

## EVALUATION OF SOME PHYTOCHEMICAL CONSTITUENTS AND THE ANTIOXIDANT ACTIVITY IN SIX ROSE HIPS SPECIES COLLECTED FROM DIFFERENT ALTITUDE OF SUCEAVA DISTRICT

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**Key words:** Rose hips fruits, polyphenol, flavonoids, anthocyanins contents, antioxidant activity

**Abstract:** In this study, species of rose hips fruits were assayed for the composition of polyphenol, flavonoids, anthocyanins contents as well as the antioxidant activity. Samples of rose hips representing six species (*Rosa pendulina*, *R. tomentosa*, *R. canina*, *R. rubiginosa*, *R. corymbifera* and *R. nitidula*) were collected from spontaneous flora of Suceava district. The smallest content of total polyphenols was recorded in *R. nitidula*, both in seeds (3.11 mg GAE/g DW) and pulp (23.99 mg GAE/g DW). The maximum concentration of total polyphenols was found in *R. pendulina*, both in seeds (17.68 mg GAE/g DW) and pulp (71.48 mg GAE/g DW). The most abundant anthocyanin content in pulp of all specie studied was in *R. canina* (16.60 mg%). The relevant differences between pulp and seed regarding flavonoid content, about 7-fold were reached at *R. tomentosa* collected from Vatra Dornei. The results revealed that antioxidant activity of pulp extract from *R. pendulina* showed the largest scavenging activity while the lowest scavenging capacity was recorded for *R. nitidula*.

### INTRODUCTION

*Rosa canina* L., known for their fruits (rose hip, brier hip, brier, rose, dogberry, dog rose, hip fruit, hop fruit, sweet brier, wild brier), grows wildly in various regions of Romania having culinary and medicinal values. *Rosa* species have attracted the attention due to their antioxidant, antibacterial and other properties (Nowak and Tuzimski, 2006). It is mostly used for the prevention and treatment of the common cold, gastrointestinal disorders, diabetes, kidney disorders, and other infections (Davis, 1972, Yeşilada, 2002). The main amount of vitamin C is located in the skin whereas the seed contain a great content of oil with antibacterial and antioxidant properties (Georgieva et al., 2014; Cheryll, 2013). In addition, in many European countries the *Rosa* species have long been used as an herbal tea, vitamin supplement or food product because its richness in ascorbic acid. Also, rose hips are a rich source of carotenoids, minerals (K and P), folate, polyphenols and various flavonoids such as anthocyanins (Uggla et al., 2005, Demir et al., 2001). On the other hand, *Rosa canina* L. is a great source of tocopherols, bioflavonoids, tannins, pectin, aminoacids, unsaturated and polysaturated fatty acids, phospholipids and gallactolipids (Chrubasik et al., 2008). However, the seeds representing 30% of the rose hip fruit and are considered a low value by product (Çinar and Dayisoğlu, 2005).

More than, rose hips extract is able to scavenge reactive oxygen species (ROS) (Daels-Rakotoarison et al., 2002) and among the various natural scavengers of ROS, polyphenols compounds have received attention. There is considerable interest in natural antioxidants from foods and biological systems because of the potential nutritional and therapeutic benefits (Vitaglione and Fogliano, 2004). In their study about nutritional value and chemical composition of eight rose hips fruit species collected from Transilvania, Roman et al. (2013) found that varieties of *R. canina* can be used as a potential source of natural antioxidants.

The medicinal value of rose hips depends primarily on the content of vitamin C and flavonoids (Kuznicka and Dziak, 1987). In the last years, the relationship between food and health has become more important and nowadays the consumers demand healthy, tasty and natural functional foods that have been grown in uncontaminated environments.

The goal of this research was to determine the chemical composition (flavonoids, total polyphenol and anthocyanins contents, as well as antioxidant activity) in pulp and seed of six wild rose hips (*Rosa pendulina*, *R. tomentosa*, *R. canina* L., *R. rubiginosa* L., *R. corymbifera* Borkh and *R. nitidula* Besser) collected from different altitude of Suceava district. Another objective was to compare the content from seed and pulp of wild rose hip species in order to establish which has better antioxidant properties.

## MATERIAL AND METHODS

### 1. Sample collection and processing

During harvest season (end of September and beginning of October 2013) samples of rose hips representing six species (*Rosa pendulina*, *R. tomentosa*, *R. canina*, *R. rubiginosa*, *R. corymbifera* and *R. nitidula*) were collected from spontaneous flora of Suceava district from different altitudes ranging between 630m and 830 m (Table 1). Rose hips were preserved according to the standard procedures being collected manually without leafs and were mixed together for a homogeneous distribution. The fruits were picked at the fully ripe mature stage as judged by their colour and were randomly chosen from 100 fruits. Some phytochemical analysis (dry weight, flavonoids, total polyphenol, anthocyanins) of powder obtained from the pulp and separate from the seeds of wild rose hip fruits were analyzed. The results were expressed as the average ( $\pm$  SE) of a total of three replicates.

Table 1. Wild *Rosa* species collecting from different altitude and areas

Sampling zona	Taxa under study	Altitude (m)
Campulung Moldovenesc	<i>R. rubiginosa</i>	630 m
	<i>R. canina</i>	
	<i>R. pendulina</i>	
	<i>R. tomentosa</i>	
Vatra Dornei	<i>R. canina</i>	807m
Dorna Candreni	<i>R. corymbifera</i>	830m
	<i>R. nitidula</i>	

### 2. Phytochemical analysis

**2.1. Dry weight** content of rose hip samples was determined using gravimetric method by evaporation at mild temperature (105°C) until they reached a steady weight. Thus, it was determined the quantity of dry matter both in pulp and seeds. The results of dry weight were expressed in g % (g/100g fresh weight) (Boldor et al., 1983).

#### 2.2. Extract preparation

Extraction was performed with methanol. Rose hips samples (pulp or pulp and separate seeds) were homogenized with 80% methanol and then they were stirred for 30 minutes (Andjelkovic et al., 2013, modified). After their centrifugation at 3000 rpm the supernatants were used for the next determinations of total polyphenol content, flavonoids content and antioxidant activity.

#### 2.3. Total polyphenols content assay

The total polyphenols content was determined by using a modified Folin-Ciocalteu method (Singleton et al., 1999). The appropriately dilute sample was added Folin-Ciocalteu reagent and mixed thoroughly. After four minutes, 15% Na<sub>2</sub>CO<sub>3</sub> was added. The absorbance of resulting blue-colored solution was read at 765 nm after two hours, against the blank (distilled water). The amount of the total phenolic content was expressed as mg gallic acid equivalent (mg GAE/g DW) ( $R^2=0.99$ ). Three readings were taken for each sample and the result averaged.

#### 2.4. The flavonoids content assay

The flavonoids content was measured following a spectrophotometric method (Dewanto et al., 2002). Briefly, methanol extract were appropriately diluted with distilled water. Initially, 5% NaNO<sub>2</sub> solution was added to each test tube; at five minutes, 10% AlCl<sub>3</sub> solution was added and then at six minutes 1.0 M NaOH was added. Finally, water was then added to the test tube and mixed well. Absorbance of resulting pink-colored solution was read at 510 nm against the blank (distilled water). Flavonoids content was expressed as mg catechin equivalent (mg CE/g DW) ( $R^2=0.98$ ). Three readings were taken for each sample and the result averaged.

#### 2.5. Total anthocyanin content assay

Anthocyanins were extracted from samples by homogenizing rose hip pulp from each species with acidified 70% ethanol solution. The mixture was centrifuged after 10 min. and the pH was adjusted at correct value 1 with HCl. The

mixture was brought to a volume of 25 mL and the absorbance was measured at 515 nm, using the Shimadzu UV-Visible spectrophotometer (Fuleki and Francis, 1968).

#### 2.6. Antioxidant activity (DPPH free radical scavenging activity) of methanol extract

Antioxidant activity (AA%) of methanol rose hip fruits was determined using stable radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH), as described by Molyneux, 2004; Shirwaikar et al., 2006. This is based on neutralization of free radicals emitted by the DPPH solution, resulting in a coloured solution. DPPH radicals have an absorption that is maximal at 517 nm, and which disappears with reduction by an antioxidant compound. The DPPH solution in methanol 0,1mM was prepared daily, and 2 mL of this solution was mixed with 20  $\mu$ L of the methanol plant extracts. After 20 min of incubation at 37°C in the dark, the absorbance was recorded at 517 nm. The control was achieved using 80% methanol and DPPH solution. The experiment was carried out in triplicate. The percentage of radical scavenging activity (AA%) was calculated according to the following equation:

$$AA (\%) = (1-A1/A0) \times 100$$

where A1 is the absorbance of the extract samples and A0 is the absorbance of the control samples (methanol)

#### 2.7. Statistical analysis

All of experiments were carried out with at least three independent repetitions. The results were expressed as the means value and standard errors of mean.

## RESULTS AND DISCUSSIONS

Biosynthesis and accumulation of secondary compounds are regulated internally within the plant and controlled by both external and genetic factors. The composition of plants may be affected by many factors including region, variety, state of ripening, soil type and condition, irrigation and weather (Oktay and Alpaslan, 2012). The most important environmental factors determining photosynthetic rates (and thus synthesis of phenolic compounds) are light and temperature, as long as water and nutrient supplies are adequate (Emmingham and Waring, 1977).

The dry weight content at wild species of *Rosa* sp. fruits studied evidenced no significant differences (Tab. 2). Therefore, in the seeds the content varied very little from 70.21 g% (*R. canina*) to 73.82 g% (*R. pendulina*). In fact, both values of were obtained at fruits collected from the same sampling area, Vatra Dornei (807m). On the other hand, the dry weight amount in the pulp ranged from 28.34 g% (*R. pendulina*) to 37.35 g% (*R. nitidula*), the rose hip being picked up from different altitudes, 807m and 830m, respectively. Similarly, a another study showed that dry weight content varied in other rose hip species from 33.85 g% (*R. villosa*) to 40.35 g% (*R. dumalis* subsp. *boissieri*) (Ercisli, 2007).

In addition, the study of Yildiz and Alpaslan (2012) found in fruits of rose hip a content of dry weight 32.5 g% which is very similar with our results. Moreover, Rosu et al., 2011, found in whole rose hip fruits that dry weight, in climatic condition of 2008, was varied between 27.53g% and 40.22 g% at *R. canina* and *R. micrantha*, respectively.

Table 2. Dry weight content in pulp and seeds of wild *Rosa* species from different altitudes (mean  $\pm$  SD, n = 3).

Sampling area	Species	Dry weight content (g%)		Altitude (m)
		pulp	seeds	
Campulung Moldovenesc	<i>R. rubiginosa</i>	36.11 $\pm$ 0.18	71.07 $\pm$ 0.45	630 m
	<i>R. canina</i>	34.74 $\pm$ 0.49	71.97 $\pm$ 0.20	
Vatra Dornei	<i>R. pendulina</i>	28.34 $\pm$ 0.86	73.82 $\pm$ 1.34	807m
	<i>R. tomentosa</i>	29.77 $\pm$ 1.23	70.94 $\pm$ 0.28	
	<i>R. canina</i>	32.13 $\pm$ 0.22	70.21 $\pm$ 0.13	

<b>Dorna</b>	<i>R. corymbifera</i>	36.02±0.47	72.00±2.78	830m
<b>Candreni</b>	<i>R. nitidula</i>	37.35±0.46	71.93±0.29	

As regard the anthocyanins content, results from this study indicated that in the pulp of rose hip species the minimum was recorded in *R. rubiginosa* (4.81 mg%) while the maximum was observed at *R. canina* (16.6 mg%) (Fig.1). In species *R. pendulina*, *R. tomentosa*, *R. canina* and *R. corymbifera* the anthocyanins contents were raised ranging between 13.08 mg% to 16.92 mg%, but in *R. rubiginosa* and *R. nitidula* this was 3 times lower (4.81 and 5.61 mg%, respectively). By the other hand, although the rose hip *R. canina* was collected from two areas with different altitudes (807m and 630m) the values of anthocyanins amount were almost the same (16.60 mg% at those from Vatra Dornei and 16.92 mg% at those from Campulung Moldovenesc). This diminishing of anthocyanins level might be due by the variation in altitude or interspecific differences. In addition, the differences in level of anthocyanins can be the result of rose hip growth region (more specifically, the various altitude) and different *Rosa* genotype which have an impact on this metabolite concentration.

Other studies like those of Connor et al. (2002) have found a significant interaction between genotype and location when they examined total anthocyanin content in American blueberry cultivars. By the other hand, Lätti et al. (2008), found an extensive variation between the northern and the southern populations of 179 bilberry (*Vaccinium myrtillus*) clones that spanned 1000 km in Finland regarding the anthocyanins content. The authors conclude that the northern climate conditions appear to favour the biosynthesis of more anthocyanins and flavonols, which have been shown to have the greatest antioxidant capacity *in vitro*. Moreover, even though the final colour of the fruit is a combination of different factors, delphinidins are known to give bluish hues in flowers and fruits. Thus, one could speculate that the blue berries growing in the north might be more blue compared with the southern populations.

Anthocyanins water-soluble compounds having a great interest in nutrition and medicine because of their potent antioxidant capacity (Garcia-Alonso et al., 2005). They are also used in dyes industry to replace synthetic pigments by natural ones (Pina et al., 2012).

Generally, environmental factors (temperature and light, latitude and altitude) have an impact on anthocyanin formation. Therefore, when studying the influence of latitudinal, altitudinal or temporal variations on anthocyanin production, environmental factors such as light and temperature are of prime importance (Åkerström, 2010).

The contents of total polyphenols, calculated as mg gallic acid equivalent (GAE) of rose hip methanolic extract was higher in pulp than seeds (Fig. 2). In fact, in pulp the total polyphenols contents ranged from 23.99 to 71.48 mg GAE/g DW whereas in seeds this was reduced varying from 3.11 to 17.68 mg GAE/g DW. Furthermore, the lowest amount was remarked in *R. nitidula*, both in seeds and pulp. On the other hand, *R. canina*, although collected from different areas show the same content of total polyphenols in seeds and values almost similar in pulp (52.34 mg GAE/g DW from Vatra Dornei and 60.39 mg GAE/g DW from Campulung Moldovenesc).

The highest concentration of total polyphenols was found in *R. pendulina*, both in seeds and pulp. Regarding the differences between polyphenolic compounds in rose hip species these could be explained by the genetic variation, because all plants were harvested at ripening.

By the other hand, Scalzo et al., 2005 was reported that the plant genotype, cultivation site and technique affect the total phenolic content in fruit.

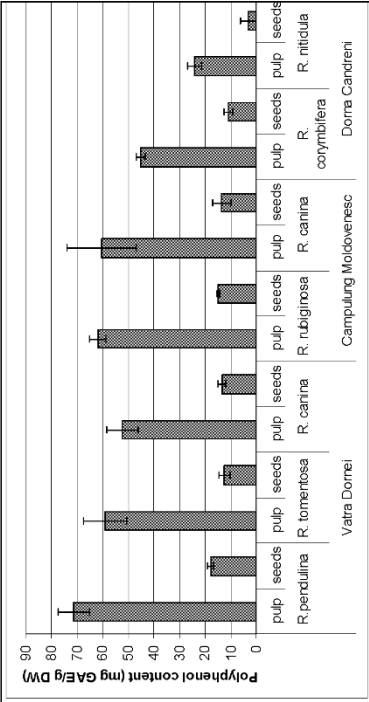


Fig. 2. Total polyphenols content in pulp and seeds of six *Rosa* species from different altitudes (mean  $\pm$  SE,  $n = 3$ ).

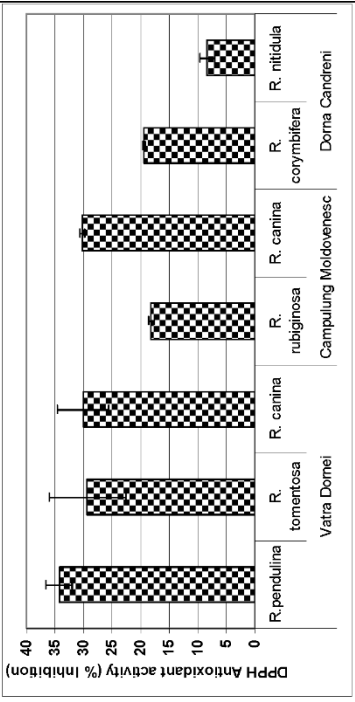


Fig. 4. Free radical scavenging activity (% DPPH inhibition) in pulp of six *Rosa* species from different altitudes (mean  $\pm$  SE,  $n = 3$ ).

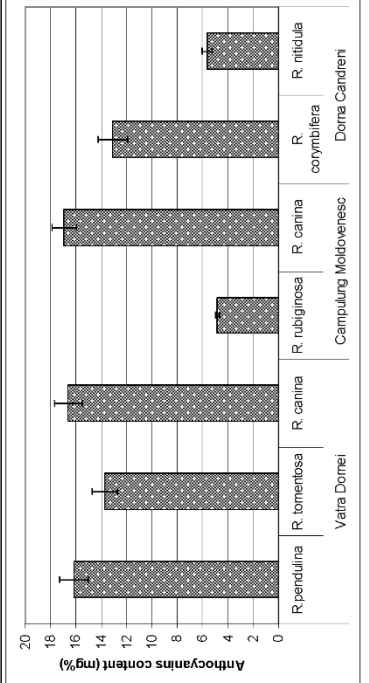


Fig. 1. Anthocyanins content in pulp and seeds of six *Rosa* species from different altitudes (mean  $\pm$  SE,  $n = 3$ ).

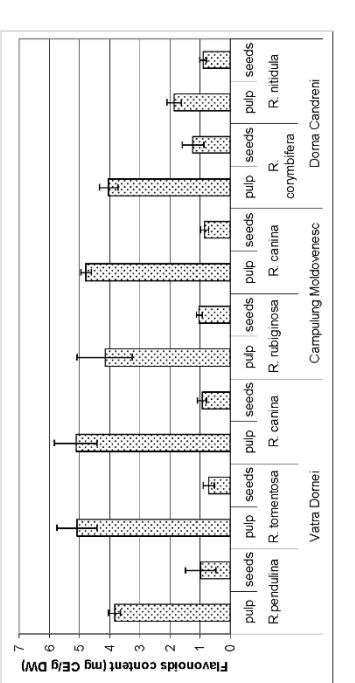


Fig. 3. Total flavonoids content in pulp and seeds of six *Rosa* species from different altitudes (mean  $\pm$  SE,  $n = 3$ ).

Phenolic compounds are important constituents because of their free radical scavenging ability facilitated by their hydroxyl groups.

By the other hand, phenolics possess a wide spectrum of biochemical activities, such as antioxidant, antimutagenic, anticarcinogenic effects as well as ability to modify gene expression (Nakamura et al., 2003). The total phenolic concentration could be used as a basis for rapid screening of antioxidant activity by inactivating lipid free radicals or prevent decomposition of hydroperoxides into free radicals (Pokorny et al., 2001). Rose hips are known to have a high phenolic content (Hvattum, 2002) but *R. rubiginosa* seeds are high in polyphenols and show strong antioxidant activity (Moure et al. 2001).

Our findings are in agreement with those of Ercisli (2007), which found a polyphenols content at *R. canina* by 96 mgGAE/100g. On the other hand, although more reduced, the values of polyphenols are in agreement with those of Yildiz and Alpaslan, (2012) which found a content polyphenols 9982 mg GAE/100g FW in rose hip. Ocksook et al., (2007) reported that total phenolic concentrations of the extracts of *R. nutkana*, *R. pisocarpa* and *R. woodsii* rose hip were 12.20 mg GAE/l, 8.77 mg GAE/l and 6.974 mg GAE/l, respectively.

In the study of Roman et al., (2013) the total polyphenols content of the *R. canina* L. hip extracts registered values between: 575.0 mg GAE/100 g for RC1 (var. *transitoria* f. *ramosissima* from Bistrita-Nasaud, Agiesel) and 326.5 mg GAE/100 g for RC6 (var. *lutetiana* f. *fallens* from Satu-Mare, Petea). In their research on chemical composition of ‘Karpatia’ *R. pomifera* fruits, Milala et al., (2013), found that among the polyphenolic compounds, the flavanols predominated, both in the flesh and the seeds; their mean quantity was 2783 mg/100 g DW and 842mg/100g DW, respectively.

As in the case of total polyphenol, the contents of flavonoid, calculated as mg catechin of rose hip methanol extract, was higher in pulp than seeds (Fig. 3). The wild rose hips fruits investigated contain an amount of flavonoids which varied in seeds between 0.72 and 0.98mg CE/g DW while in pulp from 1.86 to 5.11mg CE/g DW.

The flavonoids content in pulp of *R. tomentosa* and *R. canina* collected from Vatra Dornei as well as, *R. canina* from Campulung Moldovenesc had almost close values, 5.08mg CE/g DW, 5.11mg CE/g DW and 4.77mg CE/g DW, respectively. Moreover, although the species *R. canina* was collected from areas with different altitude (807m and 630m) the variation between flavonoids content from pulp and seeds had close values, these being about 5.4-fold and 5.6-fold from Vatra Dornei and Campulung Moldovenesc, respectively. On the other hand, the highest differences between pulp and seed regarding flavonoid content, about 7-fold were reached at *R. tomentosa* collected from Vatra Dornei. By contrast, *R. nitidula* has recorded the lower content and the differences between pulp and seed was about 2-fold.

The most common flavonoids in plants are quercetin and kaempferol and usually occur as glycosides. In rose hips there are mainly glycoside derivatives of quercetin: quercitrin (quercetin-3-O-rhamnoside), isoquercitrin (quercetin-3-O-glucoside) and hyperoside (quercetin-3-O-galactoside) (Nowak and Tuzimski, 2006).

As regards free radical scavenging activity (% DPPH inhibition) the percentages found in fruits of wild *Rosa* species harvested from different altitudes were varying between 8.31% and 34.22% (Figure 4). Therefore, the pulp extract from *R. pendulina* showed the highest scavenging activity and the lowest scavenging capacity was recorded for *R. nitidula*. The antioxidant activity of *Rosa* species collected from Vatra Dornei (807m) was not varied very much (29.32%-30.07%). The difference of antioxidant activity of *Rosa* species were remarked at *R. rubiginosa* and *R. canina* from Campulung Moldovenesc (18.17% and 30.20%) as well as at *R. corymbifera* and *R.*

*nitidula* from Dorna Candreni (8.31% and 19.31%). Even though *R. canina* fruits were picked up from two different altitude 807 m (from Vatra Dornei) and 630m (from Campulung Moldovenesc) the DPPH radical scavenging capacity was almost the same (30.07% and 30.20%, respectively).

The antioxidant capacity may be widely used as a parameter for medicinal bioactive components. The characteristic feature of antioxidants to scavenge DPPH free radical is accepted and is therefore most often selected as a reliable tool to evaluate the free radical scavenging capacity of different plant extract.

The variations of antioxidant capacity of fruits can be impacted by geographical conditions (month to month and year to year) (Lee and Coates, 1997). Wenzig et al. (2008) demonstrated that the radical scavenging activity of the two rose hips (*R. canina* L.) extracts correlated very well with their total phenolic content, while ascorbic acid contributes only little to the radical-scavenging activity due to its low concentration present in the extracts.

## CONCLUSIONS

The contents of total polyphenols, flavonoids and antocyanins as well as antioxidant activity in fruits of six wild *Rosa* species (*R. pendulina*, *R. tomentosa*, *R. canina*, *R. rubiginosa*., *R. corymbifera* and *R. nitidula*) collected from different altitudes of Suceava district were compared. The results revealed that in *R. nitidula* species were remarked the smaller contents of anthocyanins, total polyphenols and flavonoids.

Concerning the anthocyanin content the highest level was recorded in pulp of *R. rubiginosa* while the smallest was observed at *R. canina*; the difference being approximately three fold higher in rose hip, even if they were collected from the same altitude 630 m. As regards the total polyphenol and flavonoids contents showed a large variation among Romanian rose hip fruits studied. In case of *R. canina* rose hip fruits the total polyphenol content increased with the decrease of altitude while the flavonoids contents diminished with the reduction of altitude. Antioxidant activity of rosehips of studied species is shaped by the presence of anthocyanins, total polyphenols and flavonoids. The maximum capacity of neutralizing DPPH radicals was shown by extracts obtained from *R. pendulina* whereas the minimum by *R. nitidula*.

According to the results of the present study it seems that rose hips can be recommended for improving the health because of high contents of anthocyanins (*R. canina*), total polyphenols (*R. pendulina*), flavonoids (*R. canina*).

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## THE EVOLUTION OF ASCORBIC ACID CONTENT DURING WHITE CABBAGE PICKLING PROCESS

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**Keywords:** cabbage, pickling, ascorbic acid, pH

**Abstract.** The evolution of ascorbic acid content during white cabbage pickling was the purpose of this paper. White cabbage, purchased from commercial network, was the biological material. Various samples of cabbage (whole and minced) were introduced within glass jars over which was poured brine (made with non-iodized salt), for each type of cabbage. The samples were left to ferment at 18°C for 5 days, then stored at 10°C, where each brine was subjected to periodic aeration, by pouring from one container to another in prolonged contact with air. The evaluation of pH was made with a digital pH meter, and ascorbic acid content through a titrimetric method, from fresh raw material (before pickling), as well as from cabbage, minced cabbage and from their sauce, at 2 or 3 days, during the pickling process. In the whole cabbage, the ascorbic acid content significantly decreased up to the 17th day of pickling, while in the minced cabbage up to the 14th day, accumulating gradually, at about the same intervals, within their brine. At the end of the experiment (after 30 days), compared to fresh sample, the total content of ascorbic acid was reduced by 22.3% (within whole cabbage and its brine), and by 27.7% (within minced cabbage and its brine). Cabbage pickling, including mechanical processing, fermentation and periodic aeration has led to reduction of initial content of ascorbic acid especially around pH values of 4.2-4.3.

### INTRODUCTION

Cooking, pasteurization and the addition of chemical preservatives guarantee safe vegetables and fruits, but bring about a number of not always desirable changes in their physical characteristics and chemical composition (*Zia-ur-Rehman et al., 2003; Zhang and Hamauzu, 2004*). In order to reduce the drawbacks derived from above mentioned food processing technologies, novel technologies, such as high-hydrostatic pressure processing, ionization radiation and pulsed-electric fields, new packaging systems and the use of natural antimicrobial preservatives are considered (*Devlieghere et al., 2004; Gómez-López et al., 2005; Elmasser et al., 2007*).

Vegetables and fruits can be preserved, for a long time without modifying their properties, by means of conservation methods based on acidification or pickling method, i.e. lactic acid fermentation, considered as the simple and valuable biotechnology to keep and/or enhance the safety, nutritional, sensory and shelf life properties of vegetables and fruits (*Steinkraus, 1996; Buckenhüskes et al., 1997; Karovičová and Kohajdová, 2003; Demir et al., 2006; Di Cagno et al., 2013*).

Representing the easiest and the most suitable way for increasing the daily consumption of fresh-like vegetables and fruits (*Di Cagno et al., 2013*), lactic acid fermentation of vegetables has an industrial significance mainly for cabbages, cucumbers and olives (*Montet et al., 2006; Rodriguez et al., 2009*).

Between Brassica vegetable (cabbage) consumption and cancer risk is an inverse relationship (*Zhang et al., 2006; Higdon et al., 2007*). The beneficial effects of vegetables in general, and cruciferous vegetables, in particular, on human health are due to the presence of high levels vitamins (mainly ascorbic acid), and of glucosinolates, i.e. glucosides containing sulfur (*Moreno et al., 2006; Higdon et al., 2007*, cited by *Martinez-Villaluenga et al., 2009*).

According to *Aleksandrova et al.* (1992), during cabbage shredding, glucobrassicin (a cabbage glucosinolate) is transformed into indole-3-carbinol by the action of myrosinase and during fermentation, as the pH decrease, this indole reacts nonenzymatically with L-ascorbic acid to yield ascorbigen. This last compound is responsible for the anticarcinogenic properties in humans with a high intake in white cabbage (*Lysenkova et al., 2001; Smiechowska et al., 2008*).

In Romania, pickles (cabbage, cauliflower, cucumbers, green tomatoes, carrots, fruits) are widespread and appreciated, being consumed all the year, but especially in winter and spring. For many families, pickled white cabbage is a component of the daily diet (spice and/or food), being almost customary in the daily diet.

The purpose of this work was to study the ascorbic acid evolution during white cabbage pickling, to see to what extent the fermentation process influences the initial content (from the raw material) of this vitamin.

## MATERIALS AND METHODS

**Research materials.** White cabbage (*Brassica oleracea* var. *capitata* L.), purchased from commercial network and selected to meet the quality parameters necessary to conservation, was the biological material. The cabbage with a head weight of between 1-1.5 kg was fresh, well made, no damage and no attack by insects or parasites, clean, odorless and with a good taste. Prior to pickling process, fresh raw material was analyzed, determining the pH, whose value was  $7.05 \pm 0.08$ , and the ascorbic acid content ( $40.3 \pm 2.74$  mg%). For experiments it was used non-iodized crystallized salt, provided by the National Salt Company, Salina Cacică Branch, and water from the distribution network of the Suceava city.

**The preparation of pickles.** Whole cabbages were cleaned and placed in colored glass jars of 15 liters each, well stuffed (with pressure) without goals. Using non iodized salt it was prepared brine in some pots, by introducing of 25 g salt per liter of water. Although *Banu et al.* (2000) recommended as among rows of sprouts to be placed pieces of horseradish roots and dried dill weed flowers, in order to improve the flavor of the product and to give a preservative role to the broth, in this experiment there was not used horseradish, because it has an appreciable content of ascorbic acid which would influence test results. Before closing the jars, there were placed two small wooden boards (cross) to prevent cabbage to rise above, then the jars were left to ferment at 18°C for 5 days. Because the anaerobic environment may favor butyric fermentation, the cabbage brine was subjected to periodic aeration (*Guțulescu, 1973*), by pouring from one container to another in prolonged contact with air. After completion of pickling tank, pickled cabbage was stored at 10°C.

Minced cabbage was obtained from fresh cabbage, healthy, clean, without outer leaves, which was cut with a steel knife in long thin strips. The cabbage fragments were transferred within two metal flasks and were salted, separately, with non-iodized salt (25-30 g of salt per 1 kg of cabbage), and then were rubbed to leave enough juice. After approx. 30-60 minutes, the cabbage shredded and salted from containers was introduced in colored glass jars (5 liters each), squeezed by hand, and covered (2-3 cm liquid) with the soup made. Over minced cabbage were put two small wooden boards in cross. The initial fermentation temperature was 18°C for 5 days, after which the jars were transferred to a room at a temperature of 10°C. During fermentation it was added brine, made up of 15 g of salt per liter of water. The determinations of chemical and biochemical parameters were carried out to each 2-3 days, using the whole or chopped pieces of cabbage or brine.

**Research methods.** The evaluation of pH and ascorbic acid content was made from fresh raw material (before pickling) as well as from cabbage, minced cabbage and from their sauces, at 2 or 3 days, during the pickling process. **pH** was determined with a digital pH meter supplied by Hanna, and **ascorbic acid** content was determined through a method based on reduction (by ascorbic acid) of 2,6-dichlorophenol-indophenol (2,6-DCPIP) to the corresponding leucoderivate (*Artenie and Tănase, 1980; Indyk and Konings, 2000*). The result was expressed as mg ascorbic acid per 100 g or per 100 ml (mg%) product.

**Statistical analysis.** The data of experiments, consisting in four replicates for each determination, were statistically processed using SAS Version 8.02. In order to analyze the significance of differences among samples, generalized linear model analysis was carried out, and for multiple comparisons was used Duncan's multiple range test ( $P < 0.05$ ).

## RESULTS AND DISCUSSIONS

The table 1 indicates the evolution of ascorbic acid content in cabbage and cabbage brine, during pickling.

**Table 1. The ascorbic acid and pH values in cabbage and its brine, during pickling**

Time (days)	2d	5d	8d	11d	14d	17d	20d	23d	26d	30d
Sample/Par.	Ascorbic acid (mg %)									
Cabbage (C)	34.51 $\pm 3.72$ AB*	32.95 $\pm 4.15$ B*	31.56 3.93 B	31.41 $\pm 2.58$ B	27.65 $\pm 1.98$ B	24.73 $\pm 3.61$ BC	24.51 $\pm 1.76$ BC	24.82 $\pm 2.53$ BC	24.13 $\pm 3.04$ BC	24.20 $\pm 3.16$ BC
Cabbage brine (CB)	5.02 $\pm 0.86$ c*	6.28 $\pm 0.53$ bc*	6.34 $\pm 0.97$ bc	8.07 $\pm 0.65$ ab	7.92 $\pm 1.27$ ab	7.30 $\pm 0.95$ b	7.50 $\pm 0.74$ b	7.08 $\pm 1.03$ b	7.25 $\pm 0.79$ b	7.10 $\pm 0.54$ b
Total AA** (C+CB)	39.35	39.23	37.90	36.78	35.57	32.03	32.01	31.90	31.38	31.30
	pH									

Time (days)	2d	5d	8d	11d	14d	17d	20d	23d	26d	30d
Cabbage brine	6.78 ±0.16 b*	6.52 ±0.29 b	5.80 ±0.06 bc*	4.89 ±0.08 c	4.50 ±0.27 c	4.36 ±0.15 d	3.97 ±0.07 d	3.85 ±0.09 d	4.07 ±0.10 d	4.05 ±0.14 d

\*Means with different letters within a row are statistically different ( $P<0.05$ ); Par.= parameter; AA\*\*=Ascorbic acid; d=days

As compared to fresh cabbage ( $40.3\pm 2.74$  mg%), the ascorbic acid content in pickling cabbage has recorded significant decrease up to the 17<sup>th</sup> day of the analyzed interval ( $24.73\pm 3.61$  mg%), from which the values of this compound have not anymore presented significant variations ( $P<0.05$ ). The largest percentage reduction of ascorbic acid content in cabbage was after the first 2 days of pickling (14.37%) and between 14-17 days (10.4%).

Analyzing the evolution of ascorbic acid in cabbage brine, one can note that with time the concentration of this compound increased significantly until the 14<sup>th</sup> day of, then decreased after the 17<sup>th</sup> day of pickling, preserving then unchanged (without significant variations) to the end of the period (30 days).

After *Banu et al.* (2003), the maintaining of some fruit and vegetables (apples, pears, apricots, potatoes etc.) cut under water, and the pickling process of vegetables (cabbage, cucumbers etc.) causes loss of vitamins by leaching, or partial loss of nutrients of raw material (carbohydrates, vitamins, amino acids and minerals), passing the fermentation broth.

Since ascorbic acid was identified both in solid material (cabbage) and in brine, it was totaled ascorbic acid content of the product and brine at each period analyzed (Table. 1). The results show that greater losses of ascorbic acid, versus fresh sample, have emerged since the 8<sup>th</sup> day of pickling, and after 17 days the losses were biggest. Then, within 17 to 30 days, ascorbic acid values have remained stable, suffering minor changes (32.03 to 31.3 mg%).

Compared with the fresh sample, after 30 days of pickling, the total content of ascorbic acid (cabbage + brine) was reduced by 22.3%.

As seen from Tab. 1, pH values of cabbage brine decreased significantly until the 17<sup>th</sup> day ( $P<0.05$ ), after which no longer had significant variations until the end of the analyzed period (30 days).

The decreases of ascorbic acid content in cabbage from  $34.51\pm 3.72$  (after 2 days) to  $24.73\pm 3.61$  (after 17 days), corresponded to a decrease of pH from  $6.78\pm 0.16$  (after 2 days) to  $4.36\pm 0.15$  (after 17 days).

According to *Banu et al.* (2003), the oxidation of ascorbic acid depends on pH, being very rapid at pH 4.3.

In the table 2 is reproduced the evolution of ascorbic acid content in minced cabbage and minced cabbage brine, during pickling.

**Table 2. The ascorbic acid and pH values in minced cabbage and its brine, during pickling**

Time (days)	2d	5d	8d	11d	14d	17d	20d	23d	26d	30d
Sample/Par.	Ascorbic acid (mg %)									
Minced cabbage (MC)	30.71 ±3.44 B*	27.64 ±2.67 B	26.07 ±1.95 BC*	25.53 ±3.08 BC	21.42 ±1.66 C	21.41 ±2.57 C	21.38 ±1.33 C	21.34 ±2.09 C	20.96 ±0.94 C	19.85 ±1.38 C
Minced cabbage brine (MCB)	6.12 ±0.68 b*	7.78 ±0.95 ab*	8.80 ±1.09 a	8.23 ±0.97 ab	8.77 ±0.44 a	8.27 ±0.88 ab	8.16 ±1.05 ab	8.22 ±0.97 ab	8.64 ±0.63 a	9.30 ±1.14 a

Time (days)	2d	5d	8d	11d	14d	17d	20d	23d	26d	30d
Total AA** (MC+MCB)	36.83	35.42	34.87	33.76	30.19	29.68	29.54	29.56	29.60	29.15
	pH									
Minced cabbage brine	6.17 ±0.35 bc*	5.70 ±0.16 bc	5.18 ±0.05 c*	4.45 ±0.14 d	4.23 ±0.37 d	4.17 ±0.21 d	3.65 ±0.28 d	3.87 ±0.19 d	3.94 ±0.09 d	4.12 ±0.38 d

\*Means with different letters within a row are statistically different ( $P<0.05$ ); Par.=parameter; AA\*\*=Ascorbic acid; d=days

As compared to fresh cabbage, the ascorbic acid content in minced cabbage has recorded significant decrease up to the 14<sup>th</sup> day of the analyzed interval ( $21.42\pm1.66$  mg%), from which the values of this compound have not anymore presented significant variations ( $P<0.05$ ) up to the end of the experiment (30 days). The largest percentage reduction of ascorbic acid content in chopped cabbage was after the first 2 days of pickling (23.8%) and between 11-14 days (16.1%).

Analyzing the ascorbic acid of brine from shredded cabbage, one can observe that, during the pickling, the concentration of this vitamin recorded the highest values on days 8, 14, 26 and 30, with similar values, undifferentiated significantly between them ( $P<0.05$ ).

Gathering (mathematically) the values of ascorbic acid content from chopped cabbage and its brine, at each period analyzed (tab. 2), one can see that greater losses of ascorbic acid, compared to fresh sample, appeared after the first 2 days of pickling and within interval 11-14 days.

Further, between 14-30 days, the ascorbic acid content remained stable, suffering minor changes (30.19 - 29.15 mg%). As compared with the fresh sample, after 30 days of pickling, the total content of ascorbic acid (minced cabbage and its brine) was reduced by 27.7%.

Regarding the chopped cabbage brine, its pH values had significant decreases only up to the 11<sup>th</sup> day, with insignificant variations up to the end of the review period ( $P<0.05$ ).

The decrease of ascorbic acid content in minced cabbage from  $30.71\pm3.44$  (after 2 days) to  $21.42\pm1.66$  (after 14 days), corresponded to a drop in pH from  $6.17\pm0.35$  (after 2 days) to  $4.23\pm0.37$  (after 14 days).

Once begun the pickling process, the lactic acid accumulation has led to progressively decreasing of pH.

In the table 3 are reproduced  $r$  coefficient values for the correlation between pH and ascorbic acid, during the pickling process.

**Table 3.  $r$  coefficient values for correlations between pH and ascorbic acid content in cabbage and brine**

Correlations	pH CB – AAC	pH CB – AACB	pH CB – AAT (C+CB)	pH MCB – AAMC	pH MCB – AAMCB	pH MCB – AAT (MC+MCB)
$r$	0.942	-0.774	0.937	0.939	-0.676	0.939

CB=cabbage brine; AAC=ascorbic acid in cabbage; AACB=ascorbic acid in cabbage brine; AAT (C+CB)=Ascorbic acid total (cabbage+cabbage brine); MCB=minced cabbage brine; AAMC=Ascorbic acid in minced cabbage; AAMCB= Ascorbic acid in minced cabbage brine; AAT (MC+MCB)= Ascorbic acid total (minced cabbage+minced cabbage brine)

As seen from table 3, between the pH of cabbage brine and the ascorbic acid content of the cabbage, and the ascorbic acid total (cabbage and its brine), on the one hand, and between the pH of minced cabbage brine and the ascorbic acid content of minced cabbage and the ascorbic acid

total (minced cabbage and its brine), on the other hand, there were positive correlations with close values of  $r$  (0.937-0.942), because progressive lowering of pH during pickling has emphasized the oxidation process of this vitamin, especially at pH 4.23-4.36

Between pH and the ascorbic acid content in cabbage brine, on the one hand, and between pH and ascorbic acid content in minced cabbage brine were negative correlations with a higher value of  $r$  for cabbage brine, because a part (unoxidized) of ascorbic acid passed gradually in broth, accumulating there.

The conditions during pickling process (temperatures of 15-18°C and periodic aeration to avoid butyric fermentation) made that total content of ascorbic acid to decrease, both in the (whole) cabbage and in the minced one.

In the case of chopped cabbage, the reducing of the total content of this vitamin has been even more pronounced, because of the product fragmentation, which favored easier access of oxygen to the tissues, and the oxidation of a higher percentage of ascorbic acid.

Losses in vitamin C occur when vegetables (e.g. cabbage) are severely cut or shredded (Mozafar, 1994). Simply cut of cabbage, carrots leads to losses of up to 75% ascorbic acid (Banu et al., 2003), which is very susceptible to chemical and enzymatic oxidation by ascorbic oxidase during processing (Lee and Kader, 2000, cited by Martinez-Villaluenga et al., 2009).

After Martinez-Villaluenga et al. (2009), loss of vitamin C due to cabbage fermentation may be explained partially to be a result of ascorbic acid involvement in ascorbigen formation. But the decrease of ascorbic content as a consequence of its binding into ascorbigen will not achieve more than 10% of its total amount (Hrncirik et al., 2001).

## CONCLUSIONS

The pickling process of white cabbage (*Brassica oleracea* var. *capitata* L.) under certain conditions (5 days fermentation at 18°C, storage at 10°C, 1.5-2.5% salt in brine, and periodic aeration), has influenced the ascorbic acid content in this vegetable.

During pickling process, the ascorbic acid content significantly decreased up to the 17th day of pickling (whole cabbage) and up to the 14th day (minced cabbage), accumulating gradually, at about the same intervals, within their brine.

After 30 days of pickling, as compared with the fresh sample, the total content of ascorbic acid (cabbage and brine) was reduced by 22.3% (whole cabbage), and by 27.7% (minced cabbage).

Mechanical processing (cutting, shredding), progressively increasing of environmental acidity during fermentation, and periodic aeration emphasized the oxidation of this vitamin, especially at pH 4.2-4.3.

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## QUANTITATIVE CHARACTERS VARIABILITY AND THE BEECH GENETIC RESOURCES CONSERVATION IN VALEA FAGILOR NATURAL AREA

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**Key words:** quantitative characters, morphological variability, isolated beech arboreta, seedling

**Abstract:** An very important priority in the genetic resources conservation are the isolated beech arboreta which manifest cumulated effects of the limitative ecological factors and even inbreeding consequences. In the natural area *Valea Fagilor* from the National Park *Munții Măcinului* the *plus* beech trees were selected based on general criteria as growth rapidity with diameter and height measurements. *In situ* conservation of the beech genetic resources in *Valea Fagilor* natural area reclaim specific and urgent measures to stimulate the natural regeneration.

### INTRODUCTION

Beech arboreta in natural area provide a very valuable biologic material (seeds or cuttings) for an efficient and lucrative genetic forestry improvement consisting in seed orchards establishment or afforestation/reforestation activities (2,3). On the other hand, with a view to preserve all natural beech genetic resources, it is necessary to grant a particular attention to the isolated beech arboreta with a reduced number of trees, which manifest cumulated effects of the limitative ecological factors and even inbreeding consequences (1). A high variation within populations and little differentiation among populations it has been proved using provenance trials and with genetic markers (isozymes and DNA markers) (6).

The aim of this paper is to identify the proportion and the morphological variability of the beech *plus* trees in an isolated beech population from the National Park *Munții Măcinului*, Tulcea county – Romania.

### MATERIALS AND METHODS

In July 2012, in the natural area *Valea Fagilor*, we delimited 3 circular testing areas (each one of 500 m<sup>2</sup>) containing beech trees only to the altitude between 225 and 230 m and northern exposure. We identified the proportion of beech *plus* trees in each studied area. The *plus* beech trees were selected based on general criteria as growth rapidity, axle and crown height, pest and disease resistance, abundant fructification and good quality of seeds (5). For our study, we have measured the diameter and height to value the class quality.

### RESULTS AND DISCUSSIONS

According to the measurements of diameter and height performed, in the first testing area with the highest number of beech *plus* trees – 11, resulted a general average of diameter and height – 43,27 cm, respectively 34,72 m. A field specific feature is represented by a northern slope which shade the arboretum about 80% of day. As consequence, in this area it was registered the highest values of the height of trees – 34,72 m on an average and the most reduced values of the diameter – 43,27 cm on an average (table 1).

Table 1 Testing area nr. 1

Nr. ct.	Proportion of beech <i>plus</i> trees (%)	Diameter (cm)	Height (m)	Quality class
1		48	38	I
2		50	38	I
3		50	36	I
4		34	30	I
5		38	32	I
6	<b>64,70 %</b>	46	36	I
7	(11 plus trees/17 total nr)	36	36	I
8		46	38	I
9		44	34	I
10		42	32	I
11		42	32	I
Av.*		<b>43,27</b>	<b>34,72</b>	

Table 2 Testing area nr. 2

Nr. ct.	Proportion of beech <i>plus</i> trees (%)	Diameter (cm)	Height (m)	Quality class
1		40	28	I
2		42	30	I
3		52	28	I
4	<b>46,15%</b>	46	30	I
5	(6 plus trees/13 total nr.)	44	30	I
6		60	32	I
7		40	26	I
8		36	26	I
Av.		<b>45</b>	<b>28.75</b>	

Table 3 Testing area nr. 3

Nr. ct.	Proportion of beech <i>plus</i> trees (%)	Diameter (cm)	Height (m)	Quality class
1		50	26	I
2		44	24	I
3	<b>35,71%</b>	44	28	I
4	(5 plus trees/14 total nr.)	44	22	I
5		38	20	I
Av.		<b>44</b>	<b>24</b>	

\*- average

In the second testing area we selected 8 beech *plus* trees from total number of 13. After the measurements, we registered 45 cm average in diameter and 28,76 m average of height. In this area, the arboretum is shaded 50% of day (table 2).

In the third testing area we registered only 5 beech *plus* trees and the most reduced proportion – 35,71%. The notable diminution of the number and the height of trees (24 m on average) is caused by sunbathed area conditions (shade maximum 20% of day)(table 3). In time, the beech might be replaced by other forestry species (e.g. hornbeam, sessile oak or lime).

## CONCLUSIONS

The number and the proportion of beech *plus* trees in the testing areas show a constant diminution determined by the specific field features and exposure conditions with shaded or sunbathed regime.

The average values of the height and diameter of the beech *plus* trees are reverse proportionally concerning the increase and decrease in the testing areas

*In situ* conservation of the beech genetic resources in *Valea Fagilor* natural area reclaim specific and urgent measures to stimulate the natural regeneration (4). In this respect it is necessary to protect the seedling, the yearly fruit-bearing checking and testing seeds quality.

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## HEMATOLOGICAL AND BIOCHEMICAL MODIFICATIONS IN MALE PATIENTS WITH VIRAL HEPATITIS B

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**Key words:** hepatitis B, hematological indicators, biochemical indicators

**Abstract:** The study systematizes the values of main hematological and biochemical parameters at male persons wearers of hepatic B virus, monitored in the Emergency County Hospital Mavromati Botosani, by echeloning on age groups and then followed these ones deviation from the physiologic-ordinary interval. The results of clinic tests showed that, excepting the number of leucocytes and blood platelets, all the hematological and biochemical indicators kept in view got out of the normality interval's sphere, the highest pathological values registering at the older persons.

### INTRODUCTION

The hepatic affections can be of alcoholic and non-alcoholic nature, the alcoholic hepatic disease being the most frequent of all the hepatic diseases (Rossi et al., 2015), including steatosis with or without fibrosis, the alcoholic hepatitis, cirrhosis and hepatocellular carcinoma, in the Western countries representing a relevant negative co-factor in the progression of chronic hepatic lesions of different etiologies (Rehm et al., 2009; Neff et al., 2011; Mathurin et al., 2012).

The hepatitis B is a global major disease and constitutes the most severe viral form of hepatitis, being one of the main causes of cirrhosis and of hepatocellular carcinoma, approximately 350 millions of people being, in present, wearers of this virus (Lee, 2002). The disease raises serious problems in the less developed countries, reason for what it is entailed the initiation of some new therapies which has to complete the current medication, antiviral and immunomodulator, inclusive the hepatic transplant (Peng et al., 2011).

The goal of hepatitis B treatment is to prevent cirrhosis, liver decompensation and hepatocellular carcinoma. In clinical practice, treatment response is determined by suppression of serum HBV DNA levels, hepatitis B *e* antigen seroconversion to hepatitis B *e* antibody, hepatitis B surface antigen loss, normalization of alanine aminotransferase levels and improvement in liver histology (Kwon and Lok, 2011).

The present study aims to follow up the evolution of some hematologic and biochemical indexes with diagnostic value at male persons diagnosed with hepatic B virus through these ones analyze on different age categories.

### MATERIAL AND METHODS

The research was performed on 29 male persons monitored within the Gastroenterology Department of the County Hospital Mavromati Botosani, in 2008 March - September, to establish the diagnostic or for its confirmation, sampling the blood immediately after the ill's internment, determining the values of hematologic and biochemical indexes with clinical importance in the deceleration and, eventually, the establish of evolutive stage of viral hepatitis B.

Given the fact that the physiologic-ordinary interval for the hematologic and biochemical indexes is in strong correlation with the person's age, we resorted to the patients' repartition in four categories of age namely 21-30 years old, 31-40 years old, 41-50 years old and 51-60 years old, the obtained results being statistically processed, the values refund in the graphics below representing the arithmetical average of each indicator in part. For the accuracy of results and the determination of existing/non-existing of some significant difference from statistically point of view between the average of obtained results in the case of the sample of analyzed subjects and the average of ordinary values in the medical practice we calculated the standard error and the variation coefficient of the average, as well as the Student statistical signification test (Văleanu et al., 1990; Varvara, 2001; Zamfirescu and Zamfirescu, 2008).

The analyze of hematologic parameters was done with the help of Pentra 60 C+ automatic analyzer, and of those biochemical with the help of Hitachi 912 automatic analyzer.

## RESULTS AND DISCUSSIONS

From the total of subjects taken into observation a percent of 21.06% corresponds to 21 - 30 years old group, so as the other 3 categories of age to occupy each one a weighting of 26.31% (Fig.1).

A prime hematologic indicator taken into study was represented by the erythrocytes sedimentation rate (ESR), test screening that offers relations on proteic structure of blood plasma, especially on the fibrinogen, on the  $\alpha_2$ - globulines şi  $\gamma$ -globulines. The specialty literature mentions the fact that, within the hepatocellular affections, the homeostatic modifications at the peripheral blood's level present conexe functional implications, which are manifested in forms all to the aggravating the more the liver's dysfunction is much severe (Paşparan, 2009). As it can be ascertained from Figure 2, the medium values of ESR at male persons investigated don't fit between the limits of normality interval (5 - 7 mm/h), all the subjects with hepatic B virus presenting values much lower, the more drastic decrease being at the age group of 51 – 60 years old ( $2.2 \pm 0.2$  mm/h). It is known the fact that there is a correlation between the blood's deposition speed, on the one hand and the number of red cells, respectively the fibrinogen level, on the other hand. Thus, the rapport's modification between the plasmatic proteins may influence the ERS's value, in the sense that the proteins' dominance with high molecule (globulines and fibrinogen) determines the increase of ERS's values, and the increase of red cells' density leads to the decreasing of this parameter's values (Misăilă and Dumitru, 2010). Our results respect the correlation between the fibrinogen concentration and the deposition speed, but don't evidence literally the relation towards the number of red cells, supplementary factor of influence. In the same time, the low values of ESR may be explained by the installation of anemia, which impedes the formation of erythrocytary aggregates slowing the erythrocytes sedimentation Rate (Paşparan, 2009).

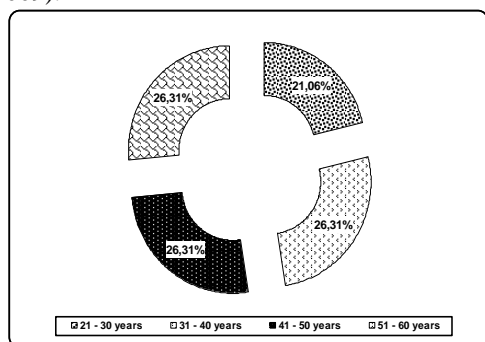


Fig. 1. The procentual distribution of the patients on category of ages

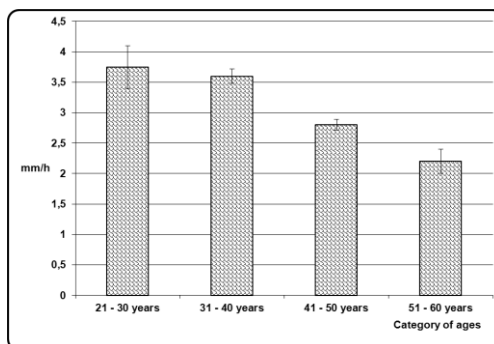
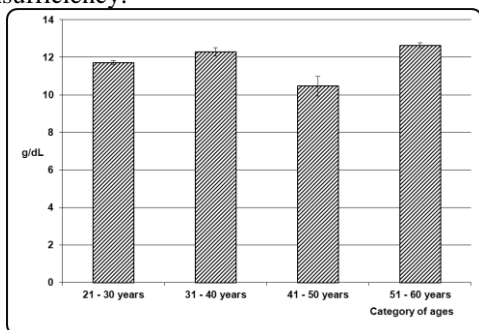


Fig. 2. The ERS average values of on category of ages

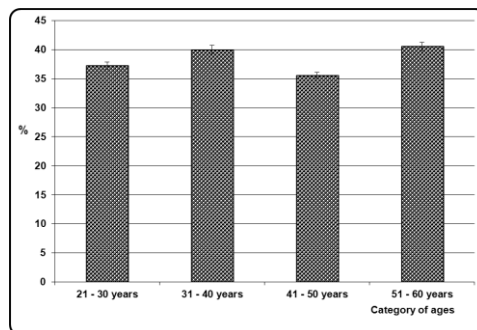
Commonly, in hepatic diseases appear perturbations as well in what concerns the iron's ordinary metabolism, with consequences on hemoglobinosynthesis and on red cells' normochromic, as well as in the deposition and in the corresponding usage of folic acid and of vitamin B<sub>12</sub>, implied in the ordinary ageing of figurate elements in erythropoiesis, all these contributing to the association of liver disease with the anemia (Sulkowski, 2003; Andreana et al., 2004; Gupta et al., 2005; Cengiz et al., 2007; Grimaldi et al., 2008; Savage et al., 2008).

In the case of hemoglobin the physiological interval values are of 13 - 17g/dL blood. At the male persons investigated by us (Fig. 3), the hemoglobinic insufficiency is broader at the 41 - 50 years old category, reaching the threshold of  $10.47 \pm 0.053$  g/dL blood, while, the other groups reached medium values between  $11.72 \pm 0.11$  g/dL (at category of ages 21-30 years) and  $12.64 \pm 0.128$  g/dL (at category of ages 51-60 years).

Similar decreasing were registered also in the case of the medium values of hematocrite (the normally values are 38 - 50%), the insufficiency in this case reaching to  $35.6 \pm 0.509\%$  at the 41-50 years old group and  $37.25 \pm 0.629\%$  at 21-30 years old group (Fig.4), thing which can be explained by the fact that the decreasing of hemoglobinic synthesis gets to a hematocritic insufficiency.

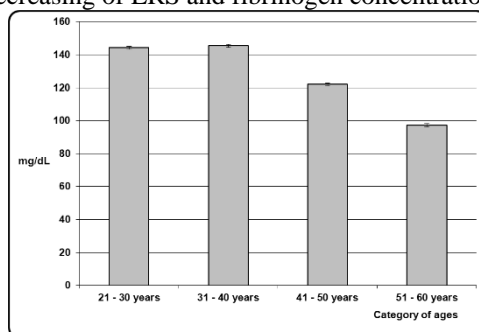


**Fig. 3. The hemoglobin average values on category of ages**

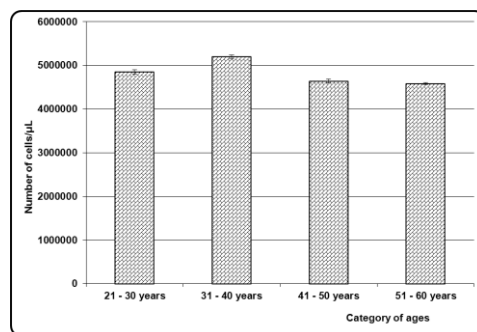


**Fig. 4. The hematocrit average values on category of ages**

In what concerns the fibrinogen quantity, it can be ascertained that this one decreases at all the groups investigated (the normal values for males oscillate between 150 and 360 mg/dL), the lowest value being registered at 51-60 years old group ( $97.2 \pm 0.374$  mg/dL), the rest of the groups presenting values that oscillated between  $122 \pm 0.316$  mg/dL and  $145.4 \pm 0.244$  mg/dL (Fig. 5), the viral hepatitis B, according to literature data (Brujan et al., 2006), being characterized by decreasing of ERS and fibrinogen concentration.



**Fig. 5. The fibrinogen average values on category of ages**



**Fig. 6. The average values of red cells number on category of ages**

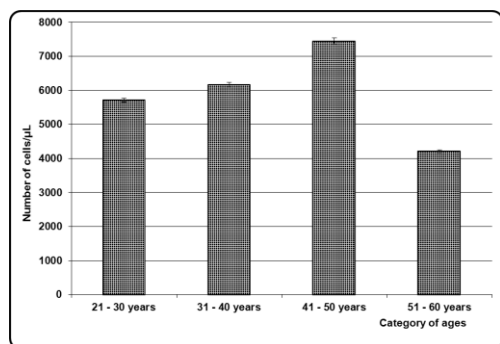
From Figure 6 comes out that it doesn't exist significant differences in what concerns the number of red cells at male persons with hepatic B virus, all the age categories presenting values under the inferior limit of the normality ceiling characteristic for the respective age, the 51-60 years

old group registering a minimum average value of  $4584000 \pm 26570.66$  red globules/ $\mu\text{L}$  blood, while, at 31-40 years old group, was dignified a medium average of  $5200000 \pm 35355.34$  red globules/ $\mu\text{L}$ .

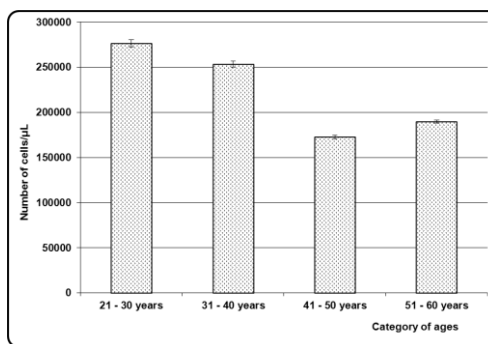
Data from the specialty literature mention the fact that leucopenia with selective neutropenia is common to viral infections, inclusively hepatic, case in which it may be come to agranulocitose all-gravely through medullar inhibition, concomitantly and compensatory installing a relative limfocitose – the ordinary response of the organism in numerous viral infections (Brujan and colab., 2006 cited by Pașparan, 2009), the medium values of the leucocytes number (Fig. 7) at all the investigated patients being situated in the physiologic-ordinary interval (4210 - 7450 white globules/ $\mu\text{L}$  blood).

A similar situation it's met also in the case of thrombocytes, the minimum average value being highlighted at the 41-50 years old category ( $172600 \pm 2070/\mu\text{L}$  blood), and the maximum at the 21-30 years old category ( $276500 \pm 3898/\mu\text{L}$  blood).

In what concerns the statistical signification of the obtained results, the hematologic parameters' values registered by us, are, in their majority, significant ( $0.01 < p < 0.05$ ) and distinctly significant ( $0.001 < p < 0.005$ ), while the hemoglobin and the fibrinogen in the case of 41-50 years old group and 51-60 years old group, respectively leucocytes and thrombocytes in the case of 31-40 years old groups, 41 -50 years old and 51-60 years old are significantly strong ( $p < 0.001$ ), and the ERS from the 31-40 years old group is insignificantly statistic ( $p > 0.5$ ).



**Fig. 7. The average values of leucocytes number on category of ages**



**Fig. 8. The average values of thrombocytes number on category of ages**

Hereinafter we calculated the medium values of the erythrocytary constants: MCV (mean cell volume) - indicator by virtue on which it is done the correct classification of the anemia types, MCH (mean cell hemoglobin) - constant which offers informations about the quantity of hemoglobin and MCHC (mean cell hemoglobin concentration) - parameter which expresses the rapport between the hemoglobin quantity and the total erythrocytary volume (Misăilă and Comănescu, 1999).

From the analyze of medium erythrocytary volume's values (Fig. 9) it is ascertained that, beside the patients with ages between 51-60 years old, which is situated in the normality interval's limits ( $88.5 \mu\text{m}^3/\text{eritrocit}$ ), at all the others, the MCV values are pathologic (approximately  $77 \mu\text{m}^3/\text{eritrocit}$ ).

In a similar way varies also the medium erythrocytary hemoglobin (at the 51-60 years old category registering a physiologic value of  $27.6 \text{ pg hemoglobin/erythrocyte}$ ) given the fact that the



MCH modifications go in parallel with those of the MCV, the easy increasing under the ordinary minimum threshold of the reference interval (25-32 pg Hb/ red cell) at the patients up to 50 years old being possible to be associated with feriprive hypochrome anemia (Manole et al., 2005).

The moderately reduced values of MCHC, at all the age categories (31.48 g Hb/100 mL erythrocytary weight at 21-30 years old category, 30.7 g Hb/100 mL erythrocytary weight at 31-40 years old category, 29.41 g Hb/100 mL erythrocytary weight at 41-50 years old category and 31.16 g Hb/100 mL erythrocytary weight at 51-60 years old category) may lead to a diagnostic of hypochromic anemia (Fig. 11).

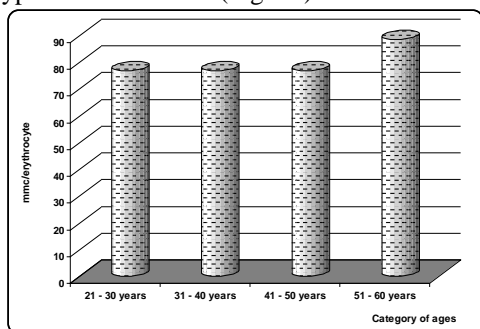


Fig. 9. The mean cell volume (MCV) on category of ages

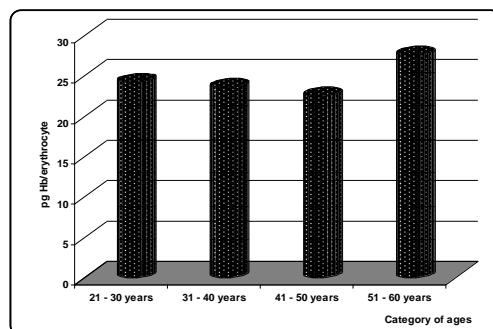


Fig. 10. The mean cell hemoglobin (MCH) on category of ages

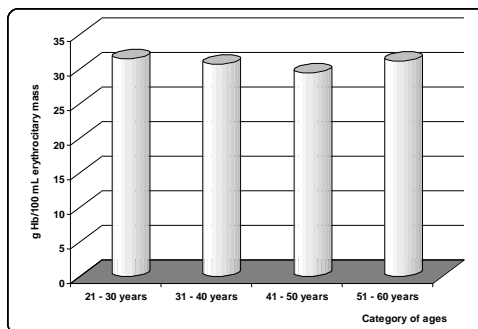
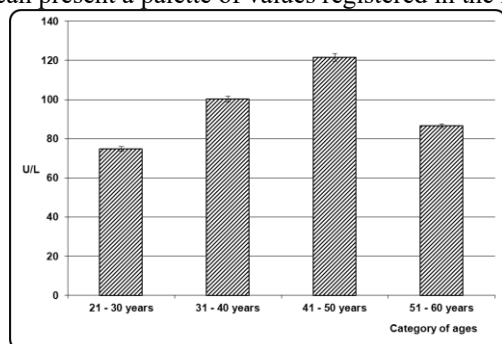


Fig. 11. The mean cell hemoglobin concentration (MCHC) on category of ages

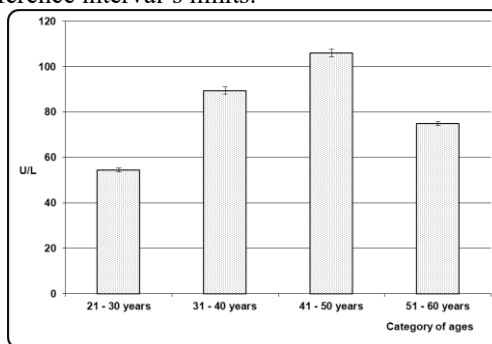
It is known the fact that the hepatic cells' suffering which leads to the decrease of the permeability of hepatocytes' membrane, but not necessary to the necrosis of these cells, it is detected on the increase in serum of some cellular enzymes. The serum level of the activity of these lesion enzymes varies based on the number of affected hepatocytes, on the lesion gravity of each cell, on the speed with which were produced the lesions and on the elimination speed from serum of the respective enzymes (Cojocaru, 2005), as a result, in the different diseases of the liver, realizing different specters of the lesional enzymes' activity, based on the etiology and on the evolutiv stage of hepatopatya (Pâslaru, 2004).

Data from literature denote an association between the increase of the aminotransferases' activity and the installation of a cytolyses estate, Nair et al., 2001 highlighting a significant increase of the alanin-aminotransferase's activity (ALAT) in the hepatic affections comparatively with that of the aspartat-aminotransferase's (ASAT). At the male persons with

viral hepatitis B taken into study, the two enzymes registered medium values a lot increased at all age categories, comparatively with the ordinary interval's limits (Figs. 12-13). Thus, the 21-30 years old group registered a medium value of the ALAT of  $74.85 \pm 1.142$  U/L, while, at the same age group, the medium value of ASAT enzyme was of  $64.55 \pm 0.86$  U/L. The 41-50 years old group reached the maximal average thresholds both in the case of ALAT ( $121.64 \pm 0.526$  U/L), as well as ASAT ( $106 \pm 0.706$  U/L), while in the case of the last analyzed age category (51-60 years old) ALAT registered a medium value of  $86.708 \pm 0.731$  U/L, while ASAT had the medium value of  $74.86 \pm 0.824$  U/L. The rapport ASAT/ALAT turned out to be sub-unitary for each age group in part, the literature data (Negură, 2008) associating these values with the acute hepatitis diagnostic, the increase of aminotransferase's activity during the antiviral therapy being a good clue of the efficiency of the applied therapy. In the same time, it should be taken into view that, during the disease's evolution, although the cirrhosis was installed and takes place the firm progression of the disease to hepatocellular carcinoma (Popescu, 2005) the aminotransferases can present a palette of values registered in the reference interval's limits.



**Fig. 12. The ALAT average activity on category of ages**



**Fig. 13. The ASAT average activity on category of ages**

The alkaline phosphatase's activity (FA) registered moderate increasing towards the superior limit of the reference interval (100-290 U/L), at all age categories taken into study. Thus, if the younger patients presented medium values of  $342.875 \pm 1.178$  U/L (the 21-30 years old group) and  $366.44 \pm 0.591$  U/L (the 31-40 years old group), at all categories beyond 40 years the enzyme registered medium values net superior to admitted maximum limit ( $452.34 \pm 0.808$  U/L - at 41-50 years old group and  $487.42 \pm 0.538$  U/L at 51-60 years old group).

And in what concerns the  $\gamma$ -glutamyl-transpherase (GGT), enzyme indicator of cholestasis, the activity acted of a similar manner, laying out, at all age groups, medium values easy increased towards the superior limit of the ordinary interval (12-48 U/L). If at the 21-30 years old group the GGT activity reached the medium level of  $52.95 \pm 0.417$  U/L, at 41-50 years old group and 51-60 years old group the medium thresholds of activity were significantly higher, the registered averages being of  $68.92 \pm 0.255$  U/L, respectively  $62.72 \pm 0.315$  U/L. From statistically point of view, the values of all serum enzymes were very significant ( $p < 0.001$ ) at all studied age categories.

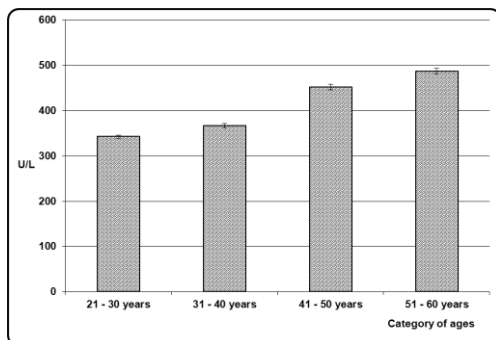


Fig. 14. The FA average activity on category of ages

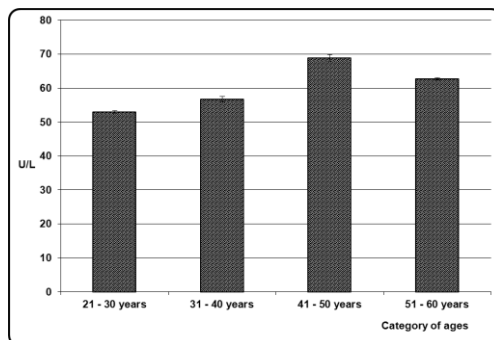


Fig. 15. The GGT average activity on category of ages

## CONCLUSIONS

- \* At the investigated patients the number of leucocytes and thrombocytes are situated, generally, in physiologic limits.
- \* The erythrocytes sedimentation rate and the fibrinogen quantity register values situated under the inferior threshold of the reference interval.
- \* The hemoglobinic and hematocritic insufficiency reaches levels more marked at the patients from the 41-50 years old category.
- \* The enzymes with diagnostic value registered significant increasing of activity at all age categories, the aminotransferases and the glutamil-transpherase being maximal at the 41-50 years old age category, while the alkaline phosphatase reaches superior quotes at the patients between 51 and 60 years old.

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## THE EVOLUTION OF PREGNANCIES FOR THE PATIENTS WITH POLYCYSTIC OVARY SYNDROME AND OVARIAN HYPER STIMULATION SYNDROME

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**Key words:** Polycystic Ovary Syndrome, infertility, clomiphene, hyper stimulation

**Abstract.** The ovarian hyper stimulation syndrome (OHSS) is a feared complication during the controlled ovarian stimulation process. In this study we followed the evolution of pregnancies for the patients diagnosed with polycystic ovary syndrome (PCOS) after they had previously undergone a controlled ovarian stimulation, using different protocols, therapy that was complicated by the syndrome of hyper stimulation. The group of participants in the study included 46 patients that were diagnosed with OHSS, aged between 25 and 39, over a period of 4 years (2011-2014). If the risk of OHSS is high, we will not use human chorionic gonadotropin (HCG) to trigger ovulation. The protocols must be carefully individualized and the stimulation cycles need to be correctly monitored. The evolution of the pregnancies with OHSS can be to term, giving birth to living eutrophic fetuses (30 patients), or there can be premature labour, before the term (8 patients – 21.1%).

### INTRODUCTION

The incidence of OHSS for the women treated with Clomiphene citrate (CC) is hard to find out, because the definitions given to the syndrome are very different from one study to another. While mild OHSS (moderate increase of the ovaries) is relatively common, severe OHSS (a massive increase of the ovaries, progressive weight gain, severe abdominal pains, nausea and vomiting, hypovolemia, ascites and oliguria) is only rarely observed.

The necessity of ovary stimulation appears when there is a diagnostic of ovulatory dysfunction, which can be established by menstrual history, a regular determination of serum progesterone (during the presumptive luteal phase), monitoring the urinary excretion of pregnanediol or through periodical transvaginal ultrasound scanning. The specific ovulation tests are useless when the menstrual history can establish the diagnostic (amenorrhea, oligomenorrhea). Once identified, infertile women, with no ovulation, deserve further evaluation before the treatment, in order to identify any other systemic diseases that would require further investigation, counselling or specific treatment. Establishing a detailed medical history and physical examination can also show other endocrine or metabolic diseases. It is justified to perform a screening for hypothyroidism, serum level for thyroid-stimulating hormone (TSH) and for hyperprolactinemia because both diseases are treated better with other medication than CC. Ovarian failure assessment is recommended for all amenorrhoeic women, regardless of age. Screening for impaired glucose tolerance or diabetes is recommended for obese women (body mass index BMI >30 kg/m<sup>2</sup>) with PCOS (Cuellar F.G, 1980; Lincoln S.R., 1999; Practice Committee of the American Society for Reproductive, 2004).

Another cause is PCOS (polycystic ovary syndrome). Pre-conception counselling for the women with PCOS should identify the risk factors for reproductive failure and should correct them before starting a treatment. It is essential to admit the presence of obesity and its centripetal distribution that can vary depending on ethnicity and geographical area and to recommend a supplement of folic acid for all the women, and also to recommend them to quit smoking. It is a well-known fact that obesity is associated with anovulation (Pasquali R., 2006), miscarriage (Heijnen E.M., et al, 2006) and the complications appeared in the last trimester of pregnancy (preeclampsia, gestational diabetes) (Boomsma C.M., et al, 2006). Pasquali states that the role of losing weight in managing PCOS should be encouraged before any pharmacological treatment (Pasquali R., 2006).

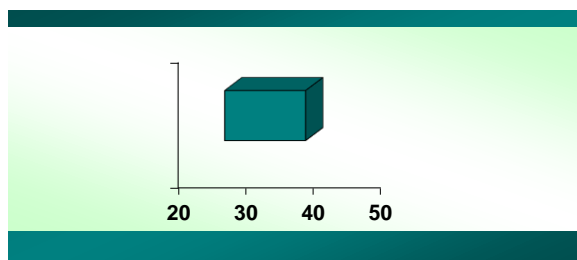
The logical treatment of the women with PCOS is to induce ovulation, in particular by administration of CC, and in case it does not work, by treatment with exogenous gonadotropins. The main complication of inducing ovulation is the rate of 10% of multiple pregnancies, especially following the treatment with gonadotropins. For this reason, the use of gonadotropins can be questioned (van Santbrink E.J., 2003). If there is no body weight loss and the treatment with anti-oestrogens or ovarian drilling does not work, we can argue that inducing ovulation with the help of the treatment with exogenous gonadotropins must be replaced by ovarian stimulation and IVF (*in vitro* fertilization) hence the risk of OHSS.

## PURPOSE AND OBJECTIVES

The purpose of inducing ovulation to the women with anovulation caused by PCOS is to re-establish fertility and to result in a living new-born. The method of inducing ovulation through therapy with gonadotropins is based on the physiological concept according to which initiating and maintaining follicular growth can be obtained with a transitory increase of FSH (follicle stimulating hormone) over a threshold-dose, for a period of time long enough to generate a limited number of developing follicles. It is essential to apply this concept ovulation is induced to women with PCOS, because they are specifically predisposed to an excessive development of multiple follicles.

## MATERIAL AND METHODS

The study group included 46 patients diagnosed with PCOS, aged between 25 and 39, over a period of time of 4 years (2011-2014).



**Fig. 1.** *The mean age of the patients included in the study*

The observation sheets of the hospitalized patients were monitored and the data supplied by them was processed. Thus it was possible to interpret the: age of the patients, social background, diagnostic when admitted, reasons for hospitalizing them, the way in which the illness started, personal physiological priors (history), personal pathological history, clinical examination, ultrasound, blood analysis, surgical intervention, pathology data, evolution and complications.

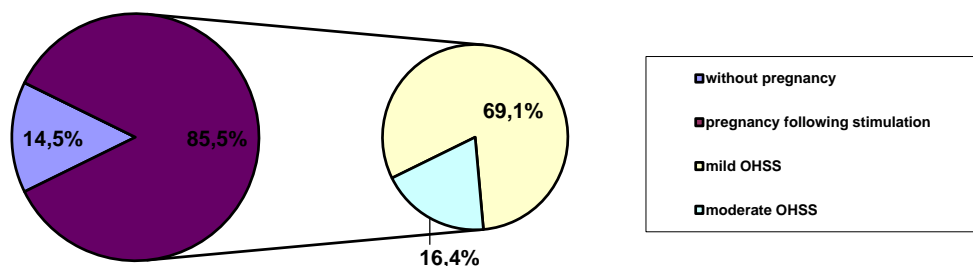
After establishing the diagnostic of **infertility and PCOS**, the doctors discussed the options and methods of treatment with every couple. At this moment there is a wide range of such options. You can choose a passive attitude, of waiting or you can choose one of the following once ovulation is induced: controlled ovarian hyper stimulation, intrauterine insemination, vitro fertilization, intracytoplasmic injection of sperm, using gametes or embryos, or surrogate mothers. Ovarian stimulation has an important role in the treatment of assisted human reproduction.

The criteria for accepting somebody in the study group were given by the appearance of the hyper-stimulation syndrome, in different degrees, for the patients with a positive pregnancy test. So, of the initial 46 patients that were initially included in the group, 38 had pregnancies following controlled ovarian stimulation, the rest of 8 did not get pregnant, but only developed a mild or moderate form of OHSS.

Clomiphene citrate was administered orally, for 5 days, starting with the second to the fifth day after the beginning of their menstrual cycle that appeared spontaneously or induced by progestogenes. The ovulation rates, the conception rates and the results regarding the pregnancy are similar, no matter if the treatment begins in the second, third or fourth day of the cycle (Practice Committee of the American Society for Reproductive, 2004). Although the dose that is necessary to obtain ovulation is correlation with body weight, there is no certain way of predicting accurately the dosage that is necessary for each woman. These medicines can be used only if the hypothalamic-pituitary axis works. The main factors that predict the negative result of the treatment are: obesity, hiperandrogenemia and age (Imani B., et al, 2002). The ovarian volume and the menstrual state are supplementary factors that help to predict the reaction to CC (Eijkemans M.J., et al, 2003).

## RESULTS AND DISCUSSION

Of the 38 patients with PCOS who got pregnant, 29 patients had a mild form of OHSS, and 9 patients had a moderate form.



**Fig. 2.** The structure of the group based on the presence of pregnancy following stimulation

Of the 8 patients with PCOS who did not result in a pregnancy, 6 had a mild form of OHSS, and two patients had a moderate form.

As clinical symptoms, the pregnant women with mild forms of OHSS showed abdominal discomfort and distension, an increase in the size of the ovaries that was shown by ultrasound, with multiple follicles, estradiol dosed to values over 3000pg/ml, progesterone over 30pg/ml.

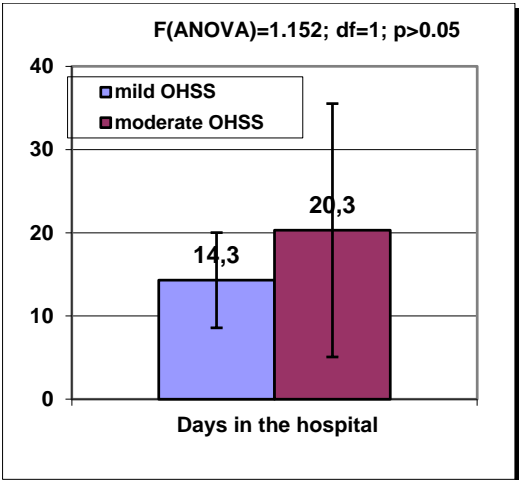
The first line therapeutic conduct was one of waiting, the patients with mild forms being monitored over a period of time of about 10 days for blood pressure, pulse, weight, and diuresis. In these cases, the clinical picture was delivered in 5-7 days.

The 9 pregnant patients with a moderate form of OHSS showed the following symptoms: ascites, urine retention, and dyspnoea, a significant increase in body weight and changes in the biochemical samples. They required close monitoring of blood pressure, diuresis, pulse, body weight and biochemical values, plus the degree of ascites. The patients were recommended bed rest and oral hydration.

None of the cases required a transvaginal puncture of Douglas pouch. Urine retention was signalled for 2 patients, with a diuresis of 100-200ml/day, considering a parenteral hydration of 2000-2500ml/day; in these cases we used furosemide for treatment.

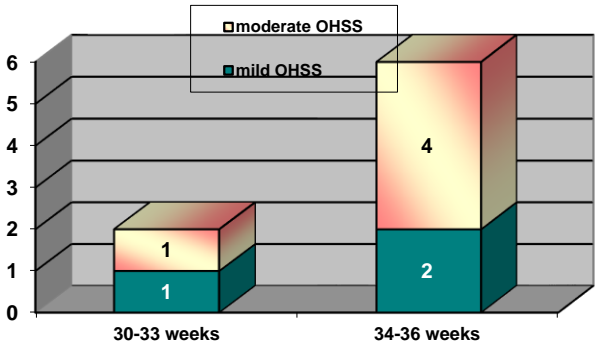
All the cases of OHSS - moderate form – were treated with albumin for obtaining a hydro electrolytic balance.

The clinical picture was made after 14-21 days.



**Fig. 3.** *The mean number of days spent in the hospital depending on the clinical form of OHSS*

The evolution of the cases was favourable, as no patient required her pregnancy to be terminated. Of the total number of pregnancies studied, 8 delivered the baby before 36 weeks.



**Fig. 4.** *Gestational age based on the clinical form of OHSS*

According to a cohort study, *The Australian Longitudinal Study on Women’s Health (ALSWH)*: the prevalence of PCOS was of 5,8% (95% confidence interval CI: 5,3%-6,4%); infertility was claimed for 72% of the patients with PCOS in comparison with 16% for the general population ( $p<0,001$ ); hormone treatments were necessary in 62%,  $n=116$  in comparison with the general population - 33%,  $n=162$ , ( $p<0,001$ ) – the rate of IVF (in vitro fertilization) was similar (Bates G.W.& Legro R.S., 2013).

In a random study, the rates of births were not different for the women treated only with CC, in comparison with the ones treated with CC and metformin (Nestler J.E., et al, 1998). In the case of the patients who do not succeed to ovulate with CC, metformin was shown to improve the ovulation and pregnancy rates in comparison with the response to CC. Many controlled random studies



showed that pre-treatment with metformin in doses of 1500 to 1700 mg per day significantly improved the ovulation and pregnancy rates in response to the administration of CC to the women who did not succeed to ovulate only with CC (Fernandez H., 2011; Zreik T.G., et al, 1999). For the obese women who did not respond to therapy with CC or for the couples who did not consider pregnancy an immediate purpose, metformin combined with diet and physical exercise for losing weight might be considered (Imani B., 2002).

HMG-human menopausal gonadotropin and pure follitropin (FSH) are the gonadotropins that are approved in treating PCOS. HMG contains 75UI FSH and 75UI LH and it is obtained from the urine of the women at menopause. FSH is obtained either from urine (uFSH), or through genetic engineering (rFSH), with no LH activity (Amin M., 2003, Krysiak R., 2006, Pritts E.A., 2002). The disadvantages of using gonadotropins for the patients with resistance to citrate of clomiphene are represented by the high costs and the risk of developing the syndrome of ovarian hyper stimulation and multiple pregnancies (Hoyt K.L., 2004). There is no data to compare directly the rates of ovulation and pregnancy with the ones obtained only with gonadotropins. The advantages of a combined therapy with CC and gonadotropins include a reduced dose of gonadotropins and a potentially decrease in the monitoring costs. The women with anovulation that are resistant to clomiphene citrate are often very sensitive to small doses of gonadotropins, and the treatment should have as main purpose the obtaining of one mature follicle every time this is possible, because, leaving IVF aside, there is no sign of deliberate supra-ovulation for the infertile women with anovulation. The alternatives to CC therapy for women who are resistant to CC include aromatase inhibitors, tamoxifen, insulin-sensitizing agents, laparoscopic ovarian drilling, the use of gonadotropins and IVF.

The most frequent complications of gonadotropin therapy are due to multiple ovule maturation, ovarian hyper-stimulation and multiple pregnancies. Women with polycystic ovary syndrome and anovulation are like to grow more follicles when they are given gonadotropins.

Heijnen et al. (2006), showed that the rate of menstrual cycle stopping is significantly higher for the patients with PCOS (12.8% versus 4.1%; RR 0.5; CI of 95%, 0.2-1.0). The stimulation lasts significantly longer for the patients with PCOS (1.2 days; CI of 95%, 0.9-1.5), even when the daily dosage of FSH is similar with that for the patients without PCOS. There were significantly more complex cumulus oocytes obtained (2.9; CI de 95%, 2.2-3.6) from the women with PCOS, but the fertilization rates were similar with those of women without PCOS. When referring to the probability of a pregnancy, the rate of clinical pregnancies for every cycle was similar ( $\approx 35\%$ ) for the patients with PCOS and those who do not have PCOS. The same thing applies for the pregnancy rate when harvesting oocytes and the embryo transfer. For the moment we lack the specific data regarding the rate of success of the transfer of one embryo to the women with PCOS. There are some proofs that show that supplementary use of metformin can improve the chances for an ongoing pregnancy and can reduce the incidence of PCOS. The most important complication of ovarian stimulation is OHSS. Still, there is no solid data regarding the incidence of OHSS in women with PCOS that undergo ovarian stimulation with the purpose of getting IVF.

## CONCLUSIONS

OHSS (ovarian hyper stimulation syndrome) appeared in the case of the patients diagnosed with PCOS (polycystic ovary syndrome) who underwent a controlled ovarian stimulation.

Dopamine agonists will be used on the patients with a risk of OHSS.

Ovulation is triggered only after the level of estradiol is known.

Oocytes cryopreservation is the only method of preventing late OHSS and will be used if the number of oocytes is over the accepted threshold.

It is important to rigorously monitor the patients with an excessive ovarian response.

OHSS (ovarian hyper stimulation syndrome) is more intense for the patients with an associated ongoing pregnancy.

When analyzing OHSS (ovarian hyper stimulation syndrome) that is associated with pregnancy it is important to follow the parameters with the purpose of obtaining a physiological plasma volume and to correct electrolyte imbalances.

The intention is to use a treatment protocol for the patients who had IVF that will impose a conduct to follow in order to get and maintain a viable pregnancy when there is OHSS present. All IVF clinics must have protocols for identifying the patients with OHSS risk and to have limits for the values of estradiol and oocytes harvested in order to prevent OHSS.

Regardless of the clinical form of the syndrome, pregnant women will be hospitalized and treated like pregnant women with an increased obstetrical risk.

The evolution of pregnancies that associate OHSS can be to term, giving birth to living eutrophic foetuses, (30 patients) or can result in premature labour, before term (8 patients).

Thus, in our group, the incidence of premature pregnancies was 21.05%.

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**MARIUS ȘEFAN: APPLIED  
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Volumul *Applied microbiology, from plant growth promotion to new neuroprotective drugs*, realizat de către Dl. Profesor universitar dr. Marius Ștefan, reprezintă o apariție de înaltă valoare pentru domeniul microbiologiei teoretice și aplicate din România.

Autorul nu enunță doar în paginile lucrării principiile de bază și direcțiile aplicative ale microbiologiei, ci filtrează informația preluată din literatura de specialitate prin propria gândire, incluzând ca suport faptic rezultate ale cercetărilor personale, obținute prin sumarea unei cantități mari de efort experimental, fapt ce oferă cititorului posibilități concrete de corectă asimilare a bogatului bagaj de date prezentat.

În cele aproximativ 200 pagini ilustrate sugestiv sunt abordate, într-o manieră sistematică, în 3 secțiuni consistente, teme de top din microbiologia aplicată contemporană, cum ar fi:

- strategiile de promovare a creșterii plantelor prin prisma cunoașterii interacțiunilor plantă-microorganism la nivelul rizosferei, cu discutarea unor situații concrete la fasole și soia, ca rezultat al cercetărilor personale;
- utilizările practice (efecte antimicrobiene) ale unor compuși naturali - uleiuri aromatice obținute prin hidrodistilare din plante medicinale/aromatice, extracte vegetale din plante spontane sau cultivate în scop alimentar ori chimici - nanoparticule de metale nobile (argint, aur);
- efectele biologice ale derivaților nicotinici din *Arthrobacter nicotinovorans*, ca suport teoretic-aplicativ pentru sinteza de noi posibile

The book *Applied microbiology, from plant growth promotion to new neuroprotective drugs*, written by Professor PhD Marius Ștefan, is a valuable appearance for theoretical and applied microbiology in Romania.

The author does not only give the basic principles and directions of applied microbiology but he also filters the information taken from literature through his own mind. He includes in the book as a factual support his own research results obtained after an important experimental effort, providing the readers real opportunities for proper assimilation of the huge amount of data presented.

In the 200 suggestively illustrated pages are addressed in a systematic manner, in 3 consistent sections, top themes of modern applied microbiology, such as:

- strategies to promote plant growth through the knowledge of the plant-microorganism interactions in the rhizosphere, discussing specific situations for runner bean and soybean, as a result of personal research;
- practical uses (antimicrobial effects) of certain natural compounds: essential oils obtained by hydrodistillation from aromatic/medicinal herb; extracts from wild or cultivated plants; nanoparticles of noble metals (silver, gold);
- biological effects of nicotinic derivatives from *Arthrobacter nicotinovorans* as theoretical/practical support for the synthesis of new potential neuroprotective drugs, with special

medicamente neuroprotectoare, cu referiri speciale la lupta cu temuta boală Alzheimer.

Informațiile astfel prezentate urmăresc detalierea mecanismele biochimice și moleculare ale proceselor și funcțiilor vitale la organismele test, având un caracter informativ pronunțat și contribuind în mod evident la îmbogățirea bagajului logic de cunoștințe al cititorului.

Ideea generală ce se poate desprinde din paginile lucrării este că utilizarea microorganismelor în agricultură, industria chimică și medicină va îmbunătăți în viitor, în mod cert, standardul de viață al omenirii, oferind soluții practice diferitelor probleme în domeniile anterior prezentate.

Titlul lucrării, în deplină concordanță cu intenția, viziunea și modul de tratare a subiectului ales spre prezentare de către autor impune cititorului avizat volumul de față ca o lucrare monografică de certă valoare științifică, menită să aducă lămuriri de ordin teoretic, susținute prin date practice, privind natura și utilizarea diferitelor clase de substanțe biologice active produse de microorganisme, lucrarea completând în mod fericit, prin datele prezentate informațiile existente în tratatele românești și străine de profil.

Textul lucrării, procesat cu deosebită grijă, este însoțit de o grafică extrem de bogată (scheme, tabele, cromatograme, fotografii, în majoritate originale), utilizează un limbaj clar și precis, iar interpretările realizate, bine corelate și armonizate cu informațiile oferite de literatura de specialitate sunt exprimate într-o manieră elegantă. Sursele bibliografice utilizate sunt reprezentate de peste 280 de titluri, dintre care 42 lucrări originale, publicate în reviste de certă valoare științifică din circuitul internațional și național; toate acestea constituie o dovadă evidentă că autorul este deplin conectat la preocupările de ultimă oră ale lumii științifice în legătură cu subiectul prezentat.

Per ansamblu, lucrarea analizată poate fi considerată o apariție inedită, care impune în biologia românească o viziune modernă asupra microbiologiei aplicate, realist interconectată cu preocupările ecologice și funcționale de interpretare ale viului, tendință ce domină actualmente climatul științific mondial.

Argumentele de mai sus, modul de abordare și tratare a subiectului, precum și originalitatea și noutatea multor rezultate proprii comunicate ca date inedite în literatura de specialitate românească recomandă volumul de față tuturor celor interesați

reference for fighting Alzheimer's disease.

The presented data follows the main biochemical and molecular mechanisms of the test organisms vital processes and functions, having a clearly marked goal and contributing to enriching the knowledge of the reader.

The general point to be drawn from the pages of this paper is that the use of microorganisms in agriculture, chemical industry and medicine will certainly improve in the future standard of mankind living, providing practical solutions to various problems in the areas listed above.

The title, in full accordance with the intention, vision and the approach of the chosen subject propose this volume to the informed reader as a valuable monograph. The book was designed to provide theoretical clarity, supported by practical data on the nature and use of various classes of biologically active compounds produced by microorganisms. The paper is happily completed by the presentation of data from Romanian and foreign treatises.

The paper, processed with great care, is accompanied by an extremely rich graphic (charts, tables, chromatograms, photographs, mostly original), using clear and precise language; the interpretations expressed in an elegant manner are well linked and harmonized with the information from literature. Bibliographical sources are represented by more than 280 titles, including 42 original papers published in national and international scientific journals. All those presented above constitutes compelling evidence that the author is fully connected to the latest concerns of the scientific world.

Overall, the analyzed paper can be considered a novel editorial appearance that impose to the Romanian biology a modern vision of applied microbiology, realistic interconnected with the environmental and functional concerns of living interpretation - a trend which currently dominates the global scientific climate.

The above arguments, the approach of the subject, as well as the originality and novelty of many personal results communicated in the Romanian literature recommend this volume to anyone interested in current issues of existence and adaptive functioning of microorganisms.

de mereu actualul subiect al existenței și  
funcționării adaptative a microorganismelor la  
mediu de viață.

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