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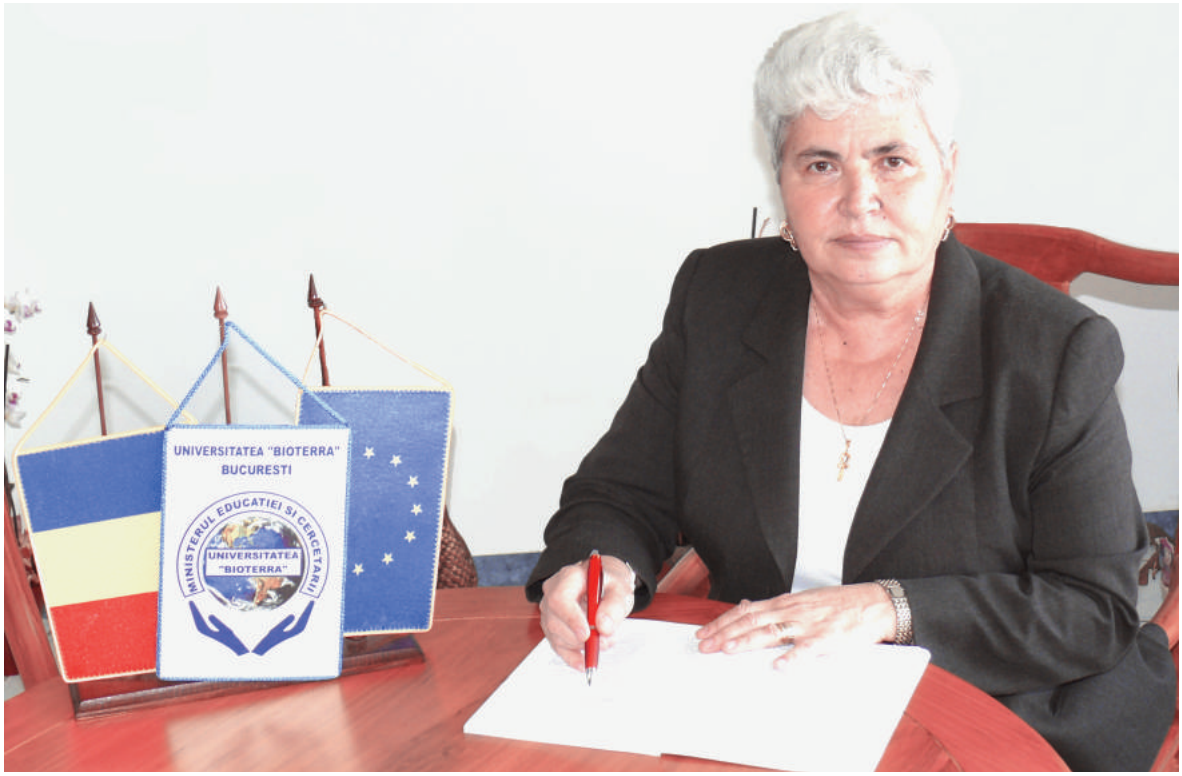

Rector's Allocution

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

*So, the Bioterra University review „**Bulletin 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, Ph.D.
Rector of the Bioterra University Bucharest

Sumary:

STUDY CONCERNING THE CULTIVATION AND CONSERVATION OPTIONS OF LEONTOPODIUM ALPINUM SPECIES

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Abstract: *The research has the main goal to find some methods and techniques to cultivate and preserve „in situ" and „ex situ" the species Leontopodium alpinum, perennial, herbaceous species, Genus Leontopodium Cass., Family Asteraceae, that has as development area the alpine plateaus of the Carpathian Mountains.*
In the last century the environment conditions degradation by pollution and excessive grazing, and also by harvesting the flower without control it leaded to the destruction of natural habitats and dramatic restriction of this species habitat development.
For this reason in Romania since 1933 Leontopodium alpinum has been declared a natural monument and it is also today protected at the national and international level with protected species status. Due to this status the collection of specimens from the wild is strictly prohibited, that makes more difficult the study of the species in the laboratory conditions in order to elaborate some culture technologies links.

Key Words: Leontopodium alpinum, protected alpine species, mountain flora.

Introduction

» *Leontopodium alpinum* is a species of a special beauty and it represents the srarest species of the whole Romania's mountain flora. Its area of origin is the calcareous plateaus and meadows from the steep and sunny slopes. In Romania the species can be sporadically met in the Maramureului, Vrancei, Bucegi, Făgăraş, Retezat, Bihor, Cozia, and Ceahlău Mountains. The highest density is in Rodnei Mountains where is was established the protected area „ Natural Reservation of Piatra Rea" having a surface of about 50 ha.
Leontopodium alpinum forms a rosette of leaves at the ground surface from the middle of which it raises a short stem of about 5-20 cm, bearing a star-shaped inflorescence covered with silver hairs which gives a special aspect.
Depending on the annual thermal regime, it blooms in July-August (Picture No. 1 and 2).

Picture No. 01 **- Leontopodium alpinum in its native habitat**

Materials and Methods

There were used 53 plants of Leontopodium alpinum grouped in 10 pots. The plants were obtained from seeds harvested from the native environment and cultivated in greenhouse conditions (Picture No. 02) by the only edelweiss grower from Romania, Nicolae Tiron.

For the transplant into the natural environment there were chosen more locations from din „ Bucegi National Park", located on the mountain about 1500 m altitude, in remote places, inaccessible and far from tourist routes (Picture No. 03).

Due to the steep slopes and rough terrain the batches of plants were hand-carried (Picture No. 04)..

Using a mini-shovel the soil has been dug and loose to a depth of 10-20 cm for plant roots to penetrate more easily. Extraction of the plants from pots has been performed carefully in order to protect the week roots. In order to reduce the physiological stress due to the transport, extraction from pot was performed at the planting place.

The specimens were planted at a depth of 8-12 cm in areas with predominantly calcareous soil (Picture No. 05).

***Picture No. 02* - Production of seedlings in greenhouse conditions**

***Picture No. 03* - Selection of locations from Bucegi National Park**

***Picture No. 04* - Transport of plants at the elevation 1500**

After planting the plots were watered thoroughly with spring water to ensure good contact between the roots and soil.
Eco-biological parameters of the batches of plants were monitored from July 2 to September 27, 2010, when the plants entered in dormancy (Picture No. 06).

Results and discussions

By the proposed objectives the study was able to effectively contribute to the conservation of rare plant species existing within protected areas, by initiating procedures aimed at growing opportunities „in situ" and „ex situ". The plants transferred in the native habitat were monitor to quantify their viability rate, development rate, ability of flowering and propagation, as well as to prevent the eventual risk factors that could endanger the future experiments.

Conclusions

» **The percentage of attachment accommodation** of the plants at the pedoclimatic conditions of the native habitat was 93%, the plants going on with the biologic circle and succeeding even to bloom from the very first year;
» **The result of the project** van determine and encourage in the future the increasing of the civic attitude of the people who love the nature in their desire to grow edelweiss;
» **Also, this action** can become a small business for the students who wish to get some extra money without affect the flora and environment

Picture No. 05 **- Transplant in the natural environment of the Leontopodium alpinum plants**

References

http://www.edelweissgrowers.com/

http://ro.wikipedia.org/wiki/Floarea-reginei

http://www.gradina-online.ro/Floarea_de_colt_A3668.html

http://atlasbotanic.florisicadouri.ro/

http://herbarium.biology.colostate.edu/onlinedatabases.htm

MOHAN Gheorghe, ARDELEAN Aurel - *Atlas botanic* –Alfa All Publishing House, Bucharest, 2010

IONEL Adrian, MANOLIU Alexandru, ZANOVSCHI Valeriu - *Cunoasterea si ocrotirea plantelor rare* - Ceres Publishing House, Bucharest, 1986

SIMIONESCU Ion - *Flora Romaniei* - Albatros Publishing House, Bucharest, 1974

Picture No. 06 **-Plants state at the end of vegetation**

MALOLACTIC FERMENTATION MLF: AGAIN IN SPECIALISTS' ATTENTION WITH NEW TECHNICAL-SCIENTIFIC APPROACHES! ASPECTS OF THE WHITE WINE MAKING (1)

Croitoru Constantin
Bioterra University of Bucharest

Abstract: *Malolactic fermentation (MLF) in white wine making is a new topic both for producers and for researchers, in the same extent as alcoholic fermentation (AF). The number of producers choosing this process of biological reduction of the high acidity of dray white wines using selected malolactic acid bacteria (SMB) and adequate nutriments is on the rise. Very recent studies show that the stage of MLF starting (by co-inocculation, by premature inoculation, by sequential inoculation and staggered inoculation) is of top importance for the achievement and completion of this biological process and also for the biological stability ensurance of the resulted wine.*

Key Words: white wine, malolactic fermentation, Sodinal Company.

Introduction

Malolactic fermentation (MLF) in white wine making remains a topical issue for both practitioners and researchers, in the same extent as alcoholic fermentation (AF). The number of producers choosing this process of biological reduction of the high acidity of the dry white wine using selected malolactic bacteria (SMB) and adequate nutrients is on the rise. Very recent studies show that the initiation stage of MLF (in the must along with the onset of AF, when the AF was performed in proportion of 50 - 60%, in the final stage of the AF or immediately after the finalization of AF of the must) is crucial for both the success and completion of the process and the insurance of the biological stability of the fermentation environment in which this process took place.

The climate of the last period overturned Romania's territory, and the heavy rains have produced and will produce changes to the vines in this active period of vegetation.

We may end up with grape harvest for white wine varieties (I primarily think of Chardonnay and less of others) which excel through a too high acidity and a low concentration in sugars, which will require the application of technological solutions in order to reduce the value of this important analytical and sensory parameter.

I am taking into account the young plantations of Chardonnay from Recaş and from Jidvei that produced wines gallooned with gold medals at some of the latest prestigious international competitions. In this context, a new approach to the issue of reducing the acidity in musts or in wines from white varieties through the biological pathway of MLF seems appropriate.

Materials and methods

Knowing the evolution of the main parameters (the SO_2 concentration, the temperature, the pH, ...) which act in the process of white wine production on a must

or a wine which requires a reduction of the titratable acidity is very important in choosing the commercial preparations of SMB and their compatible nutrient in order to guarantee optimal conditions to achieve MLF.

» **Aspects of the SO_2 content.** In white wine production, the action of sulphur dioxide used when pressing the destemmed and crushed grapes (derived from a healthy harvest of Chardonnay) is homogeneous because the treated environment acts like a single homogeneous liquid phase; during setting of the resulted must after its separation from the marc, sulfur dioxide concentrations slowly evolve according to its duration of action (table 1); it is noticed that after 3 days at 12 °C, free SO_2 concentration is 23 mg/l for an initial dose of SO_2 of 80 mg/l and only 10 mg/l for the initial intake of 40 mg/l; reported values show that the end of the setting of the must free SO_2 concentration is approximately 25 – 30 % of its original concentration which was administered at the pressing process. If it is envisaged that the fraction of molecular SO_2 is the most active fraction of free SO_2 on the microorganisms, this fraction is even more important as the pH is lower and the temperature and the alcoholic level are higher; for example, for a pH of 3,1 and a temperature of 12 °C, a must which contains 10 mg/l of free SO_2 will have only 0,24 mg/l of molecular SO_2 (according to the formula established in the literature and tables of values offered by professional institutions of specialist).

Depending on the SMB strains and their cellular physiological state, it was found that the bacterial cells have a variable sensitivity to molecular SO_2 concentration, with a critical limit allowed of 0,30 mg/l. In a comparative experiment on a must of Chardonnay kind, it was signaled a bacterial cell mortality after sowing with a strain of SMB which varied depending on the molecular SO_2 concentration of the sown must; a drastic reduction of the bacterial colony from the sown must was noticed in less than three days at a concentration of the must in molecular SO_2 of 0,35 mg/l and in less than six days at a concentration of the same must in molecular SO_2 of only 15 mg/l. This simple experiment demonstrated that the success of an early bacterial sowing with a strain of SMB depends largely on the concentration in molecular SO_2 in white wine making (table 2).

Table 1

The evolution of concentration in free SO_2 during setting of a Chardonnay must (pH 3,1) at a temperature of 12°C for two different levels of sulphitation

(Gerbaux V. and Briffox C., 2010)

Analysed parameters	Setting period (hours)	SO_2 administered in the pressing process	
		40 mg/l	80 mg/l
Free SO_2 (mg/l)	24	12	30
	48	10	25
	72	10	23
Free SO_2 proportion from SO_2 administered (%)	24	30	38
	48	25	31
	72	25	29

Table 2

The influence of molecular SO_2 concentration of Chardonnay must (pH 3,1 and 15 °C) on the implantation of a SMB population (viable cells/ml) after setting

(Gerbaux V. şi Briffox C., 2010)

Duration (days)	Molecular SO_2 concentration		
	< 0,05 mg/l	0,15 mg/l	0,35 mg/l
0	2,2 x 10^6	2,2 x 10^6	2,2 x 10^6
1	2,5 x 10^6	9,3 x 10^6	3,8 x 10^6
3	5,1 x 10^6	1,8 x 10^6	78
6	1,1 x 10^6	5	5

Table 3

The influence of the thermal regime on the SMB population after 6 days of sowing and on the MLF implementation period (sowing with a SMB population of 1 x 10^6 cells/ml at a must density of 1020 - 1030 g/l)

(Gerbaux V. şi Briffox C., 2010)

Must pH and the analyzed parameters		Temperature		
		16°C	18°C	20°C
Chablis Must 2009 (pH 3,11)	*SMB (cells/ml)*	9,5 x 10^4	6,0 x 10^4	1,7 x 10^4
	FML period (days)	38	31	27
Bourgogne Must 2009 (pH 3,26)	*SMB (cells/ml)*	6,0 x 10^6	8,6 x 10^6	1,0 x 10^6
	FML period (days)	31	28	24

» **Aspects of the temperature evolution.** Relatively recent studies (Gerbaux V. et al., 2008) have shown that the implantation of a strain of SMB in a dry white wine with higher acidity occurs in optimal conditions at a temperature of 14 – 16 °C than for a slightly higher temperature of 18 – 20 °C. In case of early bacterial sowing with a strain of SMB during AF of the must (whether or not derived from Chardonnay), the thermal impact on the bacterial implantation heavily depends on the pH value of the fermented environment (table 3).

» **Aspects of the pH evolution.** Recent research in an already presented doctoral thesis (H. Akin, 2008), led by Professor Pierre Strehaino from the University of Toulouse, on a Chardonnay must with 205 g/l sugar, an initial pH of 3,1 and an assimilable nitrogen content of 210 mg/l (of which 70 mg/l ammoniacal nitrogen and 140 mg/l amic nitrogen), where AF was conducted at 18 °C in the presence of selected strains of yeast, have shown that: in the first stage of 4 days, the pH decreased from 3,10 to 3,03 and this decrease of the pH value

is explained by the nitrogen assimilation which is available during the growth phase of the selected yeast cell; from the fourth day onwards, the pH value progressively increased to reach the final value of 3,21 and this increase of pH value is due to the proper fermentative process wherethrough the gradual increase in alcohol concentration modifies the ionic balance which has repercussions on the pH as well; the increase of pH was more significant as the AF has advanced to the point of approx. 80 %, after which these increases were progressively reduced until the completion of the fermentative process.

The pH evolution during the AF of the must has to be considered when choosing the bacterial sowing stage in case of several varieties of white musts (pre-eminently from Chardonnay) with high acidity. Choosing the SMB strain will have in view that the cellular genetic heritage and the physiological state of viable strain cells will exert a high enough tolerance even towards the smallest variations of pH (in case of approaching to the critical limit of pH tolerance value for bacterial strains of SMB cells, even a variation of only 0,05 pH units can not be ignored !). The influence of pH on molecular SO_2 concentration (and of temperature as well) will also considered.

Results and discussions

I. The SMB selection

Distribution companies can provide wine producers compatible SMB and appropriate nutrients in order to ensure the achievement of MLF with the minimum requirements listed above. Such SMB are presented in table 4 and are also distributed by SODINAL Company.

» **The nutrient selection.** Along with the recommended SMB from **table 4**, there is also a complex nutrient which is compatible with these selected bacteria, called FERMOPLUS MALOLACTIQUE. This nutrient is distributed by SODINAL Company and is corresponding to the aspects listed below.

» **The opportunity to use a nutrient at MLF of wines.** The nutrient should be used only towards amelioration, survival and growth of bacterial cells in order to accelerate the kinetics of MLF in wine and not to develope these

Table 4

Strains from the BIOLACT range

Important parameters in the inoculation phase	Biolact Acclimatée	Biolact Acclimatée PB1025	Biolact Acclimatée 4R	Biolact Acclimatée BM	Biolact CWR
Number of strains	3	1	4	2	1
Minimum pH	3,1	**2,9**	3,2	3,0	3,2
Temperature limit (°C)	18	15	18	12	-
Alcoholic degree (% vol.)	**14,5**	14	14	14	< 14
Min. temp. of inoculum	18	15	15	12	17
Total SO_2 (mg/l)	-	-	-	-	< 40

unwanted bacterial species capable of causing various diseases. It is recommended the be used in all technology situations which require the finalization of MLF of wines. Typically, the recommended optimal dose is of 30 g/hl which is administered in SMB leaven plus a preliminary dose of 5 g/hl administered in the must (wine) which is subject to biological deacidification through MLF.

» **The characteristics of a MLF effective nutrient.** It has to be a nutrient with a composition based on amino acids, vitamins, polysaccharides and mineral salts obtained from the enzymatic and thermal degradations of the autolysed yeast cell walls. It has to exercise the ability to rebalance the nutritional environment exhausted by the yeasts after AF. It should be able to reduce the latency time which is necessary for the MLF onset. It has to have a reasonable price in relation to its quality.

» **The composition.** Considering its complex composition, the product FERMOPLUS MALOLACTIQUE is a nutrient which is able to:

- rebalance the nutritional deficiencies of the wine intended for biological deacidification
- to ensure the reduction of the latency time at MLF onset
- maintain a high bacterial cell viability until the completion of MLF

The composition of this product consists of a mixture of 70 % cell walls, from autolysed yeasts, degraded through a thermo-enzymatic process (which provides all the nutritional resources required for the performance of metabolic and fementative functions of SMB) and 30 % potassium caseinate.

» **Packaging and coding.** The product, FERMOPLUS MALOLACTIQUE, is sold in large bags with a net weight of 5 kg and has the code 001253.

II. Comparative study on the state of MLF onset in white wine making

Literature (Croitoru C., 2009; Gerbaux V. şi Briffox C., 2010) envisage 4 stages to MLF onset in white wine making, which will be discussed below.

» **Triggering through co-inoculation.** This co-inoculation technique consists of simultaneously wine sowing with selected yeast suspension and also with the SMB leaven. This technique confirmed its validity in the case of musts from very healthy crops with a pH value close to 3,2 but denied the opportunity of its application in the case of some musts with pH values between 2,9 and 3,15. Using this technique, the completion of MLF may be extended up to 2-3 months. Other specialists opt for a „staggered co-innoculation" of the SMB leaven after 24 - 48 hours from the administration of selected yeast suspension (corresponding to the pH reduction period, as explained above).

This technique is very dependent on both the specificity for the metabolized substrate (malic acid) of selected SMB strain as well as the compatibility of the nutrient used in relation to this strain. In addition, it is compulsory that the administered dose of SO_2 does not exceed 30 mg/l which, in the set must, would correspond to a content of free SO_2 situated below the limit of 10 mg/l which would correspond to a concentration of molecular SO_2 below 0,2 mg/l which would affect in a lesser extent the bacterial cell viability.

» **Triggering MLF through precocious inoculation.** This technique of precocious inoculation with SMB applies to the stage in which the duration course of the AF of the must was exceeded with 50 %, corresponding more precisely with the stage in which the density of the must dropped around 1030 - 1020 g/l. Even under these conditions, the SO_2 dose administered in the destemming and crushed grapes shall not exceed 40 mg/l when

sowing with SMB leaven is required in a white must (suppose from Chardonnay kind) in which the pH is lower than the value of 3,2. This SO_2 dose has to assure the protection of the must against oxidation, so that it must originate from a healthy harvest. By applying this technique selected yeast cells will have the necessary time through exercising their metabolism to produce those carbonyl compounds which are able to fully combine the free SO_2 content of the fermentative environment; under these conditions, the success of bacterial sowing is significantly increasing, especially because the stage when SMB leaven is administered also corresponds with that maximum increase of pH previously analysed. This technique is based on a slow but continuous metabolization of malic acid (and possibly of citric acid) by the SMB leaven enriched with the compatible nutrient from the must which was subject to biological deacidification through MLF but which was also previously „fortified" with the same compatible nutrient for SMB (as shown above). By exercising their metabolism, SMB will not be able to convert to sugar metabolization because the selected yeasts will complete this metabolization before the SMB will complete the malic acid metabolization and possibly citric acid metabolization.

Comparative experiments conducted internationally have shown that by applying this technique the sensory profile of the resulted wines changes in a favourable manner and that there is no risk of unwanted bacterial deviations. However, this technique has the disadvantage that requires daily monitoring of both of the fermentation processes, AF and MLF, in order to make sure that none of them will slow down or stop; this monitoring includes both rigorous and daily controlling of the density which expresses the metabolic rate of sugars per unit time (e.g. in 24 hours) and also acidity diminution (harder to follow, if we consider that the majority of selected yeast strains also produce relatively small quantities of succinic acid and lactic acid, D and L forms of which will be discussed in the next issue) and the evolution of MLF (by significant daily control of the evolution of malic acid metabolization).

» **Triggering MLF through sequential inoculation.** This technique of sequential inoculation is applied in the final stage of the must AF (at a course stage of over 90 %). This technique is recommended when the wine still contains 10 – 12 g/l fermentable sugars. Many professionals opt for this technique because the fermentative environment becomes increasingly rich in natural nutrients due to exorbtion and autolysis processes of yeast cells under the action of increasing alcohol concentration; the content of these dead yeast cells becomes a valuable food for SMB. Until the stage of AF completion which corresponds to the complete metabolization (under 4 g/l) of sugars by selected yeasts, SMB will attack "the first course" from " the nutritional menu" they have available and prefer the most (i.e. malic acid, the first substrate in the order of preference of metabolization), they will continue, if needed, with "the second course" in case they did not have enough (i.e. with citric acid, the second preferred substrate) and only in exceptional cases (the selected yeast have not yet completed sugar metabolization and SMB show one more viable cell density high enough to require other nutritional substrates for metabolization) they turn to "desert" (in this case, the remaining fermentable sugars non-metabolised by the selected yeast). This technique requires a less stringent monitoring than the previous one, in turn, it induces longer periods of MLF (even with the use of compatible and appropriate nutrients). Triggering MLF through sequential inoculation with SMB avoids the risk of *Brettanomyces* installation in the fermentative environment that would bring considerable prejudices on the sensory profile of the infested wine.

» **Triggering MLF through staggered inoculation.** This technique of staggered inoculation is applied after the completion of AF of the must. This technique has less and less supporters, even though 10 –15 years ago it was the most appreciated technique, especially in the Bourgogne region where it was regularly applied in dry white wines from Chardonnay kind. It still maintains its followers in the case of producers to whom customers specifically ask for a Chardonnay whose sensory profile is dominated by a smell and taste typical of fresh butter („caractère beurré").

This asset of fresh butter is determined by the concentration of diacetyl resulted after the metabolizaion of the citric acid by SMB which usually occurs in the last third of the MLF. Since the technical equipment of laboratories of most wine cellars does not allow analytical control of citric acid metabolization, a pure sensory evaluation is required (with a strong empirical character) of its metabolic state by assessing the intensity and the quality of the fresh butter asset which is determined by the amount of diacetyl resulting from the metabolization of this acid.

In a wine which still contains fermentable sugars, the evaluation of citric acid metabolization is almost impossible. For this reason this staggered technique is applied only in the case of obtaining dry white wines from Chardonnay kind with a delightful asset of fresh butter.

Conclusions

Each of the four triggering and implementation techniques of MLF in white wine making has both advantages and inconvenients. Depending on the quality of the harvest, on the type of desired wine, on the technical equipment of the wine cellar and the laboratory, one of the techniques presented above can be applied. I suggest to interested parties the preliminary completion of several comparative experiments on a pilot level (demijohns of 50 l) or semi-industrial level (containers made of stainless steel of 500 - 1000 l) at the beginning of the wine making process and only after an optimum feedback the application of the chosen technique at an industrial level in the next wine making process.

The next issue of the magazine will be addressed to the behavior and the comparison of the same techniques of MLF triggering and implementation in red wine making, with a presentation of protocols for managing SMB and compatible nutrients, but also with an approach to problems regarding the completion of this process (on the evolution of volatile acidity and highlighting the forms of lactic acid) in order to stabilize the wine from a biological poin of view.

References

» Akin H., 2008 – *„Evolution du pH pendant la fermentation alcoolique de moûts de raisins: modélisation et interprétation métabolique",* Thèse de Docteur de l'INP Toulouse.

» Croitoru C., 2009 – *„Treaty of oenological science and engineering. Products of development and maturation of wines"*, Publisher AGIR.

» Gerbaux V., Briffox C., Bou-Deleris M., 2008 - *„Mise au point d'une nouvelle biomasse bactérienne pour l'ensemencement direct des vins blancs*", Revue Française d'Oenologie, 227, 6-11.

» Gerbaux V., Briffox C., 2010 – „*Faisabilité et mise en oeuvre de l'ensemencement bactérien précoce pour les moûts de Chardonnay",* Revue Française d'Oenologie, 136, 13 – 15.

MALOLACTIC FERMENTATION MLF: AGAIN IN SPECIALISTS'ATTENTION WITH NEW TECHNICAL-SCIENTIFIC APPROACHES ! ASPECTS OF THE RED WINE MAKING (2)

Croitoru Constantin
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Abstract: *Malolactic fermentation (MLF) in red wine making remains an actual topic for both wine makers and wine researchers, despite the scientific progress recorded in recent years related to the performance of selected malolactic bacteria strains (SMB). Also in red wine making, the most important aspect is the stage for the MLF onset in comparison to the stage of alcoholic fermentation (AF).*
Very recent research studies regarding the improvement of the MLF performance in red wines with SMB and adequate nutriments have revealed that this process is carried out the most rapidly at the stage of sequential inoculation (just after the separation of the free run wine from the grape pomace), and then at the stage of precocious inoculation (when the density of the grape pomace decreases to around 1020 g/l) and the stage of co-inoculation (in the debut stage of AF but after inoculation with selected yeasts), followed by the stage of tardy inoculation (in the free run wine after the complete metabolization of sugars) and very rare in spontaneous cases (without SMB inoculation).

Key Words: red wine, malolactic fermentation, inoculation, Brettanomyces.

Introduction

Malolactic fermentation (MLF) in red wine making remains a topical issue for both practitioners and researchers despite the scientific progresses recorded in the last years regarding the performances of selected malolactic bacteria (SMB) strains. In red wine making the most important aspect is represented by the stage in which FML is triggered in regard to the course of alcoholic fermentation (AF).
Very recent research studies regarding the optimization of MLF achievement in red wines with SMB and appropriate nutrients have shown that this process is completed the fastest in the stage of sequential inoculation (immediately after the separation of the free run wine from the pomace), then at the precocious inoculation stage (when the density of grape pomace decreases around 1020 g/l) and the co-inoculation stage (in the debut stage of AF but after inoculation with selected yeast), followed by the tardy inoculation stage (in the free run wine after the complete sugar metabolization) and very rare in the spontaneous cases (without SMB inoculation).

Materials and methods

I. The opportunity to accomplish MLF with SMB

There is an impressive diversity of views in the international world of specialists in oenology regarding the opportunity to accomplish MLF in red wines only under the action of a few

strains of SMB belonging to an established genus of *Oenococcus oeni (O. oeni)*.

» Aspects of the traditionalist opinion. Some specialists consider that each viticultural area has the ability to make a natural selection of useful indigenous strains of *O. oeni* existing in that area so that it is not necessary the financial effort allocated for MLF accomplishment using commercial biologicals of SMB since the indigenous strains have a superior adaptation ability to technological restrictive conditions of the fermentative environment specific to those wines.

Practical reality partially confirms this attitude because in most cases the wine cellars are unable to ensure a proper thermal condition immediately after the completion of the wine preparation process, so that the process of MLF accomplishment is postponed until the following spring when the temperature conditions often provide the triggering of biological deacidification process in the presence of indigenous microflora which certainly contains strains of *O. oeni* and other less valuable bacterial species which inevitably contribute to the achievement of the process (and sometimes to the appearance of unpleasant smell or taste as a result of the formation of biogenic amines, ethyl carbamate or as a result of a few compounds which seriously affect the sensory profile of the resulted wine).

» Aspects of the opinion of preliminary comparative testing. There are plenty of worldwide experts who are aware of the advantages of using SMB to accomplish MLF but are working in wine cellars that lack of necessary financial resources. The solution lies in the experimentation at an industrial level of the accomplishment of MLF with SMB on a specific wine lot (experimental version) however only through comparison with wine from the same lot at which MLF is carried out with bacteria from the indigenous microflora (control sample); for both versions, it is recommended the comparison of the MLF process durations and the consequences that result from it, the comparative sensory analysis, the evaluation of the costs of the experimental version and assessing the possibility of covering these costs through the price of wine as a result of improving its smell and taste characteristics. Comparing the same wine which resulted from different experiments but carried out in identical technological conditions, the opportunity of MLF accomplishment using biologicals of SMB can be properly evaluated.

» Aspects of vanguardist opinion. Another group of experts (unfortunately the least numerous, but fortunately, rapidly growing!) managed to convince decision makers from viticultural and wine making processes societies in which they operate that the generalization of MLF accomplishment is opportune only with SMB at least for red wines from the "premium" and "superpremium" category.

Companies that have generalized the MLF accomplishment with SMB were under the necessity of providing themselves with the necessary technical equipment to achieve, in optimum circumstances, this biological deacidification process; most important investments were made in the climate maintenance area of a few premises intended for this technological purpose which ensures appropriate heat treatment for the triggering, the course and the completion of MLF. It is not coincidental that these companies have acquired significant medals (the majority of gold !) at the most prestigious international competitions which they had participated in with the wines of the above mentioned categories and at which MLF was accomplished exclusively with SMB.

II. Current techniques for SMB utilization at MLF accomplishment

All these techniques differ from one another by the state in which the SMB suspension is administered related to the progress stage of AF which begins with the administration of selected yeast suspension. Other aspects of current techniques of MLF accomplishment with SMB refers to the concept of co-inoculation compatible between selected yeasts and SMB for some of these techniques and of course at the danger of installation of *Brettanomyces* yeasts for others.

» **Stages of bacterial inoculation.** Sowing the SMB can be done in different stages of AF respectively before the AF onset (Croitoru C., 2005 as a bibliographical source, but it is not subject to this article !), immediately after the AF onset (co-inoculation, simultaneously or shortly after the administration of selected yeast suspension), during AF (precocious inoculation, in the last third of the AF when the density of the destemmed and crushed grapes lowers to approx. 1020 g/l), in the final stage of AF (sequential inoculation, immediately after the separation of the free run wine from the pomace) or immediately after completion of AF (tardy inoculation, in the free run wine after the complete sugar metabolization) (Croitoru C., 2009; Bernier C., 2009) .

» **The concept of co-inoculation and compatible cohabitation.** For the first two techniques involving a more prolonged cohabitation of selected yeasts with SMB which will have to take into account the concept of compatible co-inoculation as well; this new concept was introduced very recently by Renouf V., et. al. (2008); authors presume that any couple of selected yeast strain – SMB strain involves different types of interactions that can be positive, neutral or inhibitory; considering the yeasts' activities which reflect the phenotypes of each strain, the onset of MLF can be favoured or, on the contrary, slowed down; it is important to take into account these compatibilities between selected yeast strains and SMB strains when applying the co-inoculation technique (or precociuos inoculation) in which yeast cells and bacteria have to cohabit; as for yeasts strains, the ones which produce higher concentrations of SO_2 and medium-chain saturated fatty acids will not be recommended for these techniques, a strain of yeast is choosen instead which produces these unwanted compounds in extremely low concentrations; the new concept is also applicable to SMB strains of *O. oeni* genus that have the ability to tolerate high osmotic pressures of the destemmed and crushed grapes (those with very high sugar concentrations) as well as higher AF temperatures in red wine making; these strains will clearly be recommended for the two techniques mentioned above.

» **The danger of *Brettanomyces* yeast installation.** Numerous studies (Cauchy-Alvin B., 2005; Renouf V. et. al., 2008) showed the influence of a rapid MLF onset on cotaminated *Brettanomyces* yeast cells' density; the growth of contaminated population was stopped when the MLF onset with SMB takes place; unfortunately, often in wine production, late MLF onset when the contamination level with *Brettanomyces* is already high will cause difficulty in the accomplishment of this biological deacidification process as well as the possibility of installation of some unwanted bacteria deviations. The situation is complicated when the wines were sulphited in autumn with too large doses of SO_2, so that in spring, a late trigger and a slow progress of MLF are noticed, creating the conditions of rapid development of *Brettanomyces* yeasts.

III. The co-inoculation technique

» **Generalities.** The co-inoculation process consists in the sowing of the destemmed and crushed grapes with selected yeast and SMB, simultaneously or slightly delayed (« simultaneous co-inoculation» or «quasi-simultaneous co-inoculation») by administering SMB within 24 hours after the administration of selected yeasts.
An objective of a co-inoculation is to promote SMB implantation in the fermentative environment, taking into account that the destemmed and crushed grapes, through its composition, ensures less stressful survival conditions for these microorganisms than the wine because it does not contain alcohol, and the available nutritional resources do not determine deficiencies in assimilable nitrogen and some vitamins; the destemmed and crushed grapes environment in relation to the wine environment has the advantage that the bacterial sowing provides BMS a gradual acclimatization to the fermentative environment with the increasing of its AF process.
» **Recommended technological situations.** This technique of co-inoculation is recommended especially when obtaining red wines which are intended to rapid commercialization with high pH (> 3,4) bearing in mind the fact that an additional awareness regarding their biological stabilization is compulsory.
» **Eventual possible risks.** The results obtained from co-inoculation experiments have not confirmed the initial hypotheses regarding the risk of formation of some noticeable amounts of acetic acid under the action of SMB because the acetic acid is mainly produced when the SMB or other bacterial strains have completed malic acid degradation and began the citric acid.
» **The evolution of the biological agents.** In the event of a co-inoculation, there is rapid decrease in the bacterial population followed by its increase in the stage when the yeast cells reach the death phase. Experiments have shown that bacterial cell survival is dependent on pH, and the survival rate increases once the pH increases. SMB leaven will be administered only after the density of viable cells of inoculated selected yeast intended for AF reached the maximum level (this level is reached normally after 2 - 3 days from sowing), so that yeast-bacteria interaction is avoided; after at least 48 hours from the onset of AF, SMB leaven is added which is prepared according to the protocol presented below.
» **Technological parameters of reference.** Effective management of the co-inoculation technique requires several conditions for the fermentative environment necessary for bacterial development which relates to the use of destemmed and crushed grapes with a temperature over 17°C, a pH above 3,2 and total SO_2 content below 50 mg/l. MLF monitoring is recommended so that the process will be stopped after the malic acid had been entirely metabolized or after a great amount of citric acid had been metabolised or immediately after the citric acid had been completely metabolised.
» **The necessity of rigorous analytical monitoring.** The choice of co-inoculation technique should take into account the must's analytical parameters (pH, sugar concentration, SO_2 content, assimilable nitrogen content). After the onset of AF, dosing the concentration in free SO_2 is required and the stage at which this concentration is zero, the administration of the SMB suspension is possible; in addition, SMB implantation in the fermentative environment should be controlled to ensure that this process took place under optimum conditions using the microscopic method with epifluorescence applied after 24 hours from bacterial inoculation; it is also recommended

the regular monitoring of the volatile acidity, in addition to the monitoring of must's density and its L(-)malic acid content.

» **Other specific technological aspects.** Through the co-inoculation technique, it is noted that the SMB do not attack other substrates such as sugars, but consumes only the malic acid as the pH of the fermentative environment is, in most cases, below the critical limit of 3,6. When applying this technique, the latency phase of SMB is virtually zero, the MLF duration is considerably shortened and the favorable impact on the sensory profile of wines which ensures and provides its typicity is undeniable.

It has been demonstrated that the bacterial inoculation suffers a significant loss of viable cell density due to the reduction of pH through an increase in acidity caused by the formation of succinic acid, lactic acid and pyruvic acid by the yeast cells as byproducts of their metabolism. Since the yeast cells of some strains have the ability to produce SO_2 but also to consume a large amount of the nutritional resources in this stage of AF it means that there are other technological reasons that do not recommend this alternative technique.

IV. Precocious inoculation technique

It refers to the SMB inoculation in the last part of the AF when the density of the destemmed and crushed grapes drops to approx. 1020 g/l. In regard to the co-inoculation technique, the precocious inoculation technique do not have large differences in the sense that it refers to the same technological aspects except that the administration of SMB suspension is delayed as opposed to the state of administration of selected yeast suspension; it can be said that the precocious inoculation technique can also be called the sequential co-inoculation technique which is a delayed co-inoculation of SMB in regard to the selected yeast inoculation.

In his doctoral dissertation, Nehme N. (2008) studied the interaction between a selected yeast strain of *S. cerevisiae* and a SMB strain of *O. oeni* regarding the impact of this interaction on the achievement of AF and MLF in sequential cultures (delayed co-inoculation) and mixed cultures (simultaneous or quasi-simultaneous co-inoculation); research has shown that in the case of sequential cultures, the strain of *S. cerevisiae* exerted an inhibitory activity on the strain of *O. oeni* through the resulted alcohol (a proportion of 75 % of inhibition) and through a peptidic fraction with the molecular mass ranging between 5 and 10 KDa (a proportion of 25 % of inhibition), while the metabolization level of the malic acid was up to 3,5 times higher for simultaneous co-inoculation as opposed to the sequential co-inoculation version; this research had highlighted the importance of choosing the couple of selected yeasts strains - SMB strain for maximum success of the application of simultaneous co-inoculation technique (or quasi-simultaneous) to achieve MLF.

V. Sequential inoculation technique

Sowing with SMB in the final stage of AF requires their acclimatization to the fermentative environment which is hostile (low pH, high alcohol concentration) and low in nutritional resources. Even if in the final stage of AF the yeast cells die after they release through passive release the amino acids in the extracellular environment; this release is followed later by a slow natural process of autolysis which translates into a nutrient enrichment of the wine; in some cases, the release of amino acids in the extracellular environment does not occur immediately after the death of yeasts cells and

the autolysis process requires a longer period of time (several months) in order to observe a significant enrichment of the environment in nutritional resources; for these reasons, several techniques of wine sowing with commercial biologicals of SMB require the administration of nutritional adjuvants (MLF activators) to ensure the onset, course and completion of MLF; between these adjuvants the greatest effectiveness was shown by those produced through thermo-enzymatic degradation of the cell walls originated from autolysed yeast cells to which it came upon through the experimental testing of several similar products used integrally (extracts or autolysates of yeasts) or partially processed (milled cellular capsules or with various stages of thermal degradation).

The improvement of MLF kinetics can be insured by administering the activator (e.g. FERMOPLUS MALOLACTIQUE product) directly in the wine to be sowed with SMB (the SMB choosing, see table 4 for of the first part of the article published in the previous issue in which advanced products belonging to BIOLACT range are presented) or by adding it in two stages (a part during the acclimatization phase of the SMB and the other part directly into the wine intended for sowing with SMB) according to the protocol of SMB reactivation presented below. As all activators, the activators intended for MLF must exercise a nutritional effect which will allow the bacterial biomass to increase (provided by yeasts extracts or components of cell walls thermo-enzymatic degraded) and a detoxifying effect which will ensure cell survival (provided by an intake of cell walls in lower stage of degradation).

Numerous studies conducted in recent years have shown that the administration of the leaven from several selected strains of *O. oeni* with a high affinity for the metabolization of malic acid and then citric acid can be carried out in the final stage of the AF when up to 10 - 12 g/l of residual sugars are still to be metabolised without any existing danger of unwanted bacterial deviations.

VI. The tardy inoculation technique

The technique of delayed onset of MLF has been associated with the state of administering the SMB directly in the wine just because of their compatibility. In this technique, regardless of the chosen SMB biologicals, the manufacturers and the distributors recommended the administration of the rehydratable biological material only after the completion of AF (when the reducing fermentable sugars content is very low, less than 2 g/l residual sugars).

The followers of this technique argue that a premature inoculation with SMB when the wine is left with unfermented sugar content (the stage corresponding with the slow-down of AF as a result of the yeasts entering in the decline phase) is prohibited and therefore should be avoided. In this undesirable situation, the risk of accentuation of AF slow-down appears, which can cause a bacterial deviation with serious consequences on the quality of the wine (the phenomenon of lactic tart). At this stage, inoculated SMB are characterized by a very high density of viable cells, which coexist in the presence of a viable population of yeasts with a cell density similar to the bacteria as order of magnitude, but with a much lower vitality as opposed to the vitality of bacterial cells.

The acceleration of the death phase of yeasts due to the presence of SMB in the fermentative environment has been scientifically demonstrated and practically confirmed; this biological phenomenon of acceleration of the death phase of yeasts is determined by the hydrolysis of yeasts cell walls under the action of the enzyme equipment available to SMB cells. The bacterial inoculum multiplies taking

advantage of the presence of yeast autolysis (which is an excellent source of nutrition and exceptional growth) and it ferments the residual sugars causing volatile acidity. Some SMB manufacturers and distributors try to classify MLF after its completion with SMB using direct inoculation or the preparation of a reactivated leaven; the practice of wine making process shows that the directly administering of SMB into the wine without a prior reactivation was doomed to failure in most cases, regardless of the strain's performances.

Results and discussions

A recently comparative study (see experimental versions presented at the beginning of the article compared with the control version unsown with SMB) regarding the influence of the inoculation stage with SMB on the course MLF in the case of Merlot kind had monitored the evolution of kinetics of fermentation and reported several analytical distinctions, microbiological distinctions and sensory distinctions at the resulted wines (Bernier C., 2009).

In all experimental versions (including the control version) the AF process was conducted in adequate circumstances and also performed with the same strain of selected yeast. It was found that MLF has triggered the fastest in the sequential inoculation stage (immediately after the separation of the free run wine from the pomace), then in the precocious inoculation stage (when the density of the destemmed and crushed grapes decreases around 1020 g/l) and in the co-inoculation stage (in the debut stage of AF but after the inoculation with selected yeasts), followed by tardy inoculation stage (in free run wine after the complete metabolization of sugars) and very rare in the case of control version (spontaneous MLF without inoculation with SMB).

Sequential sowing resulted MLF with the shortest duration and the shortest period between the end AF and the beginning of MLF; SMB sown samples at the beginning of AF (co-inoculation version) and in the last third of the AF (precocious inoculation version) could not completely remove the period between the completion of AF and the onset of MLF but this period was considerably reduced compared to the control sample; SMB suspensions were implanted in the fermentative environment to all experimental versions so that the risk of yeast *Brettanomyces* contamination was limited to a maximum; no significant analytical differences were found between the resulted wines; and in sensory level the highest-rated wine was the wine obtained by sequential inoculation namely the one which was the fastest in beginning and completing the process of MLF.

I. Reactivation protocol of SMB

During acclimatization, BIOLACT ACCLIMATEE selected bacteria are found in the ideal pH and nutritional conditions so that they multiply faster than indigenous bacteria from the must or wine which continues to remain in very poor growing conditions. In the example below, the total volume of wine (must) intended for MLF is of 100 hl. Regardless of the volume of wine (must) intended for the treatment to achieve MLF, the preparing of the leaven from the same wine (must) is required which is a volume of 1 % of the wine's (must's) volume intended for treatment.

Therefore, in order to sow 100 hl, a volume of 1 hl of leaven from the same wine (must) will be required. For other volumes of wine (must) intended for treatment to achieve MLF, the necessary quantities of SMB from the BIOLACT ACLIMATEE range and FERMOPLUS MALOLACTIQUE nutrient are shown in table 1.

Table 1

The quantities of SMB from BIOLACT range and FERMOPLUS MALOLACTIQUE nutrient needed to achieve MLF in different volumes of wine (must)

The volume of wine (hl) intended for treatment	The quantity (g) of BIOLACT selected bacteria	The quantity (kg) of FERMOPLUS MALOLACTIQUE nutrient
50	50	0,250 + 1,5 = 1,750
60	50	0,300 +1,8 = 2,100
100	100	0,500 + 3,0 = 3,500
150	150	0,750 + 4,5 = 5,250
200	200	1 + 6 = 7
250	250	1,250 + 7,500 = 8,750
300	300	1,500 + 9 = 10,500
400	400	2 + 12 = 14
500	500	2,500 + 15 = 17,500

The preparation of the leaven implies going through the following sequence of technological operations:

1. The preparation of a recipient with a capacity of 1 hl, in which will be added 50 liters of wine (must) and 50 liters of water free of chlorine, resulting a diluted suspension with a pH between 3,4 and 4.

2. Administering of a dose of 500 g/hl FERMOPLUS MALOLACTIQUE (nutrient necessary for growth and development of bacterial cells which is a nutritional supplement based on autolysed yeast cell walls degraded by heat and enzyme action) in a volume of 1 hl of diluted suspension, followed by homogenization .

3. If possible, ensuring an optimum temperature of 22 - 24 °C. Even a minimal temperature of 20 °C can trigger MLF but with a lower density of bacterial viable cells.

4. Sowing the homogeneous suspension with a full dose of 100 g of BIOLACT ACLIMATEE (corresponding to a volume of 100 hl wine (must), namely 1 g of bacteria in 1 hl of wine (must)) which should provide a density of approximately 2 x 10^7 viable cells/ml.

5. Maintaining the optimum temperature (20 - 22 ºC) for 24 hours for SMB acclimatization at the wine restrictive environmental conditions and for triggering the cell multiplication. Periodic homogenization of the sown wine is recommended every 3 - 4 hours with BIOLACT ACLIMATEE and enriched with FERMOPLUS MALOLACTIQUE nutrient. The assessment of homogeneity consists in periodic visual examination of the leaven content in the recipient.

6. A period of 48 hours at a temperature of 20 - 22 ºC must be insured which will allow the metabolization of approx. 2/3 to 3/4 of the initial malic acid content of the wine (must); this stage allows the progressive decrease of pH and the avoidance of pH shock. The suspension in which the malic acid had been metabolized is represented by the reactivated final leaven which is able to trigger, to undertake and to complete the MLF of that wine (must) in an optimum period of time.

Sowing the recipient with wine (must) with SMB leaven involves:

7. Administering a dose of 30 g/hl of FERMOPLUS MALOLACTIQUE adequate nutrient in the wine (must) from the recipient intended for achieving MLF with 24 hours before adding the final SMB leaven.

8. Mixing the final leaven with the wine (must) intended for MLF previously enriched with a dose of 30 g/hl of FERMOPLUS MALOLACTIQUE.

» The required amount of SMB. The dose of SMB remains constant (1g/hl) regardless of the type of product that is usedused. Usually, packages are sold with a net content of 25 g (for a volume of wine of 25 hl) or 250 g (for a volume of wine of 250 hl), which provides the necessary quantity of SMB for different volumes of wine (see table 1).

» The required amount of nutrient. The nutrient is administered in 2 distinct phases: In the preparation of the reactivated leaven, when the required amount is calculated using the formula: Q_1 = 5 g/hl x 1 % V x 100, in which: V = wine volume in hl

Example:

Q_1 = 5 g/hl x 1 % (100 hl) x 100 = 5 g/hl x 1 hl x 100 = 5 g/hl x 100 hl = 500 g = 0,500 kg

» In the feeding course of the bacteria during MLF, when the following formula applies: Q_2 = 30 g/hl x V, in which: V = wine volume (hl) intended for treatment.

Example:

Q_2 = 30 g/hl x 100 hl = 3000 g = 3 kg

» Total amount of the nutrient will be: $Q_t = Q_1 + Q_2$ = 0,500 + 3 = 3,5 kg

Note: The success of the wine's MLF onset increases if the triggering of this process of biological deacidification occurs by the end of the alcoholic fermentation when its temperature is of 20 - 22 °C, which provides a rapid cell multiplication of SMB in both the preparation of the final reactivated leaven and the course of the malic acid metabolization. Attention must be paid to the pH of the wine because a value below 3,2 should be monitored! Other limiting technological factors (alcoholic concentration greater than 15 % vol. or a free and total SO_2 content above the permissible limits of 10 mg/l and 50 mg/l) usually occur very rare in the triggering of MLF of the wine.

II. Managing the progress and the completion of MLF with SMB

After the completion of malic acid metabolization, SMB seeks other hydrocarbon resources to exercise their own metabolism. At this stage the increase of volatile acidity occurs through the metabolization of the citric acid or the appearance of unwanted bacterial deviations occurs due to the metabolization of the traces of sugars. If the period after MLF is extended, the wine becomes a favourable environment for the growth of several pathogen germs of the *Brettanomyces* and *Pediococcus* genus.

The MLF process is completed when there are only 0,1 to 0,3 g/l of malic acid, sometimes even less. Apart from exceptional cases also requiring citric acid metabolization, rapid intervention is recommended for cessation of the activity of lactic bacteria. The citric acid transformation can be appreciated or not through the formation of typical aromatic compounds. The metabolization of traces of residual sugars with the formation of volatile acids is prohibited. A normal and pure MLF only forms traces of volatile acids (+0,05 to +0,15 g/l), while in the case of lactic tart onset the volatile acidity can reach up to 0,4 g/l in a few days. Effective management of MLF completion requires a few additional analysis.

» Volatile acidity monitoring. Measurement of residual malic acid is not sufficient to

detect the presence of acetic bacteria in the fermentative environment so that the volatile acidity dosing is a complementary measure which allows rapid detection of several possible alterations caused by the presence of these bacteria.
The determination of volatile acidity can be done through the classical method (distillation) or through new methods (enzymatic method). In the case of the determination of abnormally high values (above 0,6 g/l in acetic acid) is necessary to identify the microbial agent responsible for this anomaly; the first stage is to observe, through a microscope, the bacteria present in the wine using an optical microscope (magnification x 1000) in immersion; this observation often allows the identification of bacteria and sometimes of the genus possessing these specific characteristics; in the second stage, a viable cell counting on specific mediums can be done.

» **Highlighting the forms of lactic acid.** In case of lactic tart that occurs immediately after MLF in the case of wines with high pH, the identification of the disease can be done easily by determining the stereochemical nature of the lactic acid present in wine. Enzymatic dosage of the forms of lactic acid present in wine is very simple and very fast; a content of D(-) lactic acid greater than 300 mg/l shows the occurance of unwanted bacterial deviations. Yeasts forme little amount of lactic acid begining with the metabolization of hexoses of D(-) lactate, while the lactic bacteria only forms L(-)lactate based on L(-)malate during MLF. On the contrary, when the lactic bacteria use sugars, D(-)lactic results in the case of coccus and both forms of lactate, L(-) and D(-), in the case of lactobacilli. Under these conditions, the presence of a critical content of 300 mg/l or more of D(-)lactic acid is represented by the characteristics of a multiplication of lactic bacteria which metabolizes sugars from the environment. Therefore, the rapid elimination of lactic bacteria from the wine is recommended immediately after the completion of MLF without waiting to be noticed an increase of the volatile acidity which is another proof of the installation of unwanted bacterial deviations.

» **The influence of SMB usage on the biological stability the of wines.**
In general, the microbiological stability of a wine is determined primarily by its pH value; the lower the pH value is the higher the microbiological stability is because fewer bacterial genera and species can develop and a stronger antiseptic action of SO_2 is signaled through a significant proportion of active SO_2 in the wine.
The success off a sustainable biological stability of a wine is ensured by the limiting conditions of the development of lactic bacteria that refers to the acidity level, the alcohol concentration, a low storage temperature and at the administration of a rational dose of antiseptic.
In view of the above, even if the pH value increases after MLF, the biological stability emphasizes; this phenomenon has nothing paradoxical because by the disappearance of sugars and malic and citric acids, the easily metabolised by the lactic bacteria sources of hydrocarbon disappear as well, simultaneously with the disappearance of other nutritional resources as a result of bacterial cell multiplication, to which it might be added the release by the lactic bacteria cells in the wine of several toxins which inhibit the microbial activity.
The separation of the wine from the lactic bateria sediment along with its sulfitation are also contributing to the biological stabilization.

Conclusions

The main advantages of MLF implementation with SMB will be discussed below.

» Faster MLF onset. Using SMB, chances are high that MLF will trigger faster so that the duration of the process of red wine making is reduced.

» MLF implementation in difficult wines. A major advantage is the possibility of MLF implementation in some difficult red wines (with low pH, high alcohol level and a content of free/total SO_2 exceeding 10/50 mg/l) as a result of efficacious acclimatization of SMB to the fermentative environment and the elimination of the inconvenience related to a restrictive temperature (below 17 - 20 °C).

» Limiting the risk of Brettanomyces yeasts production. By inoculating SMB directly in the destemmed and crushed grapes, which ensures their development immediately after the yeasts reach the death phase, the period of time in which there could be a vacuum of useful microorganisms is limited to a maximum; this would allow the unwanted yeasts from the Brettanomyces genus to install.

» The reduction of biogenic amines production. By using SMB, the biogenic amines production is reduced so that the resulted wines better conserve their fruitiness.

» Improving the sensory profile and the biological stability of wines. The reality of wine production demonstrates that MLF with SMB is required for all red wines structured and intended for aging because it gives them an added complexity, gracefulness and roundness taste and ensures their microbiological stability.

References

» Bernier C., 2009 – *„Influence du moment d'inoculation en bactéries lactiques sur le déroulement de la fermentation malolactique"*, Mémoire de fin d'étude pour le Diplôme National d'œnologie, Faculté d'œnologie Bordeaux 2, France.

» Cauchy-Alvin B., 2005 - *„Étude de la prédominance des bactéries sélectionnées sur la flore contaminante de type Brettanomyces"*, Revue des Oenologues, 117, 13 – 14.

» Croitoru C., 2005 - *„Reducerea acidităţii musturilor şi vinurilor. Metode şi procedee fizice, fizico-chimice, chimice şi biologice"*, Publisher AGIR, Bucharest.

» Croitoru C., 2009 - *„Tratat de Ştiinţe şi inginerie oenologică. Produse de elaborare şi maturare a vinurilor"*, Publisher AGIR, Bucharest.

» Nehme N., 2008 - *„Étude des interactions entre Saccharomyces cerevisiae et Oenococcus oeni: impact sur la réalisation de la fermentation malolactique en cultures séquentielles et mixtes"*, Thèse de doctorat, École doctorale de mécanique énergetique, génie civil et procédés spécialité: génie des procédés et environnement, Département Bio Procédés et systèms microbiens du Laboratoire de Génie Chimique, Toulouse.

» Renouf V., Gourraud C., Murat M. L., 2008 – *„Les différentes alternatives d'utilisation des levains malolactiques. Partie 1/2: Aspects cinetiques et microbiens"*, Revue des Oenologues, 128, 39 – 43.

STUDY OF SOME TOMATO VARIETIES ORIGINATING IN ENGLAND IN THE PEDOCLIMATIC CONDITION OF SOUTH-EASTERN ROMANIA, RESPECTIVELY BRAILA COUNTY

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Abstract: *The study has as the main target the testing of some Tomato varieties originating in England in the pedoclimatic condition South-Eastern Romania from Braila region. For this first stage of the project htey are not of special interest the technical economical parameters (costs, productivity, profit etc.), theese following to be the object of a different study. They have been studied six „cherry" tomatos varieties. (Solanum lycopersicum var. Cerasiforme):*

- *Cherry White (C.W.)*
- *Cherry Black (C.Bk.)*
- *Cherry Brown Berry (C.Br.)*
- *Cherry Gold Nugget (C.Gn.)*
- *Cherry Cerise (C.Cr.)*
- *Cherry Riesentraube (C.Rs.)*

The biotechnical parameters of the cultivated varieties were monitorized along the whole vegetative period, ierespectively May 29th 2011, the planting date in open field, and until October 22nd, the date of the experimental plot land clearing.

Key Words: „cherry", tomatos, Solanum lycopersicum var. Cerasiforme.

Introduction

The experimantal plot has been established in Chiscani, a locality placed 2km from Danube and 15 km South of Braila City, all varieties enjoying the same pedoclimatic conditions.
Braila County is between the following geographical coordinates: 44º 44' and 45º 30' N, 27º 04' and 28º 10' S. It is located in southeast side of Romania, occupying the north-eastern Romanian Plain and the Big Island of Braila, the largest embanked site of the Danube River.
The relief is generally flat, the only „land accident" being the rivers, and lake depressions. Among the relief units, in the North side the county includes a part of Lower Siret River Meadow, towards the West it includes small parts of Salcioara Plain and Buzaului Plain, and towards the East the Lower Danube River Meadow.
The relief of Braila County is of cumulative, sedimentary origin, the loess deposits having a thickness ranging from 10 to 20 m. About 75% of county's surface is covered of chernozem. Theese soils formed in the continental semiarid climate conditions and of loess deposits, on silt and sand, on the ground of a steppe vegetation and surface groundwater sources.
The most spread chernozem soils are:

- Brown chernozem;
- Chocolate chernozem;
- Carbonate chernozem;
- Leachade chernozem.

Braila County climate is temperate continental with shades of aridity.

Solar radiation has a relatively uniform distribution, totaling between 122,5 and 125 kcal/cm and about 2,200 hours of real sunburn, out of which 72% is in the period April-September.
The high temperatures during the summer frequently exceed 30-35ºC, being tipical for the temperate continental climate. During the cold season, along about 110 days, it occurs the ground frost, out of which 80 days occurs only at night.
Concerning the precipitation, the annual average is 465 l/m, most precipitation being recorded during May-August, and the least in autumn and winter.

Materials and Methods

Concerning the used culture materials and methods, they have been covered the following steps/technological links:
»In order to shorten the acclimatization period and to reduce the losses from transplanting, the six varieties have been imported from England as seeds, the seedlings being produced in Romania (Picture No. 01). The seeds supplier was PREMIER SEEDS, a British company.

Picture No. 01 – „Cherry" tomatos seedlings ready for planting in the field

Picture No. 02 – Land modeling

Picture No. 03 – Giving Metiocarb granules before planting

» Date of planting in pots was **May 1st 2011**, and the date of planting in the opened field was **May 29th 2011**, the efficiency being of 96 %.
» The field where it has been established the experimental plot, have been prepared in advance by making a spring plowing at 30 cm, and use of the combinator for raising and leveling. The land modeling have been manually performed by open 70 cm wide furrows (Picture No. 02).
» Before planting in the field it was given Metiocarb (2% a.i.) as granules in order to control the fen cricket *(Gryllotalpa gryllotalpa)* and other harmfull insects (Picture No. 03).
Planting distances were the same for all varieties, 70 cm between rows and 30 cm between plants on row (Picture No. 04), resulting a density of 47,619.0 plants/hectar.
» The tomatoes grown in opened field, in intensive system, having a steam suporting system and flooding irrigation furrows (Picture No. 05).
» The plants were periodically removed of side-shoots but not pinched out. Five out of the six analyzed varieties shown an excelent force of growth along the summer, reaching about 1.60 m 60 days after the planting (Picture No. 06). The sixth variety, Cherry Gold Nugget (C.Gn.) shown a determined growth..

Picture No. 04 – Planting distance between plants (30 cm)

Picture No. 05 – System of support poles fashioned from wood and bamboo crafts

Picture No. 06 – Plant height 60 days after planting

» Weeds control have been performed manually by hoeing between rows and by pulling weeds between plants on row. They were not used herbicides. They have been

performed 4 hoeings every 15-20 days.
» Concerning the irigations, thanks to the frequent rains registered in the period **May 30th July th 2011**, the water consumption was reduced, the irigations being intensified in August at a 3-4 days interval.

Results and discussions

The biotechnologic parameters of the cultivated varieties where monitored along the whole vegetative period, respectively **May 29th 2011**, date of planting in the opened field, and until **October 22nd 2011**, clearing date of the experimental plot.
The performed observations can be synthetized as it follows:
» The varieties C.Br., C.Cr., and C.W. shown a special vigour of the shoots resulted from side-shoots (Picture No. 07).

» Despite the large inflorescences, the variety C.Bk. shown a high sensitivity to pollination, the yield obtained being low (Picture No. 08).
» Although they have been periodically

Picture No. 07 – Lateral growth sprouts („side-shoots" type)

Picture No. 08 – Inflorescences of the Cherry Black variety (C.Bk.)

Picture No. 09 –Black spot on berries of Cherry Brown Berry variety (C.Br.)

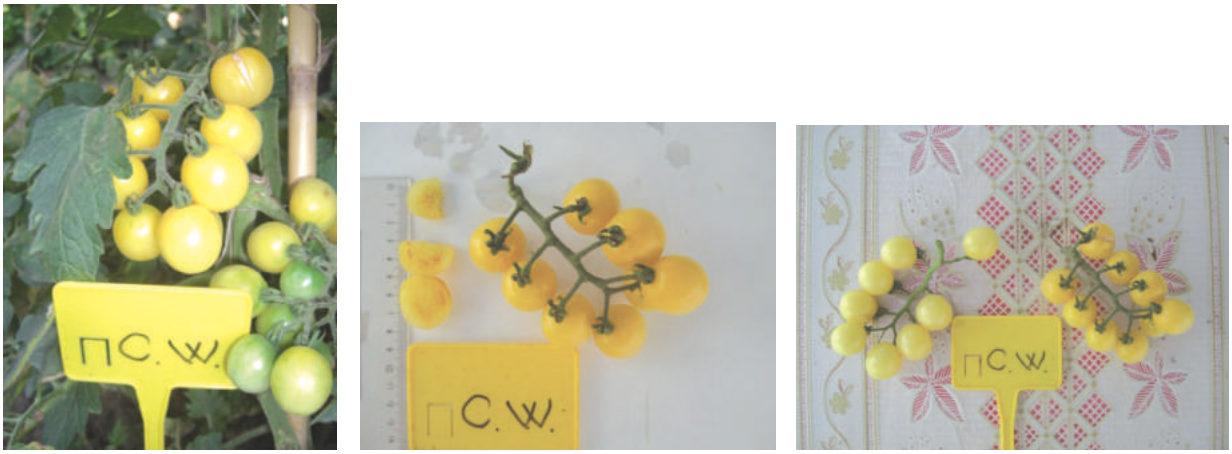
performed criptogamic treatments, the variety C.Br. shown an high sensitivity to black spot - *Alternaria spp.* (Picture No. 09).

» On reaching the physiological maturity, the berries of the variety C.Rs. shown uneven ripening and thus a commercial derogatory aspect too (Picture No. 10).

» The only variety with a detemined growth (C.Gn.), registered an average yield of 0.480 kg/plant, plant height being of only 60-70 cm. It results an average yield per hectar of 22,857.12 kg, level reached 70-75 days after planting (Picture No. 11).

» Although the berries of the C.W. variety

Picture No. 10 – Berries of the Cherry Riesentraube variety (C.Rs.)



Picture No. 12 – Berries of the Cherry White variety (C.W.) at the physiologic maturity

Picture No. 11 – Cherry Gold Nugget Variety (C.Gn.) when it reached the maximum yield.

shown uniformity and a special commercial aspect, it showed sensitivity to cracking, phenomenon amplified during the transport (Picture No. 12).

» The C.Cr. variety adapted excelent showing a high growth force, a long growing season, and a special quality of berries (Picture No. 13).

Picture No. 13 –Cherry Cerise Variety (C.Cr.)

Conclusions

» All the analized varieties adapted well in the pedoclimatic condition of SE Romania;
» The choosen culture system didn't cause special problem concerning the adaptability and productivity;
» Five out the six varieties shown a special growth vigour during the summer and a long vegetative period;
» Concerning the yield quality, the obtained results with the varieties C.Bk. and C.Br. were unsatisfactory;
» At the C.Rs. variety the berries' ripening uniformity was poor;
» The best results concerning the relation productivity / quality were registered by the varieties C.Cr., C.Gn., and C.W.

References

Atanasiu N. – *„Metode actuale de irigare în legumicultură"*,. „Horticultura", Magazine, No. 6, 2007;
Ciofu Ruxandra, Nistor S., Popescu V., Chilom Pelaghia, Apahidean S., Hogoş A., Berar V., Lauer K.F., Atanasiu N. – *„Tratat de legumicultură"*, Ceres Publishing House, Bucharest, 2004;
Drăghici Elena – *„Legumicultură"*, Granada Publishing House, Bucharest 2002;
Dumitrescu M., Scurtu I., Stoian L., Glăman Gh., Costache M., Dițu D., Roman T., Lăcătuş V., Rădoi V., Vlad C., Zăgrean I. – *„Producerea legumelor"*, Artprint Publishing House, Bucharest, 1998;
Indrea D., Apahidean S., Apahidean Maria, Sima Rodica – *„Cultura legumelor"*, Ceres Publishing House, Bucharest, 2007;
Oprea A., Galan C. – *„Tehnologii generale şi biotehnologii speciale în agricultură"*, Course, I.S.B.N. 978–973–0–06230-4, Bucharest, 2009.
Popescu V., Popescu Angela – *„Cultura legumelor în câmp şi în solarii"*, MAST Publishing House, Bucharest, 2003;
http://www.premierseeds.co.za/vegetable-seeds/tomato.html
http://stores.ebay.co.uk/Premier-Seeds-Direct/Tomatoes-/_i.html?_fsub=9717831

PROSPECTS FOR DEVELOPMENT OF RURAL TOURISM IN ROMANIA

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Abstract : *In EU countries, rural tourism is not, in fact, a new phenomenon. Over time - this time being one in decades for most EU countries - tourist accommodation in rural areas was performed in a manner more or less spontaneous or organized. Instead, what is new is the size reached by the expansion of tourism in rural phenomenon. This expansion can be explained on the one hand by launching the development of rural regions and on the other hand, the diversification of the practice of mass tourism. Therefore, rural tourism sign of Europe, one by one in the future local development policies. At first rural tourism has become slowly but consistently, especially in tourist markets in economically developed countries, industrialized countries with a high degree of urbanization. Thus in rural tourism is increasingly popular and requested the people who live and work in stressful conditions.*

Key Words: rural tourism, development of rural regions, development of rural tourism

Introduction

Rural tourism is based on three coordinates: space, people, products, because:

- space without human existence can not be support cohabitation, a space without products may not meet all travel needs of consumers;
- lack of space in people or products have only a small reception capacity;
- products that have not the space and people that have only a fleeting existence and can not ensure sustainable development locally.

The basic equation of rural tourism product is: accommodation on the farm = holiday in village + leisure in rural areas.

At first rural tourism has become slowly but consistently, especially in tourist markets in economically developed countries, industrialized countries with a high degree of urbanization. Thus in rural tourism is increasingly popular and requested the people who live and work in stressful conditions.

Practitioners of this tour can be found in various areas of the world: in almost all of Europe (European Community pays special attention to projects and programs for tourism in rural areas), North America, Australia and even Africa, standing out such flows tourism and concerns of practicing while tourism services in the most unexpected areas.

Materials and Methods

A. Motivational and strategic objectives

Rural tourism must be understood as a package of socio-cultural, sports and activities specific to the geographical environment,

made disposal, they will take place in conditions of minimal investment and nature conservation. Climate and landscape, ethnography and folklore, ceramics, crafts, hunting and fishing fund, the fund viticulture, fruit growing, existing in rural areas, make Romania to have multiple opportunities for rural tourism development.

– Strategic Objective

Analyzing the benefits derived from the practice of this type of tourism, and current situation of Romanian villages it is necessary to define Strategic Objective to be taken into account in determining any strategy, be it economic or political, at any rural communities.

The strategic objectives should aim to provide and protect the human factor, technical facilities and heritage conservation, grouped into three strands, namely:

- Stop the migration of the rural population to urban and stimulate recovery eel, eel least partially, the population of urban areas to rural areas;
- Ensuring the living conditions in rural areas and of civilization, stimulating active population stability in rural areas;
- Conservation and environmental protection areas - factor of attraction of local population and foreigners to rural areas.

Tourism in rural areas, complementary to other forms of tourism, contribute to sustaining the necessary conditions for transformation tourist tourism tour circuit to stay.

This can and should become the tourist area of residence, to spend their holiday destination all year round, because it responds not only motivation and knowledge of cultural tourism, but also other modern requirements: to leisure in nature, which otherwise found in other countries (investigations conducted among visitors to historical monuments show that attention is not only the monuments but also on their environment).

Motivation can be expressed as a convincing statement of Dr. Andre Lwoff, Nobel laureate: **"you're a European metropolis lived in noisy environments of continental Explain that here in the Upper Country, as you call it ., those in Romania, and live fulfilling feeling to know peace... The beauty and ambiance visited monasteries, quiet environment, this is what struck me was going to set there."**

B. Factors that favor the development of rural tourism in Romania

As the output of the current economic crisis and rising income, demand will improve tourism in our country, but it is possible to orient toward less traditional tourism, which will remain expensive, and may in a greater extent to rural tourism, which is cheaper.

On the possible direction would contribute and specific factors:

- ✔ mountain, hill and many other areas offer breathtaking landscapes;
- ✔ family agricultural offer natural food, prepared according to traditional recipes.
- ✔ Romanian population is largely a population who household still keeps parents or possibly nostalgia grandparents, who grew up in;

Preparation of this type of tourist offer requires a series of efforts and actions at various levels to increase satisfaction and attracting demand:

Arrangement of the agro-tourist farms

Development of infrastructure local
Creating structures organizers

C. Perspective of rural tourism development in lowland areas, hills and mountains

Alecu Ruso noticed well, or poetic sense, the harmony of natural architecture of Romania, said that when our country is one of the most blessed by God.

a) **lowland area** - includes land in S, V and E of Romania. These regions are areas of greatest agricultural importance, benefits about 3 million hectares developed for irrigation and drainage works. Network structure is the type locality collected, with a density of 2 to 6.8 km at 100 locations.

In terms of rural tourism plain area can count on a particular tourist vocation in the next 5-10 years, which can be determined by the existence in the area of object of tourist interest such as lakes, historical sites, literally, forests, etc..

b) **The area of the hill** - is defined in terms of geography, as the area that comprises the hilly terrain that is dominant with altitudes between 300-600 m. aid rural settlements have a density of 0.4 to 100 km villages and settlements network is dominated by the diffuse type, the result of natural conditions. This area lends itself to a multifunctional agriculture and structures land can be based on small family farms.

In terms of rural tourism is one such area vocation because of higher overall varied landscape and climate favorable.

c) **Mountain area** - comprising land typically located at over 600 m altitude. This area is characterized by a agrarian structure of the plot, the average area per household farm is 2.5 - 3 hectares.

In terms of rural tourism the area has always exerted a powerful attraction, especially when due to urbanization today and pollution clean nature of a human need is growing.

D. Conditions for development of rural tourism in Romania

- In our country the forms of tourism in rural areas can get a great extent.

⇨ **Urban population** - represents 55% of the total population, as such it represents an important potential for rural tourism demand if it combined with factors considerations that favor development of rural tourism in Romania;

⇨ **Free time - there**. Vacations are about one month per year and work week is only five days, after 1989. Therefore the active population employed would have to spend time both weekend and longer vacations away from home, which sometimes feels the need to escape;

⇨ **Real incomes** - but is more restrictive factor that hinder eel, even block domestic tourism demand. As long as the vast majority of urban population is forced to spend 60-80% of their income for food, we hope to remain weak for travel money. Scourge of inflation which greatly reduced the real incomes of population and purchasing power has declined from '89 (though not before this time the situation was not very good) made international tourism demand to know a strong rebound. Foreign tourism demand is hindered by economic, social and political. The quality of services we are foreigners in Romania are still below European standards. Even though in terms of national currency exchange rate and prices compared to international markets stabilized, tourists could be motivated to come to Romania, however, the fragility of political and social security and personal failure creates a braking reaction flow of foreign tourists to Romania;

⇨ **Relative inelasticity of classic tourism** - is manifested in Romania as a limiting factor, more so with this type of tourism as economic crisis lives of the transition. Given inflation and the viability of the tourism needs, prices and tariffs have become prohibitive for mass tourism potential, without the services to be improved and modernized in proportion;

⇨ **motorization families** - is a growing phenomenon. Paradoxically, although real incomes have decreased the number of cars and construction of houses has increased both the city and the countryside. Engines were in full progress, creates conditions for potential tourists traveling in rural areas. Do not forget that rural tourism is largely a form of family travel and car travel is essential for family travel, especially where no other means of transportation.

Results and discussions

Rural tourism development must integrate both the overall strategy for tourism development in the overall strategy as well as socio-economic development of this area. For this purpose it is necessary to know in advance of items:

- assessment of all categories of economic resources, financial and social place;
- defining the directions and guidelines for economic growth in the area;
- assessment of tourism development possibilities and limits.

Arranging travel the countryside is a complex action covering a wide problems including solutions to stimulate tourist traffic and the integration of tourism in rural space economy, and methods of selection and delimitation of areas, principles of operation.

– Types of visitor facilities

Localizations are influenced by the size of tourism and tourist resources spread throughout the country. So meet:

a) locations related to the existence of a single object or tourist item of great value, for example, Neamt Fortress, Bear Lake from Sovata. In these cases, arrangements are summary dimensions are closely correlated with the index of the attractiveness of that objective.
b) locations integrated into a set of conditions that gives a certain specificity of geographical areas with a specific character, for example, with the

monuments of art, architecture. In these cases, more complex facilities are taking the form of centers or tourist resorts.
c) specific locations of tourist areas relatively homogeneous, with a wide range of stretch, without a certain feature. In these cases, facilities are more numerous and can be simple or complex, and can be placed anywhere watching brief is a relationship between resources and equipment located. In this case the decision is determined decisively development of tourism demand.

Besides these types of visitor facilities, reports on tourism planning experience of the six regions of the World Tourism Organization, offers and other travel planning solutions depending on the nature of the territory in geographical space:

– Planning spa sites literally;
– Land sites mountain;
– Land of historical and archaeological sites;
– Rural land;
– Planning spa resorts, etc..

!!! site = French site, landscape meaning, position, region, geographical configuration of their own localities, and is dedicated to accept broader theory and practice in spatial planning tourism in many countries.

– Selection and delineation of tourist areas

Depending on the delimitation of tourist resources and determining on this basis, the dominant profile of each area, is a milestone in arranging travel to the territory. Visitor facilities are consistent with each area taking into account the potential surface to be decorated, climate, vegetation, cultural artifacts, labor resources and demographic situation, needs to stimulate economic and social progress of the territory

When the decision on the orientation of a tourist development area, specify the destination to be assigned to it must be such as to ensure a customer demand more diverse.

In this regard, planning policy must take into account the kind of tourism that has the best chance of development in the area and to adapt their focus to specific typology. In addition some development principles and requirements of their regions, the selection of tourist areas and are considering a number of **specific criteria**, namely:

• **natural conditions** (elements of tourist attraction, the attraction function, typology facilities);
• **cultural and historical richness** (archaeological, historical monuments, traditions);
• **socio-economic conditions** (demography planning, standard of living);
• **Infrastructure** (transport and ways and means of access, water sources, energy);
• **General equipment for culture and recreation** (means and forms of reception and accommodation, food units);
• **legislation and regulations** (have a role in stimulating or restrictive depending on the legal status of any perimeter of each development must comply with regulations on environmental protection, construction rules).

Unit types that will develop tourism in the tourist zone depend on the nature of the territory, specific resources and features, density and their value.

Landmark tourist attraction is an interesting enough to motivate tourists travel to it (natural monument, archaeological sites, etc.).

In the context of rural tourism, the basic unit of activity tourist farms and agro tourism pensions. They provide the necessary services in the area, especially the reception, accommodation and meals.

Conclusions

1. A promising start -in Romania for over two decades by promoting rural settlements ethno-folk values, cultural and scenic landscape as tourist villages was established, official rural tourism .

After 1989 the first initiatives began to appear, first at the level of animation's hearty mountain tourism, such as Radu Rey, then the mountain areas in Romania Commission and the Ministry of Tourism. Thus the law was passed approving Government Ordinance No. 62 of 1994 on the establishment of facilities for the development of rural tourism in the mountains, the Danube Delta and Black Sea. They also established ANTREC. In many rural villages appeared to profile companies that made accommodations in private homes, offering natural products and activities specific to ice.

Tourists from Germany, USA, France, Great Britain and elsewhere. The president said that the company is a member ANTREC European Federation of Rural Tourism in the American Society of Travel Agents of the U.S. Society of Ecological Tourism in the U.S., etc..

Romania has great possibilities of development of tourism, its practice is not only possible but also very necessary in this stage.

Income from this activity will contribute substantially to raising the civilization of the Romanian village and its inhabitants, the hospitality so characteristic of the Romans, could change the image of Romania in the world.

By the conclusion of partnership agreements with national, regional or local profile, it would ease access to EU funds for Romania's rural tourism, which Romania, currently can not receive more than coincidence, since there is still a member of the EU. It is also necessary to form a group of experts able to provide technical assistance and training to keep the local power bosses and owners of structures on the problems of rural tourism marketing, planning and interior partitions of the housing and training and food services classification and quality standards, catering and dining rules, rules of hygiene and ecology, behavior in dealing with tourists, information system.

2. Rural tourism and socio-economic development of the Romanian village - Romanian village - rural tourism contributes to the economic life of the village by these

– Opportunity of a policy of its long-term development, in close liaison with other sector policies, agriculture, infrastructure, environmental protection;
– Can become a support for new businesses and jobs;
– Encouraging local traditional activities;
– Dynamic factor in the process of increasing the quality of life in rural areas, etc..

3. Support and internal resources - for many years, countries like France, Austria, Germany turned to a new form of tourism

– Eco-tourism areas. Why not Romania? The wooden churches of Maramures, the monasteries of Moldova, crafts and the hospitality villages and Roman, houses with porch - these are universal values and

arguments for tourist attraction. In order to promote rural tourism, the professional inform those who want to turn to this new field, was established since 1994 ANTREC.

4. Turismul areas (a lifestyle) - one of the trends development of tourism industry is returning to nature, already manifested in all civilized countries where the current post-industries has created the need for withdrawal (for a few days) of metropolis. And for the huge tourism potential of our country,

Rural tourism is one of the most attractive tourist offers, and this from a multiple perspective: on one hand the novel or foreign tourists, which will require increasingly more intense this type of tourist service and on the other hand, villagers, who will use all his energy and skill to integrate into what will become, after efforts associations, a network covering the whole country.

Ministry of Tourism is heavily involved in rural tourism development. Important initiatives extends beyond legal forms that are required to prepare. The EU's PHARE program was attached to a component supporting rural tourism development.

Joint development efforts of rural tourism will certainly be rewarded in the near future. What concerns us all is, in fact, achieving our country's tourist vocation, which unanimously recognized, is the most beautiful country in the European geographical area. If this will be done, we have a standard of living in developed countries agree with the eel, as twenty-first century tourism industry will be.

References

» Balaure V., 2006 - *„Promotional techniques"*, support the course, University Bioterra;

» Baciuman I., 2005 – *„The economy of rural tourism and agrotourism"*, Alba Iulia;

» Bran Florina, 2006 – *„Rural Tourism - European model"*, Ed Economics;

» Bucianu C., 1999 – *„The economy of rural tourism and agrotourism"*, Alba Iulia;

» Costea R., 2002 - *„Marketing travel agents in the market economy"*, National Institute for tourism training and managemet, Ministry of Tourism;

» Deroni M., 2007 – *„The situation of rural tourism in Europe"*, department MFA Foreign Relations, Bucharest;

» Gavrilescu C., 2009 – *„Marketing"*, Bucharest;

» Radu E., 2005 – *„Service Management"*, Cantemir Christian University;

» Nistoreanu P., 2006 - *„Rural tourism - a small business with great prospects"*, Ed. ASE;

» Nistoreanu P., 1999 – *„Rural Tourism - a small business with great prospects"*, Didactic and Pedagogic Publishing House, Bucharest.

THE CONCEPT OF FOOD SECURITY IN EUROPE

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Abstract : *European Nutrition was founded in 1967 on the foundations of international conventions. In an initial phase was conditioned by a community management products. Food aid may thus vary from year to year depending on the reserves of European agriculture. Much later, after a deal between EU and ACP countries, technical and financial aid were donated to the beneficiary countries in order to gain sufficient nutrition. Until then reflect the commission's design evolved from a food aid as a tool, among others, that contribute to improved food security, access to food in concrete terms.*

Key Words: food security, management products, satisfactory diet.

Introduction

According to FAO food security is "always guarantee each individual at any place or time of access to sufficient and healthy food to allow him to have a satisfactory diet for a healthy and active life."Food security is influenced by four groups of factors:

Social-economic and political environment

– Food sector performance
– Social protection
– Health and hygiene

The main bodies involved in monitoring global security are: FAO, EC, USDA. In recent years, says there is food insecurity problems in 86 countries, 43 from Africa, 24 from Asia, 9 from Latin America and Caribbean, 7 from Oceania, three in Europe. In 2004, 35 countries have received aid emergency because of the food crisis. The causes were: military and civil conflict, post conflict situations, refugees, economic zones and climate problems. Romania, as is known, is aimed at joining the European Union on 1 January 2007. To achieve this goal, Romania must meet several conditions among which is the adoption and implementation of the Community agreement , which includes besides legislative harmonization and adoption of European standards in all areas, especially those that relate to health and people safe.

Romanian companies may have two options: either to adapt to new conditions, progress and prosper or lose their market position and are sad it bankrupt by misalignment to the new European standards.

During 2-4.06.2005 Chamber of Commerce and Industry organized by the Center Timisoara EUROINFO a European conference in the project "Food Safety, a natural consequence of product responsibility", funded by the European Commission's PHARE program.

At the conference representatives have actively participated in 5 countries: Poland, Estonia, Hungary, Bulgaria, Italy. The conference presented the following documents:

➢ management systems;
➢ HACCP principles and documents;
➢ food safety management system;
➢ Presentation of ISO 22000 standard for certifying food safety system.

Materials and Methods

Application of HACCP system in all units that make up the food chain is required under Romanian law, which requires that all companies in the food and catering motors comply with the principles of this system. Standards contribute to implementation of HACCP, developed by the CODEX ALIMENTARIUS Commission and adopted in Romania are:

➡ **SR 13462-1:2001 agro-food-hygiene**:

General principles: This standard follows the road of food production from primary stage to the final, it reaches the consumer and establish the necessary hygiene conditions for producing a safe and adequate food consumption;

➡ **SR 13462-2:2002 agro-food-hygiene:**

The Hazard Analysis and Critical Control Point (HACCP) and its implementation guide. The standard establishes principles for risk analysis and critical control points at play and how to identify these specific risks and control measures in order to ensure food safety;

➡ **SR 13462-3:2002-Hygiene foodstuffs.**

Principles for the establishment of microbiological criteria for foods at any point in the food chain from primary production to final consumption. This standard specifies the principles of establishment and application of microbiological criteria for foods, ensuring food security mainly through source control, process control and application of appropriate hygiene practices during production, processing (including labeling), handling, distribution, storage, marketing.

Application of the standards mentioned above provide compelling advantages:

✔ Maintaining a market position;
✔ a safe sanitary quality;
✔ a reduction in scrap and customer complaints;
✔ Increased customer confidence in a company;
✔ Improving a company's image.

Food safety management system integrates:

✔ HACCP principles;
✔ Applying the steps described by the CODEX ALIMENTARIUS Commission;
✔ Combining dynamics with the necessary prerequisites programs to control and reduce any risks to acceptable levels of food safety for the final product delivered in the next stage of the food chain;
✔ Hazard analysis that is essential for effective food safety management system as organized knowledge required to design an effective combination of control measures.

Safety dietary essential qualities of a food, a food is safe when the consumer does not alter and endanger the health of the body.

Food safety management system is presented in ISO 22000 international standard that specifies requirements for a food safety management system in a food chain, in which an organization:

➢ Must demonstrate the ability to control food safety hazards in order to provide safe

end products that meet food safety requirements agreed by consumers and the regulator;
➢ Aims customer satisfaction through effective control of food safety hazards, including processes to update the system.

The international standard ISO 22000 specifies the requirements that allow an organization:

➢ a plan, design, implement, manage, maintain and update a food safety management system will evaluate and assess customer requirements and demonstrate compliance with mutually agreed customer requirements related to food safety;
➢ a demonstrate effective communication with customers and other stakeholders along the food chain;
➢ will demonstrate compliance with applicable requirements of the regulatory authority with respect to food safety;
➢ will ensure that they meet established food safety policies;
➢ will demonstrate that under other stakeholders;
➢ to request a registration system or its certification of food safety management by an external organization.

International standard ISO 22000 only consider concerns about food safety issues but not discouraged by the organization and integration of other aspects of the management system elements such as quality, in general and / or environmental protection.

Organizations that wish to take into account such matters can do by implementing the international standard EN ISO 22000 with ISO 9001: 2001 and / or SR EN ISO 14001:2005.

Food safety and standards issues in the field have been widely presented and discussed at the European Conference which provided the occasion attended by economic agents to understand the priorities for developing a safe business based on the new market requirements.

Results and discussions

A. Detection of genetically modified organisms and foods derived there from

Today, varieties of soybean, cotton, corn and canola are genetically modified products on 25% of cultivated areas in the world. Genes in these plants were introduced to confer resistance to certain herbicides or toxic products used to control specific insects. In the United States produced nearly two-thirds of the yields obtained by bio-engineering globally. Other countries where crops with genetic alterations are major quantities are: Argentina, Canada, Brazil, China and South Africa.

ISO standards to be developed will be relevant globally and will facilitate international trade and services by providing a consistent international approach to detection and analysis of foods derived from genetic organisms (GMOs). This will allow the storage of seed industry, seed producers, grain, food manufacturers and analysis laboratories to meet the labeling provisions that have been developed by many countries around the world or who are currently in the planning stage, being control tool used by food control authorities in conformity with legal requirements.

B. Labeling provisions

When the United States exported for the first time in 1996, in Europe, soy genetic changes, the European Union in response to public debate, introduced obligatory labeling for foods derived from organisms with genetic changes. In April 2004 new legislation took effect that extended the labeling provisions. Other governments, following the example of the European Union have developed labeling provisions for food derived from genetically modified food. Whenever food labeling approaches with different genetic changes greatly from one country to another.

C. Detection of genetic changes in food

Understand range of DNA technology, which led to obtaining genetic organisms, allowed the development of detection methods and precise carecteristice. To determine if a food has been produced from a GMO, the method chosen was on search sites modified DNA segments and / or new proteins resulting from genetic modification. The following figure schematically illustrates the detection of materials derived from organisms with genetic modifications in food. The starting point is an adequate sampling to obtain a representative sample of the product investigated. The next step is extraction of protein or DNA in the sample studied. Protein extracts are taken at random. Extracted DNA analysis is subject to RPL - polymerase chain reaction.

Conclusions

Romanian producers are forced to obtain quality certificates from international bodies. Certificates issued by RENAR national laboratory accreditation body for certification of Romanian food, are not recognized by the European Union.

Testing laboratories, certification bodies and systems management product certification bodies can not issue valid certificates abroad. European Union withdrew its accreditation a few months ago Accreditation Association Romania - RENAR national laboratory accreditation body for testing / analysis, metrological calibration, inspection bodies and certification (quality management system / environment, HACCP, product / services and personnel).

REASONS. The suspension was decided, because there were non-compliance. Staff have the competence and deficiencies that have not provided evidence to ensure traceability of measurement results. “After all converge to the staff. It is an acute need to RENAR staff, “said association president, Prof. Fanel Iacobescu, which is part of the new management team named after finding problems by international bodies.

LACK OF SANCTIONS. Problems would not have occurred if the certification bodies would have had a duty under the rules or if RENAR would be sanctioned in the wrong, admit president. The new president does not recognize that sanctions have been applied RENAR them Romanian certification bodies have not made the right job. It is almost certain that the sanctions now come also. “We will decide in two weeks for the areas requested the assessment. It can be done in one year from the date of request. Good for entry into the EU we have many areas, “Iacobescu estimates, which estimates that international organizations that provide reliable current RENAR part management.

ABROAD. Until then, those seeking internationally recognized certificates are forced to go under another umbrella and be

certified or accredited to an international body. " Unfortunately, the letter of credit was suspended creditor monitoring. Customers are confused between a suspension of international recognition of the limited term of the right body to accredit certification bodies in Romania. Right to be a full member here, in Romania, took not one, "said Mariana Ionescu, general manager of SIMTEX - Certification Body. Now manager SIMTEX OC desire is to accredit one of the most powerful bodies in the EU.

LOCAL brake EXPORTS "Most Romanian exporters have made accreditation certification to institutions abroad, precisely because there was a lack of confidence in Romanian bodies certification, believes John Cezar Coraci, president of UGIR 1903, member organization in the College RENAR beneficiaries. He estimates that non-recognition is a major European brake Romanian export as these problems is particularly important if you want to balance the trade balance, "says John Cezar Coraci, president UGIR 1903, an organization bringing together major employers in the industry.

What are quality standards ?

RULES. Alcohol manufacturers are required to have ISO 9001 and HACCP.

ISO 9001 - Quality management systems - a set of rules and procedures that an organization always respect them. "Unfortunately, the confusion at the consumer level. The fact that a manufacturer has not certified management system tells me, as a consumer, that products are of quality. He says that control of product manufacturing and keep them well under control and that the product is always the same. This is why some manufacturers who know the confusion and write the note forces ISO 9001 certified products. It is not very fair for the product is not certified, "says general manager RENAR Cristian Dorin Nichita PhD.

ISO 14001 - This standard refers to the management of environmental issues, specifically what makes the organization to minimize adverse environmental effects caused by its activities and to continuously improve environmental results.

HACCP MANAGEMENT SYSTEM - It is a system that identifies, evaluates and control the risks relevant to food safety.

Accreditation SPEED AGAINST TIME - directors will certificates quickly. Now the fashion is the food. Alcohol-producing companies are obliged by law to obtain the tax stamp and have ISO 9001 certification risk. Another legislative regulations require food companies to have implemented a system and risk analysis and critical control points.

2000 EURO.

Both ISO 9001 accreditation costs for a form with 40 employees, the cost is around 1500 euros, plus EUR 500 for annual monitoring. Fees for certification are based on the number of auditors audit the number of days and number of personnel in the organization to be audited. There is no fixed price once.

Top ISO 9001

The biggest fans of quality management systems and of the environment are Europeans. They have 48.76% and 43.96% of the certificates. U.S. has only 37,285, even less than Spain by nearly 40,000 certificates. Great amateur certification is and China.

It has, moreover, ranking in the country with 132 926 certificates issued.

THE WORLD

China 132, 926
Italy 84 ,485
United Kingdom 50 ,884
Japan 48,989
Spain 40, 972

IN EUROPE

Romania 5183
Bulgaria 1685
Czech Republic 10 ,781
Hungary 10, 207
Poland 5753
Slovakia 2008USA 37, 285

References

» Ardelean, D. 2002 - *„Probleme de securitate alimentară şi integrarea României în Uniunea Europeană”*, Editura ASE, Bucureşti;

» Banu, C. si colab. 2002 - *„Manualul inginerului de industrie alimentară”*, vol. I şi II, Editura Tehnică, Bucureşti;

» Basdekis, J. C. 1999 - *„Ghid de dietetică”*, Editura Polirom, Iaşi;

» Diaconescu, I. 1998 - *„Merceologie alimentară”*, Editura Eficient, Bucureşti;

» Diaconescu, I. 2002 - *„Funcţiile produsului alimentar”*, Editura ASE, Bucureşti;

» Diaconescu, I. 2007 - *„Merceologie alimentară”*, Editura Qlassrom, Bucureşti, 2004;

» Viezuină, V. 2000 - *„Securitatea alimentară în lumina exigenţelor actuale”*, Tribuna Economică, nr. 2/12 ian;

» Zamfir, I. 2000 - *„Protecţia consumatorilor de la A la Z”*, Editura Premier, Ploieşti;

» Zamfir, I. 2002 - *„Protecţia consumatorilor pe înţelesul tuturor”*, Editura Dacia AS, Ploieşti;

*** FAO, 2004 - *„The State of Food Insecurity in the World 2004”*, FAO, Rome;
*** http://apps.fao.org
*** http://departments.oxy.edu/uepi.schoolfoodschecklist.htm

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