection

TRADITIONAL BALSAMIC VINEGAR, TECHNOLOGY AND TRADITION

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Aims: A minimum period of 12 years ageing is the required condition for Traditional Balsamic Vinegar of Reggio Emilia (TBVRE), a product with millenary tradition.

To age doesn't mean to let it pass this very particular product, which is constantly and accurately controlled during this essential step.

Contents: The tradition tells us that TBVRE exists at Reggio Emilia since 1046 d.C. when Bonifacio, Matilde of Canossa father's, took it like present to Enrico III, king of Germany.

This millenary tradition is still living in the families of Reggio Emilia but only, few years ago, scientific methods were applied to the technological control of this very complex transformation. We can describe three steps of control: microbiological, chemical and sensorial.

The microbiological monitoring may be applied at three important levels: the control of cooked must fermentation, the activity of acetic acid bacteria and the research of unsuitable micro organisms.

The chemical monitoring is important for the selection of raw materials and their transformation. This monitoring is also able to check the change of ageing parameter of the product (like density and acidity).

The sensory evaluation of TBVRE may be achieved at different steps of the flow process production. It is possible to check quality, defects and discords of the product, to optimize the flow of product between barrels and to predict the best potential ageing strategy of each batch of product.

The methods of building barrels, the different kind of wood used, the peculiarities of the vinegar rooms (for example their ventilation), the selection of grape and the method to obtain cooked musts, are only some of the factors that can influence the course of the ageing.

The monitoring steps at this point described are useful methods to understand in depth the effect of known factors of production but we know that the science of the TBVRE is very young and a lot of factors are actually unknown.

Conclusion: All these aspects are essential for knowing the characteristics that make unique and original every single ageing room from a physical and environmental point of view. Accurate monitoring is the only way to plan with scientific method the flow product strategy (like decanting, refill and taking) during ageing process.

Key words: Traditional Balsamic Vinegar, monitoring, production, ageing

EVOLUTION OF POLYPHENOL AND RELATED SUBSTANCES DURING VINEGAR AGING

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The final quality of vinegar is determined by the raw material used as substrate, the acetic acidification system used and eventually, wood ageing. The production of high quality vinegar requires an ageing period in wooden butts. Chemical composition and physicochemical parameters are influenced by these factors. Phenols are present in wine vinegars due to their natural content in grapes or as a result of contact with wood during the ageing process. Phenols can be accurately determined in wine vinegars by HPLC and DAD. Multivariate analysis of data revealed that phenolic compounds of wine vinegars are a good tool to classify and predict the membership of samples according to the elaboration process or indeed the type of wine used. Time of aging also changes the phenolic profile of wine vinegars

We have studied the evolution of 24 phenolic compounds in wood barrels along time (24 months). Total polyphenolic index increased along time. From the month 6, important increases in phenolic compounds were achieved, specially in aromatic aldehydes and 5-(hydroxymethyl)-2-furaldehyde.

When we applied multivariate statistical methods, a good classification was obtained of samples according to their aging time

Aging of wine vinegars can also be performed by using oak chips. In this sense we have conducted interesting experiments for evaluating their final phenolic composition and sensorial profile.

REALTIME MONITORING OF AN INDUSTRIAL *Gluconobacter* FERMENTATION PROCESS

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Increased competition in the manufacturing industries, and more rigorous regulatory requirements governing product quality and processing conditions, has driven the development of analytical techniques which allow greater control of e.g. bioprocesses. One such technique has been employed to monitor the biotransformation of D-sorbitol to L- sorbose by *Gluconobacter suboxydans*, a key step in the Reichstein process for Vitamin C (ascorbic acid) production. Analysis of the bioconversion is usually by HPLC, or equivalent methods, and, as such, is laborious, time consuming and costly. In addition, detection of one analyte (L-sorbose) in the presence of significant concentrations of its substrate (D-sorbitol) can be difficult, as the two compounds are chemically similar and discrimination between the species is problematic when standard analytical techniques are employed. Using Attenuated Total Reflectance Fourier Transform mid infra red spectroscopic analysis in at-line mode, the quantification of sorbose and sorbitol was successfully carried out in near real-time. ATR-FT-MIR analysis is rapid, non-invasive and requires no sample preparation, and potentially provides real time information allowing improved process control and increased productivity via the minimisation of side-product formation.

The organism used in the study was *G.suboxydans* ATCC621.

IMPROVEMENTS FOR AN OPTIMIZED PROCESS STRATEGY IN VINEGAR FERMENTATION

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Aims: The biological production of vinegar from alcoholic mash depends on several kinetic factors. Ethanol is a raw material that strongly inhibits the activity of the acetic acid bacteria. On the other hand, without ethanol the bacteria cannot survive. Acetic acid, which is produced by the incomplete oxidation of ethanol, also inhibits the metabolism of the bacteria. However, the bacteria are not able to grow sufficiently fast in unacidified mashes. The concurrent presence of ethanol and oxygen is the most important factor that ensures the cellular energy balance. If one of these components is lacking, this will stop immediately the energy metabolism of the obligate aerobic microorganisms and significantly decrease productivity of a fermentation plant over a long period of time. In the lecture, a short review of different activities and projects undertaken by our research and application department to analyze and improve industrial vinegar fermentation over the past few years will be given.

Methods and Results: We have identified two groups of industrial-scale submersible fermentation processes: A first group of processes that can be directly optimized by innovative process and control strategies, and a second group where process optimization can only be achieved by improved equipment performance.

The second group of processes mainly uses mashes from extract-rich raw materials (grape, cider, malt, rice, etc... wines) and achieves acidities of less than 12%. In general, these are semi-continuous repeated batch processes. The metabolic rate of the strains used in processes with extract-rich materials is extremely high, and the oxygen transfer rate in industrial-scale fermenters is limited; therefore, the productivity achieved in this process is restricted by the limited availability of oxygen. Tests have shown that process optimization could be achieved by increasing the mass transfer coefficient $k_L a$ of the aeration systems. Numerical CFD models based on Navier-Stokes equations have been efficient tools to pre-calculate the flow and mass transfer regime.

The first group of processes for the production of (mainly "white vinegar") requires a more complex process sequence, whereas the raw material to be used is a simple mix of purified alcohol, water and nutrients. These processes are either single or dual-stage repeated fed-batch processes and the metabolic rates and the viability of the acetic acid bacteria are limited by kinetic inhibition at high acidities. By using improved equipment and innovative process control, the final acidity to be achieved in these processes was 20% and more, even on industrial scale. Kinetic models based on differential equations were initially used to investigate and optimize these processes. However, the genetic instability of the bacteria strains caused changes in the metabolic flux rates as soon as the process strategy was modified and therefore a parameter change of the kinetic models. The use of cognitive algorithm and process expert knowledge has provided more flexible and reliable results and a more favorable technology to be used in process automation systems. A sophisticated process control system with implemented "fuzzy logic" was used for the optimization of productivity and acidity without man/machine interaction.

Key words: Modeling, oxygen transfer, cognitive algorithm, process strategy

MODELLING AND PARAMETRIC ADJUSTMENT OF THE WHOLE CYCLE OF A SEMI-CONTINUOUS WINE VINEGAR PROCESS

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Aims: Several strategies can be followed to optimise a specific process for wine vinegar production. Some cases need modelling the process in order to get a equation set for predicting as close as possible the system behaviour.

Methods and results: In this work, a representational model based on mass balances and kinetic equations has been looked for. To compare the predictions of the model, experimental data obtained in a 8 L Frings fermenter have been used. A semi-continuous process has been followed. A specific object oriented modelling tool (OOM), ECOSIMPRO®, as well as a Numerical Algorithm Group (NAG) library and a C++ compiler have been used.

Conclusion: The followed strategy allows a straightforward method for adjusting the parameters of the complex model used here.

Significance and impact of the study: The study shows a method to simulate and adjusting a differential algebraic equations (DAEs) based model. So, work for researchers in this area may be facilitated.

Key words: wine vinegar, modelling, optimisation, ECOSIMPRO

BIOTRANSFORMATION OF GLUCOSE TO 5-KETO-D-GLUCONATE, A PRECURSOR OF L-(+)-TARTARIC ACID

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Aims: In order to establish *G. oxydans* for industrial 5-keto-D-gluconate production, we investigated its oxidative glucose metabolism and aimed on the direction of the carbon flux to an overproduction of this metabolite.

Methods and results: One important characteristic of *G. oxydans* DSM 2343 is the oxidation of D-glucose to D-gluconic acid and further to 5-keto-D-gluconate, the direct precursor of L-(+)-tartaric acid. This strain converts glucose into 50 % of 5-keto-D-gluconate and 2-keto-D-gluconate, respectively. As this by-product formation proved to be a significant problem, the gluconate-2-dehydrogenase was inactivated. In the resulting strain no by-product formation was detected. To further improve the 5-keto-D-gluconate production, enzymes involved in 5-keto-D-gluconate formation were overproduced. The strains overproducing either glucose dehydrogenase or gluconate-5-dehydrogenase showed a 10-fold and 30-fold higher enzyme activity, respectively. In the most suitable strain more than 85 % of the glucose was converted to 5-keto-D-gluconate.

Conclusion: The resulting strain is appropriate for an industrial production of 5-keto-D-gluconate and L-(+)-tartaric acid.

Significance and impact of the study: The results provide a new method for 5-keto-D-gluconate production that will simplify L-(+)-tartaric acid production, so that the usage of L-(+)-tartaric acid could be expanded.

Key words: acetic acid bacteria, *Gluconobacter oxydans*, glucose metabolism, 5-keto-D-gluconate, L-(+)-tartaric acid

THE COMPLETE GENOME SEQUENCE OF *Gluconobacter oxydans*: DECIPHERING THE PROCESS OF INCOMPLETE OXIDATION

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Gluconobacter oxydans is unsurpassed by other organisms in its ability to incompletely oxidize a great variety of carbohydrates, alcohols and related compounds. Furthermore, the organism is used for several biotechnological processes, such as vitamin C production.

Aims: To further our understanding of its overall metabolism, we sequenced the complete genome of *G. oxydans* 621H.

Methods and results: The chromosome consists of 2,702,173 base pairs and contains 2,433 open reading frames. In addition, five plasmids were identified that comprised 235 open reading frames. Biological roles were assigned to 1,877 ORFs. The sequence data were used for metabolic reconstruction of the pathways leading to industrially important products derived from sugars and alcohols. Although the respiratory chain of *G. oxydans* was found to be rather simple, the organism contains many membrane-bound dehydrogenases that are critical for the incomplete oxidation of biotechnologically important substrates. One striking feature of the *G. oxydans* genome is that 75 open reading frames were identified that encode putative dehydrogenases/oxidoreductases of unknown functions. These findings allow to develop strategies for the employment of *G. oxydans* for a much greater variety of incomplete oxidations.

Significance and impact of the study: The genome project revealed the unique biochemistry of *G. oxydans* with respect to the process of incomplete oxidation.

Key words: *Gluconobacter*, genome sequence, pathway reconstruction, incomplete oxidation

MOLECULAR BASIS OF ACETIC ACID RESISTANCE IN ACETIC ACID BACTERIA

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Aims: The mechanism conferring acetic acid resistance in acetic acid bacteria has been hardly understood despite its importance in vinegar-manufacturing. We have previously cloned the *aar* gene cluster complementing the acetic acid-sensitive mutants of *Acetobacter aceti*. We further try to elucidate the mechanism by proteomic and genomic analyses.

Methods and results: Proteins induced by acetic acid in *Acetobacter aceti* were analysed by two-dimensional gel electrophoresis. Two major proteins with enhanced production in soluble and membrane fractions were identified as aconitase and an ABC-transporter, respectively. The ABC-transporter probably functions as exporter of acetic acid by the gene-disruption experiment. These two genes were also found in the genome of *Gluconacetobacter polyoxogenes*, high acidity vinegar-producing strain, by analysis of its complete genome sequence. Overexpression of the two genes raised the acetic acid resistance in *Acetobacter aceti* and *Gluconacetobacter xylinus* and resulted in improvement of the productivity.

Conclusion: The resistance of acetic acid in *Acetobacter aceti* is conferred by several mechanisms which might function universally in acetic acid bacteria.

Significance and impact of the study: The finding in this study gives a clue to breed a strain more suitable for vinegar fermentation.

Key word: acetic acid resistance, aconitase, ABC-transporter, *Acetobacter aceti*, *Gluconacetobacter*

A PROTON MOTIVE FORCE-DEPENDENT ACETIC ACID EFFLUX PUMP IN *Acetobacter aceti*

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Aims: Acetic acid bacteria, especially *Acetobacter* and also *Gluconacetobacter* species, have an especially intensive ethanol oxidation ability to accumulate vast amounts of acetic acid outside of the cell. Since these bacteria are able to grow in medium with a high concentration of acetic acid, they seem to have some specific mechanism to resist acetic acid. We expected that acetic acid bacteria may have an efflux pump for acetic acid as the resistant mechanism.

Methods and results: The efflux pump activity of *Acetobacter aceti* IFO 3283 was examined by measuring ^{14}C -acetic acid (or acetate) uptake with the intact cells and also with the inside-out membrane vesicles which were successfully prepared from the strain in this study.

Conclusion: Only in the presence of respiratory substrate, the inside out vesicles were able to take acetic acid/acetate up, which was shown to be pH-dependent and also sensitive to a proton uncoupler, indicating that *A. aceti* IFO 3283 has a proton-motive force-dependent efflux pump specific for acetic acid.

Significance and impact of the study: The acetic acid efflux system seems to be, at least, one of the significant mechanism responsible for the acetic acid-resistance of acetic acid bacteria.

Key words: acetic acid resistance, acetic acid bacteria, efflux pump, *Acetobacter aceti*

OPTIMISATION OF THE WINE VINEGAR PROCESS. INFLUENCE OF ETHANOL CONCENTRATION AT THE MOMENT OF DISCHARGE

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Aims: The industrial wine vinegar industry tries to get the highest possible productivity. The final ethanol concentration before discharge is amongst the operational variables which can be modified in a semi continuous typical process in order to. In this work the influence of such variable has been studied.

Methods and results: A 8 L Frings fermenter working in a typical semi continuous way has been used. The discharge was done at several final ethanol concentrations: 0.5, 1.5, 2.5 and 3.5 °GL. The mean fermentation rate as well as the productivity have been evaluated in each case.

Conclusion: The results show the increase in both fermentation rate and productivity as the final ethanol concentration goes up. The mean fermentation rate and the productivity raise a 40% and a 25% respectively when the final ethanol concentration changes from 0.5 to 3.5 °GL

Significance and impact of the study: The results suggest the possibility of getting higher productivities when optimising simultaneously the main production fermenter with the finishing one.

Key words: wine vinegar, optimisation, operational variables, ethanol in discharge

OPTIMUM OPERATING CONDITIONS IN CLOSED-SYSTEM INDUSTRIAL ACETIFIERS (CONTINUOUS OPERATION): A STUDY BY COMPUTER SIMULATION

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Aims: The growth of acetic acid bacteria in submerged culture is simulated with an overall model which takes account of the combined influences of the acetic acid, ethanol and oxygen by means of tracking expressions for the specific rate of growth and the specific rate of cell death.

The optimum operating conditions have been calculated for different rates of dilution, in each case analysing the influence exerted over the evolution of the process by the concentrations of ethanol and acetic acid in the feedstock.

Methods and Results: In the model used in this study, for the consumption of substrate and the formation of product it is assumed that the rates of consumption and formation are functions solely of the rate of growth of the micro-organism. The model has been proposed for closed systems in which the losses of ethanol by evaporation are minimal. Furthermore, it has been assumed that the consumption of ethanol and oxygen for the synthesis of cellular material, together with the consumption of ethanol and acetic acid to produce ethyl acetate by the chemical route, are negligible compared with their total consumption. The Runge-Kutta fourth order method of numerical integration has been used for all the simulations, with step widths $\delta t=0.1$ h. The evolution of the bioreactor has been analysed from the moment of start-up until the steady state conditions has been reached. The virtual experiments performed have involved modifications to the rate of dilution and to the concentrations in the feed to the reactor, using these as variables to optimise, first, the time taken to reach the steady state conditions and then, once this has been reached, the concentration of acetic acid in the effluent and the instantaneous rate of acetification of the reactor.

Conclusion: The most significant fact to be deduced from the results obtained is that it is impossible to increase the concentration of acetic acid in the product by increasing the ethanol concentration in the feed. The inhibitory effects of acetic acid and/or ethanol on the growth of the bacteria imposes a maximum limit on the acidity of the vinegar obtained under continuous operating conditions; this limit lies approximately between 65 g/L and 70 g/L.

Significance and impact of the study: These results should be useful for designing the continuous bioreactor for acetic acid production.

Keywords: Computer simulation, industrial acetifiers, acetic acid bacteria

GENERA AND SPECIES IN ACETIC ACID BACTERIA: THEIR PAST, PRESENT AND FUTURE

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Aims: The proposal and establishment of genera in acetic acid bacteria are surveyed and discussed historically, together with species of the genera.

Methods and results: Acetic acid bacteria were once accommodated in the single genus *Acetobacter* Beijerinck 1898. The second genus *Gluconobacter* Asai 1935 was proposed, however, severe confusion occurred on the priority of the generic name, *Gluconobacter* or “*Acetomonas*” Leifson 1954. The latter was a junior subjective synonym. The third genus was *Acidomonas* Urakami et al. 1989, however, the name was not accepted along with that of the subgenus *Gluconoacetobacter* Yamada and Kondo 1984, the genus *Acetobacter*. The name of the subgenus was elevated to the generic level as *Gluconacetobacter* Yamada et al. 1998 with a proposal to recognize the name of *Acidomonas*. Recently, additional four genera were described: *Asaia* Yamada et al. 2000, *Kozakia* Lisdiyanti et al. 2002, *Saccharibacter* Jojima et al. 2004 and *Swaminathanian* Loganathan and Nair 2004.

Conclusion: In the family *Acetobacteraceae*, the α -*Proteobacteria*, the total eight genera are counted.

Significance and impact of the study: These data revealed that the acetic acid bacteria have great divergence beyond our expectations.

Key words: *Acetobacter*; *Acidomonas*; *Asaia*; *Gluconacetobacter*; *Gluconobacter*; *Kozakia*; *Saccharibacter*; *Swaminathanian*

IDENTIFICATION AND CLASSIFICATION OF STRAINS ASSIGNED TO THE GENUS *Gluconobacter* BASED ON RESTRICTION AND SEQUENCE ANALYSES OF 16S-23S rDNA INTERNAL TRANSCRIBED SPACER REGIONS

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Aims: A number of strains assigned to the genus *Gluconobacter*, which are maintained at NBRC, were re-identified on the basis of restriction and sequence analyses of 16S-23S rDNA internal transcribed spacer (ITS) regions.

Methods and results: Thirty strains of *Gluconobacter* species were examined for their species-level identification. The restriction analysis with *Mbo*II and *Bsp*1286I revealed that the 30 strains were divided into seven groups. Of the seven groups, strains of four groups were re-identified as *G. oxydans*, *G. cerinus* and *G. frateurii*. Strains of the remaining three groups were not re-identified and suggested to be in separate taxa. The phylogenetic analysis based on 16S-23S rDNA ITS sequences indicated that *G. frateurii* has a heterogeneous nature taxonomically. Of the three groups, two strains of Group VII were classified as the new species and revived name, *Gluconobacter albidus* (ex Kondo and Ameyama 1958) (Yukphan et al., 2004). Two strains of Group VI and Group VIII and the problem in *G. frateurii* mentioned above are under investigation taxonomically.

Conclusion: The restriction and sequence analyses of 16S-23S rDNA ITS regions were utilized for the species-level identification in the genus *Gluconobacter*, together with DNA-DNA hybridization.

Significance and impact of the study: This study represented that the genus *Gluconobacter* Asai 1935 has diversity phylogenetically.

Key words: 16S-23S rDNA ITS, classification, *Gluconobacter*, identification, *Mbo*II, *Bsp*1286I

DESCRIPTION OF *Gluconacetobacter swingsii* SP. NOV. AND *Gluconacetobacter rhaeticus* SP. NOV., ISOLATED FROM ITALIAN APPLE FRUIT

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Aims: Identification of acetic acid bacteria.

Methods and results: Two Gram-negative, rod-shaped, non-spore-forming bacteria (DST GL01 and DST GL02) were isolated from apple fruit juice in the region of the Italian Alpes. On the basis of 16S rRNA gene sequence similarities, strains DST GL01 and DST GL02 were shown to belong to the α -subclass of the *Proteobacteria*, related to the *Gluconacetobacter xylinus* branch (98.5 - 100%). The level of similarity to the 16S rRNA genes of other validly described species of the *Acetobacteraceae* family were below 97.2%. The results of DNA-DNA hybridizations, together with physiological and biochemical tests, allowed genotypic and phenotypic differentiation between the strains DST GL01 and DST GL02 and from the 11 validly published *Gluconacetobacter* species. They therefore represent two new species, for which the names *Gluconacetobacter swingsii* sp. nov. and *Gluconacetobacter rhaeticus* sp. nov. are proposed. The type strains of these species are DST GL01^T (= LMG 22125^T) and DST GL02^T (= LMG 22126^T), respectively.

Conclusion: Many acetic acid bacteria species are strongly correlated at the phylogenetic level and have phenotypic characteristics that are similar to one another. Therefore a polyphasic study is the recommended technique for an accurate identification of acetic acid bacteria strains.

Key Words: taxonomy, new taxa, *Proteobacteria*, *Gluconacetobacter*

THE GENERA *Acetobacter* AND *Gluconacetobacter*: TAXONOMIC EVALUATION WITH THEORETICAL AND PRACTICAL IMPLICATIONS

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Aim: The species of the genera *Acetobacter* and *Gluconacetobacter* are phylogenetically closely related, even though their phenotypic and genotypic traits are very instable. This variability is due to genetic mutations but also to reversible suppression of some activities and makes the identification of the isolates at species level very tricky. New taxonomic approaches are required.

Methods and results: The evolutionary relationships among most of the species assigned to the of the genus *Gluconacetobacter* (sublineage b) and *Acetobacter*, were investigated through a comprehensive phylogenetic analysis based on 16S rRNA gene and the comparison of partial *recA* gene sequences, the evaluation of presence and distribution of insertion sequences and DNA-DNA hybridization data.

The analysis of the phylogenetic markers revealed relatively low polymorphism, although the species in the genera were found to have very low genomic correlation as established by DNA-DNA hybridisation.

Conclusion: The data obtained suggest that the evolution of the members of this family is hastened by factors that promote genetic mutations, such as mobile genetic elements.

Significance and impact of the study: This observation has a two-fold implication, in the theoretical context of taxonomy and in the practical aspects of genetic stability of strains of industrial importance.

Key words: *Acetobacter* spp, cellulose-producing *Gluconacetobacter* spp., 16S rRNA, *recA*, insertion sequences

RAPID METHOD FOR TOTAL, VIABLE AND NON VIABLE ACETIC ACID BACTERIA DETERMINATION

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Aims: In the scope of the optimisation of the wine vinegar fermentation process, accurate methods of measuring several variables are needed. A measure of the total cell concentration as well as the percentage of viable ones are specially important in order to model the process. In this work a possible option, using the direct counting in a Neubauer chamber as well as an epifluorescence staining technique, has been studied.

Methods and results: A Neubauer chamber from the firm Brand with a special deep of 0.02 mm has been used. The viable and non viable counts of bacteria were carried out with the LIVE/DEAD® BacLight™ Bacterial Viability kit provided by Molecular Probes, Inc.

Conclusion: The followed method allows a straightforward determination of total, viable and non viable bacteria concentrations. Meanwhile the total cell concentration analysis takes 15 minutes approximately, 30 minutes are needed in total for viable and non viable cells.

Significance and impact of the study: The results show an alternative for measuring quickly this variable, so the studies in this field may be carried out faster.

Key words: wine vinegar, optimisation, acetic acid bacteria, LIVE/DEAD® BacLight™, Neubauer

NUCLEIC ACID TECHNIQUES IN BACTERIAL SYSTEMATICS AND IDENTIFICATION

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The history of bacterial systematics is characterised by a permanent search for methods to establish a taxonomic framework reflecting the natural relationships of the respective organisms. The rapid progress in the development of molecular methods resulted in major restructuring of microbial taxonomy as documented in the editions of Bergey's Manual. Although comparative genome analyses showed that the major fraction of prokaryotic genomes has been subjected to lateral gene transfer during the course of evolution, there is a core of conserved ubiquitous genes which indicate a roughly congruent phylogenetic trace. The overall picture of rRNA derived phylogeny is supported by other independent markers indicating that rRNA based phylogeny roughly reflects the evolutionary history of the organisms. Consequently, it is justified to use rRNA and other conserved marker based phylogeny as backbone for adapting taxonomy. Furthermore, rRNA/rDNA based (*in situ* and *in vitro*) probe, PCR and modern microarray techniques provide valuable tools for detection and identification of microorganisms. The comprehensive data sets in combination with appropriate software allow directed design of specific probes and diagnostic primers. The major drawback of rRNA based methods concerns resolution power. At and below the species level other techniques have to be applied for taxon definition and identification. Multi locus sequence typing is an appropriate technique. However, the sets of genes or genomic regions which carry differentiating information usually have to be individually searched for different taxa. If appropriate target genes or regions are not known or reference databases are missing, directed design of strain specific probes or PCR systems is often not possible. In cases where suitable genes or regions are not known and no directed design is possible, subtraction hybridization represents an elegant method for the enrichment of strain specific DNA fragments. These can be used for identification by comparative sequence analysis or the design of (strain) specific probes or PCR systems. Polynucleotide mediated taxon specific cell immobilisation - initially depending on amplified targets such as rRNAs - has been improved to target diagnostic genome regions. More recently, the RING-FISH approach was established for *in situ* detection and identification of microbial cells based upon rRNA and single copy genomic targets.

Keywords: identification, phylogeny, probes, subtraction hybridisation, taxonomy

PHYLOGENETIC ANALYSIS AND IDENTIFICATION OF ACETIC ACID BACTERIA BASED ON VARIOUS GENOMIC SEQUENCES

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Aims: Since the acetic acid bacteria (AAB) are involved in many biotechnological processes, their species identity is important information for the technologist trying to control a bioprocess. The identification methods, based on analyses of the phenotypic characteristics of AAB, are not only inaccurate, but also very time-consuming. Therefore, the application of the molecular methods, based on the identification/characterization of specific DNA segments, could be a proper solution for quick and accurate identification of these microorganisms. With the aim to find a proper DNA target for species identification of AAB the following genomic sequences have been compared: 16S rRNA gene sequences, 16S-23S rDNA internal transcribed spacer regions and partial sequences encoding the PQQ-dependent alcohol dehydrogenase subunit I (AdhA).

Methods and results: The pairwise comparison among the 16S rRNA gene sequences shows very high similarity (93.6-99.9 %). This causes difficulties in constructing species-specific oligonucleotides for AAB. In contrast to 16S rRNA gene sequences, the 16S-23S rDNA sequences among AAB's species exhibit lower similarities. However, with the exception of two highly conserved genes, encoding tRNA^{Ile} and tRNA^{Ala}, they are highly divergent among species. Nevertheless, restriction analysis of PCR amplified 16S-23S rDNA spacer region enables quick affiliation of an acetic acid bacterium to a distinct group of restriction types. The interspecies similarities among the *adhA* sequences are lower than those based on the 16S rRNA gene sequences but higher than those based on the 16S-23S rDNA spacer region. The conserved and variable regions in the *adhA* sequences makes possible the construction of species-specific oligonucleotides. The specificity of PCR oligonucleotide constructed on the basis of *adhA* for identification of *Acetobacter aceti* was confirmed on well-defined AAB strains.

Conclusion: Restriction analysis of the PCR amplified 16S-23S rDNA internal transcribed spacer region enables quick affiliation of an acetic acid bacterium to a distinct group AAB's species. Gene encoding the subunit I of PQQ-dependent ADH is a promising target for quick identification of these biotechnologically important bacteria.

Significance and impact of the study: Suitability of different DNA targets for identification/characterization of acetic acid bacteria.

Key words: acetic acid bacteria, identification, 16S rDNA, 16S-23S rDNA spacer region, PQQ-dependent ADH

FUNCTIONAL ANALYSIS OF *adhS* GENE ENCODING SUBUNIT III OF ALCOHOL DEHYDROGENASE FROM *Acetobacter pasteurianus* SKU1108

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Aims: The function of the smallest subunit (subunit III) of quinoprotein alcohol dehydrogenase from acetic acid bacteria is still unclear. It has been suggested that subunit III, which was not found in *A. polyoxogenes*, acts as a molecular chaperon of subunit I and II. Thus it is reasonable to elucidate the function of this smallest subunit.

Methods and results: The structural gene of *adhS* gene from *A. pasteurianus* SKU1108 was amplified and the PCR product of 618 bp was cloned into pGEM-T Easy vector and used as a DNA probe for genomic DNA cloning of *adhS* gene. The 2 kb *EcoRI* DNA fragments showing strong hybridization signal from Southern hybridization with *adhS* DNA probe were cloned into pUC18. Three positive clones from colony hybridization were obtained and the recombinant plasmid was designed as pUCadhS. It was found that *adhS* was inserted into pUC18 in the same direction with *Plac*. The nucleotide sequence of *adhS* showed that 22 kDa protein was synthesized as a preprotein with NH₂-terminal 28 amino acids probably serving as its signal sequence for secretion from cytoplasm to periplasm. The *adhS* gene expression and localization of subunit III in *E. coli* was investigated by immunoblotting with anti-ADH (subunit III) antibody. It was found that subunit III was detected in soluble fraction of *E. coli*. To elucidate the function of *adhS* gene, an *adhS* disruptant was constructed by the insertion of 1.2 kb *PstI* DNA fragment carrying Km^r into *PstI* site of *adhS* gene on pUCadhS. The recombinant plasmid pUCadhS::Km was introduced into *A. pasteurianus* SKU1108 by electroporation. The *adhS* disruptant completely lost its ability to oxidize ethanol and partially lost its ability to grow on the medium containing 1% acetic acid. Effect of ethanol and acetic acid on *adhS* gene expression is under investigation by RT-PCR.

Conclusion: Cloning and expression of *adhS* gene in *E. coli* indicated that its protein was synthesized with signal sequence of 28 amino acids and located in the soluble fraction of *E. coli*. The *adhS* disruptant could not oxidize ethanol and lost acetic acid toleration.

Significance and impact of the study: The cloned *adhS* gene and *adhS* disruptant will be used as model for functional analysis of subunit III by RT-PCR as well as site-directed mutagenesis.

Key words: acetic acid bacteria, quinoprotein alcohol dehydrogenase, functional analysis, subunit III, *adhS* disruptant

THE *PQQ*-ALCOHOL DEHYDROGENASE OF *Gluconacetobacter diazotrophicus* PAL5

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Aims: Knowledge about membrane Alcohol Dehydrogenases of acetic acid bacteria (ADHs class III) is rather limited, therefore here we describe the molecular and kinetic properties of the purified *PQQ*-ADH of *Gluconacetobacter diazotrophicus*.

Methods and Results: The Triton X-100 solubilized enzyme was purified by sequential chromatography steps using QAE-Toyopearl, HA-Ultrogel and Sephacryl-200 columns. The homogeneous enzyme was composed of two subunits. SI(71 kDa) contained one of each, *PQQ* and a cytochrome *c*. SII(44kDa) possesses three cytochromes *c*. The purified enzyme "as prepared" showed cytochromes *c* in the reduced state and reduction was not increased by substrate nor by dithionite. The ferricyanide-oxidized enzyme was fully reduced by substrate. Linear alcohols and aldehydes were good substrates while ferricyanide, phenazine methosulfate, dichlorophenolindophenol or ubiquinone analogs were good electron acceptors. Quinone reductase activity was inhibited by mixothiazole.

Conclusions: The *PQQ*-ADH of *G. diazotrophicus* is a double function enzyme that catalyzes the reduction of linear alcohols and aldehydes with near efficiency. The enzyme pertains to the unusual type of ADH-III having two subunits instead of the more common three- subunit structure

Significance and Impact of study: Knowledge of molecular aspects of the enzymology of periplasmic *PQQ*-ADH-III will contribute to understand the distinctive capacity of acid acetic bacteria for the conversion alcohols and aldehydes into acids.

Grants: PAPIIT-UNAM IN204605 and CONACyT 34300N.

Key words: Quinoproteins, cytochromes, electron transport chain

BIOCATALYTIC COATINGS OF ACETIC ACID BACTERIA

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Aims: Acetic Acid bacteria are commonly used in industry for partial quantitative oxidations. However they are seldom available as “off-the-shelf” reagents, are used in dilute suspension and have insufficient stability (active-half life). In this study we describe a technology based on thin (< 100 µm), multi-layer, porous coatings consisting of partially coalesced polymer particles surrounding living bacteria, known as biocatalytic coatings.

Methods and Results: Mayer rod draw down methods and vinyl masks are used to make biocatalytic coating patches. Coatings are formulated so that they can be dried and frozen without loss of microbial activity after rehydration. Several methods have been developed to generate coating porosity. Permeability is monitored by diffusion measurements and cryogenic scanning electron microscopy (cryo-SEM). Viable cell concentration is studied by mild sonication and plate count. The oxydation of D-sorbitol to L-sorbose by *Gluconobacter oxydans* was successfully carried out in a bilayer latex-composite coating with an extended half-life of 430 hours.

Conclusions: Latex coating technology allows a robust use of *Gluconobacter oxydans* as a biocatalyst with high cell retention, reduced mass transfer limitations, extended half-life, frozen storage and possibility of remote use.

Significance and Impact of Study: The results obtained with latex-entrapped *Gluconobacter oxydans* suggest that this may be a general method to stabilize acetic acid bacteria.

Key words: biocatalytic latex coating, immobilized acetic acid bacteria, oxidation of D-sorbitol to L-sorbose, stabilized acetic acid bacteria, active half-life

ECOLOGY OF ACETIC ACID BACTERIA IN NATURAL ENVIRONMENTS AS SOURCES FOR VINEGAR TECHNOLOGY

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Vinegar (wine, cider, spirit, rice vinegar etc.) is produced by a microbial oxidation of ethanol to acetic acid. Industrial submerged vinegar production is started by an inoculation with “seed vinegar”, a microbiologically undefined remains from previous process. The characterization and classification of industrial important acetic acid bacteria strains by modern taxonomy is a prerequisite for the use of defined starter cultures in the vinegar factories. Since acetic acid bacteria could be considered for the difficulties with isolation Viable but Cultivable (VBC) it is important how to isolate pure defined strains from various sources of potential habitats where bacteria can represent high proportion in total microbial community like vinegar or in community where proportion is not high but they are still there like botryticid grapes. For this aim special isolation strategy has to be applied.

The acetic acid bacteria were supposed to be abundant also as biofilm microorganism and can be considered as desirable or spoilage organism depends on activity and designated product. The spoiling potential of biofilm communities is species specific. Interaction between acetic acid bacteria and other organisms I-e.: yeast in spontaneous and industrial fermentation i.e.: for ethanol was recognized as permanent. What is also the case in some other production practices. To detect this organisms enrichment approach is crucial since allows the detection of minor proportions of microorganisms which can not always be considered as spoiling flora.

This paper is dealing with survey of various habitats and different food products as potential sources for acetic acid bacteria i.e.: from different wine growing regions and to compare harvested data of existing published results. The aim of various studies has been cultivation, methodology and environment to screen the *Acetobacter* population and to apply rapid and reliable traditional techniques for isolation nad molecular techniques for typing acetic acid bacteria and studying their population dynamics during various traditional food and beverage production processes.

MOLECULAR TECHNIQUES OF IDENTIFICATION AND QUANTIFICATION OF ACETIC ACID BACTERIA

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Aims: Rapid and reliable techniques are necessary for the routine identification at the genera and species level and still further to the strain level. Molecular biology based techniques may fulfill the needs for these kind of techniques. These techniques will allow to study the relevance of these species in natural and industrial habitats.

Methods and results: PCR-RFLP of rDNA 16S, PCR-RFLP of ITS, ERIC and REP-PCR and Real Time PCR have been tested in Acetic acid bacteria. While PCR-RFLP of rDNA 16S shows to be a fast and reliable method for species identification, ERIC- and REP-PCR can be used for identification at strain level, but not for identification at species level. Real Time PCR is an interesting technique for counting microorganism. The design of specific primers enables the quantification at different taxonomic levels (family, genera, species). All these techniques have been also tested in samples originated in wines and grape-musts.

Conclusion: Strains and species of AAB can be identified by molecular methods. Enumeration of AAB is possible as well by Real Time PCR.

Significance and impact of the study: Molecular methods are shown as efficient tools to analyse different aspects of AAB presence and identification in natural and industrial environments.

Key words: RT-PCR, RFLP-PCR rDNA 16S, REP-PCR, ERIC-PCR

NITROGEN FIXING ACETOBACTERIA

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In agriculture, nitrogen is one of the most widely used fertilizers, with a still increasing global demand. In natural and agroecosystems the availability of this element often limits plant growth. The biological reaction, unique to Bacteria and Archea, counterbalancing the loss of N from soils or ecosystems is biological nitrogen fixation, the enzymatic reduction of atmospheric nitrogen to ammonia. Among the different groups of nitrogen fixing bacteria, three members within the *Acetobacteriaceae* family were found: *Gluconacetobacter diazotrophicus*, *G. johannae* and *G. azotocaptans*.

G. diazotrophicus (formerly *Acetobacter diazotrophicus*) is an endophytic bacterium first isolated in Brazil from sugarcane and later from other sucrose-rich plants such as sweet potato, cameroon grass and pineapple, and also from mealy bugs associated with sugarcane. Greenhouse and field experiments have shown that inoculation of sugarcane with *G. diazotrophicus* is capable to promote plant growth. Besides fixing nitrogen, this bacterium produces plant hormones such as indole-3-acetic acid, therefore it could enhance plant growth by N₂-fixation and hormone production.

G. johannae and *G. azotocaptans* were isolated from the rhizosphere of coffee plants in Mexico and recently, it was demonstrated their aromatic amino acid aminotransferase activity and indole-3-acetic acid production, suggesting a positive growth-promoting effect on plants.

Key words: Biological nitrogen fixation, *Gluconacetobacter diazotrophicus*, *G. johannae*, *G. azotocaptans*

MOLECULAR AND MORPHOLOGICAL CHARACTERIZATION OF ACETIC ACID BACTERIA FROM INDUSTRIAL FERMENTERS OF WINE VINEGAR PRODUCTION

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Aims: The most common process technology in modern industrial vinegar production used submerged culture, but only a little is known about its bacteriology. This is mainly due to the difficulties in isolating and cultivating the active microorganisms involved in vinegar production. Identification of acetic acid bacteria and characterization of predominant strains in the fast industrial submerged process of wine vinegar production was the objective of this work.

Methods and results: The characterization of microorganisms was carried out by their morphological characteristics using electron microscopy and molecular biology techniques particularly the analysis of 16S rDNA sequences amplified by PCR using *taq I* enzyme. These methods were used in different stages of the fermentation process. We found a mixture of two species which were determined to be *Acetobacter aceti* and *Acetobacter pasteurianus*.

Conclusion: Despite the fact that vinegar process has been optimized over the years, the biological transformation of ethanol into acetic acid still remains very delicate. The methodology in use is too empirical and it is vital to continue on going research on the development of the starters which would then become the trigger for launching rapid fermentation, and in the strains selection to produce the highest level vinegar and high-quality.

Significance and impact of the study: Our results suggest that the analysis by RFLP of PCR amplified 16S rDNA is a new and rapid technological approach to characterize microorganisms in wine vinegar production.

Key words: Acetic acid bacteria, industrial fermenters, vinegar, molecular and morphological analysis

DIVERSITY OF ACETIC ACID BACTERIA IN INDONESIA, THAILAND, AND THE PHILIPPINES

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Aims: A total of 331 strains of acetic acid bacteria isolated from fermented foods, fruits, and flowers were subjected to the study of the diversity of acetic acid bacteria in Indonesia, Thailand, and the Philippines.

Methods and Results: Five enrichment media at pH 3.5 were used for isolation and polyphasic taxonomy was used for identification. As results, *Acetobacter*, *Gluconacetobacter* and *Kozakia* were enriched in a medium containing glucose-acetic acid-ethanol but *Asaia* and *Frateuria* were inhibited by acetic acid. Furthermore, *Acetobacter* were mainly isolated from fermented foods, *Gluconobacter* from fruits and flowers, *Gluconacetobacter* from fermented foods, and most of *Asaia* from flowers. *Kozakia baliensis* were isolated from ragi (starter for fermented foods) and palm sugar brown in Indonesia, and *Frateuria aurantia* were isolated from fruits and flowers in Indonesia. No *Acidomonas* were isolated from the sources used in this study.

Conclusion: Modification of enrichment media is useful for the isolation of target microorganisms in nature and investigation of new biotopes led to the introduction of new genera and species of acetic acid bacteria.

Significance and impact of the study: This study indicates the rich diversity of acetic acid bacteria in Indonesia, Thailand, and the Philippines.

Key words: acetic acid bacteria, microbial diversity

ACETIC ACID BACTERIA FROM GRAPES TO WINE

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Aims: Investigations aimed to assess the presence of acetic acid bacteria (AAB) at each stage of winemaking and their impact on wine quality.

Methods and results: AAB of "botrytised" grapes were enumerated and identified. Ketonic compounds produced from sugars and responsible for bounding sulphur dioxide were determined. The involvement of *Gluconobacter oxydans* was predominant; it produced high levels of 2 oxo-fructose. In red wines, AAB were studied during aging in barrels. Enumeration on plate counts (viable and cultivable cells) showed that the level of population was closely linked to the dissolution of oxygen. However even in absence of oxygen they were always present, in very low concentration when enumerated on plates, but much higher when counted by epifluorescence. This was interpreted considering that a large proportion of the population was in a viable but non cultivable state (VNC) when deprived of oxygen. As soon as wine was aerated the population counted on plates dramatically increased and reached the same number as by epifluorescence. In the same time small amounts of acetic acid were produced.

Conclusions: For sweet winemaking, if present in high number on rotten grape berries, AAB impair the effectiveness of sulphur dioxide used for stabilization of wine. In other kind of wines, they may intervene during aging. In tanks, and mainly in barrels where the redox is higher, they are always viable, ready to grow as soon as oxygen is available. This explains why the volatile acidity always increases during aging especially in barrels.

Key words: "botrytised" wines, bound SO₂, barrel aging, VNC, volatile acidity

ACETIC ACID BACTERIA POPULATION DYNAMICS DURING WINE FERMENTATION

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Aims: The presence of different strains of Acetic acid bacteria has been investigated in wine fermentations under different vinification processes (yeast inoculation and SO₂ usage)

Methods and results: The analysis of acetic acid bacteria has been carried out by ERIC and REP-PCR methods, which showed to be suitable for routine identification of AAB isolates. Analysis during grape ripening period showed that the general AAB species is *Gluconobacter oxydans*, except when conditions allow a population increase of AAB, which is mostly due to the growth of *Acetobacter aceti*, which is the dominant species during wine fermentation. A very reduced number of strains are able to survive at the end of vinification and presumably are winery residents.

Conclusion: Molecular methods such as ERIC-PCR are suitable to analyze the population dynamics of AAB both in nature (grape ripening) and industrial (wine making) conditions. *Acetobacter aceti* is the main species that survives ethanol production, anaerobiosis and SO₂.

Significance and impact of the study: To the best of our knowledge these are the first studies that analyze the population dynamics of acetic acid bacteria at the strain level, showing that only some strains survive the harsh conditions of vinification. These strains belong to *A. aceti* species.

Key words: Strain identification, ERIC-PCR, *Acetobacter aceti*, *Gluconobacter oxydans*

ACETIC ACID BACTERIA ISOLATED FROM CABERNET SAUVIGNON GRAPES IN DIFFERENT CHILEAN VALLEYS

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Aims: Determine the different species of acetic acid bacterial associated to grapes from wine producing Chilean valleys.

Methods and Results: Healthy grapes were collected from the most important red wine producing valleys. Grapes were homogenized and then plated onto GYC medium. Two hundred isolates were selected by Gram staining and morphology. Identification of acetic acid bacteria was performed by RFLP of PCR amplified 16S rDNA and profiles were subsequently compared with those of type strains from collection of Laboratorium voor Microbiologie Universiteit Gent, Belgium. Sixty percent of the isolates showed RFLP patterns indistinguishable from *Acetobacter aceti* (53%) and *Glunocobacter oxydans* (7%). The rest of the patterns do not correspond to profiles described for acetic acid bacteria. *Acetobacter aceti* was commonly isolated in the most of the valley sampled; furthermore in some locations its frequency reached 100%. In contrast, *Glunocobacter oxydans* was only isolated from Curicó Valley, with high frequency. In Limarí Valley, no profiles corresponding to acetic acid bacteria were found and *Stenotrophomonas maltophilia* was the dominant population.

Conclusions: *Acetobacter aceti* was the most common acetic acid bacteria isolated from the Cabernet Sauvignon grapes from Chile.

Significance and impact of the study: This is one of the reports about acetic acid bacteria in Chilean grapes.

Keywords: acetic acid bacteria, Chile, Cabernet Sauvignon

SPOILAGE OF WINE BY ACETIC ACID BACTERIA – THE STORY IN A BOTTLE

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Aims: Increased risk of post-bottling spoilage of red wine by bacteria appears to be linked to certain winery practices, such as minimal filtration and non-lethal concentration of SO₂. This study was initiated to determine the nature and cause of sporadic microbial induced spoilage of bottled red wine.

Methods and Results: Acetic acid bacteria strains were isolated from both unspoiled and visibly spoiled bottles of different batches of sporadically spoiled red wine. The number of viable bacteria increased as the degree of visible spoilage increased. Bottles with visible spoilage had elevated concentrations of dissolved O₂, acetaldehyde, ethyl acetate and acetic acid. Sensory and chemical analysis data correlated well. RAPD-PCR analysis of acetic acid bacteria isolates suggested that because the same *Acetobacter pasteurianus* strain(s) was present in both the spoiled and unspoiled bottles, the strain of bacterium was not a promotive factor for spoilage

Conclusion: The comparatively higher concentration of dissolved O₂ and increased number of acetic acid bacteria in the wines present in bottles with visible spoilage suggests that O₂ has played an important role in the spoilage process.

Significance and Impact of Study: We hypothesise that the sporadic nature of spoilage can be explained by the natural variability in the O₂ permeability of cork closures. Comparison of the RAPD-PCR fingerprint of these wine isolates with strains from other spoiled beverages suggests that the wine isolates form a distinct subpopulation of *A. pasteurianus*.

Key words: acetic acid bacteria, *Acetobacter pasteurianus*, red wine, spoilage, RAPD-PCR

VINEGAR EELS: STATE OF THE ART AND PERSPECTIVES

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The vinegar eels are nematodes that grow in acids environments, as vinegar. The presence of eels in vinegar is known since a long time and literature assigns these organisms to the species *Turbatrix aceti*. The recognition of genera, and especially species, is particularly complex, in nematodes, because of the limited number of diagnostic characters. Several species, although possessing very different habitat and physiological characteristics, share so similar morphology, that their morphological identification is very difficult. The general lack of information on vinegar eels doesn't allow to unambiguously establish their effect on vinegar production. Independently from vinegar eels positive or negative effects on acetification processes, their control in vinegar production is important. The problem is particularly evident for products, e.g. Traditional Balsamic Vinegar of Modena and Reggio Emilia, which are neither filtered nor pasteurized before the use. Actually, the presence of vinegar eels, in the commercialized product, would not be accepted from the consumer.

Key words: nematodes, *Turbatrix aceti*, vinegar eels

MODULATION OF ACETIC ACID AND OTHER METABOLITES IN SOURDOUGH FERMENTATION: EFFECT ON BREAD QUALITY AND SHELF-LIFE

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During the fermentation processes for the wine, beer or bread production, the wild strains or the starters of *S. cerevisiae* encounter acid, osmotic or thermal stresses or their combinations.

The success of these important industrial processes is subjected to quality variability because of the incomplete knowledge of the metabolic regulation under stress conditions and of the transcriptional response of *S. cerevisiae* to sugar or physical stresses. The metabolic adaptation of *S. cerevisiae* can have important consequence on the aromatic profile (sensorial quality) of the fermented products as well as on their stability due to a different occurrence of acidulant like acetic acid or other antimicrobial metabolites.

Stress combinations experienced by *S. cerevisiae* and *Lb. sanfranciscensis* during sourdough fermentation or high sugar/high temperature fermentation on carbohydrate metabolism and flavour production was examined in order to evaluate the possibility of enhancing the accumulation of specific metabolites exploiting the regulatory mechanisms used by yeasts to cope with stresses. The analysis of the experimental set of data regarding both yeasts and lactic acid bacteria showed that the production of acetic acid as well as of other metabolites was significantly enhanced by osmotic and acid stress. These metabolic shift towards acetic acid production increased the microbial stability of the final products.

The growth of a bakery product spoilage yeast, *Pichia anomala*, was inhibited in products obtained from sourdoughs characterized by the application of combined stresses and a greater accumulation of acetic acid and other metabolites.

Keywords: yeasts, lactic acid bacteria, stress, flavours

ANTIMICROBIAL EFFECTS OF LEMON JUICE AND VINEGAR AGAINST *Salmonella typhimurium* AND *Yersinia enterocolitica* ON SALAD VEGETABLES

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Aims: Experiments were done with fresh lemon juice, vinegar and the mixture of them (1:1) to evaluate their efficacy in reducing the numbers of *Salmonella typhimurium* and *Yersinia enterocolitica* on fresh salad vegetables.

Method and Results: In the first experiment, fresh whole rocket leaves, shredded spring onion and carrot samples were inoculated with levels of *S. typhimurium* cells (approximately 6 log cfu/ml). In the second experiment, only carrot samples were inoculated with *Y. enterocolitica* (approximately 6 log cfu/ml) and the effects of fresh lemon juice and the mixture of lemon juice-vinegar (1:1) on carrot were investigated. After the inoculation step, vegetables were treated with the test solutions for 0, 15, 30 and 60 min and pathogens were enumerated by using direct plating on Bismuth Sulphite Agar (BSA) and on Cefsulodin-Irgasan-Novobiosin agar (CIN). The statistical analysis was carried out to demonstrate the most effective treatment and to show behaviour of different antimicrobial solutions on different vegetables.

Conclusion: It has been shown that the solutions used in the experiments have an antimicrobial effect against both *Salmonella* and *Yersinia* cells on vegetables. The antimicrobial effects of these products have changed depending on the type of microorganism, the type of vegetables and the contact time with antimicrobial solutions. On green onion samples vinegar was the most effective agent while the mixture of lemon juice-vinegar was the most effective agent for rocket and carrot samples.

Key words: *S. typhimurium*, *Y. enterocolitica*, lemon juice, vinegar, disinfection

USES OF VINEGAR IN THE FOLK MEDICAL PRACTICES OF THE MEDITERRANEAN

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Vinegar has been used since ancient times in medicine. But while quite a lot is known about this biological material in the “school traditions” of the history of the *Materia Medica*, much less has been reported on the uses of vinegars in the *folk* medicinal practices of Europe.

The paper will show a bibliographic survey conducted on the folkloric, ethnographic, and ethnobotanical literature of the Mediterranean area, and it will present original as well data, which have been recorded during five years of ethnobotanical field studies conducted among the Istro-Romanians in Croatia, the Arbëreshë and autochthonous South-Italians in Lucania (southern Italy), and among Kelmendi-Albanians living in one of the most remote areas in Europe: the North Albanian Alps bordering Montenegro.

In particular, the tradition of the Istro-Romanians – for centuries known as the most important vinegar traders in the Balkans – of producing “home-made” vinegar from Cornelian cherries and wild apples will be discussed in detail. Approx. other 40 diverse folk medical uses of vinegar, which have been recorded in the medical folklore of the Mediterranean, will be illustrated. The paper will show the most interesting uses, and it will briefly address the pharmacological studies so far and the potential relevance of these uses recorded in the medical folklore for the modern clinical therapy. Cultural anthropological issues related to the vinegar in the folk medicine will be addressed as well.

Key words: ethnobotany, ethnomedicine, *Materia Medica*, folklore, ethnography

VINEGAR POLYPHENOLS INCREASE THE PROTEOLYSIS AND DECREASE THE OXIDATIVE STRESS DURING DIGESTION BY PEPSIN

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Aims: We have demonstrated that red wine and wine polyphenols have several biochemical activities during peptic digestion. We have investigated if different vinegars and vinegar polyphenols have effect on peptic digestion.

Methods and results: Tradizional Balsamic (BTV) and Balsamic (BV) vinegars, red and white wine vinegars (RV and WV), and cyder vinegars (CV) were used. Pepsin activity and hydroperoxide formation were determined by spettrophotometric assay. The lysis of human erythrocyte membranes was induced by lactoperoxidase-H₂O₂-I⁻ or by β -amyloid protein. Most vinegars protect erythrocyte membrane from peroxidative damage, increase peptic proteolytic activity and decrease the hydroperoxide formation. The vinegars activity is related to their polyphenol content.

Conclusion: Vinegars show antioxidant and antiperoxidative activity and increase the proteolytic activity of pepsin.

Significance and impact of the study: Depending on the type and volume of the vinegar utilized in cooking and dressing the dishes, an increased protein hydrolysis and a decreased oxidative stress is obtained during peptic digestion.

Key word: Antioxidants, hydroperoxides, polyphenols, pepsin, vinegar

VINEGARS: THEIR USE IN FOODS, COSMETICS, THERAPEUTICS AND DETERGENTS

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Aims: Many different vinegars are produced in the world. Eating habits are strongly rooted, and people are very conservative with the vinegars they use. There are exceptions like for balsamic vinegar, which is universally known. Chinese vinegars are totally different from for instance European ones.

The objective of this study is to give a non exhaustive list of the vinegars of the world. For this purpose, various raw materials are discussed, and their alcoholic and acetous fermentation explained on the basis of biochemical formulas.

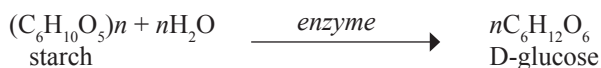
Monosaccharides are the raw materials for vinegar, for instance glucose of vegetable origin. Less known is lactose, giving the only vinegar of animal origin: whey vinegar, produced industrially only in Switzerland.

Starch being the source of sugar, raw materials derived from starch are mentioned, for instance banana, potato, chestnuts and rice. After hydrolysis of the starch into sugar, the corresponding vinegars are produced.

According to the EU MGP (manufacturing good practice), 1990 : “The name “vinegar” is reserved for the product obtained by the biological process of double fermentation, alcoholic and acetous, from liquids or other substances of agricultural origin”.

The chemical equations of these reactions are the following:

1) Hydrolysis of starch:



2) Alcoholic fermentation:



3) Acetous fermentation:



Methods and results: The first historically known vinegars and their origin are mentioned: date vinegar (Babylonians about 5000 B.C.), beer vinegar (Egyptians about 4000 B.C.), wine vinegar (Roman times).

Lists of various vinegars: Döbereiner published in 1819 an enumeration of 17 vinegars.

A modern author C. Lefebvre mentions 110 different vinegars. M.R. Adams lists 35 raw

materials. Bibliographical sources quote therapeutic, hygienic, prophylactic, medicinal, cosmetic and detergent vinegars. Wüstenfeld wrote: "People working in vinegar breweries are usually in the best health. They are protected against contagious diseases. Most of the time they reach old age".

Various denominations of origin are, for instance 1) in France: vinegar of Banyuls, Châteauneuf du Pape; 2) in Italy: *Aceto balsamico tradizionale* from Reggio and Modena, and 3) in Spain: Sherry vinegar.

Conclusion: with the constant increase of travel, more and more "exotic" vinegars will be known and bought by people. Italy is well known for wine vinegar rich in esters (ethyl acetate), and for the already mentioned balsamic vinegar.

Significance and impact of the study: The world production of vinegar at 10% acetic acid (excluding the USSR and China) was assumed to be around 1600 million liters per year, in 1983, according to Ebner and Follmann. They wrote: "There is no doubt that this production figure is among the highest for a product of primary microbial metabolism". 13 years later Ebner et al. indicated a yearly world vinegar production of 1900 million liters at 10%.

Key words: *Acetobacter aceti*, ethyl acetate, aceto balsamico tradizionale, acetous

VINEGAR FROM WHEY AS AN INGREDIENT OF DAIRY COWS RATION

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Aims: as a part of a larger project, the effects of vinegar from whey administration were investigated on milk yield and composition, nutritional status of dairy cows and physical characteristics of total mixed ration (TMR).

Methods and results: twenty Holstein cows were divided into two groups: group C, receiving the traditional TMR and group V receiving the TMR plus 10 L of vinegar·head⁻¹·d⁻¹. Throughout the experiment, lasted 75d, vinegar administration positively influenced both dry matter intake and TMR particle size distribution, resulted more balanced in group V. Milk yield was unaffected by the dietary treatment but milk urea nitrogen content was significantly lower in group V, which also showed a higher milk titratable acidity. Results could therefore suggest the positive influence of dietary treatment on rumen environment and nitrogen metabolism, as the increased body condition status in group V confirms.

Conclusion: Vinegar from whey administration could positively influence the rumen ecosystem, by improving the acceptance of low-quality hay in TMR.

Significance and impact of the study: As a dietary additive, the observed positive effects of vinegar administration to dairy cows should be evaluated as related to the cost of the treatment.

Key words: vinegar from whey, dairy cows, nutrition

ACETIC ACID BACTERIA GMO FOR THE FUTURE?

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Acetic acid bacteria are very important for industry, by the strong ability of acetate production. However, some strains also produce many other useful compounds. We have investigated cellulose-producing *Acetobacter*; *Acetobacter xylinum* (*Gluconoacetobacter xylinus*) produce large amount of cellulose from sugars. The produced cellulose (bacterial cellulose; BC) has very fine structure (1/100-1/000 compared to plant cellulose, as the diameter of fibers) and some unique mechanical properties suitable for the industrial use. Bacterial strain can be the bottleneck to construct the industrial production system of bacterial cellulose. For the construction of cellulose high producer, both of screening and strain improvement was essential. Genetic engineering is a powerful tool for strain improvement, especially for creating new physiological pathway. In this presentation, isolation and identification of the new cellulose-producing strain and some examples of gene introduction effective for the improvement of production will be shown. Future possibility of genetic-engineered strain will be also discussed.

Key words: cellulose, gene introduction, *A. xylinum* subsp. *sucrofermentans*, levan, sucrose synthase

THE NERO D'AVOLA VINEGAR: MICROBIAL, AROMATIC AND SENSORY CHARACTERIZATION

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Aims: The Nero d'Avola is new vinegar produced following traditional methods of acetification from Sicilian Nero d'Avola wine. The latter is widely described as spicy, forest fruits, mulberry, plum and ripened fruit smelling. No study has been carried out to characterize this product.

Methods and results: Several strains of presumptive acetic acid bacteria were isolated from vinegar samples. DNA was extracted, amplified by using universal primers for 16S rDNA and separated by DGGE, comparing band positions with those of type strains.

Nero d'Avola vinegar and a commercial red wine vinegar were subjected to sensory descriptive analysis by quali-quantitative evaluation of sixteen descriptors. The panel consisted of 10 trained judges. Sensory profiles were defined according to UNI U 5901950 1998 specifications; samples were analysed in triplicate.

Headspace aroma compounds of vinegar were investigated by Solid-Phase MicroExtraction (SPME).

PCR-DGGE analysis was a valid tool to study the acetic acid bacteria of vinegar.

Conclusion: Nero d'Avola vinegar was characterised, with respect to the commercial one, by spicy, cooked and maderized aromas and by cooked flavour at a statistically significant level ($p < 0,05$). The maderized aroma could be due to the aging in barrel.

Analysis of headspace aroma by SPME highlighted that the major identified compounds were ethyl-2-phenyl acetate and 2-phenylethanol.

Significance and impact of the study: The results of the present study show that the sensory and aromatic features of Nero d'Avola wine are successfully maintained in derived vinegar.

Key words: Nero d'Avola vinegar, acetic acid bacteria, sensory profile, analysis of aroma compounds

SAFEGUARDING AND PROMOTION OF TRADITIONAL BALSAMIC VINEGAR OF REGGIO EMILIA

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Aims: In order to provide a guarantee of quality for this thousand-year old vinegar tradition, in the 1980's a controlled denomination of origin was bestowed on. This is only the first step of the important way of the safeguarding and promotion of Traditional Balsamic Vinegar of Reggio Emilia.

Methods and results: With the Law no.93 of 3rd April 1986, and the Ministerial Decree no.191 of 3rd March 1987, methods of production and methods of controls were regulated. The regulations ensure that Traditional Balsamic Vinegar of Reggio Emilia is considered a food condiment, and that it is obtained from must cooked over a direct flame, coming from the pressing of grapes traditionally growing in the province of Reggio Emilia. Traditional Balsamic Vinegar of Reggio Emilia is aged in sets of different wooden barrels over a long period of time, in any case never less than 12 years. The passing of the seasons, from summer to winter, with the relative changes in temperature, assists the chemical processes and helps to personalise the product.

The 07 of July 1986 the Producer's Consortium of Traditional Balsamic Vinegar of Reggio Emilia is constituted. It has a role of safeguarding and control, it offers also assistance to producers and organises the promotional activity of the product.

With the Reg. CE no.813/2000 of 17 March 2000 the Denomination of Protect Origin from the European Union is regulated and, with the Measure Provv. 15 May 2000 of the Official Publication, disciplinary of production is in Italy defined. These laws represents today the most important level of quality in food and the certification of this quality is made by an external Organism of Control.

The Consortium has been involved in assistance to producers and promotional activity to reveal and explain the history and characteristics of Traditional Balsamic Vinegar of Reggio Emilia.

Conclusion: The Consortium's brand can be seen throughout the world on the most high-ranking and discerning tables, and identifies the exclusive label of Traditional Balsamic Vinegar of Reggio Emilia decorated with one of the tree well-known seals, Lobster Red, Silver and Gold label, according to their different sensory characteristic from 12 years old to 25 and more and more in the time.

Key words: Traditional Balsamic Vinegar of Reggio Emilia, Consortium, control, promotion

ACETIC ACID IN DESSERT WINES WITHOUT ACETIC ACID BACTERIA CONTRIBUTION

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Aims: Overripe grapes contain a rich microflora, often including acetic acid bacteria, and dessert wine usually have an higher amount of acetic acid. Thus, it is feasible to hypothesise that such amount in wine have the contribution of acetic acid bacteria.

Methods and Results: In this work we found that even from grapes and musts that did not contain acetic acid bacteria, a significant amount of acetic acid was produced during alcoholic fermentation by inoculated yeasts. The fermentation conditions that affected the acetic acid production, but also the fermentation kinetics and the overall wine composition, were the sugar concentration, the fermentation temperature, the nitrogen content and the yeast strain.

Conclusion: Acetic acid production is highly yeast strain dependent and mainly related to must sugar content, but also fermentation temperature and nitrogen additions affect its amount in wine.

Significance and Impact of Study: The yeast strain choice has to be carefully considered in dessert wine making to avoid an excessive acetic acid production.

Keywords: acetic acid, yeast, dessert wine

CONSTRUCTION OF THE BARRELS FOR VINEGAR

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Aims: RENZI FRANCESCO manufactures casks and barrels for holding Balsamic Vinegar; the company was founded in the 16th century and has been handed down through the generations. This artisan, family-run business employs very few workers and is based in MODENA (Italy) on an area covering 2000 square metres. The barrel production is briefly described.

Methods and Results: In the first working step the barrel timbers is aged in open air. Once the stavewoods were prepared and planing, they are circularly assembled side by side, to obtain the barrel form shape. Bending stavewood is formed according to the traditional method of immersion in boiling water.

Successively, the groove process is necessary at both tips of the barrel to obtain the joint of the deep ones and the assembly of the deep ones. The external smoothing and the addition of heavy duty stainless steel hoops (joined through rivets in stainless steel too) are two last steps of barrel construction. Finally all of barrels are marked and tested.

Conclusion: Painstaking labor, together with centuries of inherited experience, contributes to produce barrels of exceptional quality and craftsmanship for the preservation and ageing of fine Balsamic Vinegar.

Significant and Impact of the study: Draft of a typical production, as far as is made of the working, carried out still today exclusively by hand.

Key words: Renzi barrels, Balsamic Vinegar, barrel timber, stavewood, groove

AGEING OF ACETO BALSAMICO TRADIZIONALE: A MATHEMATICAL MODEL

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Aims: Aceto Balsamico Tradizionale (ABT, Traditional Balsamic Vinegar) is produced after a long ageing of concentrated must in five or six barrels of decreasing capacity. During this time, water evaporation occurs, but the volume is kept constant by periodical topping of the product from one cask to the following one. A mathematical approach of the phenomenon is proposed.

Methods and results: The mathematical model considers the influences of evaporation and of the toppings on an ideal solute for a period of 30 years.

Conclusion: Compared to a real data set, the proposed model satisfactorily describes the process.

Significance and impact of the study: The model provides explains easily the complexity of the trend observed during ageing and it could be used to verify the performances of the ABT cask sets.

Key words: ageing, model, evaporation, concentration

HPLC APPLICATIONS IN ACETO BALSAMICO TRADIZIONALE QUALITY ASSESSMENT

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Aims: HPLC can easily quantify the main Aceto Balsamico Tradizionale (ABT, Traditional Balsamic Vinegar) substances: sugars, organic acids, and furanic compounds. The use of the same column and the minimum sample preparation required makes this technique particularly attractive.

Methods and results: Dilution and filtration of the samples are the only operations required for sample preparation. However, sample matrix effects could make acid quantification difficult and inaccurate. For these reasons, standard addition method was used even if it required a multijection approach.

Glucose and fructose were the main components. Acid content was characterised by a great variability, with the presence of gluconic acid as peculiar compound of this fraction, while acetic acid was the most abundant component of this fraction in all samples but one. Among furanic compounds hydroxymethylfurfural was the most important substance, while the others were in considerable lower amount.

Conclusion: Reverse phase HPLC with an anionic stationary phase allowed a simple and reliable quantification of the main constituents of ABT.

Significance and impact of the study: This tool gives a wide possibility to study the product and the process, as well as it can be relevant on assessing its genuineness.

Key words: sugars, organic acids, furanic compounds, HMF, HPLC

ACCELERATED AGING OF WINE VINEGAR USING OAK CHIPS: CHEMICAL AND SENSORIAL EVALUATION

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Aims: To evaluate chemical and sensorial changes during the accelerated maturation of wine vinegar using oak chips in order to propose new economical technologies for vinegar production

Methods and results: Pretreatments applied to condition the chips include optional wetting and heating up to 180°C during 3h. Samples corresponding to different treatments, residual alcoholic degrees of the vinegar samples (0.1-2°) and time of contact with shavings (15-90 days) were analysed. Evolution of phenolic compounds determined by HPLC-DAD detection and aroma compounds by GC was followed. Sensory analysis was performed in order to evaluate their sensorial acceptance and new descriptors related with wood were tested.

At 15 days time of maceration, significant increases for most of the aromatic aldehydes (vanillin, siringaldehyde,...) occur while lactones do not increase at the same rate. Sensorial analysis revealed not significant differences between different pretreatments of shavings. A residual alcoholic degree of the vinegar samples of ca. 1° gives optimum results for extraction of components and sensorial characteristics.

Conclusion: Oak chips are valuable alternative to oak barrels for obtaining, in a short period of time (15 days), wine vinegars with a peculiar characteristic given by oak wood.

Key words: wine vinegar, aging, wood, oak chips

EFFECT OF COMPOSITION ON THE RHEOLOGICAL PROPERTIES OF TRADITIONAL BALSAMIC VINEGAR

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Aims: The influence of chemical composition (glucose, fructose, acetic acid, water, salts) on the rheological properties of traditional balsamic vinegars were studied as a function of their chemical composition at a range of temperature of 20-60°C.

Methods and results: Preliminary studies were conducted on vinegar model systems (aqueous solutions) by performing steady shear and dynamic rheological tests in order to evaluate the effect of chemical constituent alone and in combination on the rheological behavior. A fractional factorial experimental design was used to combine the composition variables (glucose content, glucose to fructose ratio, acetic acid, and potassium tartrate salt). By means of an multivariate analysis, a statistical model able to describe the effect of chemical composition on the rheological behavior of vinegar model systems was developed.

Conclusion: Among measured rheological properties, dynamic viscosity was found strictly related to chemical composition. It is reasonable hypothesize that dynamic viscosity should be related to the sensory properties of traditional balsamic vinegars.

Significance and impact of the study: Results suggest that dynamic viscosity should be used for a rapid and objective classification of traditional balsamic vinegars before the samples undergo to time-consuming and expensive sensorial analysis.

Key words: traditional balsamic vinegar, glucose, fructose, rheological properties

STUDY OF THE AGING AND OXIDATION PROCESSES OF VINEGAR SAMPLES FROM DIFFERENT ORIGINS DURING STORAGE BY NEAR-INFRARED SPECTROSCOPY

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Aims: Near-infrared spectroscopy and chemometrical techniques can be applied for controlling the ageing process in vinegar samples from different origins.

Methods and results: A correction based on water spectra was proposed for compensating the effect of lamp changing of the NIR instrument. Different classification and class modelling techniques were performed on the spectra of 96 vinegar samples taken in two different moments separate in time. Some conclusions about the physical and organoleptical effects on vinegar were drawn from the spectral zones (intervals of wavelengths) selected on the basis of the discrimination between the original spectra and the spectra taken after a variable period of storage time.

Conclusion: The application of different chemometrical techniques on the spectroscopic data is suitable for extracting all the chemical and physical information present on the near-infrared spectra of vinegar samples related to the aging and oxidation processes during storage.

Significance and impact of the study: This study is a new practical application of near-spectroscopy in the field of food safety and quality control in food industry.

Key words: vinegar, near-infrared spectroscopy (NIR), aging process, correction of spectra, chemometrical techniques

STIR BAR SORPTION EXTRACTION-GAS CHROMATOGRAPHY FOR THE ANALYSIS OF VOLATILE COMPOUNDS IN VINEGARS

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Aims: Vinegar quality is heavily influenced by flavour compounds. Several hundred compounds from different families contribute to vinegar flavour. Its chemical and organoleptic properties are determined by the acetification system, the raw material used as substrate and, in some cases, by the aging in wood.

These different raw materials and technological procedures employed result in a great variety of products of diverse quality and organoleptic properties. Considering this fact, it is logical to suppose that vinegars could be characterised and differentiated by the quantitative and qualitative analysis of their volatile components.

Methods and results: The analysis of volatile compounds is normally carried out by GC after a previous extraction and concentration stage. Even today, the extraction and concentration of flavour components, prior to their analysis, constitute a problem that has still not been satisfactorily resolved. Recently a preparation method, stir bar sorption extraction (SBSE) has been developed. The purpose of the work reported here is to optimise, using a chemometric approach, the conditions for detection and quantification of volatile compounds in vinegar by SBSE-GC.

Conclusion: SBSE is a good technique for determining the aroma compounds in vinegar with detection and quantitation limits, and linear ranges adequate.

Significance and impact of the study: The results suggest that SBSE is an appropriate tool to study the volatile compounds in vinegar.

Key words: stir bar sorption extraction; vinegar; volatile compounds; optimization; chemometric study

TEMPERATURE AND PATHLENGTH OPTIMIZATION FOR NIR MEASUREMENTS OF VINEGAR SAMPLES

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Aims: Traditionally, the study of the influence of temperature and pathlength when performing an spectroscopic measurement, is usually carried out by modifying one factor at a time. The main objective of this study is to assure the repeatability between replicates and to make the absorbance values in the spectra lay within the linear absorbance range of 0.5-2 AU. The study has been done on the practical case of the collection of near-infrared spectra of vinegar samples.

Methods and results: Experimental design and an accurate study of the results was proposed and applied to the analysis of vinegar samples by near-infrared spectroscopy in order to make the selection of pathlength and temperature in an easy and correct way and to detect the possible interactions between the factors which can lead to a mistaken interpretation of results.

Conclusion: A pathlength of 2 mm and a temperature of 43 °C were chosen as the optimal parameters for collecting near-infrared spectra of vinegar samples from different origins assuring the repeatability and linearity range for subsequent calibration processes.

Significance and impact of the study: The experimental design strategy used in this study allows reducing the number of experiments and extracting as much information as possible about the system. The same approach and methodology can be applied in future studies as a first stage for optimizing either the same or other parameters prior to the collection of spectra in different areas of the electromagnetic spectrum.

Key words: vinegar, near-infrared spectroscopy (NIR), optimization, temperature, path-length

COMBINED USE OF FTIR SPECTROSCOPY, GC-MS/SPME AND ELECTRONIC NOSE FOR THE EVALUATION OF SAFETY AND QUALITY OF BALSAMIC VINEGAR OF MODENA

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Aims: Different methods able to differentiate between traditional and industrial Balsamic Vinegar of Modena (TBVM and BVM respectively) have been proposed. Most of the techniques have been developed specifically for TBVM. On the contrary BVM is characterized by a significant variability and its quality is usually defined by its compositive and sensory analysis.

The aim of this work was to evaluate the potentiality of combined instrumental methods to assess the safety and quality of BVMs.

Methods and results: 55 samples of commercial BVM and 9 experimental ones were analyzed with ATR-FTIR spectroscopy, gaschromatography-mass spectrometry/solid phase microextraction (GC-MS/SPME), electronic nose and HPLC (with DAD and RI detectors). The multivariate statistical analysis of all the instrumental data enabled the discrimination and classification of the different samples on the basis of quality parameters.

Conclusion: The data obtained evidenced the influence of raw materials and processing parameters on the safety and quality of final products.

Significance and impact of the study: The results suggest new criteria for BVMs characterization and consequently for their differentiation according to their quality.

Key words: Balsamic Vinegar of Modena, FTIR spectroscopy, GC-MS/SPME, electronic nose, safety and quality.

Key words: vinegar, near-infrared spectroscopy (NIR), optimization, temperature, path-length

APPLICATION OF GAS - CHROMATOGRAPHY FOR THE CHARACTERIZATION OF TRADITIONAL BALSAMIC VINEGAR

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Aims: Traditional balsamic vinegars of Modena e Reggio Emilia are some of the most representative Italian traditional products. Their characterization is relevant in order to assess their authenticity and to differentiate them among similar products. In order to deepen knowledge about these vinegars, both volatile organic compounds and fixed components, such as sugars and organic acids, were investigated by gas-chromatographic techniques.

Methods and results: Flavor volatiles were analyzed in industrial and traditional vinegars and in traditional vinegars with different age. The samples were classified by means of chemometric methods on the basis of the gas- chromatographic profiles of the head- space volatile fraction, sampled by SPME.

The amount of sugars and organic acids in vinegars with different age was evaluated applying a gas-chromatographic method designed for their simultaneous determination and speciation.

The data gave useful information about the ageing process.

Conclusion: The results indicate that gas-chromatography is a very versatile and powerful technique which can be extremely useful for the study of real matrices like balsamic vinegars.

Significance and impact of the study: The results suggest instrumental methods for the characterization and the authentication of traditional vinegars.

Key words: Traditional balsamic vinegar, gas-chromatography, sugars, organic acids, volatile organic compounds

**INFLUENCE OF LONG-TERM AGEING ON SOME PHYSICAL PROPERTIES OF
“ACETO BALSAMICO TRADIZIONALE DI REGGIO EMILIA”**

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Aims: The “Aceto Balsamico Tradizionale di Reggio Emilia” is obtained from cooked and crushed grape (*must*) of local grape-wine varieties. During process, microbial metabolism and natural evaporation induce a marked increase in the concentration of derivatives of acetic fermentation, oxidation and Maillard reaction as well as a change in some physical properties (density, viscosity, etc.), affecting the typical sensorial characteristics of the final product.

Methods and results: DSC analysis and viscosity measurements have been carried out on samples of Traditional Balsamic vinegar, aged for between 3 and 25 years, as well as on some commercial balsamic dressing. A decrease in the glass transition temperature (T_g), as well as an increase in the unfrozen water was evidenced in samples aged for progressively longer time. In the 25 years-aged product, no peak correspondent to water freezing was observed. During long-term aging a significant increase in viscosity also occurred.

Conclusions: Results evidenced that Traditional Balsamic vinegar is markedly different as compared to the balsamic commercial products. Changes in composition taking place during ageing induce significant modification in both thermal and rheological properties

Significance and impact of the study: It could contribute to characterisation of both the production process and typical properties of the Traditional balsamic vinegar manufactured according to the DOP regulations (Reg. CE 813/2000).

Key words: Aceto Balsamico Tradizionale di Reggio Emilia, rheology, viscosity, T_g, quality

FORMATION OF FURFURAL COMPOUNDS IN HEAT TREATED MUST FOR TRADITIONAL BALSAMIC VINEGAR PRODUCTION

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Aims: The initial step of traditional balsamic vinegar (TBV) production is the must cooking, a heat concentration at 80-90 °C for several hours, which causes the formation of furfural compounds. Previous works showed that furfurals increase with the heat treatment time. However, the respective role that heat and concentration levels play on the formation of furfurals is not clear. The aim of this work is to show each contribution, by establishing the level of formation of HMF in must and in a model solution as a function of concentration and water activity (a_w).

Methods and results: The must was concentrated by cryoconcentration. The must and the model solution were added with different amounts of salt in order to test the effect of water activity on furfural production. All samples were treated at 95 °C for 1 h before HPLC analysis. No other furfural compound apart from HMF was found. HMF formation was correlated to the concentration level by an exponential curve, hence it seems that the rate of formation increases at higher sugar concentrations. A linear correlation was observed between the HMF level and water activity in must and model solution with different additions of salt, the sugar concentration being equal.

Significance and impact of the study: the results help to understand the influence of single variables on the formation of furfurals in must.

Key words: heat concentration, cryoconcentration, HMF, must, water activity

NUCLEAR MAGNETIC RESONANCE STUDIES ON TRADITIONAL BALSAMIC VINEGAR

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Aims: The aim of these studies is the use of Nuclear Magnetic Resonance (NMR) spectroscopy for the characterization of Traditional Balsamic Vinegar in order to determine appropriate parameters that allow the ageing and the quality assessment.

Methods and results: Spectroscopic NMR methods provide detailed information on a wide range of compounds present in a food matrix in a single experiment, offering advantages in terms of sample preparation, rapidity of analysis and selectivity. Several samples of Traditional Balsamic Vinegars have been analysed in terms of ^1H and ^{13}C NMR spectroscopy: multidimensional homo- and hetero-nuclear techniques have been used for metabolites characterization. Chemometric methods have been applied to the NMR data in terms of multivariate data analysis.

Conclusion: Traditional Balsamic Vinegar samples can be characterized and information concerning the ageing discrimination can be obtained.

Significance and impact of the study: The results highlight the potentiality of NMR methods in combination with multivariate data analysis for Traditional Balsamic Vinegar characterization.

Key words: NMR, vinegar, chemometrics, PCA, spectroscopy

CONTROL OF VINEGAR FLIES BY MEANS OF TRAPPING TECHNIQUES

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Aims: The vinegar fly *Drosophila melanogaster* (Diptera: Drosophilidae) is considered a real nuisance in vinegar-producing facilities, where it may also represent a potential threat, being responsible for infecting tanks of vinegar with the vinegar eel, the nematode *Turbatrix aceti*. Heavy populations of these flies may develop, elicited by the favourable conditions and by the impossibility to use pesticides and light traps. This study aimed to verify the efficacy of adult trapping techniques in reducing the populations of *D. melanogaster* inside vinegar-producing facilities.

Methods and results: Sticky cardboards were suspended upon vinegar barrels in previously chosen vinegar facilities: yellow ones represented the treatment and black ones the control. All cardboards were regularly inspected to count the number of trapped vinegar flies. The results showed that yellow sticky traps caught significantly higher numbers of flies.

Conclusion: Yellow sticky cardboards were useful in reducing the population of *D. melanogaster* in vinegar-producing facilities.

Significance and impact of the study: Yellow sticky cardboard traps can be recommended as an efficient, relatively inexpensive and easy-to use method for the control of vinegar flies in vinegar-producing facilities.

Key words: vinegar fly, control strategy, yellow sticky cardboards

RAPID ENUMERATION OF VIABLE AND TOTAL ACETIC ACID BACTERIA BY THE DIRECT EPIFLUORESCENT FILTER TECHNIQUE

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Aims: The detection of bacterial populations at different stages of acetification and process technology is important in vinegar quality control. Difficulties have been encountered in growing these organisms in culture media, especially when isolated directly from vinegar, even when isolation is possible, the process is often too long to be useful. Therefore, an accurate and reliable method to estimate the number of living bacteria as compared to the total bacteria is still needed.

Methods and Results: The ability of the direct epifluorescent filter technique (DEFT) to enumerate viable and total cells of various species of acetic acid bacteria was evaluated. Several fluorescent dyes were used to determine total and living bacterial cells: DAPI (4',6-diamidino-2-phenyl indole; Sigma); CTC (5-cyano-2,3-ditolyl tetrazolium chloride; Polysciences Europe, Eppelheim); LIVE/DEAD® BacLight™ Bacterial Viability kit (Molecular Probes, Inc.); DTAF (5-(4,6-dichlorotriazinyl) aminofluorescein; Molecular Probes, Inc.); DABCO (1,4-diazabicyclo-2,2,2-octano; Fluka). Optimum conditions for rapid filtration (strength of applied vacuum pressure; total volume of liquid sample), stain concentration and duration of exposure and the initial dilution of sample had to be determined before bacterial cells filtered from vinegar could be readily counted.

Conclusion: The results demonstrate the potential of LIVE/DEAD® BacLight™ Bacterial Viability kit as a rapid technique for the detection and enumeration of viable acetic acid bacteria in industrial acetifiers.

Significance and impact of the study: The rapid methods studied seems to be a good alternative determine the number of viable cells compared to the traditional culture methods, as part of quality control programmes in vinegar making. It may also be useful for studying the kinetics process.

Keywords: Acetic acid bacteria, fluorochrome, epifluorescence microscopy

CORRELATION BETWEEN ACETIC ACID RESISTANCE AND CHARACTERISTICS OF PQQ-DEPENDENT ADH IN *Gluconacetobacter europaeus*, *Gluconacetobacter intermedius* AND *Acetobacter pasteurianus*

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Aims: Acetic acid inhibits growth of the majority of microorganisms already at 0.5 %. Although most of acetic acid bacteria exhibit natural resistance to this acid, tolerance differs among the species. To study a relationship between acetic acid resistance and ethanol oxidation capabilities, characteristics of PQQ-dependent ADH were compared among species exhibiting different resistance to acetic acid.

Methods and results: The following species originating from different vinegar bioreactors were included in the study: *Ga. europaeus*, *Ga. intermedius* and *A. pasteurianus*. Among these species, *Ga. europaeus* showed the highest resistance to acetic acid (10 vol %). When cultivated in 3 L baffled shake flasks, *Ga. europaeus* exhibited higher maximal acetic acid production rate in media containing higher concentration of acetic acid. However, the specific growth rates decreased substantially with increased concentrations of acetic acid. The maximal acetic acid resistance of *Ga. intermedius* and *A. pasteurianus* was 6 vol %. PQQ-dependent ADHs purified from these strains exhibited almost the same specific activity and enzymatic properties, except that ADH of *Ga. europaeus* showed the highest resistance to acetic acid. Its activity was lost only at 15 vol % of acetic acid after 30 min incubation at 4°C. The enzymes of *Ga. intermedius* and *A. pasteurianus* lost activity at 12 and 11 vol % of acetic acid, respectively. The amount of ADH-subunit I and II visualized by staining of hemeassociated peroxidase activity was higher in both *Gluconacetobacter* species than in *A. pasteurianus*.

Conclusion: ADH from *Ga. europaeus* showed significantly higher acetic acid resistance in comparison to the enzymes from *Ga. intermedius* and *A. pasteurianus*. The higher ADH activity in membranes of *Ga. intermedius* and *Ga. europaeus* in comparison to *A. pasteurianus* suggests a higher expression of ADH in the first two species.

Significance and impact of the study: The results suggest that the amount and the acetic acid resistance of PQQ-dependent ADH in acetic acid bacteria contribute to differences in acetic acid tolerance.

Key words: *Gluconacetobacter europaeus*, *Gluconacetobacter intermedius*, *Acetobacter pasteurianus*, acetic acid resistance, PQQ-dependent ADH, vinegar

INVESTIGATION OF EXOPROTEASE ACTIVITY OF *Acetobacter aceti*: A PRELIMINARY STUDY

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Protease production by bacteria could be stimulated under sub-optimal conditions of growth, as the cells are growing in a nutritionally poor medium. In wine the nitrogen content, especially the amounts of proteins and peptides, is low. Protease excretion by wine lactic acid bacteria was previously studied. In this study we focused on exoprotease activity in *Acetobacter aceti* DSM3508^T carrying out a preliminary characterization of the functional and structural properties of the protease. The protease activity, assayed with Bovine serum albumin as substrate, was tested on concentrated supernatants of broth culture at different pH values, temperatures and ethanol concentrations. Kinetic parameters, as K_m and V_{max} were measured. The excretion of protease could have an essential role on *Acetobacter aceti* cell survival and multiplication in harsh environments. The results of our investigation might have important implications on winemaking, or in general they might be considered the start point of an applicative research on the industrial use of proteases.

ACETIC FERMENTATION MONITORING BY NIRS USING MCR-ALS

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Aims: Monitoring of kinetic and sensory parameters in acetic fermentation by near infrared spectroscopy using Multivariate Curve Resolution Alternating Least Squares method (MCR-ALS).

Methods and results: Acetic fermentation batches were performed and the spectral changes produced during the course of the process were monitored on line using an NIR spectrophotometer. Ethanol, acetic acid and biomass concentration are parameters closely involved in the kinetics of the process. Data recorded were analysed using a two-way MCR-ALS method with penalty function. Multivariate Curve Resolution (MCR) methods, specifically Alternating Least Squares (ALS), applied to spectral data from monitoring chemical reactions, provide the concentration profiles and the spectra of all the species involved in the process. Thus, spectral information as equality constraint, and the kinetic profiles for ethanol and acetic acid were obtained.

Conclusion: The addition of spectral or concentration information supplied as equality constraints allows to obtain quantitative models for different chemical parameters, the kinetic profile for biomass and spectra for ethanol and acetic acid. For all the fermentation, the overall performance of the model was evaluated by the explained variance, and the lack of fit obtained in the batches.

Significance and impact of the study: The application of MCR-ALS method allows to unravel pure compound information from the non-selective mixed original NIR spectra.

Key words: acetic fermentation, NIR, on-line monitoring, ALS

CULTURE MEDIA FOR ENUMERATION AND ISOLATION OF ACETIC ACID BACTERIA DIRECTLY FROM VINEGAR

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Aims: Isolation of acetic acid bacteria is difficult on the solid media especially when isolated directly from vinegar. The percentages of isolated bacteria are lower than the population which is observed in the microscope. The goal is to find an appropriate media to isolate bacteria from vinegar sample where the growth of acetic acid bacteria would be representative of the analysed population.

Methods and results: Four different culture media have been tested in both solid and liquid forms. These media are composed of glucose, yeast extract, ethanol (0 – 3%) and acetic acid (0 – 5%). Vinegar samples came from surface and submerged cultures. The range of dilutions done in liquid media went from 1.10^{-4} to 1.10^{-10} .

The genetic profiles found in the lower dilutions were a mixture of species, whereas in the higher dilutions the genetic profiles were identified as a single species. The solid media showed lower variability of species than liquid media

Conclusion: Growth in diluted liquid media could be reliable system for isolating pure cultures of acetic acid bacteria originated from vinegar. In the solid media the counts were as low as 1% of the counted cells under the microscope.

Significance and impact of the study: The liquid media ensured pure culture and allows to perform studies of acetic acid bacteria populations dynamics often limited due to the difficulties of isolation in the solid media.

Key words: Acetic acid bacteria, isolation media, RFLP-PCR rDNA 16S

Neosaia chiangmaiensis* GEN. NOV., SP. NOV., A NOVEL OSMOTOLERANT ACETIC ACID BACTERIUM IN THE α -*Proteobacteria

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Aims: In a study on microbial diversity in Thailand, one strain was isolated in Chiang Mai.

Methods and results: Phylogenetic trees based on 16S rDNA and 16S-23S rDNA internal transcribed spacer (ITS) sequences represented that the isolate had a close relationship to the genera *Kozakia* and *Asaia*, but constituted an independent cluster. Similarity values to other acetic acid bacteria in the two sequences were respectively 90.7-93.2% and 54.1-71.7%. The isolate was distinguished from strains of the two genera by restriction analysis of 16S-23S rDNA ITS regions with *BccI* and *HhaI*. The colony was pale-pink. Cells are non-motile. The isolate did not either oxidize acetate and lactate or produce a mucous substance, but grew well on 30% (w/v) D-glucose. The isolate grew on glutamate agar and mannitol agar, but the growth on glutamate agar was not intense. Acid was produced from ethanol. Major ubiquinone was Q-10.

Conclusion: *Neosaia chiangmaiensis* gen. nov., sp. nov. is proposed. The type strain is AC 28^T (BCC 15763^T), which was isolated from a flower of red ginger.

Significance and impact of the study: *Neosaia* was the ninth genus in the *Acetobacteraceae*. This indicates that acetic acid bacteria are diverse phylogenetically.

Key words: acetic acid bacteria, *Neosaia chiangmaiensis* gen. nov., sp. nov.; α -*Proteobacteria* taxonomy

RECLASSIFICATION OF *Gluconacetobacter hansenii* STRAINS AND PROPOSALS OF *Gluconacetobacter saccharivorans* SP. NOV. *Gluconacetobacter maltaceti* SP. NOV. AND *Gluconacetobacter nataicola* SP. NOV.

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Aims: To reclassify the taxonomical position of ten strains assigned previously to *Acetobacter hansenii* (= *Gluconacetobacter hansenii*) and *A. pasteurianus* LMG 1584.

Methods and Results: Polyphasic taxonomy included 16S rRNA sequences, DNA-DNA similarity, DNA base compositions and phenotypic characteristics were used for identification. As results, the strains studied were separated into six distinct groups by DNA-DNA similarity and other characteristics. Strains in Group I were identified as *Gluconacetobacter hansenii*, those in Group II were included in *Gluconacetobacter oboediens* and those in Group V were reclassified as *Gluconacetobacter europaeus*. Groups III, IV and VI were regarded as new species and *Gluconacetobacter saccharivorans* sp. nov. (type strain LMG 1582^T) and *Gluconacetobacter nataicola* sp. nov. (type strain LMG 1536^T) are proposed, respectively.

Conclusion: It is revealed that the strains previously classified as *A. hansenii* (= *G. hansenii*) are heterogenic species, and separated into six species on the basis of DNA-DNA similarity, 16S rRNA gene sequences, and phenotypic characteristics.

Significance and impact of the study: For the stability of nomenclature, DNA-DNA similarity is a reliable criterion for distinguishing bacterial species when clear-cut differential phenotypic characteristics are not found yet among the species

Key words: *Gluconacetobacter hansenii*, *Gluconacetobacter saccharivorans*, *Gluconacetobacter maltaceti* and *Gluconacetobacter nataicola*

ISOLATION AND IDENTIFICATION OF *Asaia* STRAINS AND OTHER ACETIC ACID BACTERIA FROM FLOWERS IN THE SUBTROPICAL REGION OKINAWA IN JAPAN

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Aims: The genus *Asaia* is an unusual acetic acid bacterium and characterized by no growth in the presence of 0.35% acetic acid and a rather limited distribution in the tropical region so far. However, *Asaia* strains are not isolated from subtropical and temperate regions. This study aims to isolate *Asaia* and other acetic acid bacteria from flowers and other materials obtained in subtropical region Okinawa in Japan.

Methods and results: The enrichment medium VI was mainly used for isolating acetic acid bacteria. This medium was designed to isolate *Asaia* strains based on their characteristics, and contained 1% dulcitol, 1% sorbitol, 0.5% yeast extract, and 0.3% peptone. Seventy-one flowers and 6 fruits were used as isolation sources and they were obtained in August 2004. Phenotypic characterization, 16S rRNA gene sequence analysis, and DNA-DNA hybridization were used for identification. Of 33 isolates, 14 isolates were identified as *Asaia* sp., 9 as *Gluconacetobacter* sp., 7 as *Acetobacter* sp., 2 as *Gluconobacter* sp., and one as *Saccharibacter* sp.

Conclusion: This study revealed the distribution of the genus *Asaia* and other acetic acid bacteria in the subtropical region. This would indicate a rich flora of acetic acid in the region.

Key words: *Asaia*, acetic acid bacteria, subtropical region

QUANTITATIVE-PCR FOR RAPID DETECTION OF ACETIC ACID BACTERIA

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Aims: Rapid detection and quantification of acetic acid bacteria in wines, vinegar and other products without culturing. Quantitative-PCR offers a sensitive, efficient and reliable approach to quantification.

Methods and results: The primers have been designed from a region of the 16S rDNA gene which is a conserved zone of AAB. To confirm the specificity of these primers, they have been tested with yeast and lactic acid bacteria which are the most common organisms present in our samples. No amplification of these two groups have been detected. Real Time Quantitative PCR has been done with a high number of reference AAB strains to obtain standard curves. In all cases all of strains produced similar results. This method allows the direct quantification of AAB in wine and vinegar samples. This technique using SYBR-Green (a double-stranded DNA intercalating fluorescence dye) is accomplished by the continuous measurement of products throughout the reaction. Thus, increased fluorescence is directly proportional to the formation of PCR products.

Conclusion: The enumeration of acetic acid bacteria in wine and vinegar is possible using this technique directly without culturing.

Significance and impact of the study: Industrial and environmental samples can be analyzed fast and easily with this technique, detecting the presence or absence of microorganism that can contribute to the spoilage of the samples.

Keywords: Quantitative-PCR, Acetic acid bacteria, wine, vinegar

MICROBIAL ECOLOGY OF INITIAL PHASES OF “VINO COTTO” PRODUCTION

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“Vino cotto” is a typical beverage obtained from prolonged fermentation (20 years or more) of cooked musts in some areas of Marche and Abruzzo regions (Central Italy). This wine is produced from autochthonous grapes cultivars such as Trebbiano. In order to monitor the diversity of yeast population in “vino cotto” as well as to identify and characterize the yeast strains according to selected biotechnological characteristics, yeasts were enumerated and isolated during the first year of fermentation at different intervals of time and on different media. A total of 47 isolates were tested for fermentation capacity, SO₂ and H₂S production, β -glucosidase and urease activity and production of secondary volatile compounds. The yeast biodiversity was evaluated by using molecular techniques. For achieving a rapid identification at species level, the PCR-RFLP assay with different endonucleases was used, whereas individual isolates were differentiated by RAPD-PCR.

Keywords: vino cotto, wine microbiology, wine yeast, PCR-RFLP, RAPD-PCR

***Acetobacter cerevisiae* BENT FOR BIOFILM FORMATION AND SURVIVAL AS PLANKTONIC AND SESSILE CELLS**

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Aims: The work was aimed to investigate the capability to develop surface biofilms by *Acetobacter cerevisiae* and the bacterial survival under the influence of different concentrations of ethanol, glucose and acetic acid.

Methods and results: Association with a surface in a structure known as biofilm is a prevailing microbial lifestyle. *Acetobacter cerevisiae* (strain AB021), recovered from Traditional Balsamic Vinegar, was subjected to a study in order to evaluate the biofilm formation and the bacterial survival as planktonics and as sessiles. The effects of different concentrations of ethanol (4%-12%), glucose (10%-30%) and acetic acid (0%-6%) were investigated by modulating their levels, according to a three factors-five levels Central Composite Design (CCD).

Conclusion: The obtained results suggested that the planktonic cells survival was negatively affected by ethanol and by acetic acid; moreover there was a positive correlation between survival and glucose concentration.

On the other hand, the biofilm formation was observed only in absence of acetic acid which was the most affecting variable on natural ability of cells to attach and grow on surfaces.

Significance and impact of the study: The obtained results supply new informations about a novel *Acetobacter* species, overall about the natural capability to develop biofilms, being this character of great technological interest.

Key words: *Acetobacter cerevisiae*, biofilm formation, survival

RECOVERY OF SODIUM ACETATE FROM AQUEOUS SOLUTIONS BY ELECTRODIALYSIS

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Aims: An integrated process using *Clostridia* for production and purification of fermentation-derived acetic acid has been proposed. Electrodialysis is used to recover acetates after microfiltration from the fermentation broth. In this work we developed a mathematical model to determine the engineering parameters necessary to design and optimize the ED purification step with reference to sodium acetate.

Methods and Results: A Nernst-Planck-derived model was used to reconstruct basic ED phenomena. Experiments were operated in a batch mode at 20° C using a laboratory-scale electrodialyser according to the following procedure: 1) to assess the physical properties of the solution undergoing the electrodialytic separation, including the ion transport numbers in solution via conductivity measurements, 2) to estimate effective solute and water transport numbers in membranes by desalination tests, and 3) to assess limiting current intensity and membrane resistance for both membranes by voltage-current experiments. Some desalination tests were also carried out to validate the estimated parameters.

Conclusions: The electrodialytic recovery of sodium acetate from aqueous solutions appeared to be quite well simulated using the developed mathematical model.

Significance and Impact of Study: Use of this mathematical model may be recommended to evaluate the economic feasibility of the ED recovery step in scalable, regional-sized plants for the production of acetic acid as a chemical feedstock.

Key words: Acetate recovery, electrodialysis, transport numbers, membrane electrical resistance, limiting electric current

VOLATILE COMPOUNDS PRODUCED BY DIFFERENT MICROBIAL SPECIES IN TRADITIONAL BALSAMIC VINEGAR

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Aims: Purpose of this work was to assess the influence of the starting medium composition on the selection of microbial groups which operate in the first step of traditional balsamic vinegar production, characterised by fermentative or oxidative metabolism.

Methods and Results: Six samples, realised by mixing different amount of cooked must, fresh must and an inoculation medium of acetic acid bacteria, were analysed by means of SPME (solid phase micro-extraction) technique during storage at room temperature. The obtained results show a different behaviour of the different samples. In those having a large quantity of cooked must a fermentative metabolism, typical of yeasts, seems to predominate leading to the production of large quantity of ethanol. On the contrary, in the sample made with a larger quantity of fresh must, the biological activity is due essentially to the presence of acetic acid bacteria, with the production of acetic acid in the first step of storage and the development of a lot aromatic volatile compounds.

Conclusion: According to the results, it is possible to hypothesize that the composition of starting medium influences deeply the selection of the microbial species in traditional balsamic vinegar.

Key words: Balsamic vinegar, microbial selection, volatile compounds, SPME

IDENTIFICATION OF A FREE-LIVING NEMATODE FROM TRADITIONAL BALSAMIC VINEGAR

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Traditional Balsamic Vinegar is a special vinegar made from cooked must with a very high sugar concentration and a low acidity. In Traditional Balsamic Vinegar, produced in old wooden barrels, we found in some cases high individual numbers of vinegar nematodes. This was generally the case in samples taken from barrels with different glucose amount. Our hypothesis is that the nematode species could be different from those occurring in other fermenting substances. The taxonomic identification revealed that the nematode is *Turbatrix aceti* (MÜLLER, 1783) PETERS 1927, formerly known as *Anguillula aceti* and colloquially as the vinegar eel, wine eel or vinegar worm. Our male specimens of the free-living-vinegar nematode *Turbatrix aceti* had the following morphologic characteristics: 1025 µm in length, spicula 35-37 µm, *gubernaculum* with dorsal outgrowth. *Turbatrix aceti* is often found in great numbers in vinegars made of apples or other fruits, sour paste or in other fermenting substances. It feeds on bacteria. The nematode is free-swimming in the liquid, reaching high individual numbers at the surface, where the oxygen concentration is higher. The maximum life span is 10 months.

Key words: vinegar nematode, *Turbatrix aceti*, *Anguillula*, agriculture, food production

VOLATILE COMPOUND AS QUALITY INDICATOR OF MICROBIAL GROWTH IN TRADITIONAL BALSAMIC VINEGAR

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Aims: The sensory properties of Traditional Balsamic Vinegar strongly depend on the particular production techniques and the use of cooked must as starting medium influences the selection of the microbial species.

In this work SPME analysis technique was used to found one or more volatile compounds of aromatic fraction with the aim to identify a quality indicator indisputably correlated with the microbial metabolism.

Methods and Results: Two different sets of Balsamic Vinegar were analysed by means SPME. One set (A), formed of 7 barrels, started in 1987 and a starter mixer of cooked must and old vinegar was used.

The other set (B), formed of 5 barrels, started in 1990 and the starter medium used was only cooked must.

The obtained results show a significant difference between the two sets. In the set A, having a mixer starter medium, a production of 1-butanol, 3-methyl and ethanol was mainly determined.

In the set B, having only cooked must as a starter medium, the production of a large quantity of ethanol was determined in the first barrel, followed by a decreasing on the others barrels; simultaneously, there was an increasing of esters concentration.

Conclusion: The obtained results leads to hypothesize that it is possible to find volatile compound correlated with the growth of specific microorganism species.

Key words: balsamic vinegar; aroma compounds; SPME; quality indicator

TECHNOLOGICAL CHARACTERISTICS OF CALABRIAN ACETIC ACID BACTERIA

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Aims: To exploit the acetic acid bacteria biodiversity in the Calabrian ecosystems.

Methods and results: Thirty-two strains of *Acetobacter pasteurianus*, two *A. aceti* and one *Gluconacetobacter hansenii* were employed in this research. They were taken from 530 strains, formerly isolated from Calabrian acescent wines (42 samples) and homemade vinegars (23 samples), identified, and pre-selected on Glucose Yeast extract Calcium carbonate Agar for acetic acid production. Micro-acetification trials were performed in duplicate on 500 mL-flasks containing 200 mL of diluted white wine. At the end of the acetification process the vinegars were analysed and the parameters varied as follows: 3.06 - 3.28 (pH), 5.23 - 7.53 g % (titratable acidity), 2.40 - 3.50 g/L (glycerol), 305 - 395 mg/L (total polyphenols).

Conclusion: A remarkable biodiversity was observed among the strains, especially for the parameter "titratable acidity".

Significance and impact of the study: This technological survey constitutes a basic step to exploit the bacterial biodiversity in the Calabrian ecosystems. Such biodiversity could be further explored with the ultimate goal to carry out selection programmes of typical strains.

Key words: biodiversity, Calabrian acetic acid bacteria, micro-acetification trials, technological characteristics

BIOCATALYTIC COATINGS OF ACETIC ACID BACTERIA: OXIDATION OF D-SORBITOL TO L-SORBOSE BY THIN LATEX COATINGS OF NON GROWING *Gluconobacter oxidans*

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Aims: A thin (~ 50 µm) porous adhesive permanent latex coating for oxidation of D-sorbitol to L-sorbose has been developed consisting of *Gluconobacter oxydans* entrapped within a acrylate/vinyl acetate polymer coating (cellcoat) sealed by a second thin polymer layer (topcoat). Vital parameters (effective diffusivity, cell concentration, intrinsic kinetics, effectiveness factor, active half-life) have been characterized following coating, drying, frozen storage and rehydration.

Methods and Results: 12.7 mm diameter bi-layer latex patches of 20 µm thick cellcoat with 19 or 36 µm thick polymer topcoats capable of retaining more than 99.5 % of cells were studied in a highly oxygenated 10 mL micro-bioreactor. Intrinsic and apparent kinetics have been determined by HPLC in a D-sorbitol pyruvate non-growth medium (SPP). Viable cell concentration was estimated to be ~ 10⁸ cfu per patch. The observed and predicted effectiveness factor was ten-fold higher then reported in the literature with hydrogel bead immobilized *G. oxydans*.

Conclusions: The above oxydation can be successfully carried out by latex-entrapped *G. oxydans* in thin coatings. The extended half-life (about 400 h), mechanical stability, cell retention, frozen storage and high reactivity make it a promising alternative to existing 1-2 mm diameter soft-bead immobilization techniques.

Significance and Impact of Study: This technology suggests new coating or membrane bioreactor configurations that could lead to improved efficiency of the Reichstein process for vitamin C production.

Key words: biocatalytic latex coating, effectiveness factor/intrinsic kinetics, *Gluconobacter oxydans*, diffusion-reaction model, oxidation of D-sorbitol to L-sorbose

***Asaia* SP. ISOLATED FROM SPUTUM SAMPLES OF IMMUNOSUPPRESSED PATIENTS**

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Aims: *Asaia* spp., which belong to the acetic acid bacteria group, were primarily isolated from flowers and fermented rice. Later an *Asaia* sp. was reported also from fruit-flavoured bottled water. Most recently a case of refractory peritonitis caused by *Asaia bogorensis* was described in a patient on automated peritoneal dialysis.

Methods and results: Twenty-five strains of pink-pigmented Gram-negative rods were isolated from sputum samples after one year of surveillance. Patients were often treated in the radiotherapy department. Strains were isolated from Sabouraud Dextrose Agar after minimum seven days of cultivation at ambient temperature. Analysis of 16S rRNA sequences led to the identification as *Asaia* sp. Biochemical tests and fatty acid analysis were used for characterization. DNA-DNA hybridization should be used to verify the species level.

Conclusion: Until now *Asaia* was considered as a solely non-pathogenic genus such as other genera from the group of acetic acid bacteria. The potential effect of the *Asaia* sp. isolated from sputum samples on the state of health of patients remains unclear.

Significance and impact of the study: This is the first report which describes the isolation of *Asaia* sp. from sputum samples.

Key words: acetic acid bacteria, *Asaia*, isolation, sputum

VINEGAR, FERMENTATION STOPPAGES: DISEASES, CAUSES, PREVENTIVE MEANS

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Aims: Pasteur (1866), recorded his study of vinegar in “Etudes sur le vinaigre” with its subtitle: “Diseases and their prevention” and in his “Etudes sur le vin”, (1873) he added the subtitle: “Diseases and their causes”.

Both titles deal with the program of fighting against disease during wine and vinegar making. Pasteur also wrote: “Wine and vinegar are very similar with respect to the causes of susceptible diseases”.

Wüstenfeld (1930), in his classical book “Lehrbuch der Essigfabrikation” writes in his conclusion to the chapter about interruption of fermentation (pp. 258-274): “One should be careful, to avoid new infections, to keep a very strict control of the vinegar plant, from the technical as well as the analytical point of view, and to work at a high total concentration”.

Hygiene: from the Greek word “hugiainein”, meaning “to feel well, in good health”.

Hygiene is an essential part of the work process, and its daily application is of paramount importance.

Methods and results: Healthy and active acetic acid bacteria are prerequisites for trouble free vinegar production. Numerous analyses under the microscope in vinegar plants, affected by fermentation difficulties, have shown the presence of wild yeast, fungi and of foreign strains of bacteria (for instance *A. peroxydans*, lactic acid bacteria, etc.).

Variation of fermentation parameters: up to a certain point, both substrates (acetic acid and alcohol) necessary for the metabolism of the acetic acid bacteria are toxic. 5-5.5% is the up limit for alcohol. Therefore such parameters as air and fermentation temperature should be kept constant ($T \pm 0, 5^\circ \text{C}$). They are relatively the most easily controllable.

Foam: indicates the presence of dead bacteria, and the resultant toxins impede fermentation. Therefore a mechanical defoamer is indispensable. Foam can be the consequence of a too low level of residual alcohol at discharge.

SO_2 in wine (limit: 15 mg/l), and Cl_2 in water (limit: 0,2 ppm), should be neutralized.

Conclusion: In order to guarantee stable fermentations, constant quality control is essential in the raw materials, as well as for the methods with which they are processed. Care is necessary in the methodology for the mash production, and the environmental elements:

- air
 - cleanliness of the tanks, pipes and filters.
- Significance and impact of the study:* all food factories risk contamination from
- raw materials
 - environment (buildings, tools, apparatus, cleaning utensils)
 - personnel (bacterial carriers, clothing)
 - insects and animals (vermin, birds, dogs, etc.).

Optimal working conditions are only possible if each of these parameters is respected.

Key words: *A. peroxydans*, hygiene, SO_2 : sulphur dioxide, Cl_2 : chlorine

ACETIC ACID BACTERIA IN TRADITIONAL BALSAMIC VINEGAR BY PCR-DGGE ANALYSIS

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Aims: The greatest limits in the study and selection of acetic acid bacteria are due to the difficulty of isolating and cultivating them. A culture-independent molecular technique, PCR-DGGE (Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis), was used in order to study the acetic acid bacteria of Traditional Balsamic Vinegar.

Methods and results: Primers WBAC1 and WBAC2 were successfully used with DNA extracted from both acetic acid bacteria strains and vinegar's samples and they gave PCR products that allowed differentiation by DGGE. The results obtained indicate that acetic acid bacteria species involved in spontaneous oxidation process can be represented by only one dominant species.

Conclusion: A change in acetic acid bacteria species during fermentation process could be supposed: *Ga. xylinus* was the main representative species in cooked must while *A. pasteurianus* became more prevalent in vinegar's samples.

Significance and impact of the study: This study clearly indicated that PCR-DGGE is a suitable tool for monitoring TBV microbial population and specifically acetic acid bacteria which are difficult to isolate applying conventional microbiological techniques.

Key words: Acetic acid bacteria, Traditional Balsamic Vinegar, culture-independent technique, PCR-DGGE

MOLECULAR PROCEDURE FOR YEASTS IDENTIFICATION OF TRADITIONAL BALSAMIC VINEGAR

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Aims: The first step of the Traditional Balsamic Vinegar production is the making of the fermented cooked must. This is developed by particular yeast, able to colonize specific niches with an elevated sugars concentration. In this research we have identified all the species that can potentially colonize the cooked must and that can be able to produce the base of start for the production of ABT.

Methods and results: For this aim we have chosen 26 types strain, belonging to many osmotolerant yeasts, selected in literature for their phenotypical traits. The twenty-six strains have been identified by PCR/RFLP of the ITS regions, using three different restriction enzymes (*Hae* III, *Hinf* I and *Cfo* I). Nevertheless, for some species, PCR products introduces multiple bands that suggest the presence of different couples of the ITS regions.

Conclusion: The number and size of restriction fragments of different species of fungi can be used as reference for identification of osmotolerant yeasts.

Significance and impact of the study: The obtained result is a specific database which has 26 strain profiles that can be applied a quick identification method of the ABT osmotolerant yeasts.

Key words: osmotolerant yeasts, PRC/RFLP of ITS regions, Traditional Balsamic Vinegar, molecular identification

SUGAR CONSUMPTION BY YEASTS IN COOKED MUST FOR TRADITIONAL BALSAMIC VINEGAR

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Aims: The cooked must fermentation is the first microbiological step of Traditional Balsamic Vinegar production and a lot of physical, chemical and sensorial properties of the final product depends from it. The yeast species which carry out the fermentation influence deeply both the alcoholic and the residual sugars concentration.

Methods and results: Nine yeast strains, isolated from cooked must for Traditional Balsamic Vinegar production, were used for fermentation of cooked must added of glucose and fructose (1:1) till final concentrations of 350, 400 and 450 g/l were obtained.

Fermentation progress was tested by determining weight loss by CO₂ release and final concentrations of glucose and fructose by enzymatic kit of Boehringer Mannheim (Darmstadt, Germany).

The alcoholic concentration in the cooked must depends on the its initial concentration and on the yeast species involved. High solid soluble concentrations inhibit the alcoholic fermentation with negative consequences on the following oxidative process and on the final acetic acid concentration.

Conclusion: The yeasts more suitable for the Traditional Balsamic Vinegar production are glucosophilic strains, able to produce alcohol in presence of high soluble solid concentrations. Among the strains isolated by cooked must and tested in this research, the strains 507 belonging to the *Saccharomyces cerevisiae* species, seems to be the best.

Significance and impact of the study: A high glucose percentage is not required because it makes the vinegar more susceptible to crystallization, while a product with more fructose is stable also at high density. Therefore is necessary to select yeast strains suitable for Traditional Balsamic Vinegar production.

Key words: yeast, *Saccharomyces cerevisiae*, vinegar, crystallization

POPULATION DYNAMICS OF ACETIC ACID BACTERIA DURING COCOA FERMENTATION

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Aims: To study the population dynamics of acetic acid bacteria during cocoa fermentation.

Methods and results: During fermentation of cocoa beans the total count of microorganisms increases in the first 24-36 h, and then stabilizes or gradually reduces. The fermentation of cocoa can be considered as being divided into three phases: phase 1 or the anaerobic development of yeasts; phase 2 or the development of lactic acid bacteria (LAB); and phase 3 or the development of acetic acid bacteria (AAB). As a first step, the sugar of the plant juice is fermented to ethanol by yeasts, thus creating a medium highly suitable for the development of AAB. The yeast activity becomes inhibited by the alcohol concentration, increasing pH, and greater aeration, conditions that are more favorable to LAB. Also, AAB occur very early in the fermentation and persist until the end. As aeration increases, AAB become more important. Their main reaction is the conversion of ethanol to acetic acid. Acetic acid mainly causes the death of the beans, whereby the biological barriers (membranes) between the cells break down, and hence various enzymes and substrates are free to mix, and the subsequent reactions produce the necessary flavour precursors for cocoa processing. The AAB are also responsible for the oxidation of acetic acid to carbon dioxide and water. This strongly exothermic reaction is mainly responsible for the rise in temperature, which can reach 50°C in some fermentations.

Conclusion: In practice, there is considerable overlap between the phases, and the relative importance of each phase varies between regions and fermentation techniques. Also, the population dynamics of each group of microorganisms may vary, in particular that of the AAB, influenced by factors such as the evolution of the acidity and temperature during fermentation and drying.

Significance and impact of the study: Studying the population dynamics of cocoa bean fermentation will unravel the contribution of acetic acid bacteria in cocoa processing.

Key words: acetic acid bacteria, cocoa fermentation, acetic acid

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