Investeşte în oameni !
FONDUL SOCIAL EUROPEAN
Programul Operaţional Sectorial Dezvoltarea Resurselor Umane 2007 – 2013
Axa prioritară: 1. „Educaţia şi formarea profesională în sprijinul creşterii economice şi dezvoltării societăţii bazate pe cunoaştere"
Domeniul major de intervenţie: 1.5 „Programe doctorale şi postdoctorale în sprijinul cercetării"
Titlul proiectului: „Integrarea cercetării româneşti în contexul cercetării europene – burse doctorale"
Numărul de identificare al contractului: POSDRU/88/1.5/S/ID 60370

ABSTRACT OF THE PhD THESIS: Reserches about the influence of the chronic and occasional wine consumption in bone healing

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Sibiu, 2014

PERSONAL PART

Preamble

This research was done in accordance with the current legislation. The studies were done taking into consideration all ethical aspects (aspects that grant them legitimacy) and obeying the law no. 677/2001 for the protection of people regarding personal data processing and free circulation.

Research objectives

Starting from the data presented in the general part of this paper and taking into account that during bone consolidation (callus formation) there are two important stages considering the vascular factors, respectively the inflammatory stage and the vascular invasion stage on the one hand, and the existence of biologically active compounds in the composition of wine (compounds that influence inflammation and vascular development) on the other hand, we can conclude that there is certainly a relation between wine consumption and bone consolidation or bone structure. Considering the aforementioned idea, in doing this research we set the following objectives:

- Setting definition thresholds for occasional and chronic wine consumption, according to literature data;
- Investigating the way in which the chronic or occasional wine consumption influences bone quality in relation to non-consumer population;
- Investigating the way in which the chronic or occasional wine consumption influences the shape of fracture lines at the level of distal extremity of the radius in relation to non-consumer population;
- Investigating the way in which the chronic or occasional wine consumption influences the secondary displacement inside the fracture focal point at the level of distal extremity of the radius in relation to non-consumer population;
- Investigating the way in which the chronic or occasional wine consumption influences the functional recovery at the wrist level after fracturing the distal extremity of the radius in relation to non-consumer population;
- Introducing the results into a statistical analysis program;
- Determination of fenolic composition for a number of 5 wines with controlate origin;
- Determination of metallic composition for a number of 5 wines with controlate origin;

Chapter 2 Material and method

In this paper, a number of 3773 cases of distal radius fracture were analyzed out of a total of 21512 cases diagnosed with limb fractures. As we can see, the percentage of distal radius fractures in the total number of fractures is of 17.53%, a little over the average of 15% reported in literature. This series of cases was registered between November 2009 and February 2014.

A number of 366 of these cases were hospitalized and underwent surgery. The rest of 3407 cases underwent conservative treatment.

Selection Criteria

Depending on the desired objective, the cases were selected based on age categories to eliminate the influences caused by the setting in of osteoporosis.

The cases which received medication that favors the setting in of osteoporosis were excluded.

During the functional evaluation, the cases for which the conservative treatment was changed to surgery were excluded.

The cases of high energy initial trauma were eliminated from the study batch.

The cases in which the plaster cast was cut to relieve the compression phenomena were not taken into consideration for the functional evaluation and the evaluation of secondary displacements.

If, after the orthopedic reduction for the patients who came late to the orthopedics-traumatology service, there were no satisfactory radiological results, then these cases were not taken into account for further evaluations.

The cases with psychiatric disorders or mental retardation as associate pathology that hindered the undergoing of an adequate functional rehabilitation program, were also excluded from the study batch.

The deaths happened during the evaluation period, the iterative fractures were excluded from the batches of functional evaluation.

The cases in which there was noticed moderate or chronic consumption of other alcoholic drinks were eliminated from the study batches.

The cases in which there was noticed occasional consumption of other alcoholic drinks were registered as non-consumers.

Therapeutic Protocol

The cases included in the current study followed the following treatment protocol:

- In the case of the conservative treatment option, the immobilization was achieved through plaster casting. For stable fractures, without displacement, immobilization with antebrachial-palmar plaster cast for four weeks was used. For unstable fractures that also had displacements, orthopedic reduction through ligamentotaxis was used under local anesthesia with Xylin 1% and immobilization with antebrachial-palmar plaster cast in a position of hand flexion and ulnar deviation depending on the type of fracture and the degree of displacement, for a period of three weeks. After the 21 days check-up, the plaster cast was changed with another in a straight position for another three weeks. In the case of distal radius fractures associated with distal ulna fracture, there was immobilization with brachial-palmar plaster cast, respecting the immobilization periods dependent on the displacements and the stability of the lines. If there was noticed a precocious secondary displacement, the following were undertaken:
- The radiological evaluation – was done both during the immobilization period and periodically during the functional recovery period. The radiological assessment consisted in radiographs made after the fracture reduction, after 21 days, at the cast removal, at the check-ups of one, two, three six and twelve months after cast removal. The rest of the examinations were fluoroscopic together with the last taken x-ray image. The critical period for the unstable fractures, that is the first 21 days, required radiological assessments in accordance with the watching protocols, at two, four, six, eight, ten and respectively, fourteen days.

In case of unacceptable deterioration of the reduction of the fracture focus in comparison to the previous check-up or to the immediate post-reduction aspect, a control x-ray image was taken. Next, the cast was removed and the reduction methods were resumed. If the control x-ray showed acceptable reduction, the conservative treatment protocol was followed. The unacceptable reductions implied bleeding reduction and internal fixation.

- The functional rehabilitation – took place only in local physiotherapy clinics in order to have control on the similarity of the gymnastics programs. During the immobilization period all cases followed an intensive program of finger gymnastics, both free and half-load exercises (the prehension was tested using a tennis ball). The use of elastic circles or other force elements in the gymnastics program was forbidden, in order to prevent the occurrence of shearing forces at the fracture focus. After the immobilization was suppressed, the cases were evaluated in functional terms, and then directed towards balneal physical therapy territorial offices. Functional re-evaluation was performed on a periodic basis, with a view to quantifying progresses after 7, 14, 21 days, and one, two, three, six and twelve months.

Distal Radius Fractures Classification

There were two classifications of distal radius fractures used in the current study. The first classification used was that of the AO Foundation (Arbeitsgemeinschaft für Osteosynthesefragen). This is an alphanumeric classification which characterizes all bone fractures in the body. Although for our study it is of only didactic interest, we used this classification because it is global, world-wide accepted, and it provides the possibility of good statistical quantification of the followed cases.

The second classification used in our study was the Kapandji classification, which, although it is older and has certain drawbacks regarding the establishment of very clear therapeutic protocols, it is at many levels more suitable for the objectives of the current research. This classification divides distal radius fractures into 12 types depending on the fracture line and stability degree. As shown in other studies, the fracture line depends on the trauma energy and bone frailty. Hence, if trauma energy is invariable, then the complexity of fracture lines is in inverse ratio to bone strength and in direct ratio to its frailty, which suits completely the objectives of this study.

Type 0 fracture in Kapandji classification is the complete fracture of the inferior extremity of distal radius, without displacement. It is considered to be a stable fracture without the risk of secondary displacement.

Type 1 fracture in Kapandji classification is the complete fracture of the inferior extremity of distal radius with posterior displacement. In this type of fracture with displacement, the distal fragment of the focus has an angular posterior displacement. Thus, between the two fragments there is an obtuse angle opening towards the dorsal face. In this type of fracture, the line is unique without compaction or comminution at the posterior cortical level. After reduction, it is considered to be a stable fracture as the previous type.

Type 2 fracture in Kapandji classification is similar to the previous type, but in this case there is comminution (fragmentation or compaction) at the level of posterior cortical. Also, there may or may not be a third fragment dislocated at the level of inferior radioulnar joint. It is considered to be an unstable fracture.

Type 3 fracture in Kapandji classification is the fracture in which the distal fractured fragment displays an additional fracture line which divides it into two fragments in frontal plane, an anterior one and a posterior one. This fracture is also called fracture with frontal or bimarginal T. In is an unstable fracture.

Type 4 fracture in Kapandji classification is the fracture in which the distal fractured fragment displays an additional fracture line which divides it into two fragments in sagittal plane, a medial one and a lateral one. This type of fracture also belongs to the category of unstable fractures. However, it is considered that, after reduction, the ligamentotaxis provides a higher degree of stability comparing to the previous type.

Type 5 fracture in Kapandji classification is also called the radial styloid fracture. In this type of fracture only the radial styloid is affected. The fracture line inside the fracture

focus may be simple or comminuted. The comminuted lines usually imply high energy trauma. This type of fracture is considered to be stable.

Type 6 fracture in Kapandji classification is the posterior marginal fracture. If the fracture line affects less than 25% of the articular surface, then the fracture is considered stable after reduction (through ligamentotaxis). If more than 25% of articular surface is affected, then the fracture is considered unstable.

Type 7 fracture in Kapandji classification is the anterior marginal fracture. Generally speaking, this is considered to be an unstable fracture. However, there are authors who state that if the fracture line affects less than 25% of the articular surface, then the fracture is considered stable after reduction (through ligamentotaxis) while, if more than 25% of articular surface is affected, then the fracture is considered unstable. In our study we considered this type to be unstable.

Type 8 fracture in Kapandji classification is the complete fracture of the inferior extremity of distal radius with anterior displacement. In this type of fracture with displacement, the distal fragment of the focus has an angular anterior displacement. Thus, between the two fragments there is an obtuse angle opening towards the ventral face. In this type of fracture, the line is usually unique but there may be compaction or comminution at the anterior cortical level. After reduction, it is considered to be an unstable fracture.

Type 9 fracture in Kapandji classification is considered to be the largest fracture. It is schematically described as being the x-shaped fracture of the distal radial extremity. In fact the distal fragment of the focus presents two fracture lines in each of the two planes: frontal and sagittal. Thus, the distal extremity of the radius is divided into 4 fragments: medioventral, mediodorsal, lateroventral and laterodorsal. It must be said, however, that this anatomic-pathological aspect rarely occurs in current practice because, in turns, each of the 4 fragments have their own fracture lines, the distal radial extremity looking like an exploded epiphysis. As far as stability is concerned, this type of fracture has a high degree of instability; there is a very high risk of secondary displacements.

Type 10 fracture in Kapandji classification is defined as any of the previous types to which a fracture of the lower extremity of the ulna is added. The most frequent associations are with the types 2, 4 and 9. This type of fracture has a high degree of instability, being considered a fracture of the lower third of the forearm.

Type 11 fracture is in fact the vicious callus of the distal radius.

The Functional Assessment of the Cases

The cases included in this paper were evaluated periodically after the immobilization was suppressed. The functional assessments were on a weekly basis in the first month and then took place after 2, 3, 6 and 12 months. The parameters that were quantified were: pain perception, the amplitude of the flexion-extension movement and the amplitude of the adduction-abduction movement. The functional evaluation was needed to be able to assess the clinical evolution during the functional rehabilitation period.

Additionally, we used the MAYO score (Mayo Wrist Performance Score) for assessing the wrist function. This score follows four aspects in evaluation the fist: **pain perception, functional status, the movement amplitude** comparing to the opposite normal limb or, if this is not possible, the movement amplitude through measuring the described arches, and **the prehension**. It is very important to assess the functional status for the dominant limb.

The points are awarded as follows:

- Section 1 – Pain perception
  - Non-painful fist 25 p
  - Small occasional pain 20 p

- Moderate, tolerable pain 15 p
- Severe, intolerable pain 0 p

- Section 2 - functional status
  - completely reintegrated in work field 25 p
  - limited work capacity 20 p
  - able to work but unemployed 15 p
  - unable to work because of pain 0 p
- Section 3a – Movement amplitude comparing to normal limb
  - 100 % 25 p
  - 75-99 % 15 p
  - 50-74 % 10 p
  - 25-49 % 5 p
  - 0-24 % 0 p
- Section 3b – Movement amplitude assessed only for affected member
  - More than 120^{o} 25 p
  - $90^{o} - 120^{o}$ 15 p
  - $60^{o} - 90^{o}$ 10 p
  - $30^{o} - 60^{o}$ 5 p
  - Less than 30^{o} 0 p
- Section 4 - Prehension
  - 100 % 25 p
  - 75-99 % 15 p
  - 50-74 % 10 p
  - 25-49 % 5 p
  - 0-24 % 0 p

Score interpretation:

- over 90 points - very good (excellent)
- 80-89 points - good
- 60-79 points - satisfactory
- Sub 60 points - unsatisfactory

For the Mayo score, prehension was measured using a hydraulic dynamometer.

Separately, prehension was quantified with the help of a method I imagined and used for some time. This is done as follows: the patients from the sitting down position, with the forearm flexed on the arm at 90^{0} and propped on a hard plane in such a way that the wrist is free at the edge of the hard plane, and with the palm in complete pronation, held in hand a tennis ball with a hook on which various weighs were hung. The evaluation of the prehension function was done in the following way:

- "-" if the patient did not succeed to hold the 500g weigh for 20 seconds
- "+" if the patient succeeded to hold the 500g weigh for 20 seconds
- "+ +" if the patient succeeded to hold the 1000g weigh for 20 seconds
- "+ + +" if the patient succeeded to hold the 2000g weigh for 20 seconds

Assessing the alcohol consumption

To achieve the goals set by the current research, we have to clearly define the categories of wine consumers. The best evaluation method is through assessing the alcohol

quantity ingested by the subjects. This was done starting from the studies of research teams concerning the thresholds of alcohol ingestion in relation to its harmfulness. The harmfulness is mainly caused by the effect of the ethanol on the human liver.

Thus, we defined four study batches respecting the following definitions:

1. The chronic alcohol consumer is the subject who ingests an amount of at least 60g of pure alcohol every day. Taking into account that wine has an average concentration of 11% of volume, and the density of the pure alcohol is 0.8, it results that a daily amount of 750ml of wine would have to be consumed to get over the threshold;
2. The moderate wine consumption is defined as the daily ingestion of an amount of 30 to 60g of pure alcohol. Converting this amount into ml of wine, we got a value between 400 and 750ml to define the moderate consumption of this liquor;
3. The occasional wine consumer is defined as the subject who ingests an amount of pure alcohol that is less of 30ml pure alcohol. This consumption, converted to ml of wine, implies an amount of no more than 400ml. It has to be said that for this category of subjects, there has to be alcohol consumption for at least four days each week;
4. The sporadic consumer or the non-consumer represents the category of subjects who drink alcohol only sporadically, does not commit excesses and for which the alcohol consumption is present in no more than three days a week.

Observations:

- The amounts of alcohol previously mentioned refer to a daily average of alcohol consumption and does not imply the obligation of daily consumption;
- The amounts of alcohol previously defined are to be reduced to a half for female subjects, according to the international conventions concerning the assessment of alcoholism and its effects;
- Some authors accept the definition according to which the occasional consumer is the one who does not exceed the amount of 10g of pure alcohol every day, accepting also the daily consumption. As far as we are concerned, we included this latest category of consumers in the occasional consumers group since the object of the study is to assess the effects of the metabolically active compounds and not to evaluate the effects of alcohol on the subjects. From this point of view we consider that the daily ingestion of a glass of wine brings about enough resveratrol, estrogens or quercetin to be able to be considered constant consumption of substances that may influence bone metabolism.

Chapter 3 Results and Discussions

The study of the complexity of the fracture lines

As shown in the previous partial studies, there is a high rate of wine consumption among the subjects coming from rural areas. These people drink mainly wine produced in their own households.

In this paper we recorded 3773 cases of fractures of inferior distal extremity. Out of these, 2111 (55.95%) were women and 1662 (44.05%) men, the cases distribution being normal within general population. As far as age groups distribution is concerned, there is a tendency of this type of fracture towards the third age group.

The fractures were divided into study batches according to the Kapandji classification and the categories of wine consumers.

Kapandji Classification	Non-consumers of wine	Occasional wine consumers	Moderate wine consumption	Chronic wine consumption	Total cases
Type 0	226	132	73	76	507
Type 1	148	99	66	73	386
Type 2	343	123	51	50	567
Type 3	346	51	41	38	476
Type 4	341	66	48	46	501
Type 5	25	10	21	40	96
Type 6	14	13	11	13	51
Type 7	17	20	19	12	68
Type 8	7	8	7	7	29
Type 9	810	68	79	94	1051
Type 10	39	0	1	1	41

From the complexity point of view, fractures of types 3, 4, 9 and 10 are considered complex, while the others are considered simple. The distribution of fractures according to their complexity is rendered in table.

Fracture type	Non-consumers of wine	Occasional wine consumers	Moderate wine consumption	Chronic wine consumption	Total cases
Simple fractures	780	405	248	271	1704
Complex fractures	1536	185	169	179	2069

From the tabel, it is apparent that in the case of complex fractures, the incidence is lower for wine drinkers. The aforementioned data were introduced into a statistic analysis program.

Functional Results

The functional results were assessed for only 6 of the Kapandji classification types of fractures of distal radius extremity; these are types 0, 1, 2, 3, 4 and 9. The other types of fractures, even if their results were quantified, will not be the subject of these discussions since we consider that the batches are not representative in order to provide accurate assessment.

Functional results for the batches with type 0 fracture according to the Kapandji classification

In the batch for which the functional results of Kapandji type 0 fractures were quantified, after eliminating the cases who abandoned the functional rehabilitation program, there were 412 cases left. 184 of them were sporadic consumers or non-consumers of wine or consumers of other alcoholic drinks, 108 declared they were occasional wine drinkers, 59 moderate drinkers and 61 declared they were over the threshold for alcohol chronic consumers (through wine ingestion). We mention that the plaster immobilization period for these cases was of 30 days.

The functional results for these cases after 12 months were as follows:

Batch no. 1 (non-consumers or sporadic consumers of wine)

- The flexion-extension arc was between 55° and 130°, with the median at 120°;
- The arc of the adduction-abduction movement was between 30° and 60° with the median at 52°;
- The Mayo score was between 70 and 100 points with the median at 95 points;
- The prehension: "+ + +" 170 cases; „+ +" 11 cases; „+" 2 cases; "–" for 1 case.

In this batch there was one case (chronic drinker of hard liquor) who displayed neuro-algodystrophy.

Batch no. 2 (occasional wine consumers)

- The flexion-extension arc was between 55° and 130°, with the median at 120°;
- The arc of the adduction-abduction movement was between 30° and 60° with the median at 53°;
- The Mayo score was between 80 and 100 points with the median at 95 points;
- The prehension: "+ + +" 86 cases; „+ +" 22 cases.

There were no complications in this batch.

Batch no. 3 (moderate wine consumption)

- The flexion-extension arc was between 90° and 130°, with the median at 120°;
- The arc of the adduction-abduction movement was between 50° and 60° with the median at 54°;
- The Mayo score was between 90 and 100 points with the median at 95 points;
- The prehension: "+ + +" 54 cases; „+ +" 5 cases.

There were no neuro-algodystrophy cases in this batch.

Batch no. 4 (chronic wine consumers)

- The flexion-extension arc was between 55° and 130°, with the median at 120°;
- The arc of the adduction-abduction movement was between 30° and 60° with the median at 53°;
- The Mayo score was between 80 and 100 points with the median at 95 points;
- The prehension: "+ + +" 45 cases; „+ +" 14 cases; "+" 2 cases.

The neuro-algodystrophy did not occur in any of the cases in this batch.

As it can be seen, the functional rehabilitation gave good and very good results for all 4 batches. All batches displayed similar values without big differences between the quantified parameters. There must be said, though, that for the moderate wine drinkers, the results were closer to *"restitution ad integrum"*.

Functional results for the batches with type 1 fracture according to the Kapandji classification

After eliminating the cases who abandoned the functional rehabilitation program and those whose reduction was inacceptable or could not be achieved after secondary displacements, the batch with Kapandji type 1 fractures of the inferior radius extremity included 313 cases. 120 of them were sporadic consumers or non-consumers of wine or consumers of other alcoholic drinks, 80 declared they were occasional wine drinkers, 54

moderate drinkers and 59 declared they were over the threshold for alcohol chronic consumers (through wine ingestion). We mention that the plaster immobilization period for these cases was of 21+21 days according the protocol.

The functional results for these cases after 12 months were as follows:

Batch no. 1 (non-consumers or sporadic consumers of wine)

- The flexion-extension arc was between 45° and 130°, with the median at 110°;
- The arc of the adduction-abduction movement was between 25° and 60° with the median at 45°;
- The Mayo score was between 60 and 100 points with the median at 90 points;
- The prehension: "+ + +" 44 cases; „+ +" 69 cases; „+" 5 cases; "–" for 2 cases.

In this batch there were two cases (chronic consumption of hard liquor) who displayed neuro-algodystrophy.

Batch no. 2 (occasional wine consumers)

- The flexion-extension arc was between 80° and 130°, with the median at 110°;
- The arc of the adduction-abduction movement was between 30° and 60° with the median at 46°;
- The Mayo score was between 70 and 100 points with the median at 90 points;
- The prehension: "+ + +" 60 cases; „+ +" 18 cases; "+" 2 cases.

There were no complications in this batch.

Batch no. 3 (moderate wine consumption)

- The flexion-extension arc is between 90° and 130°, with the median at 120°;
- The arc of the adduction-abduction movement is between 50° and 60° with the median at 54°;
- The Mayo score is between 90 and 100 points with the median at 95 points;
- The prehension: "+ + +" 46 cases; „+ +" 8 cases.

There were no neuro-algodystrophy cases in this batch.

Batch no. 4 (chronic wine consumers)

- The flexion-extension arc was between 65° and 130°, with the median at 150°;
- The arc of the adduction-abduction movement was between 30° and 60° with the median at 50°;
- The Mayo score was between 70 and 100 points with the median at 90 points;
- The prehension: "+ + +" 37 cases; „+ +" 20 cases; "+" 2 cases.

The neuro-algodystrophy did not occur in any of the cases in this batch.

As it can be seen, the functional rehabilitation gave good and very good results for all 4 batches. All batches displayed similar values without big differences between the quantified parameters. The fractures from the group of moderate wine drinkers apparently had a much better evolution, similar to that of the fractures without displacement.

Functional results for the batches with type 2 fracture according to the Kapandji classification

In the batch with Kapandji type 2 fractures of the inferior radius extremity, after eliminating the cases who abandoned the functional rehabilitation program and those whose reduction was inacceptable or could not be achieved after secondary displacements, there were 460 cases left. 278 of them were sporadic consumers or non-consumers of wine or consumers of other alcoholic drinks, 101 declared they were occasional wine drinkers, 41 moderate drinkers and 40 declared they were over the threshold for alcohol chronic consumers (through wine ingestion). The plaster immobilization period for these cases was of 21+21 days according the protocol.

The functional results for these cases after 12 months were as follows:

Batch no. 1 (non-consumers or sporadic consumers of wine)

- The flexion-extension arc was between 35° and 130°, with the median at 105°;
- The arc of the adduction-abduction movement was between 25° and 60° with the median at 42°;
- The Mayo score was between 60 and 100 points with the median at 90 points;
- The prehension: "+ + +" 103 cases; „+ +" 116 cases; „+" 53 cases; "–" for 6 cases.

In this batch there were 6 cases (chronic drinker of hard liquor) who displayed neuro-algodystrophy.

Batch no. 2 (occasional wine consumers)

- The flexion-extension arc was between 60° and 130°, with the median at 115°;
- The arc of the adduction-abduction movement was between 30° and 60° with the median at 40°;
- The Mayo score was between 60 and 100 points with the median at 90 points;
- The prehension: "+ + +" 34 cases; „+ +" 55 cases; "+" 12 cases.

There weren't any cases of neuro-algodystrophy in this batch.

Batch no. 3 (moderate wine consumption)

- The flexion-extension arc was between 80° and 130°, with the median at 110°;
- The arc of the adduction-abduction movement was between 40° and 60° with the median at 50°;
- The Mayo score was between 70 and 100 points with the median at 90 points;
- The prehension: "+ + +" 22 cases; „+ +" 19 cases.

There were no neuro-algodystrophy cases in this batch.

Batch no. 4 (chronic wine consumers)

- The flexion-extension arc was between 80° and 130°, with the median at 110°;
- The arc of the adduction-abduction movement was between 40° and 60° with the median at 50°;
- The Mayo score was between 70 and 100 points with the median at 90 points;
- The prehension: "+ + +" 20 cases; „+ +" 19 cases; "+" 1 case.

The neuro-algodystrophy did not occur in any of the cases in this batch.

As it can be seen, the functional rehabilitation gave good and very good results for all 4 batches. All batches displayed similar values without big differences between the quantified parameters. However, the fractures from the groups of moderate and chronic wine drinkers apparently had a better evolution.

Functional results for the batches with type 3 fracture according to the Kapandji classification

After eliminating the cases who abandoned the functional rehabilitation program and those whose reduction was inacceptable, the batch with Kapandji type 3 fractures of the inferior radius extremity included 387 cases. 281 cases were sporadic consumers or non-consumers of wine or consumers of other alcoholic drinks, 41 declared they were occasional wine drinkers, 34 moderate drinkers and 31 declared they were over the threshold for alcohol chronic consumers (through wine ingestion). The functional results for these cases after 12 months were as follows:

Batch no. 1 (non-consumers or sporadic consumers of wine)

- The flexion-extension arc was between 45° and 130°, with the median at 100°;
- The arc of the adduction-abduction movement was between 30° and 60° with the median at 44°;
- The Mayo score was between 20 and 100 points with the median at 80 points;
- The prehension: "+ + +" 119 cases; „+ +" 101 cases; „+" 53 cases; "–" for 8 cases.

In this batch there were 8 cases of neuro-algodystrophy.

Batch no. 2 (occasional wine consumers)

- The flexion-extension arc was between 45° and 130°, with the median at 100°;
- The arc of the adduction-abduction movement was between 30° and 60° with the median at 44°;
- The Mayo score was between 20 and 100 points with the median at 80 points;
- The prehension: "+ + +" 19 cases; „+ +" 15 cases; "+" 7 cases.

There were not any cases of neuro-algodystrophy in this study batch during the functional rehabilitation period.

Batch no. 3 (moderate wine consumption)

- The flexion-extension arc was between 60° and 130°, with the median at 100°;
- The arc of the adduction-abduction movement was between 30° and 60° with the median at 48°;
- The Mayo score was between 60 and 100 points with the median at 90 points;
- The prehension: "+ + +" 20 cases; „+ +" 13 cases; "+" 1 case.

There were no neuro-algodystrophy cases in this batch.

Batch no. 4 (chronic wine consumers)

- The flexion-extension arc was between 60° and 130°, with the median at 100°;
- The arc of the adduction-abduction movement was between 30° and 60° with the median at 42°;
- The Mayo score was between 60 and 100 points with the median at 90 points;
- The prehension: "+ + +" 15 cases; „+ +" 15 cases; "+" 1 case.

Neuro-algodystrophy cases did not occur in the evolution of the chronic wine drinkers with fracture of inferior radius extremity of type 3 according to Kapandji.

As it can be seen, the functional rehabilitation (in absolute values) gave satisfactory and good results for all 4 batches. The results of chronic and moderate wine drinkers were slightly better. The poorest functional results were registered for the batch no. 1, the group of non-consumers, sporadic consumers and consumers of other alcoholic drinks.

Functional results for the batches with type 4 fracture according to the Kapandji classification

In the batch for which the functional results of Kapandji type 4 fractures were quantified, after eliminating the cases who abandoned the functional rehabilitation program, there were 407 cases left. 227 of them were sporadic consumers or non-consumers of wine or consumers of other alcoholic drinks, 52 declared themselves as occasional wine drinkers, 40 moderate drinkers and 38 declared they were over the threshold for alcohol chronic consumers (through wine ingestion). The functional results for these cases after 12 months were as follows:

Batch no. 1 (non-consumers or sporadic consumers of wine)

- The flexion-extension arc was between 45° and 130°, with the median at 105°;
- The arc of the adduction-abduction movement was between 30° and 60° with the median at 43°;
- The Mayo score was between 40 and 100 points with the median at 85 points;
- The prehension: "+ + +" 86 cases; „+ +" 139 cases; „+" 50 cases; "–" for 2 cases.

There were 2 cases with neuro-algodystrophy in this batch.

Batch no. 2 (occasional wine consumers)

- The flexion-extension arc was between 45° and 130°, with the median at 100°;
- The arc of the adduction-abduction movement was between 30° and 60° with the median at 45°;

- The Mayo score was between 50 and 100 points with the median at 85 points;
- The prehension: "+ + +" 18 cases; „+ +" 26 cases; "+" 8 cases.

There were not any cases of neuro-algodystrophy in this study batch during the functional rehabilitation period.

Batch no. 3 (moderate wine consumption)

- The flexion-extension arc was between 60° and 130°, with the median at 100°;
- The arc of the adduction-abduction movement was between 30° and 60° with the median at 48°;
- The Mayo score was between 60 and 100 points with the median at 90 points;
- The prehension: "+ + +" 21 cases; „+ +" 18 cases; "+" 1 case.

There were no neuro-algodystrophy cases in this group.

Batch no. 4 (chronic wine consumers)

- The flexion-extension arc was between 60° and 130°, with the median at 100°;
- The arc of the adduction-abduction movement was between 30° and 60° with the median at 42°;
- The Mayo score was between 60 and 100 points with the median at 90 points;
- The prehension: "+ + +" 11 cases; „+ +" 24 cases; "+" 3 cases.

Neuro-algodystrophy cases did not occur in the evolution of the chronic wine drinkers.

Functional results for the batches with type 9 fracture according to the Kapandji classification

After eliminating the cases who abandoned the functional rehabilitation program and those whose reduction was inacceptable, for the Kapandji type 9 fractures of the inferior radius extremity 849 cases were registered. Out of which, 654 cases were sporadic consumers or non-consumers of wine or consumers of other alcoholic drinks, 52 declared themselves as occasional wine drinkers, 67 moderate drinkers and 76 declared they were over the threshold for alcohol chronic consumers (through wine ingestion). The functional results for these cases after 12 months were as follows:

Batch no. 1 (non-consumers or sporadic consumers of wine)

- The flexion-extension arc was between 15° and 130°, with the median at 85°;
- The arc of the adduction-abduction movement was between 5° and 60° with the median at 25°;
- The Mayo score was between 20 and 100 points with the median at 80 points;
- The prehension: "+ + +" 201 cases; „+ +" 214 cases; „+" 218 cases; "–" for 21 cases.

There were 16 cases of neuro-algodystrophy in this lot. This complication occurred exclusively for the subjects who admitted they were chronic hard liquor drinkers.

Batch no. 2 (occasional wine consumers)

- The flexion-extension arc was between 30° and 130°, with the median at 85°;
- The arc of the adduction-abduction movement was between 14° and 60° with the median at 29°;
- The Mayo score was between 40 and 100 points with the median at 85 points;
- The prehension: "+ + +" 15 cases; „+ +" 21 cases; "+" 11 cases; "-" for 5 cases.

There was only 1 case of neuro-algodystrophy in this study batch during the functional rehabilitation period.

Batch no. 3 (moderate wine consumption)

- The flexion-extension arc was between 60° and 130°, with the median at 90°;
- The arc of the adduction-abduction movement was between 25° and 60° with the median at 35°;
- The Mayo score is between 60 and 100 points with the median at 90 points;

- The prehension: "+ + +" 19 cases; „+ +" 34 cases; "+" 11 cases; "-" for 3 cases.
  There were no neuro-algodystrophy cases in this batch.

Batch no. 4 (chronic wine consumers)

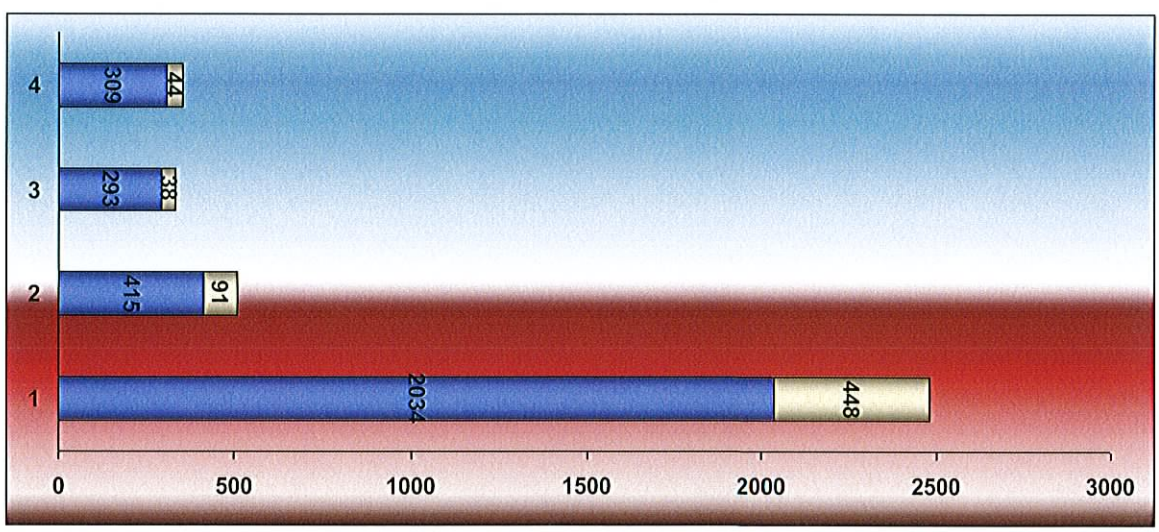
- The flexion-extension arc was between 60° and 130°, with the median at 90°;
- The arc of the adduction-abduction movement was between 25° and 60° with the median at 35°;
- The Mayo score was between 60 and 100 points with the median at 90 points;
- The prehension: "+ + +" 22 cases; „+ +" 34 cases; "+" 15 cases; "-" for 5 cases.

Two neuro-algodystrophy cases occurred in the evolution of the chronic wine drinkers with fracture of inferior radius extremity of type 9 according to Kapandji.

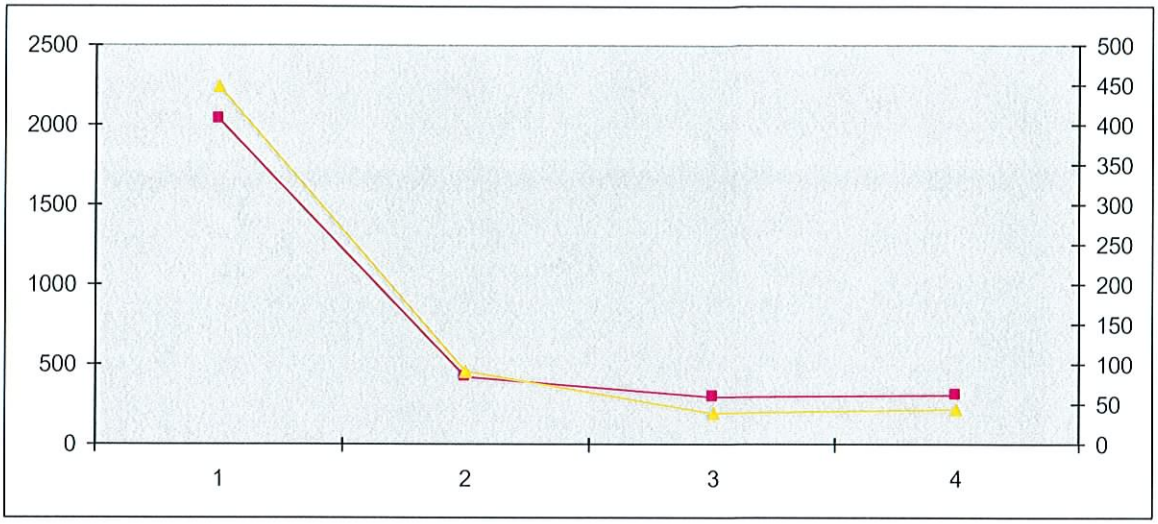
As it can be seen, the functional rehabilitation (in absolute values) gave satisfactory and good results for all 4 batches. The results of chronic and moderate wine drinkers were slightly better. The poorest functional results were registered for the batch no. 1, the group of non-consumers, sporadic consumers and consumers of other alcoholic drinks.

The secondary displacement

In the course of the evolution of the cases, a series of secondary displacements occurred inside the plaster cast. Depending on the moment of their occurrence and the possibilities of conservative correction, these displacements were kept or not in the general batch for study. Thus, if a secondary displacement occurred in less than 10 days from the trauma moment, the reduction and immobilization were resumed. If not, the case underwent surgery and was implicitly excluded from the batch. If the secondary displacement was small, respecting the criteria of good reduction, the case was x-ray monitored during the next period, further therapeutic actions being held back. If, after the reduction of the secondary displacement of the fracture focus, we noticed, at the moment of the radiological check, an unacceptable reduction from the point of view of reduction criteria, the case was redirected towards surgery and excluded from the study lot. The total number of displaced fractures which needed reduction was 3051. Out of these, 621 cases had secondary displacements as follows: 448 cases were sporadic consumers or non-consumers of wine or consumers of other alcoholic drinks; 91 declared themselves as occasional wine drinkers; 38 moderate drinkers and 44 declared the overpass of the threshold for chronic alcohol consumption (through wine ingestion). The data are shown in the following charts:



Batch distribution of cases with secondary displacements



Comparative distribution of cases with and without secondary displacement

As shown in the two charts, there is certainly a difference between the rates of secondary displacements of the 4 categories of consumers. Thus, for the non-consumers and the occasional wine consumers, the rate of this complication reaches about 21-22%, while for the moderate and chronic wine consumers the rate of secondary displacements is about 13-14%. Probably, the differentiating mechanism is at the level of the formation and organization of the fracture hematoma, since there is generally agreed that various wine compounds have anti-inflammatory propreties.

Chapter 4 Statistical Analysis

In order to get a preliminary idea on the relationship between the fracture complexity and the different types of wine consumers, we are using an exploratory descriptive technique: the correspondence analysis. The results provided by this technique are of a similar nature to the ones of the factorial analysis. The Correspondence analysis is a technique that is used only for categorical variables, allowing us to explore the structure of the variables and the relationships between them.

Kapandji Classification	Wine non-drinkers	Occasional wine drinkers	Moderate wine consumption	Chronic wine consumption	Total cases
Type 0	226	132	73	76	507
Type 1	148	99	66	73	386
Type 2	343	123	51	50	567
Type 3	346	51	41	38	476
Type 4	341	66	48	46	501
Type 5	25	10	21	40	96
Type 6	14	13	11	13	51
Type 7	17	20	19	12	68
Type 8	7	8	7	7	29
Type 9	810	68	79	94	1051
Type 10	39	0	1	1	41

Total number of cases: 3773

The results of the correspondence analysis

To construe the results, we have to consider the meaning of some values to be used in the interpretation of the results:

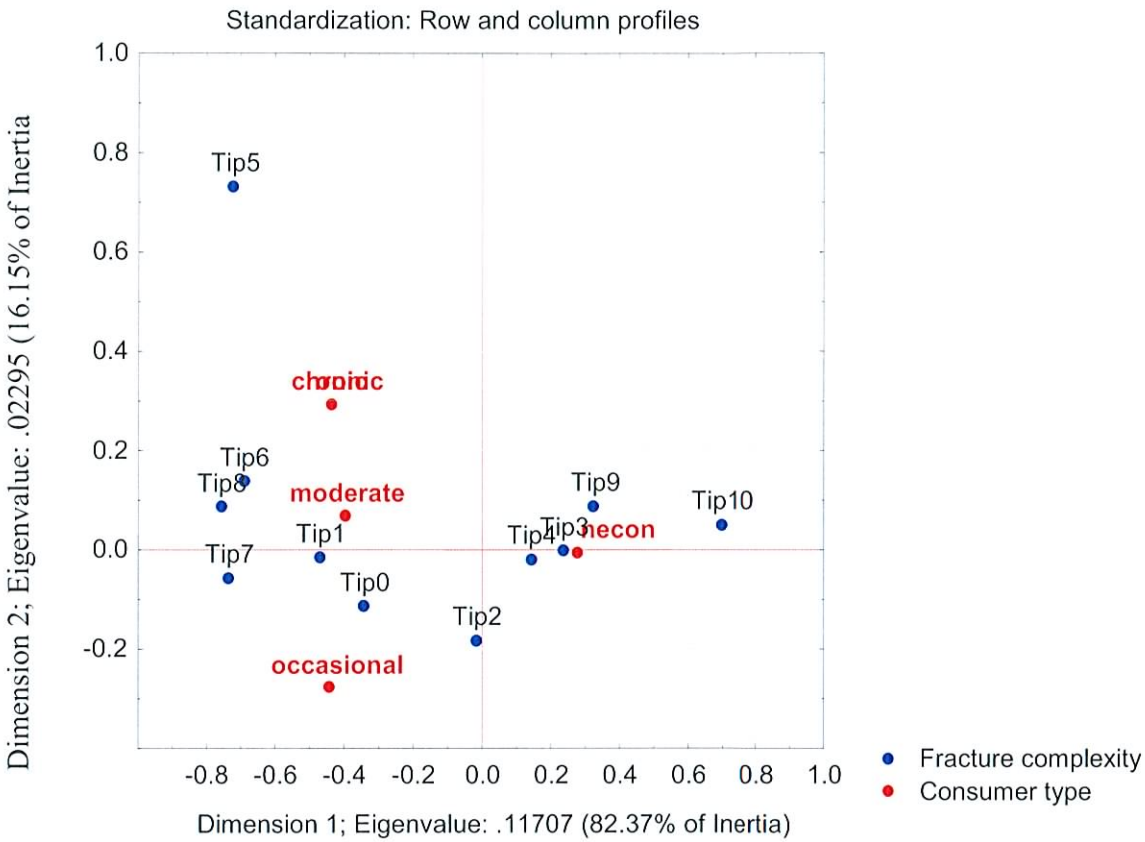
Mass = the total on each row and respectively each column, for the relative frequency table

Quality = the representation quality of a point – the higher the value, the better represented that point is

Relative inertia = the ratio out of the total inertia explained by that point

Relative inertia for each dimension = the relative contribution of that point to the inertia explained by that dimension

Cosine^2 = the point – dimension correlation. The higher its value is, the more important the determination of the dimension by that point is.



The coordinates of the columns and the contributions to the inertia

	Col. No.	Coordin. Dim.1	Coordin. Dim.2	Mass	Quality	Relative Inertia	Inertia Dim.1	Cosine^2 Dim.1	Inertia Dim.2	Cosine^2 Dim.2
Non-con.	1	0.27	0.00	0.61	1.00	0.32	0.39	1.00	0.00	0.00
occasional	2	-0.44	-0.27	0.16	1.00	0.30	0.26	0.72	0.51	0.28
moderate	3	-0.40	0.07	0.11	0.93	0.14	0.15	0.90	0.03	0.03
chronic	4	-0.44	0.30	0.12	0.99	0.24	0.20	0.68	0.46	0.31

The coordinates of the rows and the contributions to the inertia

	Row N0	Coordin. Dim.1	Coordin. Dim.2	Mass	Quality	Relative Inertia	Inertia Dim.1	Cosine^2 Dim.1	Inertia Dim.2	Cosine^2 Dim.2
Type 0	1	-0.35	-0.11	0.13	1.00	0.13	0.14	0.90	0.07	0.09
Type 1	2	-0.47	-0.01	0.10	1.00	0.16	0.20	1.00	0.00	0.00
Type 2	3	-0.02	-0.18	0.15	0.93	0.04	0.00	0.01	0.21	0.92
Type 3	4	0.23	0.00	0.13	0.99	0.05	0.06	0.99	0.00	0.00

	Row N0	Coordin. Dim.1	Coordin. Dim.2	Mass	Quality	Relative Inertia	Inertia Dim.1	Cosine² Dim.1	Inertia Dim.2	Cosine² Dim.2
Type 4	5	0.14	-0.02	0.13	0.99	0.02	0.02	0.97	0.00	0.01
Type 5	6	-0.72	0.73	0.03	0.99	0.19	0.11	0.49	0.60	0.50
Type 6	7	-0.70	0.14	0.01	1.00	0.05	0.06	0.96	0.01	0.04
Type 7	8	-0.74	-0.05	0.02	0.88	0.08	0.08	0.88	0.00	0.00
Type 8	9	-0.76	0.09	0.01	0.98	0.03	0.04	0.97	0.00	0.01
Type 9	10	0.32	0.09	0.28	1.00	0.22	0.25	0.93	0.10	0.07
Type 10	11	0.69	0.05	0.01	1.00	0.04	0.04	0.99	0.00	0.01

The first dimension, the horizontal one, has its own value of 0.11707 and explains 82.37% of total inertia. It largely separates the non-consumers of wine from the moderate ones.

The second dimension, the vertical one, has its own value of 0.2295 and explains 16.15% of total inertia. This dimension differentiates the simple fractures from the complex ones.

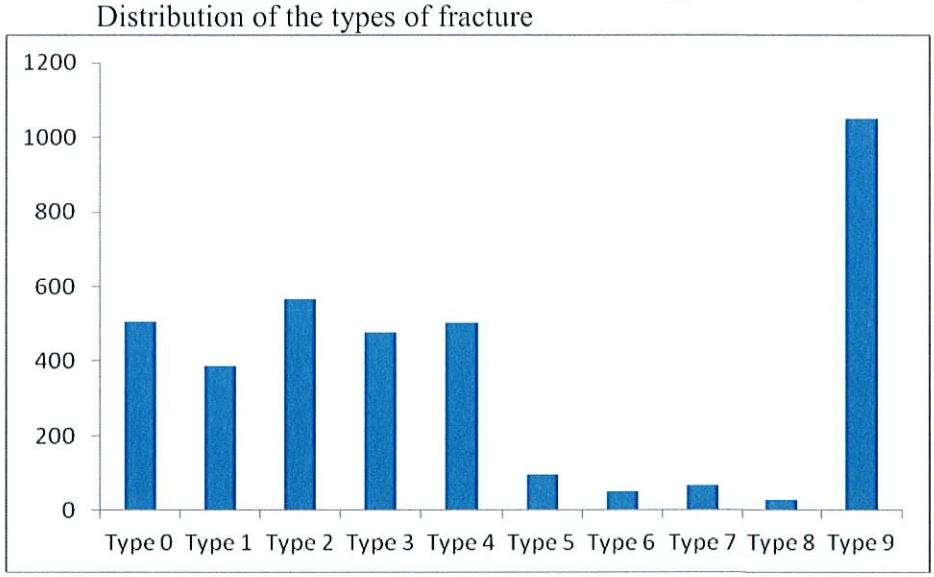
The big picture shows us that the non-consumers of wine tend to usually have complex fractures, while the other types of wine consumers tend to have simpler fractures: chronic drinkers have fractures of type 6 and 8, the moderate drinkers 1, 6 and 8 and the occasional ones 0 and 2.

Considering the fact that we are dealing with a longitudinal evaluation in which repeated measurements were made at certain time intervals, we need a more complex model to study the relation between the complexity of the fracture and the amounts of wine consumed by the future patients. A statistical method that very much suits our goal is that of generalized estimation equations. This method has a number of important advantages over other statistical models used in longitudinal studies:

- The variant does not have to be homogeneous;
- The errors can be correlated;
- The cases do not have to be independent;
- There is no need for a multivariate distribution (it treats the deviations from normality well).

We chose a model with ordinal answer with the linking function cumulative logit that is applied to the cumulative probability for each category of answer (of the dependent variable). In our model the dependent variable is the type of fracture (frak).

Distribution of the types of fracture



The independent variables are:

- lot, that keeps the distribution of wine consumers (with four categories: 1 non-consumers; 2 occasional drinkers; 3 moderate drinkers; chronic consumers). The chronic consumers represent the reference category for the study of the relationship with the fracture complexity for the other categories.
- Flexion (numerical variable)
- Adduction (numerical variable)
- Mayo (numerical variable)

By restructuring the data base, we decided to keep only the results of the first and last evaluations for our model.

The distribution of wine consumers

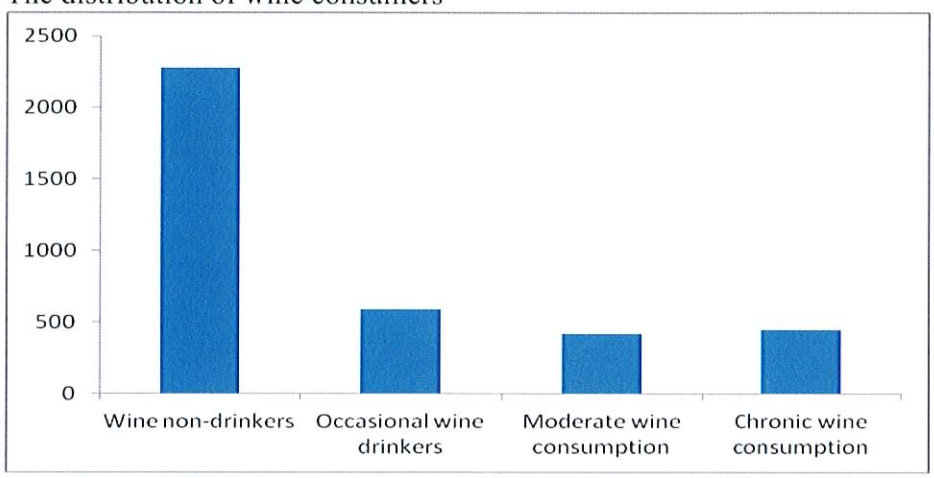


Table The results of testing the effects

Source	Type III		
	Wald Chi-Square	df	Sig.
lot	**9.421**	**3**	**0.024**
flexion	**13.083**	**1**	**0.000**
adduction	**17.654**	**1**	**0.000**
mayo	0.004	1	0.950

Having a probability of less than 0.05, only lot, flexion and adduction have significant effects.

The results of estimating the parameters

Parameter		B	Std. Error	95% Wald CI		Hypothesis Test			Exp(B)
				Lower	Upper	Wald Chi-Square	df	Sig.	
Threshold[a]	[frak=1]	-1.027	.2249	-1.468	-.587	20.873	1	.000	.358
	[frak=2]	-.129	.2201	-.560	.303	.343	1	.558	.879
	[frak=3]	.552	.2223	.116	.987	6.157	1	.013	1.736
	[frak=4]	1.297	.2338	.839	1.756	30.808	1	.000	3.660
	[frak=5]	2.288	.2587	1.781	2.795	78.235	1	.000	9.859
[lot=1]		**.725**	**.2777**	**.181**	**1.269**	**6.818**	**1**	**.009**	**2.065**
[lot=2]		**.714**	**.2836**	**.159**	**1.270**	**6.346**	**1**	**.012**	**2.043**
[lot=3]		.302	.2853	-.257	.862	1.122	1	.289	1.353
[lot=4]		0[b]	.	.	.	.	.	.	1

flexion		**.002**	**.0005**	**.001**	**.003**	**13.083**	**1**	**.000**	**1.002**
adduction		**-.005**	**.0011**	**-.007**	**-.002**	**17.654**	**1**	**.000**	**.995**
mayo		-2.01E-02	.0003	-.001	.001	.004	1	.950	1.000
Scale	1								

[a] Threshold = threshold parameters that define transition points between the categories of the dependent variable. These are always included in the model.

[b] fixed at 0 because this element is redundant.

The non-consumers and the occasional consumers of wine are associated to a higher probability of having a fracture of higher complexity. Every one of them has approximately two times more chances of suffering from complex fractures than the chronic wine drinkers. The moderate wine drinker does not display fractures of a different complexity than the chronic drinker.

As far as flexion and adduction are concerned, these have opposite but very weak effects when increasing with one unit: flexion increases the chances of a complex fracture with 1.002, while adduction decreases the chances of complex fracture with 0.995.

At first we found out, using an exploration technique – the correspondence analysis, that the non-consumers of wine are associated with fractures considered to be complex. In a later stage we could state, using the general estimation equations and taking into account the repeated assessment of the evolution of the healing process, that the non-consumers and the occasional consumers of wine have about two times more chances than the chronic consumes to be associated with a fracture of a higher degree of complexity. In the last stage we tried to determine more accurately the significant differences that occur between the types of wine consumers for the variables: flexion, adduction and the Mayo score, measured at the last assessment for each of the categories of complexity.

We experience a situation in which none of the variables has normal distribution. Quick confirmation can be gotten by a simple visual analysis of the histograms of these variables. We are presenting the histograms of the variables relevant for our study for all types of consumers depending on the complexity degree of the fracture. These variables are to be used in testing the differences between the types of consumers.

Almost no histogram comes close to the shape of the Gauss's curve, suggesting that the type of comparison to be used should be a nonparametric one. Considering the fact that we are in the presence of multiple comparisons (the significance thresholds are going to be adjusted), we chose the Krusksal – Wallis test for independent samples. In order to make the presentation of the results simpler, we are going to keep only those comparisons that are significant from the statistical point of view.

K=0 All null hypotheses (identical distributions for all categories of consumers) are accepted.

K=1

Null hypothesis	**Test**	**Sig.**	**Decision**
Flexion variable has the same distribution along the lot categories.	Kruskal – Wallis for independent samples	0.002	The null hypothesis is rejected
Adduction variable has the same distribution along the lot categories.		0.000	The null hypothesis is rejected
Mayo variable has the same distribution along the lot categories.		0.015	The null hypothesis is rejected

Variable	**Samples**	**Test statistics**	**Std. Error**	**Std. test statistic**	**Sig.**	**Adj. Sig.**

flexion	chronics– moderates	20.556	5.758	3.570	0.000	0.002
	occasional– moderates	-18.356	6.813	-2.694	0.007	0.042
Adduction	occasional– moderates	-23.511	6.862	-3.426	0.001	0.004
	Non-consumers– moderates	-22.433	6.200	-3.618	0.000	0.002
	chronics– moderates	15.694	5.800	2.706	0.007	0.041
Mayo	chronics– moderates	17.833	5.606	3.181	0.001	0.009

The following alternative hypotheses are accepted:
Flexion: The chronic consumers have significantly higher values than the moderate ones and the moderate ones have significantly higher values than the occasional consumers.
Adduction: The moderate consumers present significantly higher values than the occasional ones and the non-consumers and significantly lower than the chronic consumers; the chronic consumers have significantly higher scores than the moderate ones.
Mayo: The chronic consumers register higher values than the moderate ones.
K=2

Null hypothesis	Test	Sig.	Decision
Flexion variable has the same distribution along the lot categories.	Kruskal – Wallis for independent samples	0.253	The null hypothesis is accepted
Adduction variable has the same distribution along the lot categories.		0.000	The null hypothesis is rejected
Mayo variable has the same distribution along the lot categories.		0.245	The null hypothesis is accepted

Variable	Samples	Test statistics	Std. Error	Std. test statistic	Sig.	Adj. Sig.
Adducțion	occasional – non-consumers	14.957	5.606	2.668	0.008	0.046
	Occasional - moderates	-28.426	6.472	-4.392	0.000	0.000
	occasional - chronics	-28.972	6.472	-4.477	0.000	0.000

The following alternative hypotheses are accepted:
Adduction: the occasional consumers have considerably higher values than the non-consumers and significantly lower than the moderate and chronic consumers.
K=3

Null hypothesis	Test	Sig.	Decision
Flexion variable has the same distribution along the lot categories.	Kruskal – Wallis for independent samples	0.296	The null hypothesis is accepted
Adduction variable has the same distribution along the lot categories.		0.000	The null hypothesis is rejected
Mayo variable has the same distribution along the lot categories.		0.001	The null hypothesis is rejected

Variable	Samples	Test statistics	Std. Error	Std. test statistic	Sig.	Adj. Sig.
Adduction	Non-consumers–	-23.861	5.920	-4.030	0.000	0.000

	moderates					
	Occasional - moderates	-20.472	5.920	-3.458	0.001	0.003
Mayo	Occasional - moderates	-20.489	5.853	-3.500	0.000	0.003
	Non-consumers– moderates	-16.433	5.853	-2.808	0.005	0.030

The following alternative hypotheses are accepted:
Adduction: The moderate consumers present significantly higher scores than the occasional ones and the non-consumers.
Mayo: The moderate consumers register considerably higher values than the occasional ones and the non-consumers.
K=4

Null hypothesis	Test	Sig.	Decision
Flexion variable has the same distribution along the lot categories.	Kruskal – Wallis for independent samples	0.220	The null hypothesis is accepted
Adduction variable has the same distribution along the lot categories.		0.000	The null hypothesis is rejected
Mayo variable has the same distribution along the lot categories.		0.005	The null hypothesis is rejected

Variable	Samples	Test statistics	Srd. Error	Std. test statistic	Sig.	Adj. Sig.
Adduction	Non-consumers– moderates	-26.625	5.899	-4.514	0.000	0.000
	Occasional - moderates	-16.931	5.733	-2.953	0.003	0.019
Mayo	Occasional - moderates	-18.726	5.654	-3.312	0.001	0.006

The following alternative hypotheses are accepted:
Adduction: The moderate consumers present significantly higher scores than the occasional ones and the non-consumers.
Mayo: The moderate consumers register considerably higher values than the occasional ones.
K=9

Null hypothesis	Test	Sig.	Decision
Flexion variable has the same distribution along the lot categories.	Kruskal – Wallis for independent samples	0.012	The null hypothesis is rejected
Adduction variable has the same distribution along the lot categories.		0.000	The null hypothesis is rejected
Mayo variable has the same distribution along the lot categories.		0.006	The null hypothesis is rejected

Variable	Samples	Test statistics	Std. Error	Std. test statistic	Sig.	Adj. Sig.
flexion	occasional - chronics	-13.990	6.703	-2.087	0.037	**0.221**
	Occasional - moderates	-16.538	6.339	-2.609	0.009	**0.055**
	Non-consumers– chronics	-13.167	6.570	-2.004	0.011	**0.270**

Adduction	Non-consumers– chronics	-20.583	6.696	-3.074	0.002	0.013
	Non-consumers–moderates	-22.393	6.318	-3.545	0.000	0.002
Mayo	Non-consumers–moderates	-18.964	6.260	-3.029	0.002	0.015

The following alternative hypotheses are accepted:

Flexion: in this case the adjusted values of the significance threshold are higher than 0.05 while the asymptotic significance levels are lower than 0.05. A separate evaluation of the presence of significant differences using the Kolmogorov-Smirnov and Mann-Whitney tests confirm the occurrence of these significant differences. Chronic consumers register considerably higher values than the occasional ones and non-consumers and the moderate consumers present higher values than the occasional ones.

Adduction: The non-consumers present significantly lower scores than the chronic consumers and the moderate ones.

Mayo: The moderate consumers register significantly higher values than the non-consumers.

WINE CHEMICAL ANALYSIS

IDENTIFYING AND QUANTIFYING THE PHENOLIC COMPOUNDS IN WINE

Introduction

The phenolic compounds have an important role in producing the aromas in wine, but at the same time, a role in consolidating the consumers' health. The most usual ones in grapes are the hydroxybenzoic and hydroxycinnamic phenolic acids that through esterification with wine alcohols give the specific aromatic compounds as ethyl and methyl vanillates, ethyl cinnamate (methyl trans-3-phenylpropionate). A predecessor of ethyl cinnamate is the ferulic acid that gives wine fruitiness, together with thiolic compounds like mercapto-pentanone that react with oxidized phenolic compounds (tynones). The aromatic character of wine is in close relation to the area where the grapes come from, but it also depends on the soil structure and the edaphic and climatic factors. The current paper aims at evaluating the phenolic compounds in the wines of the prestigious wine region of Iasi.

Materials and methods

- Wines of the varieties: Fetească regală (FR), Fetească albă (FA), Italian Riesling (RI) – white wines, production of 2010, Iași Vineyard;
- Wines of the varieties: Fetească neagră (FN) și Băbească neagră (BN) – red wines, production of 2010, Iași Vineyard.

The identification and quantification method for the phenolic compounds consisted in direct injection into the chromatographic column of the HPLC Agilent 1200 chromatograph equipped with auto-sampler and UV-VIS detector set at 220nm.

Balancing the column takes 10 minutes in average, then the injection may take place. The chemical compounds and the solvents used had analytic purity, while for dilutions we used ultrapure water made by Milli-Q purifying system (Millipore, Bedford, MA) with maximum resistivity of 18,2 MΩ/cm.

The benchmarks for the phenolic compounds (gallic acid, syringic acid, *p*-coumaric acid, (+)- catechin, quercetin, rutin and vanillin, (-)- epicatechin, resveratrol) were purchased from Sigma-Aldrich company (Steinheim, Germany).

The basic benchmark solutions were made by accurately weighing about 10mg of each phenol in a 25 ml volumetric flask, then dissolved in 10ml of methanol. The working benchmarks were made by the dilution of the basic benchmark solutions in the same solvent.

Both the basic standard solutions and the working benchmarks were kept at a temperature of 4°C.

The separation of the phenolic compounds was made through a previously optimized method, on an Aquasil C18 column (5 µm, 250 x 4.6 mm), mentaining a flowing velocity of the mobile phase of 1ml/min, at a temperature of 30°C.

The mobile phase consists in two solvents, a solvent A: water/acetic acid (98:2 v/v) and a solvent B: methanol/solvent A (60:40 v/v) based on a program of graded elution as follows: initial condition 0% solvent B, from 0% to 10% solvent B after 5 minutes, followed by 10-20% solvent B after 15 minutes, 20-30% solvent B for 15 minutes and 30-40% solvent B for another 15 minutes, then returning to the initial conditions.

The identification of phenolic compounds was made by comparing the retention time with the standard times.

The individual quantification of phenols was made by using the calibrating curves gotten for each standard.

Results and discussions

The assessment of some ecoclimatic parameters in Iasi Vineyard

The average temperatures in the region of Iasi reached 9.7°C in 2010, 9.2°C in 2011 and 9.3°C in 2012.

We can state that the average value for these three years under study was 9.4°C.

During the vegetation period, the sum of temperatures was 3187°C in 2010, 3129°C in 2011 and 3160°C in 2012, so the average value for the three years monitored reached 3159°C.

As far as the number of insolation (incoming solar radiation) hours is concerned, it was 1462 in 2010, 1434 in 2011 and 1452 in 2012. This was favorable for the grape harvest and the wines produced were of very good quality.

The precipitations were in moderate amounts, smaller in 2010, having an average of 545mm, 573mm in 2011 and an optimum of 565 in 2012. This resulted in an annual average value of 562mm, for the three years under observation.

The precipitations during the insolation period had average values of 354mm in 2010, 378mm in 2011 and 366mm in 2012, one of the best years for grape harvest.

The average value of this factor for the three years in question was 366.

The oenological aptitude index recorded values of 4407 in 2010, 4402 in 2011 and 4401 in 2012, the average for the three years being 4433.

The wines produced in Iasi Vineyard are usually light, little extractive, not very acid, with a lower concentration of alcohol, but full of aroma and savor.

In this vineyard, the viniculture is favored and productive on the sunny plateaus and the basins of the secondary valleys.

In general the vineyards have northern exposure, but they are sheltered from the air streams from north or north-east, being under the foehn effect of the southern and western air masses which descend from the high plateau towards the Bahlui and Prut Valleys.

Identifying and quantifying the phenolic compounds in the white and red wines produced in Iasi Vineyard

The phenolic compounds estimated in the white wines (Italian Riesling, Fetească regală, Fetească albă) produced in 2010 and the red ones of the same year (Fetească neagră and Băbească neagră) are presented in the table.

Table The identification and quantification of phenolic compounds in the white and red wines under study, production of 2010 (Fetească regală, Fetească albă, Italian Riesling, Fetească neagră and Băbească neagră)

TOTAL mg/L ppm		FR	FA	IR	FN	BN
		177.002	**189.089**	**185.451**	**646.045**	**678.033**
Anthocyanins	Malvidin	112.334	121.325	128.783	429.887	442.732
	Petunidin	22.675	23.443	22.578	82.131	89.412
	Delphinidin	13.771	14.774	10.931	49.812	48.391
	Cyanidin	7.213	7.998	5.834	18.778	19.721
	Peonidin	21.009	21.549	17.325	65.437	77.777
Flavans	**TOTAL**	**0.178**	**0.273**	**0.287**	**2.652**	**2.983**
	Epicatechin	0.122	0.197	0.123	1.389	1.667
	Catechin	0.031	0.056	0.097	1.117	1.121
	Gallocatechin	0.009	0.011	0.56	0.025	0.056
	Epigallocatechin	0.016	0.009	0.011	0.121	0.139
Flavones and Flavonols	**TOTAL**	**0.368**	**0.428**	**0.513**	**18.400**	**18.768**
	Quercetin	0.019	0.023	0.056	5.872	5.114
	Kaempferol	0.011	0.013	0.023	1.334	1.211
	Myricetin	0.034	0.029	0.037	0.995	0.892
	Isohramnetin	0.011	0.021	0.009	0.763	0.552
	Rutin	0.293	0.342	0.388	9.436	10.999
Phenolic acids	**TOTAL**	**31.721**	**33.825**	**46.667**	**49.979**	**63.471**
	caftaric acid	0.111	0.123	0.152	0.994	0.781
	gallic acid	0.324	0.297	0.444	4.456	5.721
	caffeic acid	0.229	0.528	0.429	2.997	3.323
	vanillic acid	10.923	11.452	11.449	12.093	13.756
	syringic acid	20.134	21.425	34.193	29.439	39.89
Micro-phenolic compounds	**TOTAL**	**30.189**	**28.976**	**26.476**	**85.145**	**100.907**
	shikimic acid	29.078	27.983	25.561	41.223	51.892
	Resveratrol	1.111	0.993	0.915	43.922	49.015

We can observe that the total sum of anthocyanins in the wines in question has very different values depending on grapes variety, the lowest being for the white wines.

Thus for the variety of Feteasca regala we have a value of 177.002mg/l, for Feteasca alba, 189.089mg/l and for the Italian Riesling, 185.451mg/l. So, the lowest value is for Feteasca regala, while the highest is for Feteasca alba.

Concerning the amount of anthocyanins in the red varieties, we notice that it presents generous values between 646.045mg/l for Feteasca neagra and 648.033 mg/l for Babeasca neagra.

Analyzing the content of anthocyanins through the perspective of anthocyanic fractions, we can notice that *malvidin* is between 112.334 mg/l for Feteasca regala and 128.783 mg/l for Italian Riesling.

For the red wines, there are values of 429.887mg/L for Fetească neagră and 442.732 for Băbească neagră.

The resulted values define both the grape variety from which the wine was made as well as their origin area.

The *petunidin* was identified in almost equal amounts for the white wines: 22.578 mg/L for the Italian Riesling and 23.443mg/L for Fetească albă. The red wines had almost four times higher values than the white wines: 82.131 mg/L for Fetească neagră and 89.412 mg/L for Băbească neagră. *Delphinidin* values were with 45% lower than those of petunidin for the white wines and with about 53% for the red ones. Thus, this anthocyanin is between 10.931mg/L and 14.774mg/L for the white wines and between 48.391 mg/l and 49.812 mg/L for the red wines. The *Cyanidin* is a compound with small values: 5.834 mg/L for Italian Riesling, 7.213mg/L for Fetească regală and 7.998 mg/L for Fetească albă. There is to be noticed that the value for Italian Riesling is with 20% lower than that for the other two white wines. The red wines have concentrations between 18.778 mg/L for Fetească neagră and 19.721 mg/L for Băbească neagră. The *peonidin* is close to petunidin in values, the differences being of about 9-10%. These values are in the interval 17.325 mg/L to 21.549 mg/L for the white wines and in the range of 65.437 mg/L to 77.777 mg/L for the red wines.
The flavans represent an important segment concerning the aromatic potential of the wines, even though the values reported are of 0.178 mg/L and 0.287 vfor the white wines and 2.652 mg/L and 2.983 mg/L for the red wines.

Looking for the flavans composition in the wines under study, it can be observed that the most substantial values are for *epicatechin* which is between 0.122 mg/L for Feteasca regala and 0.197 mg/L for Feteasca alba. The red wines are 10 to 12 times richer in epicatechin than the white ones, so the values are between 1.389 mg/L for Feteasca neagra and 1.667 mg/L for Babeasca neagra. *Catechin, gallocatechin and epigallocatechin* present values between 1.117 mg/L and 1.121 mg/L.

The flavones and flavonols are present in small quantities in the white wines: 0.368 mg/L and 0.513 mg/L. On the other hand, the red wines have18.400 mg/L for Feteasca neagra and 18.768 mg/L for Babeasca neagra. In this case the *quercetin* and the *rutin* display the highest values (5.114 mg/L – 5.872 mg/L and respectively 9.436 mg/L- 10.999 mg/L).
The phenolic acids have growing values, starting with 31.721 mg/L for the white Feteasca regala and doubling for the red Babeasca neagra. Thus, the *caftaric acid* varies between 0.111 mg/L for Feteasca regala and 0.152 mg/L for Italian Riesling. The red wines have caftaric acid accumulations that vary from 0.781 mg/L for Babeasca neagra to 0.994 mg/L for Feteasca neagra. The *gallic acid* is under 1 in the white wines, reaching 4.456 mg/L and 5.721 for the red ones.

The *vanillic acid* has similar values for all the varieties studied, being quantified between 10.923 mg/L and 13.756 mg/L. The values foe Feteasca alba and Italian Riesling are very close, about 11.450 mg/L. Another phenolic compound which is important for the character of a wine is the *syringic acid.* The values determined in this case were 20.134 mg/L and 21.425 mg/L for Feteasca regala and Feteasca alba and with 70%higher for Italian Riesling wines. The red wines have interesting values, for instance, the wine Feteasca neagra has with 29.439 mg/L less syringic acid than Italian Riesling. The wine Babeasca neagra doubles the value of Feteasca regala, reaching 39.890 mg/L. In fact, all these identified compounds (caftaric acid, gallic acid, caffeic acid, vanillic acid and syringic acid) lead to an aromatic range specific for each wine in particular.

The micro-phenolic compounds participate actively in the harmonious finalization of wine aroma, also having important implications in the health of the human body. It is observed that these compounds reach 26.467 mg/L (Italian Riesling), the lowest amount, up to 30.189 mg/L (Feteasca regala), the highest amount for the white wines studied. The red wines have 85.145 mg/L micro-phenolic compounds (Feteasca neagra) up to 100.97 mg/L (Babeasca neagra), these values being 3-4 times higher than for the white wines.

The *shikimic acid* presents values between 25.561 mg/L for Italian Riesling and 29.078 mg/L for Feteasca regala. These values are with 40-50% lower than those for the red

wines. There are 41.223 mg/L and respectively 51.892 mg/L shikimic acid in Feteasca neagra and Babeasca neagra. The *resveratrol* is one of the compounds that affect health positively, its effects being well known and appreciated. It is in small amounts in the white wines (0.915 mg/L and 1.111 mg/L), being much more present, however, in the red wines: 43.922 mg/L for Feteasca neagra and 49.015 mg/L for Babeasca neagra, influencing positively their quality. All these components together with others lead to making especially fine wines with a beautiful ruby-red color, characteristic taste and fruity aroma: sour cherries, cranberries, raspberries, vanilla and peanuts.

IDENTIFYING AND QUANTIFYING THE METALS IN WINE

Introduction

The wine is made of a multitude of elements and micro-elements, many of them coming from the vine or grape, others occurring during the technological processes used or from the reaction taking place. An important aspect for the character of a wine is its multi-elemental composition, which depends on the geochemistry of the soil. The studies so far demonstrated the presence of a very strong correlation between the content of cations (K, Zn, P, Cu, Ca, Al, Sr, Mn, Fe, Mg, and Na) in wines and in the soil from the area of origin. Geana et al. (81) proved that elements such as Mn, Cr, Sr, Ag, Ni and Co can be good indicators for the region of origin of the varieties of wine grapes.

Materials and method

- Wines of the varieties: Fetească regală (FR), Fetească albă (FA), Italian Riesling (RI) – white wines, production of 2010, Iași Vineyard;
- Wines of the varieties: Fetească neagră (FN) și Băbească neagră (BN) – red wines, production of 2010, Iași Vineyard.

In order to identify and quantify the heavy metals in wines, the official method of analysis (OIV 2012) was used, which uses atomic absorption spectrometry in flames. The wine samples were previously subjected to microwave digestion, where 2.5 ml of wine sample is introduced into a digestion Teflon container, adding 2.5 ml of nitric acid. After closing the container, thisis placed into the digestion oven set to run the following program: 20 min to reach 180°C, then 15 minutes to cool down. After digestion and cooling down to lab temperature, the resulted solution is transferred into a volumetric flask of 50 ml. This is filled to the sign with ultrapure water. The calibrating was made with standard solution multi-element XVI CertiPur.

Results and discussions

The table shows the results of the identification process for the five wines selected. It is to be noticed that selenium and uranium have less than 0.5 µg/L concentrations, while cadmium has a value lower than 0.1µg/L for all working samples. These values are not quantifiable in relation to the method's accuracy.

Table The identification and quantification of metals in the white and red wines under study, production of 2010 (Italian Riesling, Fetească regală, Fetească albă, Fetească neagră and Băbească neagră)

Element µg/L	FR	FA	IR	FN	BN
Pb	40.127	21.565	39.223	54.309	67.902
Cd	<0.1	<0.1	<0.1	<0.1	<0.1
Se	<0.5	<0.5	<0.5	<0.5	<0.5

Sr	381.123	327.296	456.897	860.564	567.109
Zn	379.891	187.665	415.228	326.712	388.990
Mn	601.223	396.891	578.954	967.009	893.234
Co	2.997	2.678	8.998	3.712	5.789
Cr	156.189	121.998	196.876	213.198	223.107
Ni	19.891	27.123	34.178	25.784	21.111
Rb	600.304	701.778	697.605	1456.789	1567.982
Ag	7.301	6.235	7.004	7.981	7.453
U	<0.5	<0.5	<0.5	<0.5	<0.5
Cu	599.235	325.657	567.945	524.332	499.891
Be	6.783	7.001	7.108	7.223	7.222

We can notice that the wine samples presented lead concentrations of 21.565 μg/L for Fetească albă, 39.223 μg/L for Riesling italian, close to Fetească regală with 40.127 μg/L. The red wines displayed values with about 45-50% higher than the white ones, thus Fetească neagră accumulated 54.309 μg/L of lead and the wine Băbească neagră 67.902 μg/L. Comparing the five types of wine, the lowest lead concentration was in Fetească albă, and the highest in the red Băbească neagră.

Nickel was between 19.891 μg/L and 34.178 μg/L. Among the white wines, Fetească regală had the lowest values, while the highest was in the case of Italian Riesling. The wines Fetească regală and Băbească neagră had close values, the same being for Fetească albă and Fetească neagră. In four out of five wines we notice that the nickel values are lower than the lead ones with 20% in average.

Large amounts of metals as nickel and lead are not recommended, the values determined in this study, being within the boundaries set by law. This admits a maximum of 0.15 μg/L of lead, so the wines in this study follow the OIV regulations. There are important amounts of strontium and zinc in the wines studied. The occurrence of zinc may be associated with the anti-fungi treatments of the vine. The results for zinc can be classified in relation to the type of wine as follows:

FA< FN< FR< BN< IR

The lowest values of zinc were in Feteasca alba: 187.665 μg/L, and the highest reached 415.228 μg/L for Italian Riesling. However, the values do not exceed the maximum admitted by the O.I.V. which is 0.5 μg/L.

The results for zinc can be classified in relation to the type of wine as follows:

FA<FR<RI<BN<FN

One can observe that Feteasca alba presents the lowest concentrations of strontium (327.296 μg/L), while the maximum of 860.564 μg/L is reached by Feteasca neagra.

Coper is an essential element, but when in excess it can become toxic. This element is usually associated with the addition of pesticides in viticulture as well as with the wine making process. During the vinification, the amount of copper may vary, reaching low values due to the formation of insoluble precipitates or high values caused by the corrosion of the equipment used. The variation of the amount of Cu from the wine sample analyzed is relatively low, this element having similar values in all five cases studied. The lowest value is for Fetească albă, (325.657 μg/L) followed by Băbească neagră (499.891 μg/L), then Fetească neagră (524.332 μg/L), Italian Riesling with 567.945 μg/L, the most substantial value being for Fetească regală (599,235 μg/L).

The manganese is one of the elements that confer the notion of "terroir", but the values achieved reflect the region of the vineyard and the intimate structure of the soil where the aforementioned varieties of grapes were cultivated. The values were between 396.891 μg/L, minimum, for Feteasca alba and 967.009 μg/L, maximum, for the red wine Feteasca neagra. It

is noticeable that both red wines present values close to 900, which certifies the statement about the vineyard origin.

One can observe the evolution of some elements like cobalt, silver and beryllium, systematized together since they have similar values. Cobalt is between 2.997μg/L, and 8.998μg/L for the white wines, and between 3.712μg/L and 5.789μg/L for the red wines. Silver stays almost constant, having values close to 7μg/L with little oscillations.

The decreasing trend of silver in the analyzed wines is shown below:

FA<RI<FR<BN<FN

The same can be stated about the beryllium, this having values between 6.783μg/L and 7.223μg/L.

FR<FA<RI<BN<FN,

noticing that both values for the red wines are slightly higher than those for the white ones.

Rubidium is one of the elements that is to be found in the soil, giving the grapes a stronger, more intense color. The white wines accumulated amounts of rubidium varying from 600.304 μg/L for Fetească regală, to 701.778 μg/L for Fetească albă; in between being the Italian Riesling: 697.605 μg/L. On the other hand, the red wines presented almost double the values. Thus, Feteasca neagră accumulated 1456.789μg/L, while Băbească neagră accumulated rubidium in sum of 1567.982μg/L.

Conclusions

1. There is certainly a difference between the rates of the secondary displacements of the four categories of consumers. Thus, for the non-consumers and the occasional consumers of wine, the rate of this complication is about 21-22%, while for the moderate and chronic wine consumers, the secondary displacement rate tends to be 13-14%.
2. The non-consumers and the occasional consumers of wine are associated to a higher probability of suffering from a fracture of a higher complexity. Every one of them has approximately two times more chances of having more complex fractures than the chronic wine consumers. The moderate wine drinker does not present fractures of a different complexity than the chronic drinker.
3. As far as flexion and adduction are concerned, these have opposite but very weak effects when increasing with one unit: flexion increases the chances of a complex fracture with 1.002, while adduction decreases the chances of complex fracture with 0.995.
4. There is certainly a positive influence in bone healing at the people that chronically and occasionally consume wine compared to other population groups.
5. The phenolict compounds determined in the white varieties presented values that lead to the typical aromas of these wines. For the red wines, these compounds differentiate the varieties, the gallic acid, catechin, epicatechin, rutin, quercetin and resveratrol being key elements of their aromatic character.
6. The amounts of phenolic compounds are a lot smaller for the white wines than for the red wines, the concentrations of anti-oxidant components being up to ten times lower. Analyzing the two categories of wines, white and red, we can assert that, from the

phenolic compounds point of view, it is advisable for health to drink moderate amounts of red wine.

7. The substantial amount of micro-phenolic compounds in the red wines recommends them for curative consumption.
8. The metals found in the analyzed wines can originate from various sources like soil configuration, agro-viticulture practices or the technological equipment used. The values determined in this study do not exceed the values admitted by the current wine legislation, so the wines can be consumed without any danger from this point of view;
9. The metals occurrence in wines contributes to the formation of their aromatic and color character, having definite influences on the "terroir" feature of the area of origin specificity. Elements as cobalt, silver and beryllium, even if they do not present significant values, influence the chemical composition of wine, these elements not being characteristic for the white or the red wines;
10. Wine color is clearly influenced by compounds as rubidium, even the name (Feteasca neagra and Babeasca neagra) of the variety being relevant in this case.
11. The values obtained and the other studies that show sometimes conflicting results about wine consumption require to establish and to introduct a standard wine for research and a technological line related.

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