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mail : mustang.expres@gmail.com
web : www.editura-mustang.ro
phone : 0040 722 293 135

Technical Editors:

GALAN Cătălin
catalin.galan@gmail.com

Professional Translation:

LĂZUREANU Cătălin

Office:
Nicolae Șuțu Street, no. 11 – 15
Central building, second floor, room 9,
sector 1, Bucharest, Romania
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Rector's Allocution

*We have the special pleasure to let you know that the Review of our University, „**Buletin of Scientific Information**", having ten years of consecutive issue, it achieved the recognition of the **National Council for Scientific Research in Higher Education**, being comprised in the category „**National Reviews – C Category**"*

*So, the Bioterra University review „**Buletin of Scientific Information**" works as a real platform for the information and exhibition of the most recent and valuable research in the agricultual field and connected sciences (food industry, agro-tourism, ecology, agricultural economics etc.).*

This way I express my gratitude the contributors to our review, authoritative academic and univeritary names of whose studies are found in the selection done by the scientific board of the review, co-workers with whom we have strong relations of partnership and mutual support in the development and course of some conjointed research projects.

I wish to the review many and consistent issues.

Prof. Floarea Nicolae, PhD
Rector of the Bioterra University of Bucharest

Summary:

INCIDENCE OF GENETICS AND MOLECULAR BIOLOGY ON THE SENSORIAL PROFILE OF WINES. THE EMPHASIS OF THE TYPICAL VARIETAL PROFILE OF THE SAUVIGNON BLANC VARIETY BY THE SELECTION AND USE OF A YEAST STRAIN WITH SUPERIOR BIOLOGICAL CHARACTERISTICS

Croitoru Constantin, Nicolae Ion, Mihăilă Daniela
Bioterra University of Bucharest

Abstract: *The metabolism of the yeast's cell can be modified by genetic engineering in order to improve some of its oenological properties that have favourable effects on wines' sensorial profile. The selection of some yeast strains which posses in their cells enzymes that free typical aroma grape variety starting from their precursors such as wines from Sauvignon blanc grape variety, represent one of the principal interests of biological sciences in oenology.*
Such a recently selected yeast strain was IPER R; its enzymatic cellular equipment is rich in carbonsulphurilase and alcoholacetiltransferase enzymes; the carbonsulphurilase transforms cysteine sulphur precursors (cysteine-3-MMP and cysteine-3-MH) into their principal varietals free aroma of the Sauvignon blanc grape variety (4-MMP and 3-MH); in its turn, alcoholacetiltransferase transforms the most simple aromae compound (3-MH) in its acetate (3-MHA) by endorsing the complexity and the typical olfactory of the resulted wine.
In the performed comparative experiments we tested the strain IPER R ant the nutrient FERMOPLUS ENERGY GLU (FEG). The results have shown that the elaboration of some wines from the Sauvignon grape variety with a remarkable sensorial profile (by the increase of the smell intensity and of the fruity-floral character, accompanied by the emphasis of tasty sensations of freshness, oiliness, roundness and lengthiness) need the compulsory use of the IPER R yeast strain during the alcoholic fermentation (AF) of musts in association with a correspondent and compatible nutrient such as FEG.

Key words: *sauvignon blanc variety, molecular biology, genetics, typical varietal profile.*

Introduction

Amongst the research areas that are adjacent to oenology, classical biological techniques such as crossing (hybridization), mutagenesis and directed or adaptive evolution of yeast strain are of major importance *(Pretorius I.S., 2000, quoted by [2])*. Latest genetic approaches *(Dequin S., 2001, Schuller D. and Casal M., 2005, quoted by [2])* have been integrated by these classical techniques and they made possible the results displayed in table 1.
In principle, all properties of oenological selected yeast strains can be improved by the above mentioned classical techniques.
There are yet three inconvenients which limit the number of strains that can be generated: the impossibility in improving other characteristics that those already present in parental strains; the difficulty in improving

Table 1:

Exemples of new selected yeast strains that were obtained through genetic engineering and the potential applications in the wine making area

(Croitoru C., 2009 [2], adaptation from Benoit D. and Bauer F. F., 2008)

Name	Selling	References
The streamlining of fermentative processes		
The introduction of the malolactic enzyme and the transport of malate in the *S. cerevisiae* yeast in order to induce simultaneously AF and MLF.	Homologated strain in USA and Canada	Volschenk H.et all. , 1997; Husnik J. I. et all. , 2006
The streamlining of stabilization and clearance treatments for wines		
The secretion of pectolitic enzymes (poligalacturonases, xilanases an cellulases with different origins).	No	Fernandez-Gonzales M. et all. , 2005; Louw C. T. et all. , 2006; van Rensburg P. et all. , 2007
The emphasis of odourantand tasting characteristics of wines		
The secretion of β-glucosidase enzymes of different fungus and yeast origins in order to increase the production of free odourant monotherpehnols and to emphasize the varietal typical odourant character of the variety the wine comes from.	No	Arevalo-Villena M. et all. , 2005; Van Rensburg P. et all. , 2005
The general emphasis of odourantcharacteristics of wine by manipulating the metabolic paths that are associated with the production of odourantcompounds.	No	Lily M. et all. , 2006a, b
The production of healthier wines		
The reduction of alcohol content: the secretion of glucozidase enzyme by a *S. Cerevisiae* strain which was genetically modified in order to transform a part of the glucose in gluconic acid and consequently reduce the final concentration of ethylic acid.	No	Malherbe D. F. et all. , 2003
The production of a smaller urea quantity during AF which contributes to the decrease of ethyle carbamate concentration – very well known cancer provoking substantce.	It has received the status of innocuous organism in USA (522^{EC} strain)	Coulon J. et all. , 2006
De novo production of resveratrol. This compound is an antioxidant which is naturally present in red wines: it is associated with a more reduce frequency of cardiovascular disorders.	No	Becker J.v. W. et all. , 2003
Better microbial control		
The production of bacteriocines (i.e. periocine and leucocine) during AF in order to eliminate the lactic acid bacteria.	No	Schoeman H. et all. , 1999
Recently discovered killer toxins production (Comitini et all. , 2004a, b) in order to control the debasing yeast s' development such as *Dekkera bruxellensis, Zygosaccharomyces sppp., Schizosaccharomyces pombe* and *Saccharomycodes ludwigii.*	No	It is to be settled.

a certain character above certain level (any avant-garde improvement may need the support of external genetic material); the random character of each applied classical strategy due to a very small quantity of descendants with the desired oenological characters.

Consequently, due to the application of hybridization or mutagenesis it is necessary to make an intensive selection in order to identify the yeast strains which posses the desired oenological characters *(Benoit D. and Bauer F. F., 2008, quoted by [2])*.

The information which was generated by the biological sciences allow the improvement of the efficiency of the above mentioned classical techniques but the current wine consumers' reluctance toward genetically modified organism limit the forecast evolutions for selected yeast strains that undergo genetic modification in order to reach a specific oenological objective *(Cellobero E. et. all, 2007, quoted by [2])*.

This paper focuses on the comparative study of two selected yeast strains and on a nutrient – all of them being investigated in view of optimizing the biotechnology for improving wines that emanate from the Sauvignon blanc variety.

Materials and methods

In order to achieved our assumed goal, it is necessary to test the nutrient and the selected yeast strains in identical technological conditions (same fermented must, same volumes in containers with same geometry, the same thermal regime for fermentation, the same dose of selected yeast, ...) which could allow considerable improvement of the sensorial profile of wines that emanate from the Sauvignon blanc variety by stressing the typical character and the odourant and tasting expressivity which is established by comparative tasting.

» Aspects regarding the used nutrient.

In order to better understand the necessity of elaboration of some last generation nutrients with more complex composition is necessary to state the specific objectives to yeast nutrition taking into account the life cycle of any viable cell and to know the factors that influence the cellular multiplication.

The objectives of yeasts' nutrition aim to: favour the yeasts' cells multiplication during rehydration; favour the implantation of yeasts' cells in the starting stage of AF by supplementing the assimilable nitrogen content of the must; favour the rapid finalisation of the AF without risking to cut-off the fermentative process.

» The stages in cellular activity cycle.

There are 3 main stages in the life cycle of every active dry yeast cell when it is inoculated in the fermentative medium (must or distammed and crushed grapes); these stages are rehydration, multiplication and AF. There stages can be represented as in the graphic curve from figure 1 which shows that the stage 1 is for the latent period which is the same with the rehydration of yeasts;

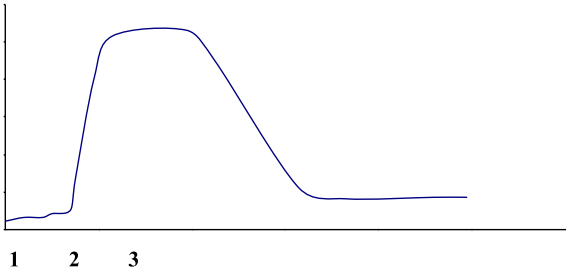


***Fig. 1* – The stages in cellular progress specific for the life cycle of any dry active yeast cell at the inoculation in the fermentative medium (must or distammed and crushed grapes)**
1 – Latent stage; 2 – Multiplication stage; 3 – Fermentation stage

stage 2 is for the cellular multiplication process of yeasts and stage 3 is for the AF carrying process.

»Factors that favour the cellular multiplication. Gluthation's role.

Cellular multiplication is the stage during which cells reproduce. An in depth analysis of yeasts cells' multiplication highlights the necessity to assure some aerobiotic conditions. Nevertheless, the molecular oxygen, O2, has a nefarious incidence the yeasts' cells because it burns the intracellular proteins (this process is also known as „aging of cells").

In order to prevent this oxidative process, during the cellular multiplication the viable yeast cell uses all available gluthation as a consumed resource in order to prevent nutritional proteic losses because of the oxygen. In most of the case the gluthation concentration present in the interior of yeast cells is insufficient for the prevention of oxidation of cellular proteins.

Beside the early ageing of viable yeast cells, there may appear order undesired oxidative phenomena. The oxygen's presence at intracellular level will allow the attack on the amino acids from the interior of the yeast cell causing their irreversible oxidation; in these conditions, a series of amino acids can no longer serve as nutritional resources for the yeast cell; oxygen attacked amino acids during cellular respiration are methionine, histidine, tyrosine and tiroxine; generally, methionine is oxidised into sulfoxide methionine which detonates a series of chain reaction which allow the formation of a protein with oxidative characteristics.

The oxidative process of these amino acids provoke an oxidative metabolism for the yeasts' cells which determine at their turn the same process on their offspring (forming) affecting negatively the varietal odourant constituents (from the must and the distammed and crushed grapes which were destined for the fermentative process) in terms of decreasing concentration.

The contribution of directly assimilated amino acids and gluthation have a favourable impact by facilitating the cellular multiplication and by limiting the nefarious effects yield by the oxidation of proteins and amino acids inside the viable yeast cells. Such contribution was made possible when using an adequate nutrient such as FEG during AF.

» The characterization of the used nutrient.

The FEG product is a fermentation coadjuvant made from cellular walls resulting from the thermal and enzymatic autolysis of inactive yeast cells that were obtained in an original technological process. It is a nutrient with an energizing and enriched with gluthation character which is recommended in wine making industry for any difficult AF of must or distammed and crushed grapes, but also for refermentations. It is a preparation from yeasts cellular walls mainly (99.4 %) with a high gluthation content, amino acids and vitamins together with the B1 vitamin (0.6 %). A dose of 10 g/hl from this product gives to the must a 0.6 mg/l vitamin B1 content.

» Aspects regarding the used yeast strain.

The FERMOL IPER R selected yeasts strain exhibits some very important oenological characteristics: its implantation in the fermentative medium needs a more accurate monitoring; it also needs a nitrogenous nutrition and it behaves itself remarkably in the presence of a complex autolysed yeast derivatives-based nutrient such as FEG; it presents intracellular enzymatic equipment (high content of carbon sulfuridase which is a β-lyase) which is capable to produce high concentrations of volatile thiols with agreeable odourant character (3-MH, 4-MMP); it is a adapted yeast strain to AF

in reductive conditions; the wines obtained from this yeasts strain have a very intense odourant character associated with freshness and persistence notes in the oral cavity; it is recommended to the AF of musts coming from Sauvignon blanc variety, Colombard and Syrah. Recent studies have shown the importance of the selected yeast strain on the development of varietal aromae from the Sauvignon blanc variety by the action of his enzymatic equipment on the odourant precursors during the AF of must [4].

» The precursors of cysteine-derivated volatile thiols.

The musts coming from the Sauvignon blanc variety have an almost neutral odourant character because the specific variety typical aroma shows itself during AF as a consequence of the action of enzymatic equipment of the selected yeasts strain. The responsible substances fro these orourant touches are some poly functional thiols, 4-methyl-4-mercapto-pentan-2-one (abbreviated 4 MMP) and 3-mercaptohexanole (abbreviated 3 MH), which are found in must coming from this variety in form of S-conjugated of the amino acid cysteine [11].

» The 4 MMP compound.

Is it found in the boxwood leaves and in the defoliated lentil sticks; its threshold of smelling perception is of 0,8 ng/l in model solution and of 40 ng/l in the Sauvignon blanc variety wines with remarkable tipicity. This observation allows the demonstration that the association of „boxwood-green broom" sensorial descriptors corresponds to a chemical reality.

» The 3 MH compound.

Its odourant character reminds of the grapefruit and passion fruit smells in which this compound was first identified. Its smelling perception threshold is of 60 ng/l, but it is always present in a few hundreds ng/l or even more µg/l concentration in Sauvignon blanc wines.

» The 3 MHA compound.

It possesses a complex boxwood aroma associated with the smell of grapefruit peel and with that of passion fruit. Its smelling perception threshold is of 4 ng/l but some wines from the Sauvignon blanc variety may have even a few hundred in ng/l. Its concentration decreases during maturation by generating the 3 MH compound.

From sensorial point of view these S-conjugated compounds of cysteine are responsible for the specific Sauvignon blanc retronasal sensation with is perceived when tasting the grapes or the must that comes from this variety; the enzymes from our oral cavity posses a specific β-lyase activity which is capable of liberating agreeable volatile odourant thiols in a few seconds by starting from their cysteinilized precursors.

The forming mechanism of agreeable volatile odourant thiols consists in the enzymatic hydrolysis of cystenilized aromatic precursors under the influence of FERMOL IPER R selected yeasts as it is shown in the below represented reactions (Fig. 2). The eliberation of volatile thiols take place under the action of the CS-lyase enzyme ([5], [10]), and the transformation of 3-MH in 3-MHA takes place under the action of the alcoholacetyltransferase [9], as it may be observed in the figure 2.

Recent studies have shown that the liberation, conversion and sensorial perception of polyfunctional volatile thiols go on a more complex biochemical sequential network; in figure 3 it is represented the complex biochemical networked by which 3-MH and 3-MHA compounds are formed by highlighting some elements that interfere with intracellular processes; because of all these it is necessary a profounder biological study if it is indented to maximum revaluate

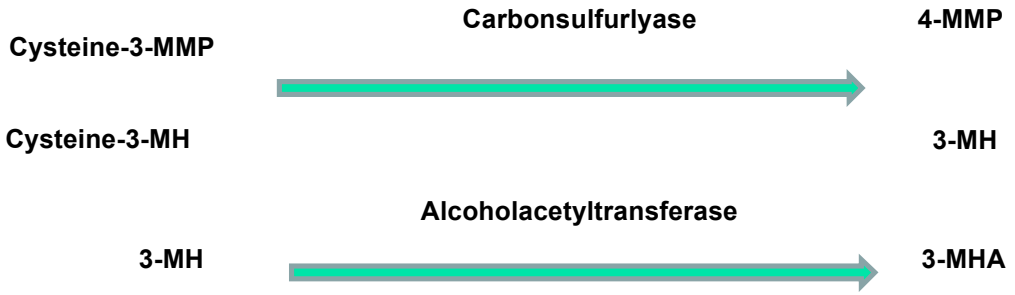


Fig. 2 **– The agreeable odourant volatile thiols synthesis by the enzymatic hydrolysis of ysteined precursors under the action of FERMOL IPER R selected yeast cells**

the smelling-tasting potential that comes from the liberation and the conversion of poly functional volatile [3].

The scheme in the figure 3 highlights the forming of 3-MH and 3-MHA taking into account the deduced transport of the gluthation conjugated precursor and its potential biochemical degradation ways; the paths indicated in black have been investigated in the context of poly functional thiols' forming, those indicated in interrupted grey line have been deduced from the gluthation circulation studies and that indicated with a white line represents a hypothetical route for transport.

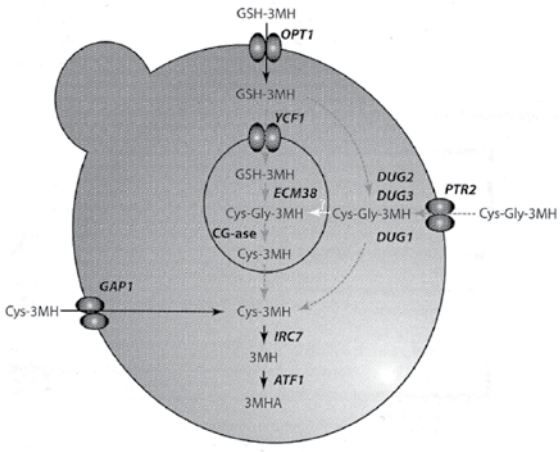


Fig. 3 **– The biochemical and complex genetically forming paths for 3-MH and 3-MHA [3]**

There were detected a lot of implicated genes in this complex process in the cells of some selected yeast strains; we indicated here a single example that aims at the penetration of conjugated precursors in the yeast cell with the help of amino acids and peptides that had the transport role.

Subileau M. R. et all, 2008 [8] identified that the cysteinilized precursors follow partially the GAP1 path that presumes the intervention of a general nitrogen acid permease after which it can be observed the decrease in the production of 3-MH and 3-MHA in the respective cell yeast strains which undergo the GAP1 genetic mutation; these gene is repressed because it is preferred the nitrogen sources by the selected yeast cells [1].

Other permeases have been implicated in the cysteine's metabolisation and might as well be implicated in the 3-MH cysteine's metabolisation; of particular interest is the very recent characterisation of the encoded protein with the metabolisation of 3-MH cysteine; of another particular interest is the very recent characterization of the encoded protein with the YCT1 gene that represents a transporter with high affinity for cysteine that may seem as the principal route to metabolize cysteine in the conditions of a fermentative medium rich or poor in nitrogenous [6].

» Aspects regarding the fermentation condition.

It was used the same Sauvignon blanc variety must ((218 g/l sugars and a titrable acidity of 6,2 g/l tartric acid) which was racked by refrigeration, separated in equal 80 hl equal volumes and fermented in identical stainless steel recipients with a capacity of 100 hl.

The antioxidant protection of the crop and of the must was assured with the product ANTIOXIN W (a blend of potassium metabisulfite and ascorbic acid) in a dose of 10 g/hl. The AF of the must which was cleared and separated by refrigeration was triggered at 12 °C and it was developed at 14 – 18 °C by a progressive slow increase, although the specialized literature recommends the thermal interval of 18 – 20 °C as being the most suited interval to highlight the Sauvignon blanc variety typical aroma [7].

The compared selected yeasts' strains (the common one and the up presented one) have been utilized in doses of 20 g/hl and the presented nutrient in dose of 5 g/hl. The fermentation kinetics was monitored by daily measures of must density and temperature.

Results and discussions

» The elaborated comparative experiment is present in the figure 4 which highlights the fermentation diagrams of the three variants. Compared to the experimental

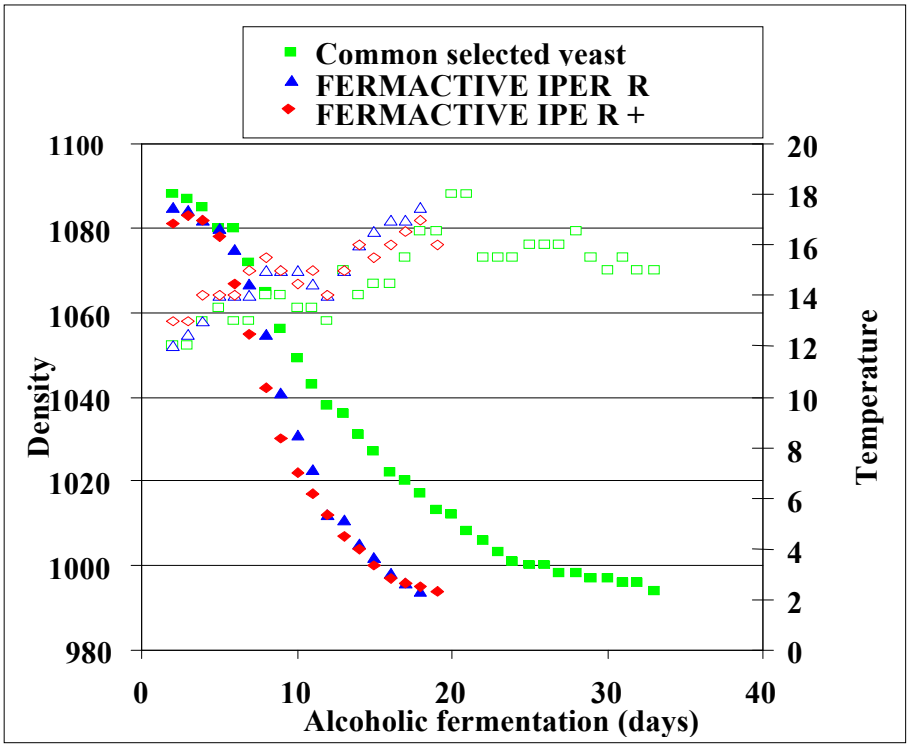
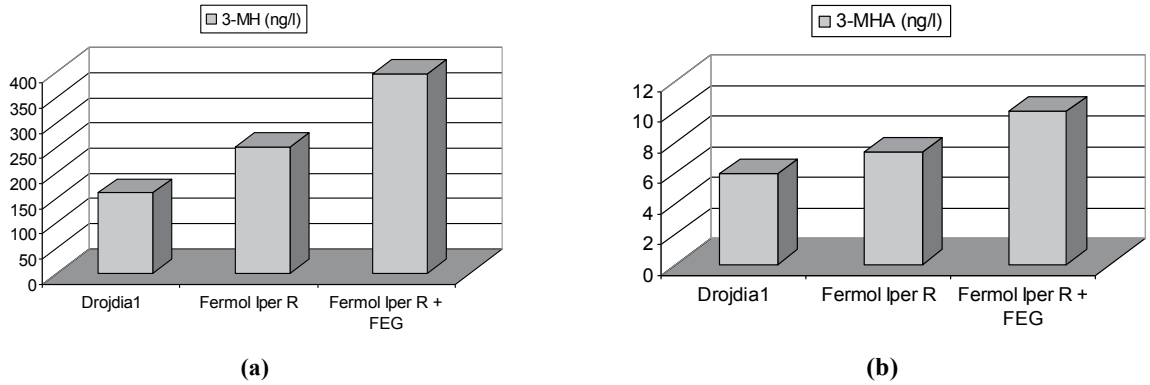


Fig. 4 **– Quantified fermentation kinetics through the evolution of density and of the thermal regime in the case of a must from the Sauvignon blanc variety which fermented in different experimental conditions**: *1 – AF with common selected yeast strain; 2 – AF with FERMOL IPER R selected strain; 3 – AF with FERMOL IPER R selected strain and nutrient FEG*



(a) **(b)**

Fig. 5 **– Comparative evolution of two representative compounds such as 3-MH (a) which is characterized by the "grapefruit" sensorial descriptor and 3-MHA (b) which is characterized by citric and tropical fruits sensorial descriptors in the case of a must from the Sauvignon blanc variety which fermented in different experimental conditions:**
1 – AF with common selected yeast strain;
2 – AF with FERMOL IPER R selected strain; 3 – AF with FERMOL IPER R selected strain and nutrient FEG

variants 1 and 2, at the 3rd variant is used a FEG adequate nutrient which determined a clip of the fermentative process, but also a decreasing of its final stage when the viable cells determine a more reduced rate of sugar metabolization in the unit of time (Fig. 4).

» The importance of associating the selected yeast strain with an adequate nutrient.

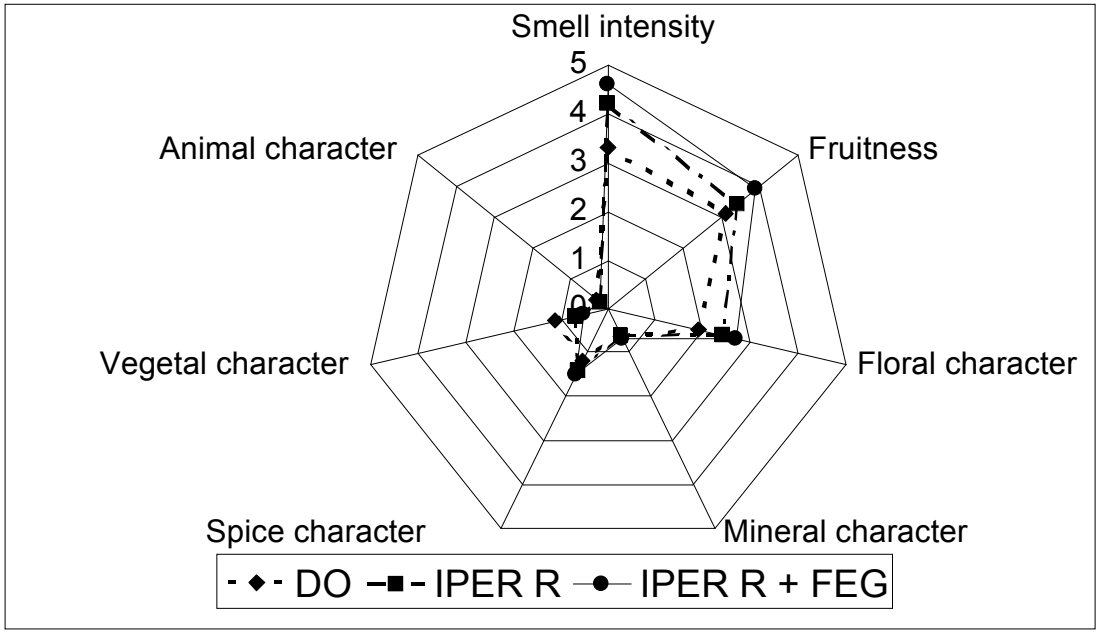
In the case of the comparative experiment already discussed regarding the AF of a must coming from Sauvignon blanc variety, the FERMOL IPER R selected yeast strain will free a higher concentration of agreeable odourant volatile thiols from their precursors at the variant which realized its fermentative process in the presence of the FEG complex nutrient (fig. 5 a and b).

» The sensorial benefits of a successful association.

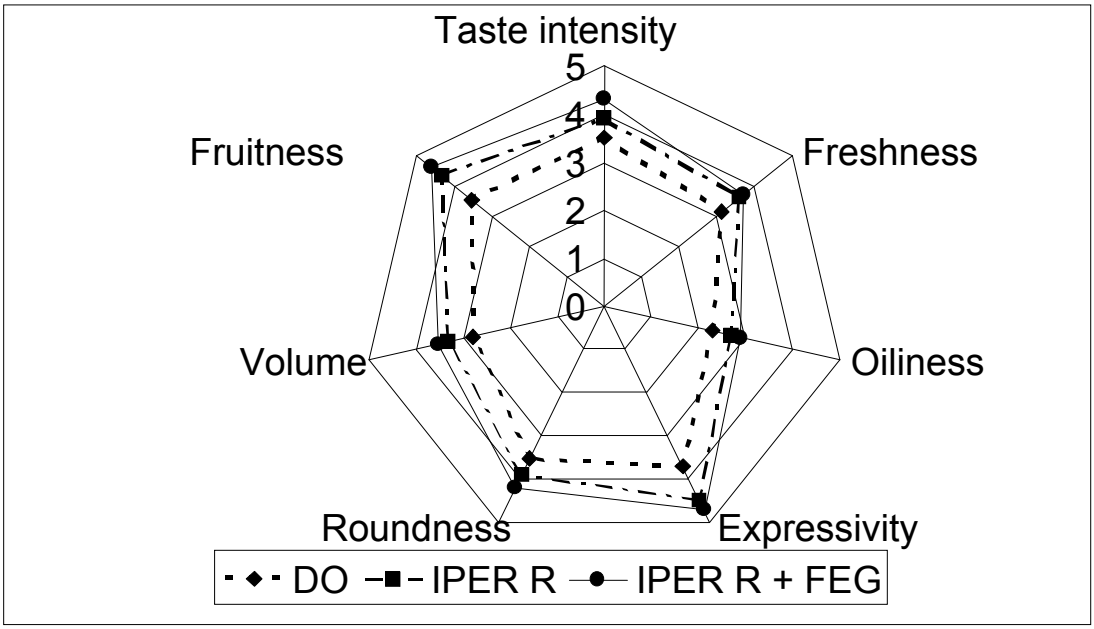
The use of FERMOL IPER R selected yeast strain and the nutrient FEG at an AF process has allowed a 55 % increase of the 3-MH odourant varietal compound concentration for a Sauvignon blanc wine. In the figure 6 there are presented the comparative sensorial diagrams for the wines that correspond to the three presented variants.

Conclusions

The FERMOL IPER R selected yeasts' strain which has cellular enzymatic equipment is capable of realising the enzymatic hydrolysis of the cysteine precursors which free the typical aroma constituents of the Sauvignon blanc variety expresses better its metabolic and fermentative functions in the presence of an adequate and compatible nutrient such as FERMOPLUS ENERGY GLU in such a matter that typical character and expressivity of the sensorial profile coming from the Sauvignon blanc variety are profoundly influenced.



(a)



(b)

Fig. 6 **– The odourant (a) and gustative (b) profiles in the case wines coming from the Sauvignon blanc variety which fermented in different experimental conditions:**
DO – FA with common selected yeast strain; IPER R – FA with the FERMOL IPER R selected yeast; IPER R – FA with the selected strain FERMOL IPER R and nutrient FEG

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BACTERIAL GENETIC MUTATIONS. THE CAUSE FOR XXI-ST CENTURY EPIDEMICS. PATHOGENIC ESCHERICHIA COLI

Cărădan Simona Laura, Atudosiei Nicole-Livia, Niculaie Liana
Bioterra University of Bucharest

Abstract: *After birth, animal bodies progressively colonize a large number of microorganisms that are located on the body and various mucous membranes, especially the digestive tract and skin. Human and animal body collect a total of 10 cells of microorganisms (Dobzhanky 1974), grouped into two major categories: normal microbiota (associated with indigenous or native organisms) and foreign or alien microbiota with microorganisms from the external environment, some can be pathogenic.*
Escherichia coli can be classified in two categories, it is part of the normal microbiota of finding the body especially in the digestive tract, but at some point may come from external environment, with structural changes or genetic, in different ways (ingested, inhaler).
Resistance of bacteria is relatively small when it comes through chemical or physical methods of sterilization, E. coli having an optimum temperature between 10-30 degrees C, the resistance between 5-50 degrees C is destroyed by disinfectants and antiseptics in normal concentrations, genetically modified strains are not destroyed by antibiotics with broad spectrum of action.
E. coli was discovered by Austrian scientist Escherich in 1886, researcher who gave the scientific names of bacteria. From measured by morphological, bacteria are rod shaped, Gram negative and it grows on ordinary culture media, this is the explanation of its spread in most animals organisms in natural environments.

Key words: *bacteria, infection, genetic mutations.*

Introduction

Escherichia coli (figure 1) is a bacterium that has been systematically framed by the following scheme:

» Domain Bacteria;
» Phylum Proteobacteria;
» Class Gammaproteobacteria;
» Order Enterobacteriales;
» Family Enterobacteriaceae;
» Genus Escherichia.

Figure 1
Escherichia coli (Gram negative, red stain)

This bacteria is representative for pathogenic microorganisms, are quite common in the living world on which there have been many researches on the structure of its resistance to external factors and the mode of action if the contamination followed by diseases living organisms.

Structure refers mainly to bacterial cellular wall, which serves to protect the genetic material responsible for copying and character of the infectious microorganism. Characteristic of bacterial cellular wall is given by the presence of substances - murein, component which gives stiffness and thus cell integrity upon contact with potentially aggressive external factors to the cell (hydrochloric acid in gastric juice, disinfectants, some antibiotics).

As the cellular wall thickness (wall thickness mureinics) is higher, so the cell is stronger, survival, latency and dissemination of the bacterial cell is more efficient, which increases exponentially virulence capacity. In electron microscopy was observed that the cellular wall of E. coli looks multilayered, being composed of two membranes: outer membrane (phospholipids 35%, 15% protein, lipopolysaccharide 50%) and internal membrane (mureinic wall).

Phospholipids are the basic layer, it interfering and support the other two components of the cell outer membrane. Proteins serve to regulate cellular wall permeability and are responsible for intra and extracellular transport through membrane channels.

Lipopolisaccahides are located in the last layer of the cellular wall, they are actually bacterial toxins, called endotoxins, which are issued only after these cell loses its integrity and expresses its infectious or toxic nature. As a species does not disappear, it must survive extreme environmental conditions, so that all organisms have developed mechanisms or adaptive behavior; all organisms rely on a structure of resistance or defense: E.coli specialized its mureinic wall.

What affects the animals organisms, especially human bodyes, is that bacteria can be harmful, so that it survives is not a bad thing, but when it finds nutritional conditions to develop, to proliferate and create complications in the body must be given medical treatment and strict personal hygiene measures and food.

Materials and methods

1. Pathogenesis and clinical manifestations

E.coli is a pathogen opportunist germ, as long as the bacteria does not change at the genetic level, it remains part of the body's normal flora (normal microbiota or associated).

For people with chronic diseases (gastrointestinal, renal or liver), not just E.coli, but also other microorganisms can cause diseases that trigger the system and organ complications, complications that can worsen leading to death.

E.coli has undergone genetic changes (chromosomes) of any nature, can have serious effects, giving complications to children and elders; genetically modified microorganisms are acting on the human immune system, which is not immunized with E.coli antibodyes with different genetic structure than that of normal microbiota, can not defend, so regional epidemics occurr, ragionale and in worst case global epidemics.

The pathogen character of E.coli, is given by the existence of several bacterial strains:

a) - Enterotoxigenic E.coli (ETEC), characterized by secretion of enterotoxins after intestinal colonization (location of bacteria in the intestine), causing a watery diarrhea, especially children as the age of 3 years, especially in underdeveloped countries;

b) – Enterohaemorhagic E.coli (EHEC) cause hemorrhagic diarrhea, toxins secreted have tropism for vascular endothelium;
c) - Enteroinvazive E.coli (EIEC) cause inflammation in the colon with ulcerative lesions;
d) - Enteropathogens E.coli (EPEC) produces watery diarrhea in children younger than 2years;
e) - Enteroagregative E.coli (EAEC) produces persistent watery diarrhea in infants and immunocompromised persons (AIDS);
f) - Urinary pathogen type (UPEC) is located in the intestins, peritoneal region and distal urethra is involved in 90% of cases of urinary infections in women;
g) - Diffusely adherent E.coli, produces watery diarrhea in older children.

Bacteremia type, can colonize the vaginal mucosa, which in the case of a natural birth can contaminate the newborn, in which can lead to neonatal meningitis. Classical clinical manifestations of infection with E.coli are recognized by the appearance of abundant watery diarrhea and bloody, fever, nausea, vomiting, severe abdominal cramps, untreated in time events may lead to enterohaemorrhagic syndrome (EHEC) which produces worse hemolytic uremic syndrome (HUS).
EHEC is diagnosed clinically by abdominal cramps, vomiting, bloody diarrhea followed by dehydration that attracts complications in all systems of human organs; occurs due to production of bacterial toxins, which develops the optimum temperature of 37 degrees C.
HUS produces hemolytic anemia (shortage of red blood), thrombocytopenia (plateletpoor), but also acute renal failure by kidney failure.
Extraintestinal infections occur through the migration of bacteria to other tissues or organs, usually via the blood, at which there are favorable conditions for proliferation(nutrition and breathing).

2. Epidemiology
Transmission of infectious agents is through direct contact with infected people (coming from epidemic areas), as happened in the north-west of Europe, or in contact with different products and vehicles coming from infected areas.
Epidemic outbreaks occur mainly in areas with poor hygiene, the most efficient way of transmission is by fecal-oral, respiratory default. Source of infection is the human or areas and food unclean or contaminated.
The natural habitat of E.coli is human or animal gut, where disseminate easily can reach in treated drinking water insufficiently depurated and decontaminated.

3. Therapy and Diagnosis
Diagnosis is established by several methods:
» Clinical diagnosis: by observing symptoms of the suspected persons to be infected with E.coli, in case nausea, vomiting, fever, watery diarrhea, symptoms occur the diagnosis must be confirmed by laboratory analysis of faeces, urine and venous blood;
» Microbiological diagnosis: is based on samples of faeces (stool) harvest from the patient, are prepared biological culture media in Petri dishes, which are put in the thermostatto 37 degrees C and after a period of 48 hours shall be considered microscopic culture medium, the results are expressed by presence or absence of pathogen agent;
» Serological diagnosis is made in specialized laboratories to determine exactly which strains of E.coli pathogen expressed character. Infection with E.coli is prescribed only after antibiogram because there are many situations that must be taken into account:

a) - strain of E.coli can be quite resistant, which is why antibiotics should not have the desired effect;
b) - class of antibiotics includes several types of drug substances, for which sensitivity is to reduce the number of antibiotics and to indicate exactly those substances that act on stem;
c) - patients tolerance or intolerance to antibiotics.
Antibiotics are prescribed in relation to sensitivity, the most effective as penicillins, cephalosporins and fluoroquinolones. For patients with diarrhea and vomiting states recommend treatments to slow intestinal peristalsis and the patient supine.

4. Bacterial resistance mechanisms. DNA mutation.
Watanabe (1960) described R-plasmids: are genetic elements extracromosomic which gives bacteria carrying resistance to multiple antibiotics simultaneously. R-plasmids have a complex genetic structure, being composed of several genes, most notably the transfer resistance R and the transfer factor of resistance or FTR.
In E.coli the two genes are recombined and form a large plasmid that carries both antibiotic resistance genes and genes transfer of antibiotic resistance.Sometimes antibiotic resistance is caused by mutations in chromosomal genes, but most often is the consequence of acquisition of multiple resistance R plasmid.
Multiple resistance to antibiotics is plasmidial and has the ability to transmit both vertically (from generation to generation) and horizontal genetic transfer mechanisms between different types of species of pathogenic.
Mutation is a change in genetic material, which is transmitted to offspring, the DNA molecule is the target of factors destructive internal and external (endogenous or exogenous). Mutations induced by endogenous factors are called spontaneous mutations and those induced by exogenous factors are called mutations induced. Spontaneous mutations occur in certain areas of the DNA chain (consisting of a sequence of purine nitrogen bases: adenine, guanine and pyrimidine, cytosine, uracyl, thymine), also called point mutations, which result from the substitution of a pair of nitrogenous bases.
Mutations induced (caused by exogenous factors) occur at a rate superior to the spontaneous mutation rate. Factors external mutagens are represented by chemical agents (analogues of bases, alkylating agents, agents deamination and acridine derivatives) and radiation.
UV radiation is mutagenic, DNA absorbs radiation with wavelengths of 260 nm, to which most bacteria do not survive and those that remain viable have mutated with high frequency. Ionizing radiation with the highest mutagenic rate are the γ and X, which works by breaking chemical bonds of molecules involved.

Results and discussions

» *Prevention, infection control and decontamination methods:*
Following the European outbreak of E.coli, the World Health Organization WHO together with other non-governmental associations, made remarks about personal hygiene and food standards in order to pathogen contamination prevention.
As for contamination with E.coli and other bacterial, viral or fungal infections, prevention methods recommended by improving and strengthening the immune

system, the easiest way is eating. Everyone (especially those living in crowded areas and those living in areas in reach or underdeveloped countries) must have a diet based on fruits and vegetables, fermented dairy and meat protein.

Fruits, vegetables and dairy products bring added vitamins and minerals, which have an important role in maintaining normal digestive flora. As is known, the bacteria E.coli, is beneficial for human bodies, but the cause of the European epidemic was the emergence of a new bacterial strain, due to genetic changes, without knowing the origin. Pathological cases caused by E.coli occurred for two reasons: on one hand the human body has antibodies against the new E.coli (there were no contacts with the bacterial strain and it was not immunized with it), on the other side some people with chronic illnesses or those with immune deficiencies (immunosuppressed or immunocompromised AIDS) do not have a body strong enough, to fight antigens (pathogen agents), most often occurring complications in the chronic organ systems and can reach up to death.

People with chronic disease presents in terms of metabolic disorders, affected organs do not function at normal capacity, have restrictions on medical treatment, which is why a simple microbial infection can be fatal. The best way to destroy pathogenic E.coli in healthy people, is to maintain a healthy digestive flora as digestion by eating fruits and vegetables consistently, cheese and fermented milk, especially to avoid treatment with antibiotics, if possible.

Conclusions

Bacterial infections should be considered seriously since the early stages of fever or muscle pain, in which prevention cannot be applied.

Lately WHO wanted and made a pretty good coverage put of the prevention methods, infection control with E.coli including enterohaemorrhagic disease symptoms, in order to prepare the population of E.coli epidemic phenomenon.

June of 2011 saw a significant number of pathogenic E.coli contaminations in Europe, and also a small number of deaths.

Pathogenicity of the bacteria E.coli in Europe mainly manifested in two forms, two of the previously described: EHEC and HUS, the percentage given by the WHO as they estimated in early June (Figure 2 and Figure 3).

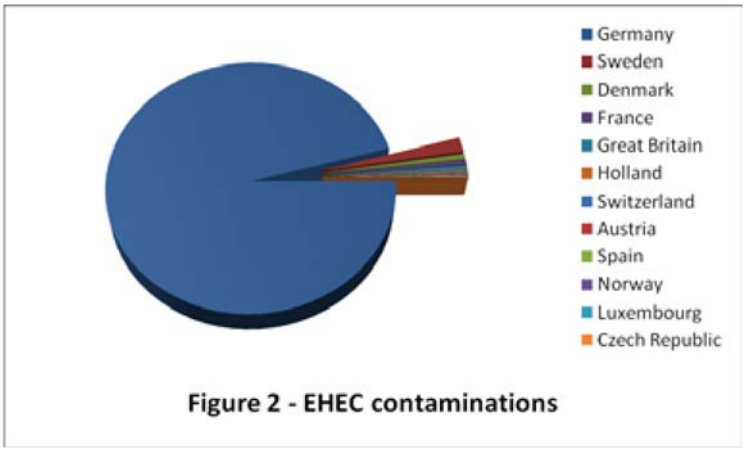

Figure 2 - EHEC contaminations

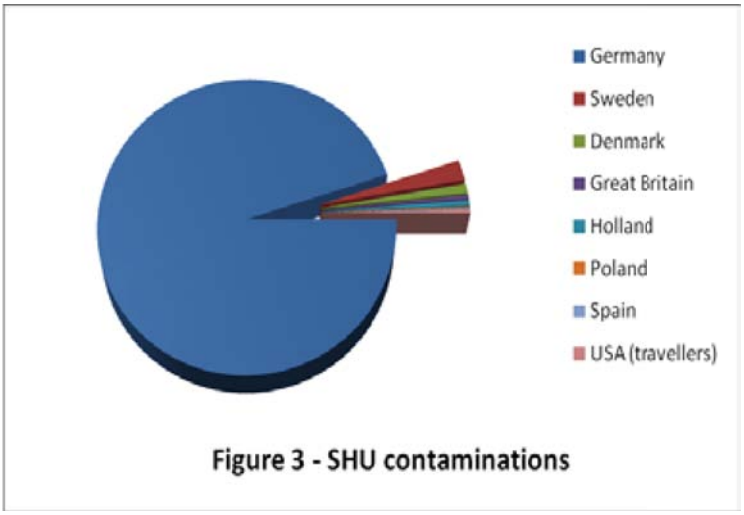

Figure 3 - SHU contaminations

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INFLUENCE OF FERTILIZERS ON THE LEVEL AND QUALITY OF MAIZE

Coțianu Răzvan Daniel, Stăncila Ileana Geanina
Bioterra University of Bucharest

Abstract: *Maize is a nitrogen-intensive crop, the largest part of this element is absorbed into the first part of the vegetation, with maximum intensity to the formation of floral organs. Towards maturation of seed, 70% of nitrogen goes from bean leaves, but at this stage of culture needs this element for further enhancing photosynthesis and grain protein content. The many factors that influence the effectiveness of fertilizers creates difficulties in establishing dose more so, since some factors are more difficult to control and taken into account (climatic conditions), increases the production of fertilizers obtained by applying varying from one area to another.*

Key words: *fertilizers, effectiveness, climatic conditions*

Introduction

Recovery depends on the top of the fertilizer application method than theirs. For autumn fertilizer management is less efficient for the economy and energy, spring maize fertilization is recommended, along with sowing (on line).

Fertilization level is influenced by pre-plant, so as to reduce nitrogen pulses with 30-40 kg / ha, which means an energy savings of 771-1028 kWh / ha. Corn produces large crops, but with low grain protein content, it will decrease the amount of food and feed. Using nitrogen increases both yield and protein quality of grain.

For example, under the Albota at fertilization, grain protein content was 8%, and by application of 200 kg N/ ha increased from 10.2% protein. Phosphorus (40-160 kg / ha), by contributing to plant growth and development, increased production of protein / ha. In our country's conditions, fertilization increases the protein maize (11-12%), contributing to the genetic potential of cultivated hybrid.

Materials and methods

Knowing the effects of chemical fertilizers applied to cereals generally, depending on climatic conditions, the dose ratio of fertilizer and nutrients, allows determining the amounts to be allocated judiciously to achieve optimal economic effect, a higher quality of production and viability in terms of energy crops.

The results covered by this paper aims to contribute to useful information for economic and energy efficiency in the maize crop specific experimental conditions.

In this regard, the following are the objectives of the research conducted succinctly:

- Determination of total crop growth and average growth due to unilateral application of compound fertilizers based on phosphorus and nitrogen;
- Effect of NP fertilizers on economic efficiency;
- Effect of NP fertilizers on energy efficiency.

For corn crop came from experimental data Albota and Simnic resorts. Simnic a P2O5/ha agrofond 80 kg were studied four

options: V1 - 0lbs N / ha, V2 - 80kg N / ha, V3 - 160kg N / ha and V4 - 240 kg N / ha. If Albota resort on a 100 kg agrofond K2O/ha P2O5/ha and 80 kg received a dose of 120 kg N / ha split applied at different times for each experimental variant part.

Results and discussions

The corn crop to determine the economic and energy efficiency, experimental results were used in institutes and research stations, after 2000, because they are accurately measured - production, fertilizers, water quantity, etc. and can make comparisons between the versions.

In Table 1 are made of corn yields in the central area of Oltenia (SCDA Şimnic), the constant fund raising dose of phosphorus and nitrogen.

In the variant fertilized with minimal cost, could achieve a production of 4540 kg / ha. Cost per 1 kg was 0.33 lei profit was 316 lei/ ha and the profit rate is 21.06%. If not using chemical fertilizers, energy consumption is reduced, 1178 kWh / ha, 259 kWh per tonne back. Energy balance is also very favorable, with 19 524 kWh/ ha.

At a minimum power consumption, energy efficiency reached 17.57, ie, energy consumed per unit of energy units is obtained as 17.57 grains of corn (to take into account the primary energy production). Taking into account the energy obtained from the production of straw (4.25 kWh/1 kg straw) means that it can double, because grain production to around 4000 kg / ha, straw production is equivalent to -11.

Analyzing the second variant, that are used 80 kg P2O5 and 80 kg N / ha, production increased by 24.6%, but this increase is not sufficient to reduce product unit costs in order to reduce production cost and increase profit per hectare. Conversely, the cost increases and profit decreases kg. In terms of energy due to the introduction of fertilizers, consumption kWh / ha reached 3761, and a ton of product consumption is 664 kWh. Net energy is 22,048 kWh / ha and energy efficiency 6, 86.

In the third variant, doubling the amount of N, from 80 to 160kg/ha, production increased only by 11.7% or 530 kg / ha, which further influenced the increasing cost and reducing profit per hectare.

Energy consumption increased to 5862 kWh / ha, returning 947 kWh /t. The energy produced reaches 28 226 kWh / ha, net energy is 22,364 kWh / ha, while energy efficiency is 4.81.

In the fourth variant, the tripling of the amount of nitrogen (240 kg N / ha) decreased from previous production to 170 kg or 3.7%. In these circumstances the cost of production increases and more profit per hectare is reduced and its rate also decreases, reaching 0.54%.

Instead, as a result of tripling the amount of nitrogen, increases energy consumption 7914 kWh/ ha and at 1314 kWh per tonne yield also decreases to 3.46. In this case there are three avenues to be explored to draw a conclusion.

The first way would be getting free fertilizer and irrigated production, so at minimal cost.

A second way is to use moderate amounts of fertilizer and maximum effect. This is because the use of 240 kg N/ha, production began to fall from the variant with 160 kg N/ha.

A third way is by providing prices that reward work effort and encourage producers to obtain high yields.So, more production, even with a higher cost, mass higher profit per hectare. In the case presented to the first variant, the third variant, the yield per hectare

increased by 199 lei /ha. In the podzolic soils (Arges county) can be obtained by taking the best production of chemical fertilizers.
Thus, the data presented in Table 2, experiments conducted at SCDA Albota, that production can be almost doubled.

In unfertilised variant could get a production of 4200 kg / ha, with a relatively low income (40 lei / ha), being the profit rate of 2.43%. From this level of crop production is becoming profitable.

Table 1:

Economic indicators and energy to make corn crop under different doses neirigare and nitrogen in the central area of Oltenia (SCDA Şimnic)

Nr. crt.	Specification	U.M.	Unirrigated			
			N-0 P_2O_5 - 0	N-80 P_2O_5 - 80	N-160 P_2O_5 - 80	N-240 P_2O5 - 80
1.	Average production	kg/ha	4540	5660	6190	6020
2.	Increased production	kg/ha	-	1120	1650	1480
		%	100	124.6	136.3	132.6
3.	Expenditures	lei/ha	985,50	1486,60	1714,82	1832,89
4.	Expenditure on manpower	lei/ha	403,35	411,47	405,57	384,39
5.	Other expenses	lei/ha	111,15	152,13	169,71	177,72
6.	Total production expenses	lei/ha	1500,00	2050,20	2290,10	2395,00
7.	Cost of production	lei/ha	0,33	0,36	0,37	0,39
8.	Profit per kg	lei/ha	0,07	0,04	0,03	0,01
9.	Profit per ha	lei/ha	316,00	213,80	185,90	13,00
10.	Price per kg	lei/ha	0,4	0,4	0,4	0,4
11.	The value of grain production	lei/ha	1816,00	2264,00	2476,00	2408,00
12.	Profit rate	%	21,06	10,42	8,11	0,54
13.	Labour productivity	man-day/ha	15.00	16.00	16.00	16.00
		man-hour/t.d.c.	26.43	22.61	20.67	21.26
		mechanic-hour/t	2.99	2.96	2.97	3.05
14.	Consumption of fuel (diesel)	l/ha	61.50	63.80	65.50	65.20
		l/t	13.54	11.27	10.58	10.83
15.	Energy consumption per ha	kWh	1178	3761	5862	7914
16.	Energy consumption per tonne	kWh	259	664	947	1314
17.	Energy obtained (grain production)	kWh /ha	20702	25809	28226	27451
18.	Energy balance (net energy)	kWh /ha	19524	22048	22364	19537
19.	Energy efficiency	obtained/ consumption	17.57	6.86	4.81	3.46
20.	Energy consumption per ha	Mj	4243	13547	21115	28506
21.	Energy consumption per tonne	Mj	934	2393	3411	4735
22.	Energy consumption per ha	Mcal	949	3031	4725	6378
23.	Energy consumption per tonne	Mcal.	209	535	763	1059
Variant 0,6 lei/kg						
24.	Profit per kg	lei/kg	0,27	0,24	0,23	0,21
25.	Profit per ha	lei/ha	1224,00	1345,80	1423,90	1217,00
26.	Price pe kg	lei/kg	0,60	0,60	0,60	0,60
27.	The value of grain production	lei/ha	2724,00	3396,00	3714,00	3612,00
28.	Profit rate	%	81,60	65,64	62,17	50,81

Table 2

Economic indicators and energy to make corn crop in the area of Arges, with different periods of application of nitrogen (SCDA Albota)

Nr. crt.	Specification	U.M.	Unfertilized	P2O5 100 + K2O 80		
				40 kg N autumn 80 kg N spring	40 kg N spring 80 kg N at 7-8 leaves	40 kg N autumn 40 kg N spring 40 kg N at 7-8 leaves
1.	Average production	kg/ha	4200	6900	6200	6900
2.	Increased production	kg/ha	-	2700	2000	2700
		%	100	164,28	147,61	164,28
3.	Expenditures	lei/ha	1060,42	1814,34	1814,11	1847,09
4.	Expenditure on manpower	lei/ha	458,05	455,77	408,92	456,79
5.	Other expenses	lei/ha	121,53	171,14	178,18	184,12
6.	Total production expenses	lei/ha	1640,00	2441,25	2401,21	2488,00
7.	Cost of production	lei/kg	0,39	0,35	0,38	0,36
8.	Profit per kg	lei/kg	0,01	0,05	0,02	0,04
9.	Profit per ha	lei/ha	40,00	318,75	78,79	272
10.	Price per kg	lei/kg	0,40	0,40	0,40	0,40
11.	The value of grain production	lei/ha	1680,00	2760,00	2480,00	2760,00
12.	Profit rate	%	2,43	13,05	3,28	10,93
13.	Labour productivity	man-day/ha	13.90	19.40	17,80	20.00
		man-hour/t.d.c.	31.77	22.49	20.00	23.18
		mechanic-hour/t	2.75	2.90	2.96	3.13
14.	Consumption of fuel (diesel)	l/ha	46.65		54.80	57.45
		l/t	13.33	8.10	8.83	8.32
15.	Energy consumption per ha	kWh	942	5189	5150	5231
16.	Energy consumption per tonne	kWh	269	752	830	758
17.	Energy obtained (grain production)	kWh /ha	15960	31464	28272	31464
18.	Energy balance (net energy)	kWh /ha	15018	26275	23122	26233
19.	Energy efficiency	obtained/ consumption	16.94	6.06	5.49	6.01
20.	Energy consumption per ha	Mj	3393	18690	18550	18842
21.	Energy consumption per tonne	Mj	969	2708	2992	2730
22.	Energy consumption per ha	Mcal.	759	4182	4151	4216
23.	Energy consumption per tonne	Mcal.	217	606	669	611

In terms of obtaining a price of 0.6 lei/ kg, may return to start at 3000 kg / ha up. Productions 2000 - 2500 kg / ha, they get very many farmers - even most - especially in hilly areas or soils with low fertility, are unprofitable. In variants fertilized with nitrogen, applied in different eras, a fund of 100 kg P2O5 and 80 kg K2O/ha given in autumn, it is noted, however, a rebound of production when nitrogen is applied only in spring, even when administered in two stages.

Administration of 1/3 N in the autumn has greater effect. From an economic perspective, costs are reduced to variants with partial nitrogen in the fall administration, and profit per hectare than 250 lei / ha. The profit rate is 13.05 and 10.93% to 3.28% for the variant with only nitrogen management in spring.

Conclusions

In the central area of Oltenia, irrigated corn, increase the maximum production (36.3%) was obtained by application of 160 kg N/ha, 80 kg P2O5 fund. 1 kg N were obtained 10.3 kg grain increase. Largest profit was made from unfertilised variant (rate of return = 21.06%), whereas costs were lower.

The price of 0.6 lei/kg, the rate of return to this variation (unfertilized) reached 81.60%, almost double the version with the highest dose of N (240 kg/ha). Energy efficiency is higher in unfertilised variant (17.57). In the case of podzolic soils, crop production becomes more profitable to 3,000 kg / ha.

Thus, the substance of 100 kg P2O5 and 80 kg K2O/ha, applying N in two rounds (40 kg/ ha in autumn and 80 kg/ha in spring) results in production of 6900 kg/ha, with a profit rate of 13.05%, the highest. Energy efficiency for variant but remains unfertilized (16.94).

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APITHERAPY WITH DRONE LARVA

Moraru Ionuț
Pro Natura Company Bucharest

Abstract: *Beehive products represent a rich source of nutrients and principles necessary to the normal development, have great potential for hormonal rebalancing (may reduce discomfort associated with menopause, andropause, delayed puberty). Improves sexual dynamics and the resistance of the body and mind to physical and mental efforts. Supports natural defense of the organism, increasing the immune response.*

Key words: *Immunostimulator, antibiotic, antifungal, antiviral, hepatoprotective, antioxidant, increases sexual performance, drone larva.*

Introduction

Drone larvae atomized (fig. 1) contains a complex of natural substances: proteins (including all essential amino acids), carbohydrates, lipids (mainly phospholipids), mineral salts and trace elements, vitamins (beta-carotene, riboflavin, nicotinic acid, choline, thiamine, phytomenadione, precursors of sexual hormones). Stimulates metabolism and the general factors of natural defense of the organism.

Propolis potentiate the actions of the product due to the biologically active principles that it contains: flavones, enzymes, volatile oils. It is a good antiseptic, antibiotic, antiviral and antifungal agent. Supports the immune system functioning, change the biological response. It is an excellent antioxidant. It was demonstrated that the effectiveness of the combination atomized of drone larvae - propolis is enhanced by the association with bee pollen, which is considered an excellent "functional food".

Bee pollen has a hepatoprotective action due to the involvement of inositol in the liver metabolism; nicotinic acid causes the decrease in cholesterol; the tocopherols may reduce the liver lesions due to the specific lack of lysine in the diet; the poliphenols play the role of natural antioxidants; diminishes the intensity of aging processes.

Has beneficial effects in pediatrics, eases the influenza symptoms. Also, pollen increase physical and mental capacity, activates metabolic function, strengthens the cardiovascular and respiratory systems by creating a neuro - vegetative and hormonal balance. Increases the sexual energy (through its content of gonadotropic hormones that are stimulating the sexual glands).

The product is a rich source of nutrients and active principles necessary for a normal and harmonious body development, have great potential for hormonal rebalancing (reduce discomfort associated with menopause, andropause, delayed puberty). Improves sexual dynamics and the resistance of the

body and mind to efforts, supports the body's natural defense, increasing the immune response. Natural source of antioxidants (reduced intensity of the processes associated with aging), supports the body during periods of convalescence.

Materials and methods

After conducting preclinical studies (acute and chronic toxicity, specific activity) and clinical studies (performed on lots of sportsmen or in University Clinic) of the atomized larval drone (a natural product, beekeeping, biologically active, resulting from the grinding and filtering drone larva collected in a larval stage, namely the day before closing the cells with their full nutritional content found on harvesting) that is found in the following goods: API PROP POLLEN, Masculin, ROYAL TONIC Potent, ROYAL TONIC Athletes, ROYAL TONIC Geriatric, ROYAL TONIC Adults, Osteocalcin.
For these studies the following results have been obtained:

Preclinical studies conducted on rats were carried out with atomized larval drones (ALD). No acute and chronic toxicity.

Following effects were observed in the male rat genital tract:

- increased trophicity of the entire genital tract as a result of improved vascularisation;
- boosting spermatogenesis in the testicles (stimulating maturation of the spermatogonia) compared with the untreated group;
- no anti-metabolic phenomena;
- decrease or disappearance of stasis in the seminal vesicles and prostate; normal structure of the testicles;
- mild interstitial congestion;
- the multiplication and ripening (spermatogonia spermatocyte, sperm) was slightly accelerated preserving the stimulating testicular effect also after discontinuation of the product. ALD does not intervene in the testosterone contents of the blood;
- it prevents weight loss of the blocked testicular in the abdomen and the testicle testosterone has not decreased.

Results and discussions

In the female rat genital tract following effects were observed: ovogenesis stimulation, as well as stimulating multiplication and physiological maturation of the ovarian follicles; the reproduction process showed that there is gain weight in newborns. None of the newborns showed malformations. ALD acts as hormone replacement treatment for secondary menopause (induced surgical = oophorectomy), lowers the Green score and influences positive evolution of calcium, cholesterol and triglycerides in serum (gynecologic Clinic University Hospital Bucharest).

Clinical effects on people who practice professional sports showed that ALD tested on active football players influenced positively the aerobic and anaerobic power of muscular contraction, decreased urinary mucoproteines (marker of the metabolic fatigue), as well as decreased the level of post - effort fatigue (Bucharest ANEFS study).

Conclusions

Showed that the drone atomizate does not intervene in the testosterone contents of the blood;it prevents weight loss of the blocked testicular; the testicle testosterone has not decreased).
ALD might acts as antiaging drug for increasing physical performance and for better adaptability to competitive stress; it is a hormonal compensation for men and women and for couple sexual dysfunction (impotence, premature ejaculation, frigidity, disorders climacterium and andropause).
For optimal quality of drone brood and its best thereapeutic action it should come from drones from organic bee hives.

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GENERAL PRINCIPLES OF THE ENVIRONMENTAL COMMUNITY LAW

Trandafir Cornel, Olteanu Cristina Elena
Bioterra University of Bucharest

Abstract: *In the process of elaboration and application of community regulations on environment protection, a series of general principles have crystallized and are affirming, under different forms. Initially mentioned in the periodical action programs, they found the juridical expression in the provisions of art. 130 of the Treaty of Rome, introduced by the Sole Act in 1987, which conferred them a mandatory application.*
Thus, paragraph 2 of such article states that the Community's action in the matter should be founded on three principles: preventive actions, correction of pollution at source and „polluter pays" principle. Certainly, it is not about new principles, as they have been defined in the second and third action program in the field of environment. Also, the same article established that the exigencies regarding environment protection are a component of the other policies of the Community. Such requirement marked a new stage in the development of environmental community law.
The dimension of environment protection marks now, on a mandatory basis, all the community policies, together with the founding of a separate field.

Key words: *pollutant pays principle, protection areas, regulations on environment protection*

Introduction

» Principle of environment degradation prevention

The general principle of environment degradation prevention, based on the idea that the prevention activity is less expensive than the repair of ecological damages presenting in many cases an irreversible character, has known specific progresses within community law.

The preventive character of community policy within the field was such defined even in the first action program in 1973: *the best environment policy consists in avoiding, from the very beginning, the creation of pollutions or degradations than fight against their subsequent effects.*

At their turn, the subsequent programs (especially those in 1977 and 1983) have emphasized that the best environment policy consists in avoiding, ab initio, the creation of pollutions or damages of environment; it was affirmed the necessity of taking into account as soon as possible the incidents on environment in every technical process, of planning and decision and for such purpose there were provided some procedures for the evaluation of such incidents.

The requirements of such principle were expressed, on the one hand, in the introduction of preventive obligations, and on the other hand by promoting some activities or procedures that would lead to avoiding some negative modifications of environment quality.

Thus, for example, the directive over the evaluation of a project consequences on the environment, submits some project categories to an evaluation regarding the impact on environment. Such evaluation has to be taken into consideration within the authorization process of certain projects. The document constitutes the main instrument of fight against environment degradation associating the requirement of taking into consideration the ecological problem at the decision planning process in all the fields of activity and especially in agriculture, industry, energetic sector, transports, tourism and regional development.

Materials and methods

» The document established a series of obligations

Thus, the head of work for a public or private project that can have a notable incidence over environment is obliged to provide the competent authorities with a study of possible incidences over environment. The competent authority shall, at its turn, take into account the received information and the obtained approvals, when it will take the decision of authorizing the proposed project; also, the involved public must be consulted and can propose alternatives to the project.

Projects that due to their nature, size or localization are susceptible of having notable incidences over environment shall be subject to a preliminary evaluation.

The same preventive principle is also reflected in directive 82/501 (named Seveso) regarding risks in case of major accidents within industrial installations. It obliges exploiters to achieve an analysis of risks that exist in relation to the functioning of installations and to foresee the means of fight of a possible accident (so-called hazard study). Such analysis shall be among the documents necessary in case of procedures for authorization of installations.

Also, the rule of prevention shall operate also in the field of international transportation of hazardous waste. Thus, according to a directive, in order to make a transportation of waste between the community states or between the European Union and third party states, the holders of such waste must notify to the specialized administrations of transit states some information on their nature, means of transportation, delivery contract concluded with the addressee and their means of elimination, being expressly provided that such waste shall be neutralized in the reception country without endangering the environment quality. The transportation can only take place by presenting the confirmation for reception of such notification.

For the same purpose, the launching on the market of new chemicals is subordinated to prior notifications indicating their specific features and risks over the environment. In addition to such concrete applications of the principle of prevention, the community regulations obliges the member states to elaborate cleansing plans and programs (air, water), to designate protection areas, (air, water, nature, etc.), to prepare plans regarding the elimination of waste and others.

Results and discussions

» Principle of fight against pollution by action against its sources

Especially during the last decade it is remarked a special development of community regulations aimed at fighting against or correcting pollution by action on its sources.

All these evolutions have lead to the formulation of a principle specific to community law.
Such regulations are grouped in two large categories:
a) - regulations regarding pollutant activities (e.g. those on vehicles, industrial activities using titanium dioxide, large combustion installations);
b) - regulations referring to pollutant substances (norms on the contents of aquatic remains in mercury, cadmium, lithium, etc.).

» Pollutant pays principle
Already recognized as a fundamental principle of the international environment law, the "pollutant pays" principle also found a specific reflection in the system of juridical community norms. Thus, in the first action program dated November 22, 1973, it is emphasized the application of the "pollutant pays" principle as defined by OCDE, providing some appropriate adaptations to the respective environments, types and sources of pollution, as well as the respective regions. A recommendation dated November -7, 1974 of the EEC Council has explained the principle on imposing the costs and intervention of public powers in the field of environment; a document of the same type dated March -3, 1975, includes in attachment detailed rules regarding the application of such principle.
Resumed in the second action program, the principle appeared in the following program as strategy, for the purpose of achieving a better use of resources, as the enforcement at pollutants of costs related to environment protection provided by such principle, incites them to reduce the pollution whose cause consists of their operations and to search for less polluting products and technologies. This is indispensable for avoiding a distortion of competition.
Even if such charges are susceptible in some cases of putting companies in difficulty, the Commission has admitted in 1974 and 1980 that the member states, throughout a period that ended in 1987 and in certain conditions, are granting assistance for the purpose of facilitating the introduction of new regulations that may provide an adequate protection of environment.

Conclusions

» Principle of integration of ecological considerations in every community policy
Pursuant to such principle, the community strategies and measures, no matter the field they aim, should take into consideration the requirements of environment protection. Application of such requirement, introduced through the Sole Act, has practically lead to the modification of EEC objectives that were seen until then from an economic perspective only; now, four sectors appear as priorities from such point of view: agriculture, energy, regional policy and cooperation for development.
As it regards the agriculture, it should be emphasized the reformation of financial assistance mechanisms and restructuring the development of agricultural operations in a conception that would not damage the quality of environment.
The energetic policy has to be reevaluated in its ensemble from the point of view of the consequences on environment, as it regards both the aspects on conservation of energetic resources and the rational use of energy. An important sector shall be represented by the nuclear industry, the Community having a major role for such purpose, especially in order to surmount the cleavage created between the member states that adopted different policies in such matter.

As it regards the regional policy, the objective consists of incorporating the ecological aspects in the systems of community assistance. The Commission has already adopted external instructions allowing a better ecological evaluation of projects and programs financed by the Community.
Eventually, the cooperation between the European Union and developing countries should reflect preoccupations aimed at environment protection. Thus, it is more and more obvious that in certain states from disfavored areas of the world, the development problems represent ecological problems and vice-versa; there are also problems of world dimensions (as compensation), for the solving of which the Union can play a decisive part, especially within the already established institutional structure (Convention of Lome, special relations between the European Union and the countries of Central and South America, or with ASEAN, etc).

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ORGANIC WINES. THE PROS AND CONS. LEGAL, TECHNOLOGICAL AND SENSORIAL ASPECTS. PERSPECTIVES AND LIMITS IN CONSUMER'S PREFERENCE VIEW

Croitoru Constantin, Nicolae Floarea, Atudosiei Livia-Nicole, Galan Cătălin, Lazin Valentina
Bioterra University of Bucharest

Abstract: *The authors analyze the proposals on the opportunity of utilisation of some technological additives and of some oenological practices employed for organic wines' elaboration. There are presented critical aspects regarding the use of SO2 and wine elaboration, clarification and stabilization products, as well as some aspects regarding the necessary composition's enrichment (acidification and sugar content) in some vintage years.*
This paper presents authors' opinions on oenological products which are not allowed in organic wines, the particular case of allergenic products and SO2, and oenological practises (acidification, enrichment and other treatments) that may be forbidden for organic wines. There are highlighted aspects regarding sensorial quality of organic wines and their profile in consumers' preferred perspective.

Key words: *organic wines, sensorial aspects, SO2 equivalent, critical analysis.*

Introduction

Organic (or biological) agriculture which includes also viticulture and wine making is defined as a holistic production system that ameliorates the health of agro eco systems together with their biodiversity, biological cycles and biological soils' activity.
This system has its foundation mainly on the revaluation of culture methods and less on the use of products that are outside the production domain and taking into account that there are necessary local systems which are more adapted to the regional specific conditions (IFOAM, 2005).
In this context the organic (or biologic) wine is obtained exclusively from biological grapes that are a result of organic viticulture which excludes the use of synthetic products (fertilizers, pesticides or herbicides); the biological grapes are obtained in wine-growing districts for which are used only biological production methods that were defined at European level according to EC regulations no 834/2007 and no 889/2008 regarding the biological production and labelling of biological products; given these conditions, the only general rules for wines coming from biological grapes are those stipulated in the EC regulations no 479/2008 (annexes 4 and 5) and no 1622/2000 which define the authorized oenological practices, products and treatments for wine-making in Europe.

Materials and methods

The general evaluation of perception on present allowed oenological products and practices for organic wine-making implies

broad studies for consumers, producers and traders' preferences.

Due to one on these specific studies that was conducted in a lot of European countries that consisted in filling in an online questionnaire with items on the use of oenological products and practices for organic wine-making, there were obtained a variable number of answers for each country – this number reflecting the interest for the issues in the corresponding country; the outcomes of this study were presented as tables and graphics during Biofach work session on February 19th – 22nd, 2009 at Nürnberg in Germany.

A significant part of the outcome presented at that congress is going to be critically analysed in the sections that follow and there are going to be expressed the authors' opinions and proposals regarding the current issues.

Results and discussions

After a short characterization of the obtained results, there were presented the authors' opinions on the current issues highlighting the legal, technological and sensorial aspects backed up by argued previsions on their characteristic quality as informed organic wines consumers.

a) Critical analysis of forbidden oenological products in organic wines but accepted in most standards for normal wines.

The results of the study regarding these oenological products are presented table 1.

Table 1:

Forbidden oenological products in organic wines, but accepted in the majority of standards for normal wines. The evaluation of favourable answers to their prohibition

Forbidden oenological products in organic wines	**Analysed countries with their respective number of questionnaires which were filled in on the internet**						
	Italy ***143***	**France** ***162***	**Germany** ***254***	**Austria** ***40***	**Switzerland** ***25***	**Spain and Portugal** ***31***	**Other countries** ***10***
Thyamin hydrochloride (0,6 mg/l)	37 %	39 %	6 %	33 %	44 %	35 %	20 %
Diammonium phosphate (1g/hl)	37 %	36 %	6 %	33 %	32 %	39 %	20 %
Ammonium sulphate (1g/hl)	36 %	32 %	5 %	38 %	40 %	35 %	40 %
Diammonium sulphite (0,2 g/l)	44 %	39 %	7 %	35 %	24 %	35 %	50 %
Yeast derivatives (40 g/hl)	26 %	31 %	3 %	20 %	8 %	26 %	30 %
Metatartaric acid (100 mg/l, in wines)	29 %	43 %	13 %	28 %	16 %	42 %	30 %
Copper sulphate (1g/hl, 1 mg/l, in wines)	32 %	39 %	7 %	23 %	32 %	32 %	10 %
Resin from Alep pine	33 %	36 %	19 %	40 %	16 %	48 %	40 %

» **Characterisation.** The majority of oenological products are perceived favourably except sulphites (negatively apreciated so that the majority of the decision factors from the European wine making countries take into consideration the reduction of the maximal admitted concentrations for these respective factors) and gelatine (also incriminated by most wine consumers).

From the percentages presented, it can be observed that the thiamine, the copper sulphate, the diammonium phosphate, the yeast derivatives and the resin from the Alep pine have been less incriminated by the respondents of this study while the ammonium sulphate, moreover the metatartaric acid and especially the diammonium sulphate have been penalized drastically.

» **Opinions.** Due to laboratory and pilot experiments we observed that the ammonium sulphide favours the SO2 production by yeasts during the AF process of musts so that its use might be uncertain; in his turn, the diammonium sulphide is not too approved for normal wine-making also because it might increase the SO2 content by its decomposition in acid environment; as for the metatartaric acid, this is suspected for the forming of some cancer causing derivatives in the thermal transformation process by which is issued but it can be easily substituted with other tartaric stabilisation procedures; as for copper sulphate and the resin of Alep-pine, they should remain prohibited if we take into account the exceptional sensorial quality that the organic wines must have.

We consider an exaggerated measure to forbidden some nutrients for yeast such as the thiamine hydrochloride and diammonium phosphate and even less justified the prohibition of yeast derivatives that will allow in the future a real revolution in the world of technological auxiliaries that are used to chemically, physically and biologically stabilize wines and especially organic ones.

b) The critical analysis of forbidden oenological products in both organic wines and of some standards for normal wines.

The results of the study regarding this category of oenological products are presented in table 2.

» **Characterisation.** At this oenological products' category, the respondents have evaluated negatively in considerable proportions a series of technological auxiliaries such as sorbic acid, potassium ferrocyanide, dimethyl dicarbonate (DMDC), calcium phytate, PVPP and copper citrate; moreover, the use of lysosyme has a controversial character due to some potential allergenic effects although this biological product is very appreciated when considering biological stabilization of wines due to the reduction of necessary doses of SO2; the calcium tartrate had a positive evaluation as well as vegetal proteins, manoproteins from yeasts' derivatives and wood chips.

» **Opinions on products intensely incriminated.** The respondents' fears regarding the previously incriminated products have been slightly exaggerated because:

- the sorbic acid is used not only for the biological stabilisation of wines that have sugar reserves whose proportion of the total volume of organic wines should be as smaller as possible if we take into account the unfavourable high glicemic index of these wines on human health;
- the ferrocyanide is extremely rarely used for the metallic stabilisation of wines that are obtained in others vessels but the stainless steel ones, as for a wine rich in heavy metals

Table 2:

Forbidden oenological products in organic wines and in the majority of standards for normal wines. The evaluation of favourable answer for their prohibition

Forbidden oenological products	Analysed countries with their corresponding number of on line filled in questionnaires						
	Italy *143*	France *162*	Germany *254*	Austria *40*	Switzerland *25*	Spain and Portugal *31*	Other countries *10*
Sorbic acid	56 %	62 %	59 %	65 %	44 %	45 %	40 %
Potassium sorbate	59 %	64 %	42 %	55 %	48 %	48 %	30 %
Potassium ferrocyanide	73 %	78 %	58 %	60 %	64 %	52 %	70 %
Dimethyl dicarbonate (DMDC)	68 %	65 %	39 %	53 %	60 %	52 %	50 %
Calcium phytate (8 g/hl, in wines)	57 %	66 %	31 %	53 %	44 %	39 %	50 %
Calcium tartrate (200 g/hl, in wines)	44 %	56 %	15 %	33 %	32 %	45 %	20 %
Copper citrate (20g/hl)	52 %	61 %	27 %	38 %	40 %	45 %	40 %
PVPP (80 g/hl)	52 %	59 %	40 %	50 %	56 %	32 %	50 %
Lysosyme (500 mg/l)	44 %	54 %	38 %	55 %	44 %	39 %	40 %
Vegetal proteins	36 %	46 %	15 %	40 %	20 %	32 %	20 %
Manoproteins from yeasts derivatives	38 %	49 %	18 %	45 %	28 %	35 %	50 %
Oak chips, cubes and staves	42 %	59 %	25 %	50 %	48 %	42 %	30 %

we cannot talk about an organic or biologic wine;

- the calcium fitat is very similar to the potassium ferrocyanide being extremely rarely used to the stabilisation against the iron casses so it applies the same reasoning;
- the using of DMDC and copper citrate (as antiseptics) it is not yet generalized not even for the biological stabilisation of normal wines so that they do not present an interest for the imposed standard of sensorial quality for organic wines;
- the PVPP which prevents the yellowing of white and rose wines but is induced together with the oxidised and oxidising poliphenols so that are found in the sediments on the bottom of the vessel, are rarely used and the sensorial quality of that wine will be clearly under any expectations.

Having the context of the above explanations, we conclude that potassium ferrocyanide, calcium phytate, DMDC (synthesis product) and copper citrate should not be allowed to be used in the production of organic wines, but sorbic acid should not be banned because there exists not another replacement for the biological stabilisation of this type of wines when they contain a certain sugar reserve.

» **Opinions on products slightly incriminated**. Regarding the prohibition of the other oenological products for the elaboration of organic wines, we consider that it is exaggerated because:

- the calcium tartrate is of wine nature being a constituent of wine and because of its use for the acceleration on tartaric stabilisation it precipitates together with the excess of tartaric salts from the wine;
- the manoproteins from yeast derivatives are extracted products from the cellular walls of natural autolysed yeasts which leave behind in the treated wine valuable active biological components and with favourable impact on the sensorial profile and on the physic-chemical stabilization;
- the alternative products from oak wood and other essences have the same chemical composition as the barrels of wood vessels

accepted for maturation; a special attention should be given to vegetal proteins given the following appreciations.

c) The critical analysis regarding some oenological products with allergenic character.

In the category of technological auxiliaries with allergenic character there are several other categories of oenological products.

» **Characterisation.** We consider as wholesome the acceptance of some products of animal origin (casein, egg albumen, milk albumin and potassium caseinates) for the elaboration organic wines because after the treatment of wines with these products, they are found in the sediments from the bottom of the vessel together with the trained suspensions from the treated wine's composition and the possible remained and solubilised components do not represent a danger for the health of consumers.

Some products of vegetal origin (proteins with gluten from cereals) and of biological one (lysosyme) which are not yet allowed for organic wines might be very useful to the elaboration of organic wine taking into account the reasons presented below; but in this given context the legitimate question arises – is the their authorisation for organic wines going to affect the image of these wines regarding their effect on health? In the case of these products not being allowed authorisation it appears the problem of finding a new alternative.

» **Opinions.** We believe that the lysosyme which is extracted from egg albumin and has the capacity to attack and degrade the cellular wall of lactic bacteria should be accepted because the egg albumin has already been accepted; also, by using lysosyme it is considerably reduced the dose of SO2 which is necessary to ensure the biological stabilisation of wine.

As for the proteins with gluten from cereals, these should be accepted for organic wines only if they are both excepted for normal wines; nevertheless, taking into account their use as clearance agent for wines which contribute also to the poetic stabilisation, we express a very favourable opinion because by their training of suspension, these proteins will be found in the sediment on the bottom of the vessel so that their afterglow in wine as incriminated as it may be is insignificant and could not exert an incidence of consumers' health.

d) Critical analysis regarding the particular case of sulphites.

Although they also exert an allergenic character, the sulphites have already been accepted for the elaboration of organic wines; following this the problem of drastically reduce the doses of SO2 which are admitted for these wines is put with strong acuteness. The ORWINE project experts have proposed 3 scenarios regarding this problem:

- the elimination of SO2 use for the elaboration of organic wines (Scenario 1);
- the discard of admitted SO2 concentration limitation for the elaboration of normal wines (Scenario 2);
- the progressive limitation of current admitted SO2 concentration which may assure the obtaining of guarantee biologically stabilized wines and superior and reproducible quality for them (Scenario 3).

The details regarding the progressive reduction of current admitted SO2 concentrations according to Scenario 3 are presented in table 3.

Table 3:

The project of progressive reduction of current admitted SO2 concentrations in organic wines

Categories of wines	Current accepted content (mg/l)	20 % reduction	30 % reduction	40 % reduction	50 % reduction
		Step 1	Step 2	Step 3	Step 4
Red wines < 5 mg/l sugars	160	128	112	96	80
White wines < 5 mg/l sugars	210	168	147	126	105
Red wines > 5 mg/l sugars	210	168	147	126	105
White and rose wines > 5 mg/l sugars	260	208	182	156	130

» **Characterisation.** By analyzing the diagram in the figure 2 we may observe that the Italian respondents agree on a reduction to 50 % of current admitted SO2 concentration, the French choose a reduction between 20 and 30 % and show strong reluctance towards the bulk wines and those which are destined for a long maturation, the Germans do not agree on the reduction of current allowed SO2 concentrations, the Swiss accept a reduction between 20 and 30 % and the Spanish and the Portuguese voted for a reduction of maximum 20 %.

» **Opinions.** In our vision, a progressive reduction of current SO2 doses is very possible and welcomed not only for organic wines but also for normal wines, especially for those dry ones because the antioxidant

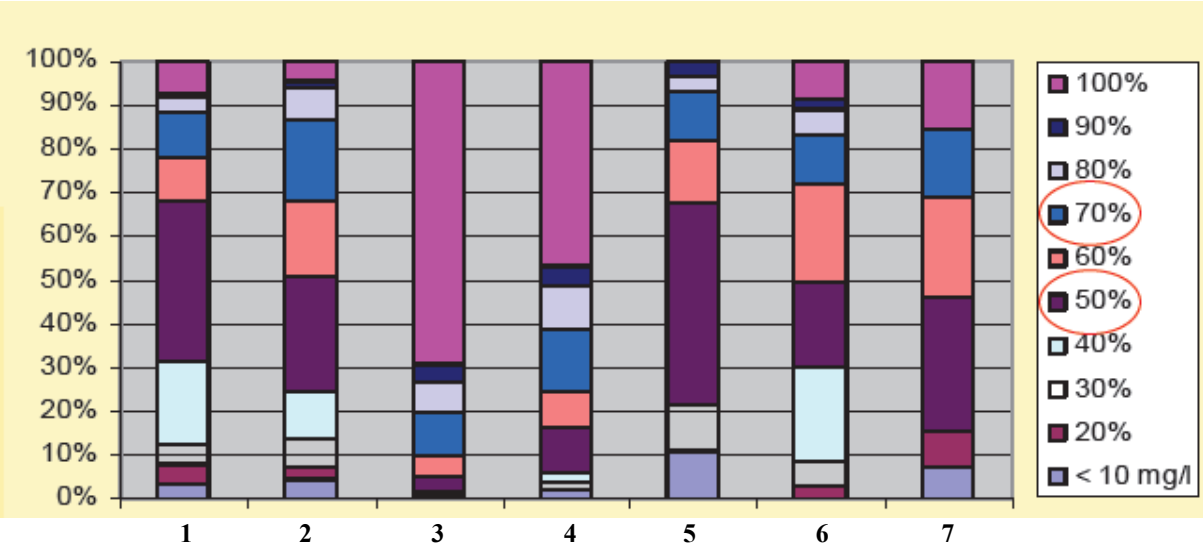

Fig. 2

Proposed SO2 limits for dry organic white wines (% values computed from the current admitted concentration for normal dry white wines) in the countries that participated to the study

1 - Italy, 2 - France, 3 - Germany, 4 - Austria, 5 - Switzerland, 6 - Spain şi Portugal, 7 - Other countries

SO2 equivalent: 100% (210 mg/l); 90% (189 mg/l); 80% (168 mg/l); 70% (147 mg/l); 60% (126 mg/l); 50% (105 mg/l); 40% (84 mg/l); 30% (63 mg/l); 20% (42 mg/l); 10% (21 mg/l).

protection which is transmitted to wines by this product may be enabled by other less incriminated products such as ascorbic acid and gallic tannins; very recently there has been elaborated a bicomponent product based on gallic tannin and ascorbic acid which protects the chromatic and odorant characteristics for the treated wines by rapid abstraction of oxygen.

Also, the possibility of optimal administration of the thermal regime during AF together with the use of selected yeast strains allow the procurement of some wines with a very reduced combining capacity of the SO2.

e) Critical analysis of some practices regarding the modification of acidities and alcoholic concentration, the tartaric stabilization, the filtration of wines and antifoaming that may be banned for organic wines.

The oenological practices that are presented in table 4 are not allowed for wines in UE based on the CE settlements no. 1493/1999, 1622/2000 and 479/2008, on the other hand these practices have been positively evaluated by the Committee of OIV Experts and they are already used in European winemaking countries that are not a part of the UE and in other winemaking countries from other continents.

» **Characterisation.** It can be observed a rather hostile attitude towards the acidification with lactic and malic acid and the tartaric stabilization with CMC, a curiously hostile towards the ultra and nanofiltration and exaggeratedly hostile towards the use of oleic acid as antifoaming agent, changing resins of ions for the correction of musts and wines' pH and also the reduction of alcoholic concentration of wines in the rotary evaporator (table 4).

Table 4:

Oenological practices that may be prohibited for organic wines. The evaluation of favourable answers towards their prohibition

Oenological practices that may be prohibited for organic wines	Analysed countries with their respective number of online filled in questionnaires					
	Italy *143*	France *162*	Germany *254*	Austria *40*	Switzerland *25*	Spain and Portugal *31*
The acidification of wines and musts with lactic acid (max. 4 g/l)	48 %	63 %	40 %	68 %	40 %	52 %
The acidification of wines and musts with malic acid (max. 4 g/l)	49 %	61 %	36 %	60 %	48 %	52 %
Tartaric stabilisation by CMC (carboximethylcellulose)	56 %	65 %	40 %	63 %	56 %	65 %
The addition of oily acid in musts as an antifoaming agent	70 %	73 %	69 %	85 %	76 %	61 %
The use of changing resins of ions for the modification of pH of musts and wines	65 %	65 %	61 %	70 %	64 %	58 %
Ultrafiltration and nanofiltration of wines	50 %	57 %	45 %	65 %	56 %	39 %
The reduction of alcoholic concentration of wines in rotary evaporator	56 %	65 %	72 %	83 %	64 %	61 %

» **Opinions pros.** We consider that the acidification of musts and acids with malic and lactic acid should not be incriminated because these acids are found naturally in wines so that they cannot affect neither the wine composition nor the consumer's health; also, an acidity correction, especially to white and rose wines always assure an equilibration of the gustatory profile with the olfactory one and enriching the sensorial profile of the treated wines.

» **Opinions cons.** The tartaric stabilization of wines with CMC is a practice that has been recently accepted by OIV and it will need at least 3 to 5 years to be generalized in the winemaking production; in this context, we consider that is not opportune yet to accept it for the tartaric stabilization of organic wines taking into account that is unknown is technological efficiency evolution on normal wines and yet alone its impact on consumers' health.

Ultra and nanofiltration should be forbidden because by their use wines are impoverished of some macromolecular compounds that contribute decisively to the olfactory and gustatory features as some of them greatly influence the „terroir" tipicity from which these wines come from; these unconventional techniques could be accepted only for some normal wines' categories, but never for organic wines. On the use of oily acid as an antifoaming agent we cannot issue an opinion because this oenological product is not used in the Romanian winemaking and it is rarely used by other European winemakers.

The use of changing resins for ions should also be forbidden for the correction of pH because it affects the composition of the treated wine but it could be accepted for the obtaining technology of rectified concentrated musts that can further be used at the correction composition of some musts which are framed for organic wines.

In its turn, the reduction technique for alcoholic concentration of wines in the rotary evaporator should also be banned for all normal wines and for organic wines too obviously and may be accepted only for the procurement of some medical wines (that need an alcoholic concentration reduction and a concentration of other valuable constituents such as vitamins, mineral salts and mostly poliphenols and stilbenes, mainly resveratrol.

f) The critical analysis of some practices regarding the correction of sugar concentration and other constituents of must and wine that may be accepted for organic wines.

The results of the answers' evaluation from the table 5 highlight a general acceptance of sucrose corrections, concentrated must and rectified concentrated must of organic provenience and a strong objection for corrections by classical physical treatments and unconventional ones (vaporisation, reversed osmosis, cryoconcetration) that are applied to musts and wines.

» **Opinions regarding the additions.**

In our opinion, sucrose addition, concentrated must or concentrated must and rectified concentrated must of organic provenience do not affect the value of an organic wine by any mean; sucrose or alimentary sugar, coming either from beetroot or cane although it is not of oenological provenience, should be accepted for organic wines procurement taking into account that during AF they are wholly metabolized by the viable yeast cells into alcohol, CO2 and other secondary products; we share also the same vision for the concentrated and rectified concentrated musts; we express this opinion because the fundamental feature of any traded wine in the opinion of the most neophyte consumer

is its reproducible quality that consists in being able to choose from the shelf the same wine with a constant sensorial quality from year to year, regardless of the climate in the vegetation period of the respective crop.

» **Opinions on physical treatments.** We consider that the following cannot be accepted for the direct elaboration of organic wines: concentrated musts by thermical treatments (cryoconcentration and vaporization) or unconventional ones (reversed osmosis) because all these modify the compositional natural structure of wines that will result from these kind of musts; even less may be accepted the cryoconcentration of a wine because by this technological operation it is produce a profound modification of its composition and one implicitly of its sensorial profile.

» **Scenarios characterisation.** Taking into account the importance of the above approached problems, the ORWINE experts have forecasted 4 scenarios regarding the sugars content correction (table 6).

Table 5:

Oenological practices regarding the correction of sugars concentration and other constituents of musts and wines. The evaluation of favourable answers for their acceptance

Oenological practices that may be accepted for organic wines	**Analysed countries with the corresponding number of fiiled in questionnaires on the internet**						
	Italy *73*	**France** *80*	**Germany** *164*	**Austria** *31*	**Switzerland** *14*	**Spain and Portugal** *17*	**Other countries** *9*
The addition of organic sucrose coming from beetroot or cane	51 %	31 %	13 %	10 %	64 %	47 %	13 %
The addition of concentrated must with organic provenience	33 %	39 %	38 %	19 %	29 %	29 %	0 %
The addition of rectified concentrated must of organic provenience	33 %	35 %	41 %	29 %	36 %	24 %	25 %
Concentration by must's reverse osmosis	51 %	51 %	65 %	58 %	21 %	47 %	75 %
Concentration by must's vaporisation	45 %	54 %	65 %	65 %	36 %	47 %	50 %
Cryoconcentration of must	40 %	54 %	70 %	71 %	36 %	47 %	63 %
Cryoconcentration of wine	52 %	63 %	74 %	84 %	43 %	41 %	50 %

Table 6:
Proposals regarding the composition correction of crops at sugars concentrations after scenarios 3 and 4 with respect to scenario 2 for European wine-growing areas A, B and C

Considered reduction	Area A	Area B	Area C
No reduction (Scenario 2)	3,5 %	2,5 %	2 %
30 % reduction (Scenario 3)	2,45 %	1,75 %	1,4 %
50 % reduction(Scenario 4)	1,8 %	1,3 %	1 %

Scenario 1 prefigures the exclusion of correction, scenario 2 accepts the correction as well as for normal wines but using organic products, scenario 3 implies the correction only with organic products and a 30 % reduction with respect to the accepted correction for normal wines and scenario 4 stipulates the correction of sugars content only with organic products and a 50 % reduction with respect to the accepted correction for normal wines.

These proposals have taken into account the field reality which means that the wine-making countries from the meridional regions of Europe request the limitation of these corrections whereas wine-making countries from septentrional regions are against the limitation of these corrections.

In this situation, we consider the proposals as being reasonable, even for crop years in which vegetation weather might have been unfavourable and we accredit the idea that organic wines cannot be obtained in crop years in which the quality of crop is poor (it needs sugars content correction above the admitted limit) which means promotion of a superior level of exigency to that specific to wines with name of controlled origin.

g) Appreciations on the sensorial quality of organic wines.

We had the possibility to taste this kind of wines at French reputed producers; there are their terse sensorial characterisations:

» **Bio wines from Bourgogne.** Ghislaine et Jean-Hugues Goisot, Bourgogne Cotes d'Auxerre 2008: a Chardonnay wine with peerless freshness and oiliness and with an excellent quality/price rate; Domaine Thibaut Liger-Belair, Vosne Romanée Aux Réas 2007: a thick and elegant Chardonnay with an amazing constant of the sensorial profile from one year to another.

» **Bio wines from Alsacia.** Domaine Léon Boesch, Gewurztraminer, Zinnkoepflé grand cru 2007: broad, gererous, distinct wine and with an exceptional freshness of its „terroir"; Domaine Dirler-Cadé, Riesling grand cru, Kessler Hesse Wanne 2007: a high class wine which exalts the papillae by amplitude and density and also by a majestic and delicious character and which may progress a great deal at sensorial level in the next 10 years.

» **Bio wines from the Rhône's Valley.** Domaine Combier, Crozes-Hermitage rouge 2007: a red wine of high quality. Maturated in exceptional conditions that may earn more complexity after 2-3 years in barrel; Domaine de la Monardière, Vacqueyras rouge vieilles vignes 2007: a very dense red wine, which has been „arrested" by its own tannins, slightly introverted al the olfactory exam but which definitely can progress by ageing in bottle and almost surely becoming the trademark of its domain and even of its provenience area.

h) Perspectives and limits in the view of customers' preferences.

The above mentioned French producers achieve reduced volumes of organic wines which explain their remarkable sensorial quality but also their association with the „terroir" character which is specific to those respective wine-growing domains. Taking into account that the price for a 0.7 or 0.75 l bottle of organic wine bought directly from the producer exceed the inferior limit of 10 € and sometimes they reach the superior limit of 40 €, the consumers' preferences for this kind of wines in the next 5-10 years will follow a quite slow growth; this progressive growth of organic wine consumption will be limited due to price and this limitation will be fixed at some level or other depending on those respective wines to stand out by a reproducible sensorial quality from one year to another which represents the fundamental characteristic in the view of consumers preferences when choosing a wine from selling rack.

Conclusions

After the critical analysis of the approached problems, we consider that:
» It is necessary to adopt a viticulture and an oenology that should be more preventive that curative in order to obtain organic grapes – all these by eliminating phytosanitary products for treatments and elaboration of organic wines by a limitation of the intervention with chemical products for clearing and/or stabilization of wines.
» There should be a more drastic limitation of SO2 content of wines in the maturation period (storing period) until their bottling by favouring the acceptance of a broader category of oenological auxiliaries in treatment and by no means the reversed situation of a limitation of these auxiliaries and accepting high limits for SO2 content.
» It is impossible to obtain an absolute consensus between the European winemaking countries regarding the uniform reduction of SO2 content due to geographic or mentality reasons and the difficulties faced up by trying to chime in with national legislation.
» It is possible the elaboration of a biotechnology for elaboration and maturation of organic wines and also of some technologies of clearing, stabilisation and filtration of these wines which are based only on the use of biological products and with biological and oenological origin, corroborated with the use of some treatments and biological, mechanical and physical procedures which exclude the access to chemical treatments and products.

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INTERNATIONAL PARTNERS

EGIPT

Khadiga Mohamed Gaafar – Higher Institute for Specific Studies
May Mohamed El Batran – Higher Institute for Specific Studies
Mohamed Mahmout El Batran – Higher Institute for Specific Studies

ITALIA

Balestri Gino – D.A.G.A. Sezione Scienze Zootecniche, Università di Pisa
Ceccanti B. – Istituto per lo Studio degli Ecosistemi (ISE), Pisa
Doni S. – Istituto per lo Studio degli Ecosistemi (ISE), Pisa
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Macci C. – Istituto per lo Studio degli Ecosistemi (ISE), Pisa
Mani Danilo – D.A.G.A. Sezione Scienze Zootecniche, Università di Pisa
Martini Andreea – Dipartimento di Scienze Zootecniche, Università di Firenze
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"Enrico Avanzi", University of Pisa
Peruzzi E. – Istituto per lo Studio degli Ecosistemi (ISE), Pisa
Pistoia Alessandro – D.A.G.A. Sezione Scienze Zootecniche, Università di Pisa
Poli Piera – D.A.G.A. Sezione Scienze Zootecniche, Università di Pisa
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