Poster Session I

Traditional Vinegar Fermentation

PI-2: Traceability of phytosanitary product in the production of a Sherry wine vinegar.

Enrique Durán, Remedios Castro, Ramón Natera, Carmelo G. Barroso.

Analytical Chemistry Department, Faculty of Sciences, University of Cádiz. P.O. Box 40, E- 11510. Pol. Río San Pedro, Puerto Real, Cádiz. Spain.
E-mail: enrique.duranguerrero@uca.es

In spite of the trends towards the use of an ecologic agriculture, even nowadays it is necessary to employ phytosanitary products with the aim of guaranteeing both the quality and the quantity of too many cultures. Because of this quality, the absence of residues of these chemical compounds in the final product is required. Moreover, the legislation is shortening the maximum pesticide quantity permitted, with a clear predisposition to reach the "zero tolerance"[1]. On the other hand, today exists an increasingly demand from the agroalimentary sector to the production of "ecologic products", and therefore to "ecologic vinegars". To obtain this guarantee of origin and quality of the vinegar, the different legislations demand a control of the traceability of the product, requiring various certificates that guarantee the non-existence of phytosanitary products in each step of the production process. Because of this, it is interesting the development of analysis methods for the determination of this kind of compounds during all the vinegar production process.

In the present work, the monitoring of the evolution of the different phytosanitary products employed in the production of a Sherry wine vinegar has been carried out. The study covers the complete process, from the grape ripening to the vinegar fermentation. For the liquid samples analysis, a method based on SBSE-GC-MS and previously developed[2] was used. For the grape samples, the use of two different extraction methods (ultrasound assisted extraction and microwave assisted extraction) was considered. Both methods were correctly optimized by means of factorial designs, and finally were compared between them. Considering the obtained results, the ultrasound extraction method was chosen to make the extraction of the solid samples. After the extraction process, the different extracts were analysed by means of SBSE-GC-MS.

The achieved results show that the decrease of the phytosanitary product residues during the grape ripening, most of them being removed completely before the final product, or reaching levels lower than those permitted by the Spanish legislation[3].

1. C.M. Cooney, ***Environ. Sci. Technol.***, **30(9),** 336-337 (1996)
2. Duran et al., ***J. Chromatogr. A, 1165,*** 144-150 (2007)
3. Spanish Legislation: BOE, 23 of May (2008). Modification of R.D. 280/1994.

PI-3: Accelerated aging studies with microoxygenation and chips in the production of a Sherry wine vinegar

Enrique Durán, Ramón Natera, Remedios Castro, María Jesús Ruiz, M. Carmen Rodríguez, Carmelo G. Barroso.

Analytical Chemistry Department, Faculty of Sciences, University of Cádiz. P.O. Box 40, E- 11510. Pol. Río San Pedro, Puerto Real, Cádiz. Spain.
E-mail: enrique.duranguerrero@uca.es

Conventional Sherry wine vinegar aging process resorts the using of oak wood barrels in which the vinegar is introduced to be aged. In these barrels, the vinegar is in close contact with the wood, which porosity provides to the medium a contribution of oxygen that conditions its evolution during the aging.

In this work, an aging method which managed to accelerate the process as well as to obtain a vinegar with similar analytical and organoleptic characteristics to the traditional vinegar has been developed. To achieve the optimal conditions, several experiments using "suitable" wine vinegar for the production of Sherry vinegar, have been carried out. In these experiments, the addition of chips together with the use of microoxygenation was used, modifying both the amounts of chips and the volumes of flow of oxygen.

The experiments were carried out in a local vinegar production company, using tanks with 5000 litres of capacity and heights upper of two metres, in order to guarantee the complete oxygen dissolution process. During the development of the different experiments, samples of the tanks were periodically taken, in order to determine the polyphenolic composition, volatile composition, medium-FT-IR spectra and colour analysis. Moreover, sensorial analyses of all the samples were carried out by means of a trained panel.

After evaluating the analytical and organoleptic data, the final experimental conditions were fixed. These final conditions could be substitutes of the conventional aging method thanks to the production of vinegar with similar organoleptic characteristics but obtained in a minor time.

PI-4: Chromosomal polymorphism and ploidy divergence in *Zygosacharomyces rouxii* strains isolated from Traditional Balsamic Vinegars

Lisa Solieri, Stefano Cassanelli, Paolo Giudici

Department of Agricultural and Food Sciences, University of Modena and Reggio Emilia, Via Amendola 2, 42100 Reggio Emilia, Italy.
E-mail: lisa.solieri@unimore.it

The genus *Zygosaccharomyces* traditionally describes osmophilic yeasts that differ from the those of the genus *Saccharomyces* for their vegetative growth, in which sexual conjugation between cells or a cell and its bud, precedes the sporulation[1]. Diploidization through zygote formation means that vegetative life cycle is mainly haploid. In the last past decade phenotypical and molecular approaches based on cloning procedure, single-gene PCR amplification and karyotyping highlighted a high variability within *Zygosaccharomyces rouxii* species, mainly between type strain CBS 732 and some wild strains isolated from miso and traditional balsamic vinegar (ATCC 42981, ABT 301, and ABT 601, respectively)[2,3,4]. Recently random fragments sequencing from strain ATCC 42981 and allele-specific PCR reaction from strain ABT301 and ABT 601 have revealed that all these wild strains have two copies of several genes, including ITS-5.8S rDNA, 26S D1/D2 regions, *ZSOD* and *HIS3*[4,5]. Genome size is one of the most fundamental genetic properties of living organisms. However no study has been attempted to evaluate genome size and ploidy for *Z. rouxii* strains. In this work genome size and ploidy level estimation, as well as *ZSOD* gene chromosome mapping were used to elucidate the taxonomic relationships among these wild strains compared to *Z. rouxii* CBS 732^T.

In particular, we applied flow cytometry to determine DNA content of cells in G0/G1 phase. The genome size was estimated 11.89±0.21 Mb (12.1±0.22 fg) for strain CBS 732^T, 21.08±0.37 Mb (21.56±0.37 fg) for ATCC 42981, 25.68±1.11 Mb (26.27±1.14) for ABT 301, and 36.00±0.37 Mb (36.80±0.38 fg) for ABT 601. Moreover pulsed field gel electrophoresis (PFGE) confirmed the genome size divergence with polymorphic PFGE patterns consisting in different number of chromosomal bands: 6, 7, 10, and 11 for CBS 732^T, ATCC 42981, ABT 301, and ABT 601, respectively. By densitometric analysis of PFGE profile, the haploid genome size has been obtained as sum of chromosomal bands size. These data have been combined to DNA content from flow cytometric analysis in order to calculate the ploidy level for each strain. The results showed CBS 732^T has a haploid DNA content, whereas wild strains ATCC 42981, ABT 301, and ABT 601 a diploid DNA content.

Finally the change in ploidy has been also confirmed by doubling of genes involved in osmotic adaptation (*ZSOD*, *HOG*, *GPD*, and *GCY1*), as well as by polymorphic rDNA genes occurring in ATCC 42981[5,6] and in ABT 301 and ABT 601[4], but not in CBS 732^T [2]. For this purpose chromosome mapping of *ZSOD* genes (encoding Na^+/H^+-antiporters involved in halotolerance) showed that, differently from haploid CBS 732^T, diploid strains ATCC 42981, ABT 301 and ABT 601 have two partially divergent *ZSOD* copies located on different chromosomes.

In conclusion genome size and ploidy are two fundamental and informative traits mainly for yeasts with complex diploid/haploid shift in their life cycle. Our complementary molecular strategies highlighted a naturally occurring variation in genome size and ploidy level within *Z. rouxii*, suggesting that there is a complex picture in genome organization and the strains now recognized as *Z. rouxii* could belong to more than one species.

References

1. Barker, B.T.P., ***Trans. R. Soc. Lond. B.*, 194**, 467–485 (1901).
2. Kinclova, O., et al.,. ***J. Biotechnol.*, 88**, 151–158. (2001)
3. Pribylova, L., et al., **Yeast, 24**, 171-180 (2007)
4. Solieri, L., et al., ***Yeast,* 24**, 403–417 (2007)
5. Gordon, J., Wolfe, K.H. ***Yeast*, 25**, 449-456 (2008)
6. Iwaki T., et al., ***Microbiol.* 145**, 241-248 (1999).

PI-5: Improvement of traditional vinegar production by selected acetic acid bacterium strain: *Acetobacter pasteurianus* as acetification starter

M. Gullo, L. De Vero and P. Giudici

Dept. Agricultural and Food Sciences, Via Amendola, 2, 42100 Reggio Emilia, Italy, University of Modena and Reggio Emilia
E-mail: maria.gullo@unimore.it

Recent microbiological studies investigated the acetic acid bacteria microflora of traditional balsamic vinegar (TBV) highlighting the occurrence of *Gluconacetobacter europaeus* as widespread indigenous species, followed by *Acetobacter pasteurianus*, *Acetobacter aceti* and *Acetobacter malorum*[1,2]. However no correlation between occurring species and quality of product have been examined. In this study a selected starter culture (SSC) was designed, implemented at laboratory scale and applied to vinegar factory by scale up procedure. *A. pasteurianus* strain AB0220 was selected as SSC on the basis of phenotypical and technological traits suitable for TBV must oxidation. The SSC was implemented through three stages: starting steps in laboratory conditions (Stage 1); scale-up performed through a tanks system (Stage 2) and a barrels system (Stage 3) in factory conditions. Main analytical parameters were monitored by pH, titrable acidity, ethanol and soluble solids trend. Moreover molecular identification based on 16S rDNA region analysis (PCR-DGGE and sequencing) and ERIC-PCR were performed respectively to assess species occurrence and evaluate strain persistence during the whole process. In particular the dominant AAB species population was estimated by PCR-DGGE analysis allowing to distinguish two different species groups along the 3 stages. 16S rDNA sequencing confirmed DGGE results, showing high percentage of sequence homology (99 and 100%) with *A. pasteurianus* at stage 1 and 2 and *Ga. europaeus* at Stage 3. Finally, ERIC-PCR fingerprinting assay performed at the end of each acetification stage showed an electrophoretic profile similar to that of AB0220 at stage 1 and 2, whereas a different pattern at stage 3. This result supports DGGE data suggesting a change in the population during the 3 acetification stages. On the basis of these evidences, our hypothesis is that the persistence of inoculated strain during stages 1 and 2 was assured by the scale-up procedure. New must was periodically added to increase the SSC volume and microbial population was not exposed to constant effect of acetic acid. Instead, the static conditions of stage 3 resulted in a constant increase of acetic acid concentration, negatively affected AB0220 growth. In this environment, indigenous *Ga. europaeus* cells, which is less sensitive to physiological stress caused by acetic acid[3], found optimal growth conditions to become dominant.

In this study for the first a SSC for TBV production was implemented and applied at factory scale. Results demonstrated that selected *A. pasteurianus* strains are suitable to start the acetification of TBV ensuring the acetification course in unsuitable conditions for other AAB such as *Ga. europaeus.* On the contrary, *Ga. europaeus* strains are able to oxidise cooked must in presence of constant of acetic acid concentration corresponding to the final stages of TBV oxidation. Results suggest that the introduction of SSC could be a valid innovation in TBV production, contributing to safety and quality of the product and improving the reliability and stability of technological process.

1. Gullo and Giudici, ***Int. J. Food Microbiol.*, 125,** 46-53 (2008)
2. Gullo et al., ***Int. J. Food Microbiol.*, 106**, 209-212 (2006)
3. ***Sievers et al.,*** System. Appl. Microbiol., *15,* ***386-392 (1992)***

PI-6: Valorisation of onion waste to onion vinegar: monitoring of acetic fermentation of onion alcohol by NIRS

C. Pizarro, I. Esteban-Díez, S. Rodríguez-Tecedor and J.M. González-Sáiz

Department of Chemistry, University of La Rioja, c/Madre de Dios 51, 26006 Logroño (La Rioja), Spain
E-mail: sofia.rodriguez@alum.unirioja.es

Wastes and by-products of the onion-processing industry pose an increasing disposal and environmental problem and represent a loss of valuable sources of nutrients. The present work is part of an ambitious project, carried out in cooperation with other research groups, aimed at examining the feasibility of new approaches for upgrading onion waste to value-added products. In particular, this work focused on the production of vinegar from worthless onions as a potential valorisation route which could provide a viable solution to multiple disposal and environmental problems, simultaneously offering the possibility of converting waste materials into a useful food-grade product and of exploiting the unique properties and health benefits of onions.

The first essential step in onion vinegar production, i.e. effective ethanol production from worthless onions and the necessary monitoring of the alcoholic fermentation of onion juice to control the process in real time, has already been studied and described in detail in previous studies published recently[1,2]. This study is the natural continuation of the above-mentioned works, insofar as it focuses on the valorisation of onion waste to promote its transformation into an added-value product such as onion vinegar and, more specifically, on the second and definitive step of the fermentation system analysed: the effective production of onion vinegar from onion alcohol previously obtained from the alcoholic fermentation of onion juice via acetic fermentation by Acetobacter[3]. In the search for a suitable methodology for monitoring this process in real time, separate PLS-regression models were constructed based on NIR spectra of samples collected during acetic fermentation in order to model the key parameters involved in the acetic fermentation of onion alcohol. Additionally, a wavelength selection method based on PLS regression such as iterative predictor weighting (IPW) was applied to test the hypothesis that a minimum number (maximum parsimony) of informative predictors (NIR absorption bands closely related to the response of interest) can predict a certain response variable with at least the same accuracy or even improved reliability and robustness such as using the whole NIR wavelength range. Biomass, substrate (ethanol) and product (acetic acid) concentration were predicted in the acetic fermentation of onion alcohol with high accuracy using IPW-PLS models with a root-mean-square error of the residuals in external prediction (RMSEP) lower than 2.5% for both ethanol and acetic acid, and a RMSEP of 6.1% for total biomass concentration (a very satisfactory result considering the relatively low precision and accuracy associated with the reference method used for determining the later). The potential use in routine monitoring of the reliable and simple calibration models proposed (developed from a minimum number of informative predictors) could substantially reduce the analytical time, costs and work of assessing key parameters in fermentation. Thus, non-destructive, accurate and near-real time determination, fundamental for the control and global optimisation of the whole process, would be possible.

1. J.M. González-Sáiz et al., *J. Agric. Food Chem.*, **55**, 2930-2936 (2007)
2. J.M. González-Sáiz et al., *Biotechnol. Bioeng*., DOI 10.1002/bit.21939
3. J.M. González-Sáiz et al., *Anal. Bioanal. Chem*., **391**, 2937-2947 (2008)

PI-7: Hypotensive effect of malt black vinegar on spontaneously hypertensive rats

M. Odahara[1], T. Tsuruki[2], Y. Ogino[2], M. Kimura[2], N. Nakamura[1] and K. Kimoto[3]

[1]*Research Laboratory, Kewpie Jyozo Co., Ltd., JAPAN*
[2]*R&D Division, Q.P. Corporation, JAPAN*
[3]*Faculty of Home Economics, Tokyo Kasei University, JAPAN*
E-mail:makoto_odawara@kewpie-jyozo.co.jp

Recently, the consumption of "Kurozu" (black vinegar) has increased in Japan, as the health benefits of vinegar have become better known. The quality of Kurozu is defined by the Quality Labeling Standard for edible vinegar in the JAS (Japanese Agricultural Standard) system. Kurozu is classified into "Kome-Kurozu" (unpolished rice black vinegar) and "Ohmugi-Kurozu" (barley black vinegar). Kome-Kurozu has been extensively studied for health functions such as the hypotensive effect. On the other hand, studies on Ohmugi-Kurozu are limited[1]. Therefore, we investigated the effect of malt black vinegar (Ohmugi-Kurozu made from barley malt) on blood pressure in spontaneously hypertensive rats (SHR)[2].

SHR were given malt black vinegar orally either in a single dose of 3 g/kg body weight or by feeding for 8 weeks (10% (w/w) in diet). In each experiment, malt black vinegar showed a significant hypotensive effect compared with a distilled water control. Wort and acetic acid, the main components of malt black vinegar, also lowered the blood pressure of SHR, suggesting that these components are involved in the hypotensive effect of malt black vinegar. We also compared the hypotensive effects of malt black vinegar and unpolished rice black vinegar and found that the former exhibited a hypotensive effect equal to or higher than that of the latter. Furthermore, we investigated the mechanism of the hypotensive effect of malt black vinegar *in vitro*. Malt black vinegar, whose pH was adjusted to 8.3, inhibited angiotensin I-converting enzyme activity (IC_{50} = 3.9 mL/100 mL). These results helped clarify the hypotensive effect of malt black vinegar.

1. Ichikawa, M. et al., ***J Nutr. Sci. Vitaminol, 49***, 428-433 (2003)
2. Odahara, M. et al., ***Nippon Shokuhinn Kagaku Kougaku Kaishi, 55***, 81-86 (2008)

PI-8: Preparation of Rice Vinegar with High Content of γ-Amino Butyric Acid

C. X. Chen, F. S. Chen

College of Food Science and Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, P. R. China
E-mail: chenfs@mail.hzau.edu.cn

γ- amino butyric acid (GABA) has been proven to possess many physiological functions, such as adjusting blood pressure, promoting brain blood flow, making nerve cells nutritious, increasing growth hormone secretion, preventing obesity, improving climacteric syndrome and so on[1,2]. Recently, some foods riched GABA were perpared[3,4].

In this study, the rice vinegar with high content of γ- amino butyric acid (GABA) was prepared in liquid-solid fermentation process by using sprouted rice as raw material, red yeast rice as saccharifying agent. 500g sprouted rice of 28.49mg GABA /100g, smashed into pieces of 40 mesh sieve was mingled with 500mL distilled water, and sterilized at 121℃ for 30 min. After cooling, the effects of adding amounts of red yeast rice, Xiaoqu(raw starter complex), water, glucoamylase and sodium glutamate, and fermentation time, temperture and pH were studied on GABA content in fermented mash through Plackett-Burman experiment. The results showed that adding amounts of red yeast rice, water and glucoamylase were main factors during fermentation. On this basis, the regression equation of response surface methodology according to Box-Benhnken center-united experiment design was $Y=147.1-7.4A+2.2B-1.8C-14.1A^2-3.7B^2-2.6C^2-0.2AB-2.1AC+0.4BC$ (Y, GABA in fermented mash, mg/100mL; A, the amount of red yeast rice, %; B, the amount of water,%; C, the amount of glucoamylase,%). According to this equation, the estimated value of GABA was reached at 147.09 mg per 100mL fermenting mash at the optimal conditions of 500g smashed sprouted rice, 4g red yeast rice, 335mL adding water, 1.6mL glucoamylase preparation, fermented at 28℃ for 5 days, while the average experimental GABA value was 145.18/100mL, very close to the the estimated value. Then, 350 mL fermented mash was mixed well with 750g wheat bran and 500g rice chaff, and inoculated with 17mL culture medium (OD_{600nm} =0.8) of *Acetobacter pasteurianus* CICC 7009, and fermented for 7d at 28℃ by turning over every 5-7 h, then matured for 8d in the sealed container. After that, the rice vinegar coantaining 100mg/L GABA (acidity 2.12g/100mL, counted by acetic acid) was prepared, when the above vinegar mash was immerged in 5000mL distilled water. Meanwhile, GABA in rice vinegar prepared by the conventional fermentation condition was only 2.7 mg / L.

1. Ye W., ***Progress In Physiological Sciences*****, 17**, 187-189 (1986)
2. Takahashi H et al., ***Jpn J Physiol.*****, 8**, 378-380 (1958)
3. Li B. et al., ***Food and Fermentation Industries.***, **32**, 28-30 (2006)
4. Park K. et al., ***Bioresource Technology*****., 98**, 1675-1679 (2007)

PI-9: Functional role of acetic acid bacteria in cocoa bean fermentation

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

*Research Group of Industrial Microbiology and Food Biotechnology, Department of Applied Biological Sciences and Engineering, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium (*Present address: Barry Callebaut NV, Wieze, Belgium)*
E-mail: ldvuyst@vub.ac.be

Cocoa beans are the seeds of the fruit pods of the cocoa tree. They are used for the production of chocolate. The cocoa beans undergo a fementation prior to further processing. In the pods, the cocoa beans are embedded in a mass of mucilaginous white pulp, which mainly consists of pectin, citric acid, and carbohydrates. After removal of both beans and pulp from the pods, the pulp-bean mass is piled into heaps, covered with plantain leaves, and a spontaneous fermentation starts. To study their biodiversity, population dynamics, and metabolomics, several spontaneous heap fermentations were carried out[1,2,3]. Culture-dependent and –independent molecular methods and various chromatography and mass spectrometry techniques were used for species identification and target metabolite analysis, respectively. A succession of microbial activities took place, encompassing a restricted species diversity of yeasts, lactic acid bacteria, and acetic acid bacteria (AAB). The AAB population consisted of several *Acetobacter* species, namely *A. pasteurianus* (the predominant one)[1,2] and the novel species *A. ghanensis*[4], *A. senegalensis*[1,5], and *A. fabarum*[6]. Their isolation frequency was cultivation medium-dependent. The AAB were responsible for the oxidation of ethanol, which was formed by the yeasts out of sucrose, into acetic acid under conditions of high ethanol concentrations (due to yeast activity), satisfactory oxygen availability (due to air penetration into the fermenting cocoa bean mass, caused by reduction of viscosity and drainage of the cocoa pulp, in turn due to pectinolytic activity of the yeasts). Acetic acid was needed to penetrate the cocoa beans, kill the embryo, and destroy their internal structure to initiate biochemical transformations necessary for flavour and colour development. Also, acetic acid itself contributed to flavour development. It was further shown that acetic acid could be further oxidized to carbon dioxide and water; also, lactate and mannitol could be oxidized to carbon dioxide and water. All oxidation reactions were responsible for a considerable temperature increase, which finally haltered the fermentation process of the cocoa bean mass. Hence, AAB played an important functional role during cocoa bean fermentation, as they contributed to the formation of cocoa flavour precursors and the control of the cocoa bean fermentation process.

1. Camu, N. et al. *Appl. Environ. Microbiol.* 73**, 1809-1824 (2007).**
2. Camu, N. et al. *Appl. Environ. Microbiol.* 74**, 86-98 (2008).**
3. Camu, N. et al. *J. Sci. Food Agricul.* 88**, in press (2008).**
4. Cleenwerck, I. et al. *Int. J. Syst. Evol. Microbiol.* 57**, 1647-1652 (2007).**
5. Ndoye, B. et al. Int. J. Syst. Evol. Microbiol. 57**, 1576-1581 (2007).**
6. Cleenwerck, I. et al. *Int. J. Syst. Evol. Microbol.* 58**, 2180-2185 (2008).**