CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Vol. 2 (1)
June 2010

North University of Baia Mare Publishing House

Carpathian Journal of Food Science and Technology, ISSN 2066-6845
http://stiinte.ubm.ro/multimedia/Chimie/index.html

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Publishing House: North University of Baia Mare Publishing House, http://editura.ubm.ro/

CONTENT

IMPACT'S ASSESSMENT OF THERMAL PROCESSING AND STORAGE CONDITIONS ON ENZIMATIC ACTIVITY AND HMF CONTENT IN HONEY

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Abstract

The aim of the paper is to study the impact of heating and storage conditions on the diastase activity and hydroxymethylfurfural (HMF) in honey. In this end, three types of honey (chestnut, acacia and polifloral) were used in experiments. After a transient heating stage, the honey samples were kept for 2 hours and 3 hours at 50^{o}C and 80^{o}C and then analyzed to determine the diastase activity and the HMF content. Also, identical honey samples were storage during 12 days and 50 days in different conditions: refrigeration, dark at 20^{o}C and light at 20^{o}C and analyzed to find the coefficient of diastase activity and the HMF content. Experimental data indicate that from diastase activity point of view, the most aggressive treatment is honey heating at 80^{o}C for 3 hours when enzymatic activity is complete destroyed for all honey types. Comparing with others processing and storage conditions, 3 hours heating of honey at 80^{o}C has also a major impact on honey's quality due formation of high amounts of HMF, above the 40 mg/Kg the maximum admitted limits by international regulations. In terms of HMF formation, the most heating affected honey is chestnut honey and the lowest impact was recorded for polifloral honey. Considering experimental data, the impact rank according to processing and storage conditions was performed.

Keywords: *acacia honey, polifloral honey, chestnut honey*

1. Introduction

Honey is produced by honeybees from nectar of different melifer flowers as well as from honeydew. Its physico-chemically-microbiological properties depend by floral origins (floral origins with everything that implies), climatic conditions but also by the processing steps. Between all steps that honey go through from producing to consumption, the most important from preserving their properties the thermal treatment and storage conditions are considered the most important. Thermal treatment of honey is applied in order to reduce the viscosity in order to facilitate the dosifiers and jar's filling, avoiding the post bottling crystallization and to destroy the microorganisms that contaminate it. Honey's storage conditions also have a major influence on it quality and safety. The most used parameters to emphasize the quality and safety of honey are diastase activity and the hydroxymethylfurfural (HMF) content. The activity of the diastase is closely related to its structure and can be modified by denaturation, brought about by heating [1]. HMF results mainly by hexoze dehydration at pH lower than 5 and Maillard reactions [2]. Due the fact that Maillard reactions occur at high temperature, we can consider that HMF content is a very good indicator of honey freshness. Others factor that takes part in Maillard reactions and implicitly influences the HMF formation are the sugar content of honey [3], temperature and time heating [4], use of metallic containers [5].

The aim of the paper is to investigate de impact of thermal treatment and storage conditions on quality of honey considered as references diastase activity and HMF formation.

2. Materials and methods

2.1. Samples

Three honey types were selected for the study. The declared botanical origin by the producers was considered: Chestnut (*Castanea sativa L.*), Acacia (*Acacia*) and Polifloral (hay field). All samples were from 2009 production.

2.2. Heating treatment

The experiment was conducted under isothermal treatment. The honey were transferred in coil which were submerged in hot water bath in such way that after 5 minutes, corresponding to transitory stage, honey reached the studied temperature (Table 1). After the isothermal treatment elapsed, the coil were withdrawn, rapidly cooled in ice water and analyzed to determine the coefficient of diastase activity and the HMF content. Oneway analysis of variance (ANOVA) was performed to examine the effects of heating at different temperatures and storage conditions on the above mentined parameters. The F-test was used to estimate the statistically significant differences (P-value <0.05).

2.3. Storage

After extraction, the same honey samples were stored in glass holder in different conditions as the Table 2 indicates.

2.4. HMF analysis

Winkler method [6] was used for analysis of HMF in honey samples. The method is based on the direct proportionality between the HMF concentration and the intensity of red colored complex obtained when HMF reacts with barbituric acid in the presence of paratoluidine. The absorbance of honey solution was reading at 550 nm using a CINTRA 5. The value of absorbance increased up to a maxim and then decreased. The blank sample contained 1 cm^3 of water instead of barbituric acid. The HMF content was calculated according with the equation:

$$HMF = \frac{E}{S} 192 \ \text{mg/Kg honey} \qquad (1)$$

where:

E – maximum absorbance red at spectrofotometer

S – thickness layer (cm)

192 – absorbance conversion factor in HMF equivalent

2.5. Coefficient of diastase activity

It was measured considering the volume (cm^3) of 1% starch solution transformed in dextrins during 1 hour, under temperature of 45^{o}C by the α-amylase from 1 g of honey (in the presence of Cl^- as activator of enzyme), according to Schade method [7]. The starch is decomposed under the enzyme action as resulting in discoloration of solution from blue to intermediates colors (violet, pink, no color) proportionally with the amount of decomposed starch and implicitly with the intensity of enzyme's action.

2.6. Total free acidity of honey

Free acidity of honey's samples was determined by titration with 0.1 M sodium hydroxide solution to pH 8.30.

2.7. Mineral content

The honey sample was ashes, dissolved in acidic solution (HCl: HNO_3 3:1) and analyzed by atomic absorption spectrofotometric method (Perkin Elmer AAS 800) [8]. For each sample the content of heavy metals has been measured by three times and the result was presented as average.

2.8. Total dry mater

Honey samples were dried in an oven at 105^{o}C up to constant weight.

2.9. Sugars content in honey

Elser method [9] was used for analysis of sugars (glucose and fructose).

Table 1

Paramaters of isothermal heating treatment of honey samples

Honey Type	**Heating temperature**	**Heating time**
Chestnut (*Castanea sativa L.*)	50^{oC}	2 hours
Acacia (*Acacia*)	50^{oC}	2 hours
Polifloral (hay field)	50^{oC}	2 hours
Chestnut *(Castanea sativa L.*)	80^{oC}	2 hours
Acacia (*Acacia*)	80^{oC}	2 hours
Polifloral (hay field)	80^{oC}	2 hours
Chestnut (*Castanea sativa L.*)	80^{oC}	3 hours
Acacia (*Acacia*)	80^{oC}	3 hours
Polifloral (hay field)	80^{oC}	3 hours

Table 2
Honey storage paramaters

Honey Type	**Storage temperature**	**Storage time**
Chestnut (*Castanea sativa L.*)	refrigeration 4°C	12 days
Acacia (*Acacia*)	refrigeration 4°C	12 days
Polifloral (hay field)	refrigeration 4°C	12 days
Chestnut (Castanea sativa L.)	dark 20°C	12 days
Acacia (*Acacia*)	dark 20°C	12 days
Polifloral (hay field)	dark 20°C	12 days
Chestnut (*Castanea sativa L.*)	light 20°C	12 days
Acacia (*Acacia*)	light 20°C	12 days
Polifloral (hay field)	light 20°C	12 days
Chestnut (*Castanea sativa L.*)	refrigeration 4°C	50days
Acacia (*Acacia*)	refrigeration 4°C	50days
Polifloral (hay field)	refrigeration 4°C	50days
Chestnut (Castanea sativa L.)	dark 20°C	50days
Acacia (*Acacia*)	dark 20°C	50days
Polifloral (hay field)	dark 20°C	50days
Chestnut (*Castanea sativa L.*)	light 20°C	50days
Acacia (*Acacia*)	light 20°C	50days
Polifloral (hay field)	light 20°C	50 days

3. Results and discussions

3.1. Physically-chemically characteristic of honey samples

Some chemically characteristics of studied honey samples are presented in Table 3.

The concentrations of K, Na, Ca and Mg are very high in all honey samples, as others authors mention [10]. The high presence of these elements in soils and plants, as major constitutive elements, justifyes theirs higher concentrations in honey. Among the elements, K presents the top concentration (1115 mg/Kg) in chesnut honey, followed by the hay field honey (1095 mg/Kg) and acacia honey (890 mg/Kg). The lowest concentrations are in case of Mg in acacia honey (78 mg/Kg). Concentrations of Fe, Zn, Cu, Mn, Ni and Cr are much lower than the concentrations of above mentioned elements, but they are at the same magnitude order with those indicated by others authors [11]. The highest Fe concentration is recorded for hay field honey, 7.1 mg/Kg, folowed by the chestnut honey with 2.4 mg/Kg and acacia honey with 1.3 mg/Kg. Maximum Zn concentration appears in case of acacia honey (2.8 mg/Kg), closely followed by the hay field honey with 2.4 mg/Kg and chestnut honey (1.7 mg/Kg). High total acidity of acacia honey (2.3^0T) and hay field honey (2.1^oT), indicating a corrosive potential, and keeping honey in galvanized containers might be considered the sources of Zn contamination in honeys [12]. In case of Cu, the lowest concentration was recorded in acacia honey (0.02 mg/Kg) and the highest in hay field honey (2.4 mg/Kg). Mn concentrations have comparable values for all honey types: 0.8 mg/Kg in chestnut honey, 1.1 mg/Kg in case of acacia honey and 1.2 mg/Kg in case of hay field honey. Minimum Ni concentration are recorded in case of chestnut honey (0.01 mg/Kg), while in acacia honey the concentration of Ni is 60 times higher (0.6 mg/Kg) and in hay field honey by 2990 time higher (29.9 mg/Kg). In case of hay field honey also are recorded the maximum values for Cr and Al concentrations. In case of Cr, the concentration in hay field honey (12.4 mg/Kg) is 11.27 time higher than in chestnut honey (1.1 mg/Kg) and by 24.8 time higher than in acacia honey (0.5 mg/Kg). An extreme Al concentration can be noticed in hay field honey (109 mg/Kg), by 363.3 time higher than those recorded in chestnut honey (0.3 mg/Kg) and by 218 time higher than the Al concentration in acacia honey (0.5 mg/Kg). As conclusion, we can say that with exception of Mg, the hay field honey presents the highest concentrations of elements Na, Ca, Mg, Fe, Zn, Cu, Mn, Ni and Cr among all studied honey types. The presence near the hay field to an industrial plant or a high crowded road, the winds that transport sedimentable powders concentrated in mentioned elements or high concentrations of elements in the soils that meliffer plants are growing, can explain the top concentrations in honey [13].

3.2. Coefficient of diastase activity

The highest initial diastase activity was recorded to polifloral honey (Table 3). In Figure 1 and 2 are presented the variation of coefficient of diastase activity related to temperature heating during of two hours.

Table 3
Chemically characteristics of honey samples

Characteristic of honey sample	Chestnut (*Castanea sativa L.*)	Acacia Honey (*Acacia*)	Polifloral Honey (hay field)
Total dry matter (%)	83.5±0.2	81.7±0.1	80.9±0.2
Total acidity (^{o}T)	1.2±0.1	2.3±02	2.1±0.3
Coefficient of diastase activity	11.7±0.4	11.2±0.2	13.88±0.3
HMF (mg/Kg)	7.6±0.1	8.3±0.1	10.2±0.1
Glucose (%)	20.1±0.1	22.4±0.1	27.4±0.6
Fructose (%)	32.2±0.6	37.2±0.2	41.2±0.3
K (mg/Kg)	1115±30	890±20	1095±30
Na (mg/Kg)	210±10	178±1	101±2
Ca (mg/Kg)	123±10	239±10	409±12
Mg (mg/Kg)	92±12	78±10	139±2
Fe (mg/Kg)	2.4±0.1	1.3±0.1	7.1±0.2
Zn (mg/Kg)	1.7±0.1	2.8±0.1	2.4±0.1
Cu (mg/Kg)	1.2±0.1	0.02±0.1	2.4± 0.2
Mn (mg/Kg)	0.8±0.1	1.1±0.1	1.2±0.1
Ni (mg/Kg)	0.01±0.1	0.6±0.2	29.9±0.3
Cr (mg/Kg)	1.1±0.2	0.5±0.1	12.4±1.2
Al (mg/Kg)	0.3±0.1	0.5±0.1	109±10

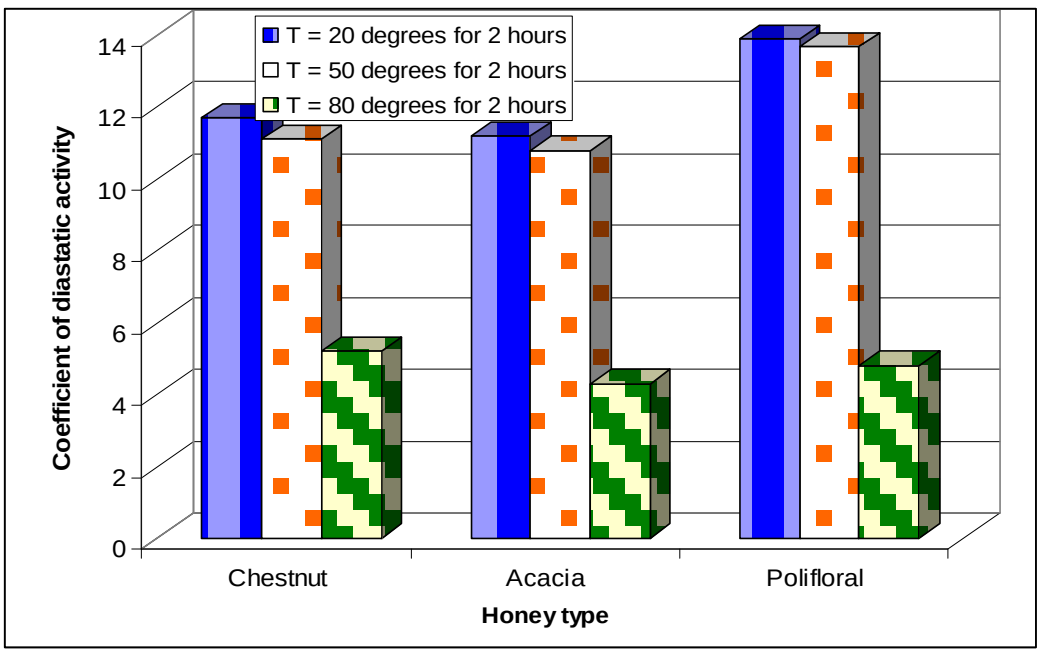


Figure 1. Variation of diastase activity with temperature during of two hours heating

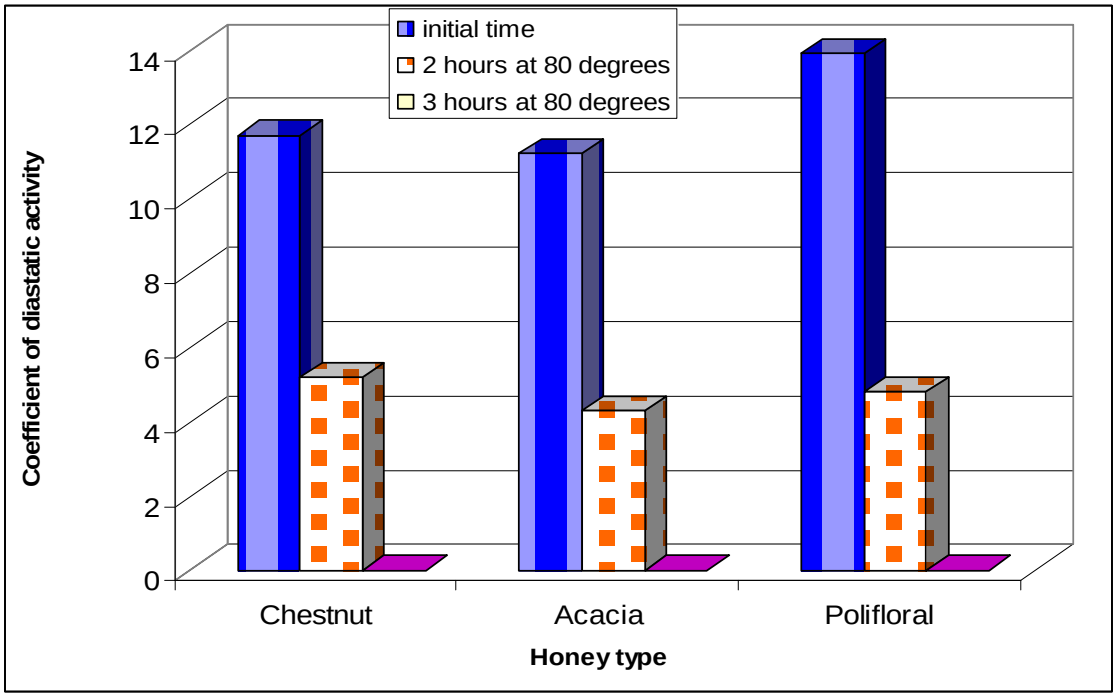


Figure 2. The influence of time heating on coefficient of diastase activity

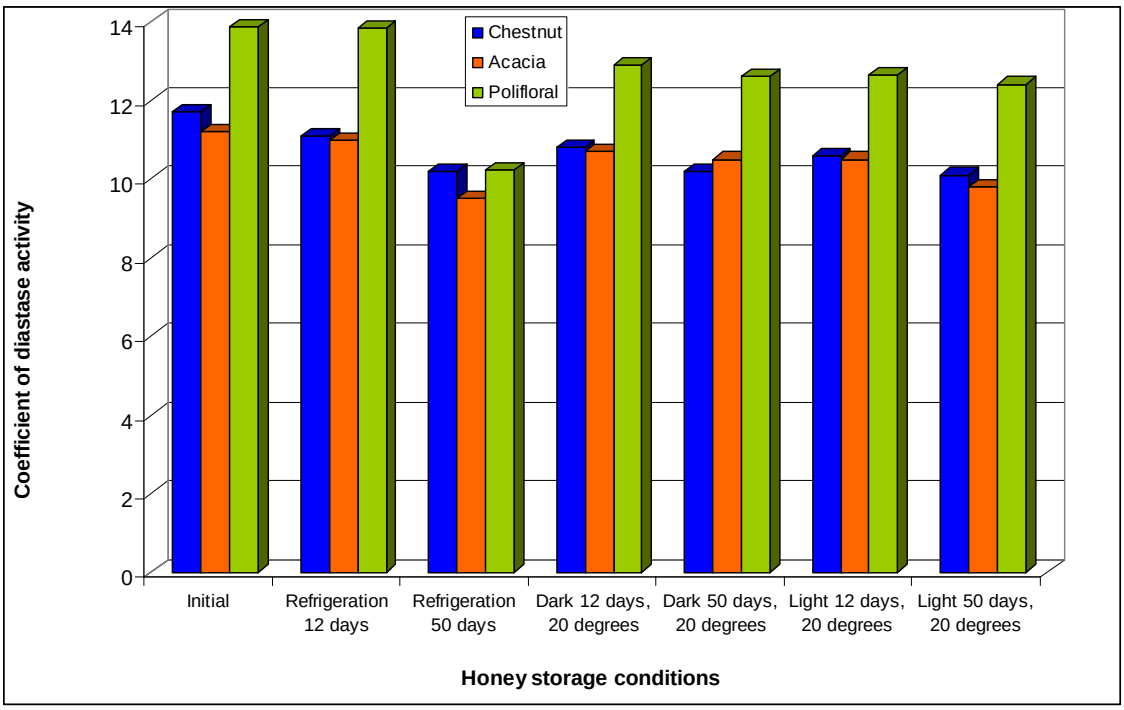


Figure 3. The impact of honey storage conditions on enzimatic activity

The decrease of diastase activity with increasing of heating time can be noticed for all honey samples. The decrease from initial value is insignificant in the first two hours of heating at 50^{o}C for all honey samples. Thus, in case of polifloral honey the decrease is 1.44% (from 13.88 to 13.68), in case of acacia honey the decrease is 3.57% (from 11.2 to 10.8) and 5.12% (from 11.7 to 11.1) in case of chestnut honey. The hypothesis that the 40^{o}C – 50^{o}C temperature range is optimal for α-amylase enzyme activity can be considered (14). In case of acacia and chestnut, a higher initial acidity, contributes to a rapid decrease of diastase activity. Increasing the temperature by 1.6 time (from 50^{o}C to 80^{o}C) led to a major decrease of diastase activity in all considered samples. The maximum fall down is recorded in case of chestnut honey, 63.24% (from 11.7 to 4.3), followed polifloral honey with 62.89% (from 13.88 to 5.2) and acacia honey with 61.60% (from 11.2 to 4.3). Increasing of temperature brought the structural changes in enzyme molecules, expressed in changes of enzymatic activity. This behavior agrees with that reported by Richardson and Hyslop [15] on the reversible inactivation of enzymes according to treatment conditions. Considering such a major decrease of diastase activity with increasing of temperature, we can assess that the complete inactivation can occurred around 90^{o}C as literature indicates [16]. Increasing the time heating from 2 hour from 3 hours provokes a complete and irreversible inactivation of enzymatic activity, as Figures 1 and 2 indicate. After 3 heating hours the enzymatic activity was not detected for any honey sample.

Considering storage conditions for honey, they also influence the honey diastase activity as Figure 3 indicates. In the first 12 days of refrigeration, the lowering comparing with initial is with 5.1% for chesnut honey (from 11.7 to 11.1), 1.78% in case of acacia honey (from 11.2 to 11) and with 0.4% in case of polifloral honey (from 13.88 to 13.82). Prolongation of refrigeration up to 50 days continues the decrease with 12.8% in case of chestnut honey (from 11.7 to 10.2), 15.1% in case of acacia honey and with 13.97% in case of polifloral honey (from 13.88 to 11.94). The influence of temperature on coefficient of diastase activity is underlined by the decrease of enzimatic activity in case of honey storage in darkness but at 20^{o}C. After 12 days storage, the chesnut honey decreases its enzimatic activity with 7.69% (from 11.7 to 10.8), the acacia honey with 4.46% (from 11.2 to 10.7) and the polifloral honey with 7.06% (from 13.88 to 12.9). After another 38 storage days, the enzimatic activity is reduced with: 16.2% in case of chestnut honey (from 11.7 to 9.8), 16.96% in case of acacia honey (from 11.2 to 9.3) and 19.30% in case of polifloral honey (from 13.88 to 11.2). If the honey is stored at 20^{o}C in daylight, the enzimatic activity is reduced after 12 days as follows: 19.6% in chestnut honey (from 11.7 to 9.4), 21.4% in acacia honey (from 11.2 to 8.8) and 26.15% in polifloral honey (from 13.88 to 10.25). After 50 day storage at 20^{o}C in daylight, the values become: 28.20% in chestnut honey (from 11.7 to 8.4), 30.35% in acacia honey (from 11.2 to 7.8) and 34.43% in polifloral honey (from 13.88 to 9.1).

The comparative analysis of processing's impacts on the enzimatic activity's of each honey type is presented in Figure 4 (A-C). The major impact in terms of enzimatic activity's decreasing is represented by heating at 80^{o}C. After 3 hours, no enzimatic activity was reported for any honey sample, which represents a 100% decrease (complete inactivation of enzime activity). In opposition, the lower

impact is represented by the 12 days of refrigeration.

3.3. HMF content

As the Figure 5 indicates the HMF contents in all honey samples are strongly influenced only by intensive thermal treatment. Honey heating at 80^{o}C for 2 hours and 3 hours also increases the HMF content above the maximum admitted limits (MAL) of 40 mg/Kg as recommended by Codex Alimentarius (16) and Council of European Union directive (18). Under the heating at 80^{o}C, the most affected is chestnut honey, due the fact that the HMF content increases about 17.97 times after 3 hours heating and by 15.96 times after 2 hours heating (Figure 6). The most resistant seems to be the polifloral honey, considering an HMF rate increase by 8.45 times after 3 hours heating and 7.48 times after 2 hours heating. On the other hand, no impact in terms of HMF increase was observed for refrigeration (both 12 days and 50 days).

Consideration of diastasic activity and HMF formation as reliable parameters in assess the honey quality is demonstrated by the direct correlation between them, as the Figure 7 indicates. With exception in case of refrigeration, the impact on studied honey samples are in the same order.

Heating honey at 80^{o}C during of 3 hours provokes the maximum negative impact in terms of complete enzimatic activity destruction and maximum HMF increasing content. In opposition, the refrigeration and darkness storage have the minimum impact.

80^{o}C 3h	80^{o}C 2h			Daylight 50 days 20^{0}C			Daylight 12 days 20^{0}C			Darkness 50 days 20^{0}C			Refrigeration 50 days			Darkness 12 days 20^{0}C			50^{o}C 2h			Refrigeration 12 days		
C=A=P	C	P	A	P	A	C	P	A	C	P	A	C	A	P	C	C	P	A	P	A	C	C	A	P

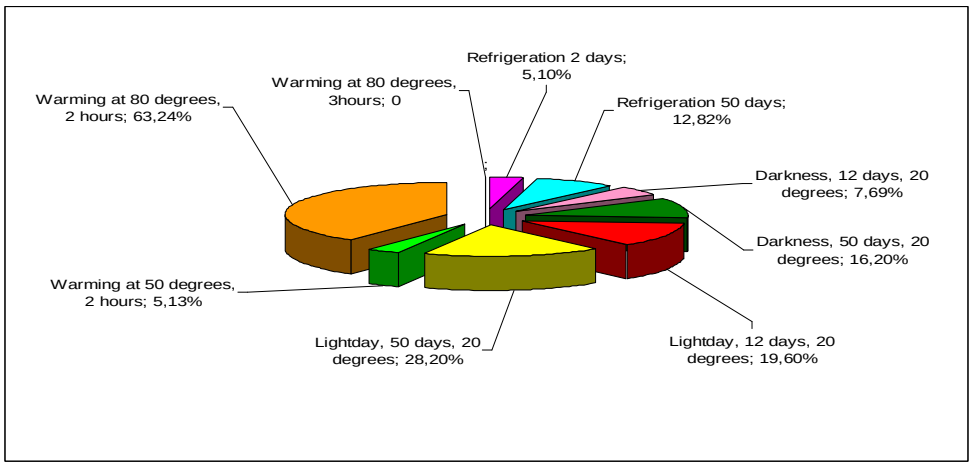
Decreasing the processing's impact on enzimatic activity of honey

80^{o}C 3h	80^{o}C 2h			Daylight 50 days 20^{0}C			Daylight 12 days 20^{0}C			Darkness 50 days 20^{0}C	50^{o}C 2h			Darkness 12 days 20^{0}C		Refrigeration 12 days and 50 days
C=A=P	C	A	P	C	A	P	C	A	P	C = A = P	P	A	C	C	A = P	C=A=P

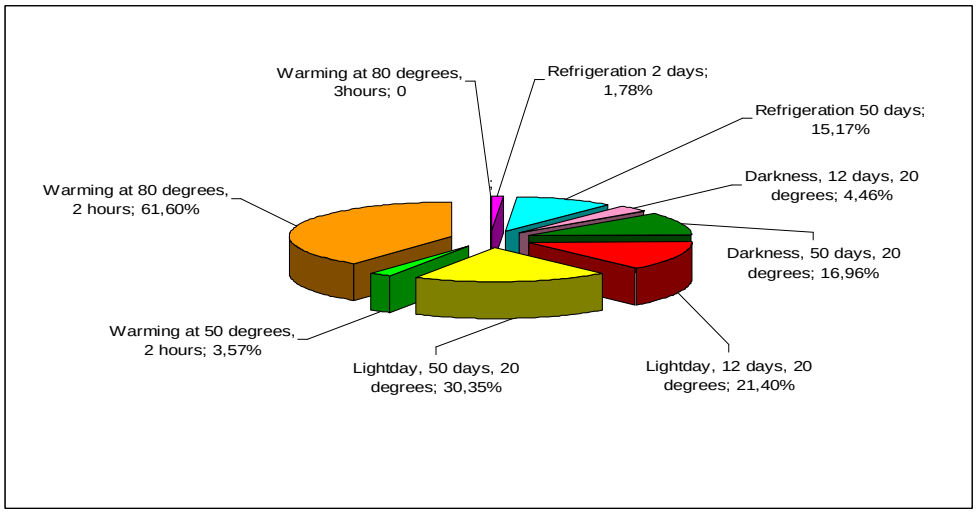
Decreasing the processing's impact on HMF content in honey

Legend: C – Chestnut honey; P – Polifloral honey; A – Acacia honey

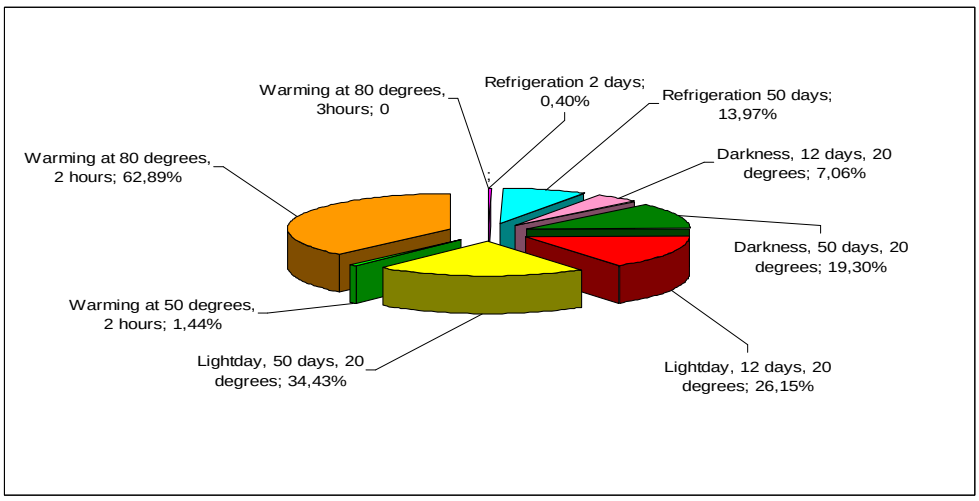
Figure 7. Correlation between diastasic activity and HMF content



A – Chestnut honey



B – Acacia honey



C – Polifloral honey

Figure 4. The impact of processing treatment on studied honey's enzimatic activity

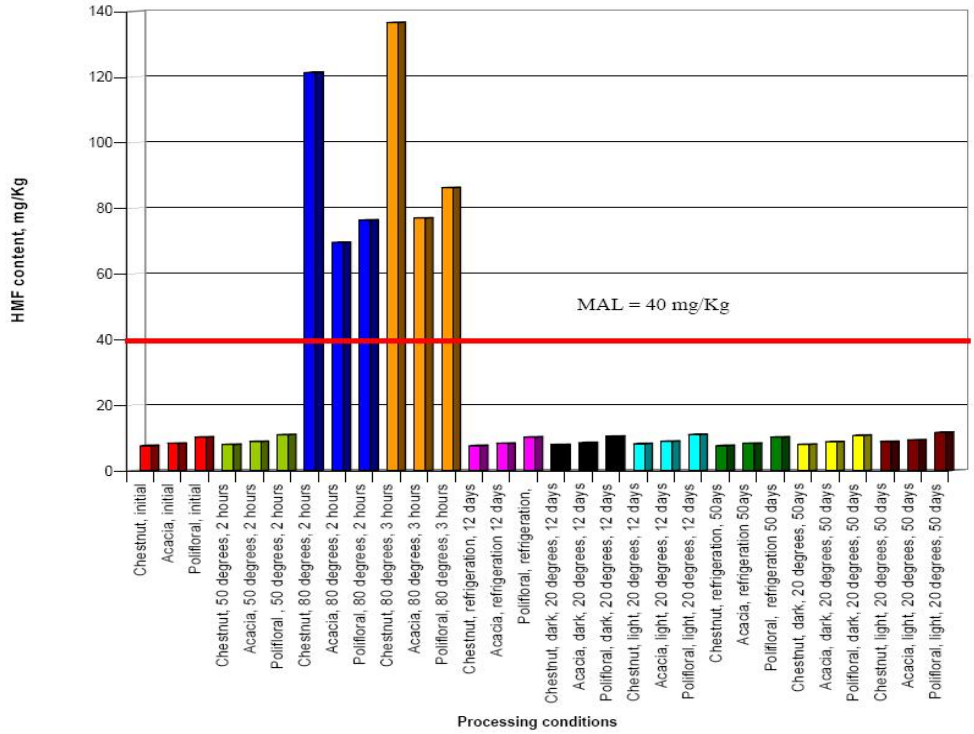


Figure 5. The impact of honey's processing conditions on HMF content

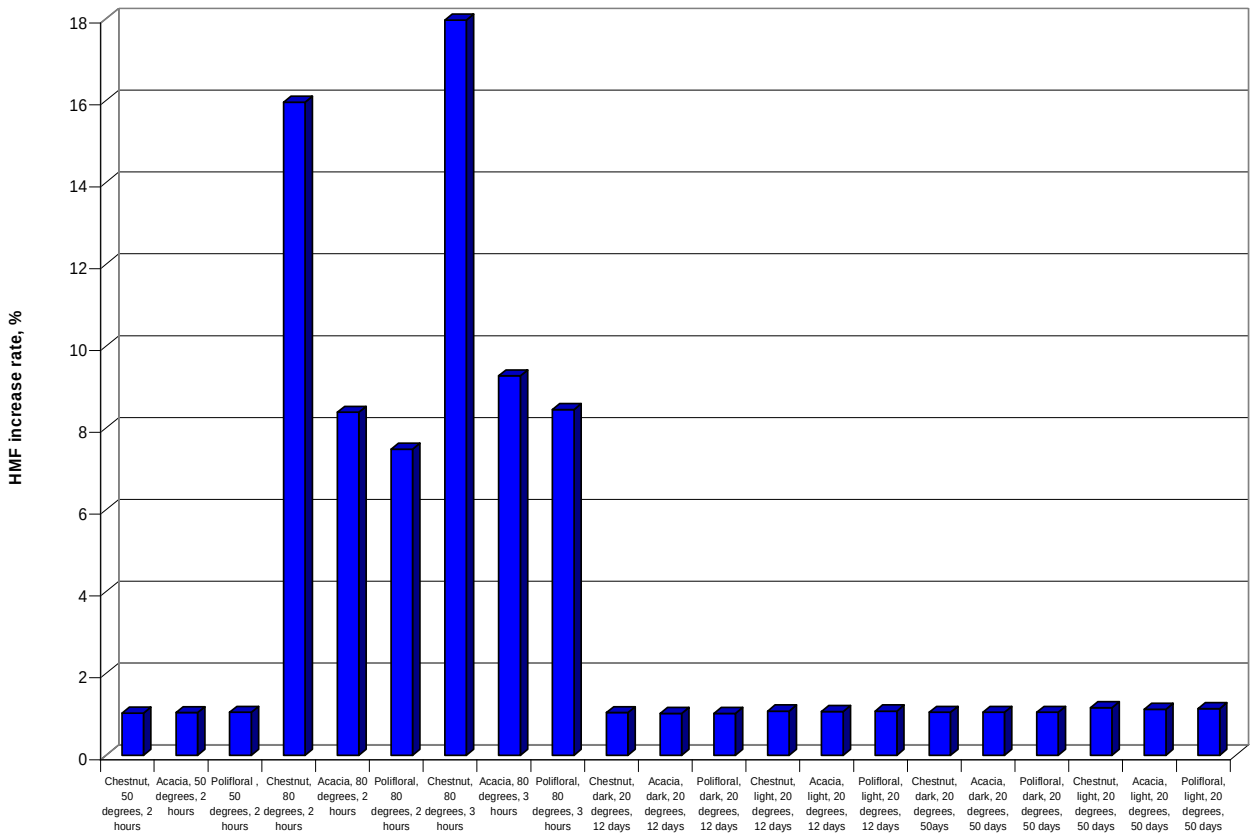


Figure 6. The HMF increase rate on studied honey samples

4. Conclusions

From diastase activity point o view, thermal processing of studied honey up to 50°C did not influence significant the enzimaticv activity. Increasing of temperature from 50°C to 80°C have a major impact on it. Between all studied honey types, the most resistent honey under heating appears to be acacia and polifloral honeys of which diastatic activity decrease by 2.6 time comparing with 2.7 time in case of chestnut honey.

Storage conditions influence also the enzimatic activity of α-amylase, considering the storage temperature, time range and the presence/absence of daylight. Between all honey samples, we can notice polifloral honey to be more resistent in terms of diastase activity under 12 and 50 days storage in daylight at 20°C, 50 days storage in darkness at 20°C and heating at 50°C for 2 hours. Chestnut honey proved to be more resistent to 80°C heating for 2 hours, to storage 12 days in darkness at 20°C and under refrigeration for 12 days. Acacia honey seems to be more rezistent under 50 days of refrigeration, comparing with others honey types.

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THE PHYSICO-CHEMICAL QUALITY OF MILK IN MARAMUREŞ COUNTY

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Abstract

The notion of "*quality of milk*" includes the requirements of nutritive, hygienic sanitary, technological and commercial order. A crude raw material of quality milk can be obtained only by the satisfaction of the producers, operators and legislative and decisional public factors. The quality of the finite product is the sum of the qualities of all the stages of operation through which the raw material is taken, being possibly achieved through a collective effort and common acceptance of competition qualitative criteria. A number of 288 samples of milk raw material have been analysed, coming from the collecting centres of a milk factory in Maramures County. The samples have been taken in conformity with the current legislation and analysed using the LactoScope FTIR. The major polluters of vegetation are especially Pb, Cd, Cu, Zn, Fe, Mn. Pollution is mainly realized through the polluted soil, but also through the polluted atmosphere, the level of pollution in this way varies between 10.37 – 23.37%.

Keywords: *milk, acidity, density, freezing point, water,protein, fat, lactose, S.U.T., S.U.D*

1. Introduction

The EC countries with tradition in exploiting the cow milk have tried the unification of social categories mentioned above through the elaboration of an adequate legislative framework and the founding of institutions designed to implement the decisions taken and to observe that the decisions are followed [1].
According to Baldwin et al. [2], the quality of a product is corresponding if this reunites four criteria:
a) the nutritive criterion – which refers to the content of the food in proteins, glucides, lipids, mineral substances and vitamins;
b) the sensorial criterion – refers to the gustative, olfactory, visual, tactile and chromatic qualities;
c) the hygienic or salubrity criteria refers to the natural toxicity of the product, its physic-chemical and microbiological contamination;
d) the aesthetic criterion – refers to the criteria enumerated above, to which the way of operating, packing and labelling the product are added.

In the current economic conjuncture and the situation of international markets the quantity and the price as factors for the regulation of demand and offer does not ensure the market supremacy [3, 4].

The quality of the finite product is the sum of the qualities of all the stages of operation through which the raw material is taken, being possibly achieved through a collective effort and common acceptance of competition qualitative criteria [5-9]. The physico-chemical qualities of crude raw material milk are regulated by STAS 2418-61.

2. Materials and methods

A number of 288 samples of milk raw material have been analysed, coming from the collecting centres of a milk factory in Maramures County. The samples have been taken in conformity with the current legislation and analysed using the LactoScope FTIR. The samples have been statistically analysed using the Microsoft, Excel.

The determination of the acidity, cryoscopy point, water, S.U.T., proteins, fats, lactose and S.U.D. we made with the FTIR lactoscope, an apparatus that uses the infrared spectrum technique as a way of determining these parameters. This latoscope is used in laboratories and food industry companies, being calibrated to obtain, simultaneously, in less than a minute, all these parameters.

The analyses do not need a primary prework which would determine an alteration of the physic-chemical properties of milk. The apparatus registers minimum 3 determinations for each sample, the final value being the arithmetic average of the 3 determinations, a low level of errors being thus registered. S.U.D was determined by the difference between S.U.T. and the percentage of fat.

3. Results and discussions

The major polluters of vegetation are especially Pb, Cd, Cu, Zn, Fe, Mn. Pollution is mainly realized through the polluted soil, but also through the polluted atmosphere, the level of pollution in this way varies between 10,37 – 23,37% (fig.1-9). Lead is a ubiquitous element, being an element of the earth shell and the soil, from where it is taken by plants and animals. Its provenience in the ambient environment is directly proportional to the pollution provoked by the use of the metal in industry, vehicles' exhaust, but also by its presence in almost every objects of current use: pipes, canes, cosmetics, paints, etc. The lead evacuated in the atmosphere by different sources can get directly into the organism with the inspired air, or indirectly, after the depositing on the surfaces or soil, then water, food, etc., as well as after the intake from the soil by plants, some species absorbing more than others, especially in certain parts. Cadmium is a very toxic metal, without any biogenic role, and its compounds don't have any pharmaceutical use, being one of the elements known as cancerous. This represents a source of pollution for the environment, together with lead and zinc, those coming from foundry and mining. Cadmium gets into the environment especially trough the industrial emissions, contaminating the air, water, soil and plants.

4. Conclusions

The major polluters of vegetation are especially Pb, Cd, Cu, Zn, Fe, Mn. Pollution is mainly realized through the polluted soil, but also through the polluted atmosphere, the level of pollution in this way varies between 10,37 – 23,37%. Lead is a ubiquitous element, being an element of the earth shell and the soil, from where it is taken by plants and animals.

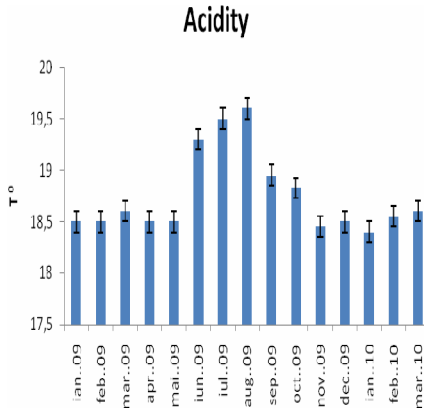

Figure 1. Variation of milk acidity in January 2009-March 2010

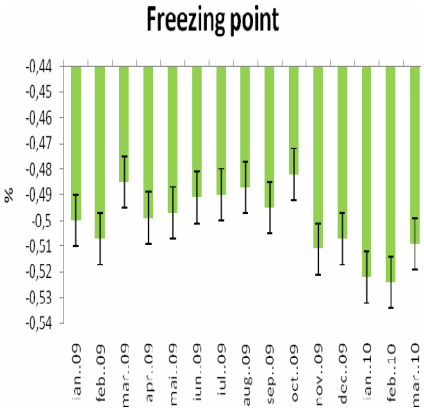

Figure 2. Variation of milk freezing point in January 2009-March 2010

Figure 3. Variation of milk density in January 2009-March 2010

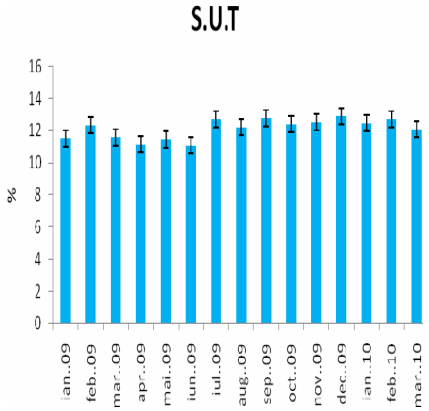

Figure 4. Variation of milk water in January 2009-March 2010

Figure 5. Variation of milk S.U.T in January 2009-March 2010

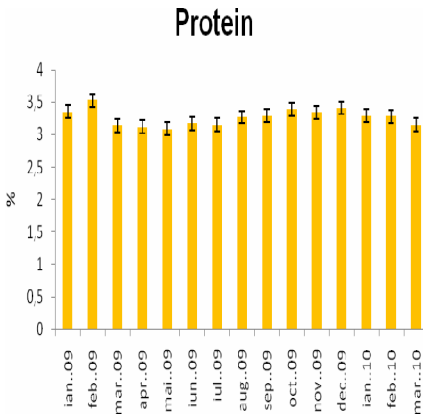

Figure 6. Variation of milk protein in January 2009-March 2010

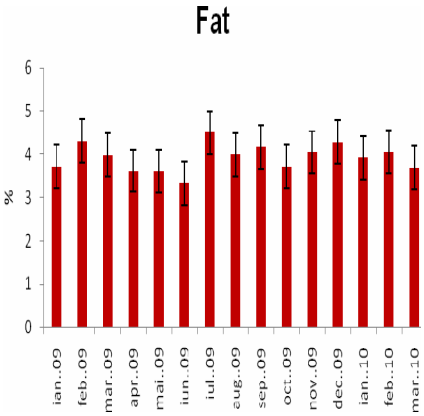

Figure 7. Variation of milk fat in January 2009-March 2010

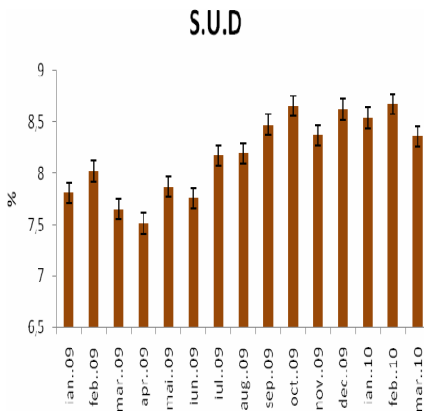

Figure 9. Variation of milk S.U.D in January 2009-March 2010

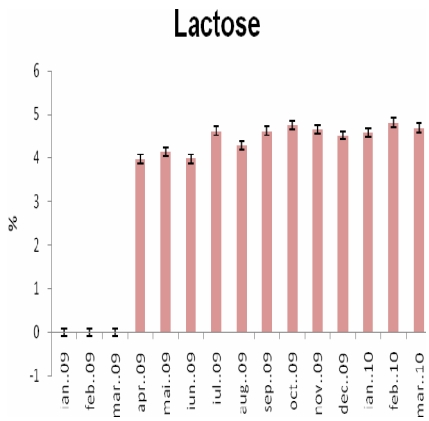

Figure 8. Variation of milk lactose in January 2009-March 2010

Its provenience in the ambient environment is directly proportional to the pollution provoked by the use of the metal in industry, vehicles' exhaust, but also by its presence in almost every objects of current use: pipes, canes, cosmetics, paints, etc. The lead evacuated in the atmosphere by different sources can get directly into the organism with the inspired air, or indirectly, after the depositing on the surfaces or soil, then water, food, etc., as well as after the intake from the soil by plants, some species absorbing more than others, especially in certain parts. Cadmium is a very toxic metal, without any biogenic role, and its compounds don't have any pharmaceutical use, being one of the elements known as cancerous. This represents a source of pollution for the environment, together with lead and zinc, those coming from foundry and mining. Cadmium gets into the environment especially trough the industrial emissions, contaminating the air, water, soil and plants.

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MONITORING THE TOTAL IRON CONTENT IN FOOD

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Abstract

The aim of the paper is to monitorize the total iron content in some food categories presented in the Romanian people'diet in order to obtain information about the most indicate food for anaemia. The studied food categories are liver, meat, vegetables, fruits, bread and cereals, chicken egg, nuts and seeds, bee's products. Due the fact that some food are consumed after a thermal treatment, the impact of thermal processing (frying, boiling, roasting) on total iron content comparing with no-processed food has also studied. Experimental results indicate that the highest levels of total iron content are in raw cow liver, olive and raw pork liver. The lowest iron content was found in trout meat, floral honey, white bread, horseradish, brown Champignon mushrooms and parsley roots. Frying and roasting proved to be the most destructive thermal treatments comparing with boiling in terms of total iron content decreasing. High levels of total iron were founded in food that content (beta-carotenoides) and low levels in food that have compounds with negative impact of iron bioavailability (phytic acid). The further paper will present the proportion of iron bioavailability in human body.

Keywords: *anemia, special food, food defficiencies*

1. Introduction

Anemia is a common nutritional disorder caused by low content of iron in human organism. According to some documents, it affects around 3.5 billion people [1]. Infants, schoolchildren, adolescents, pregnant and lactating adult women are considered the most vulnerable groups [2]. During the time were developed some strategies for anemia reducing [3], especially in children cases, but their effects are considered low. The newest strategies are focused on producing and consuming the fortified food and on to provide additional micronutrients to regular home made food. They are completed with education programs that encourage improving complementary feeding by food diversity [4]. Considering these aspects, became very important to know the level of nutrients contained in the food, in order to choose the most appropriate food that correct nutritional disorders.

The aim of that paper is to monitorize the total iron content in different food categories usually included in Romanian people diet. Further investigations will emphasize the contents in iron bioavailability in the above mentioned food.

2. Materials and methods

2.1. Food samples

The experiment was conducted on some food categories frequent founded in people's diet: fresh liver (pork, chicken, cow, weanling) and liver pate, meat (pork, cow, fish), vegetables (onion, garlic, mushrooms, radish, carrots, red beets, horseradish, celery, parsley, pepper, peas, soybeans, beans, spinach, cabbage, broccoli, potatoes, olives), fresh and preserved fruits (apples, kiwi, bananas, oranges, grapefruits, dates, raisins, figs), bread and cereals, chicken egg, nuts and seeds (peanuts, nuts, hazelnuts, pumpkin,

poppy), bee's products (pollen, honey). Also was analyzed the total iron content in the special syrup (iron syrup) recommended for children with anemia. The raw food sample was washed in distillated water, dried in oven at 105°C up to constant weight and ashes 8 hours at 450°C.

2.2. Thermal treatment

Some food samples were thermal treated (fried, boiled and roasted) in order to emphasize the impact of thermal treatment of total iron content. 30 g of food sample were fried for 10 minutes at 180°C in a stainless steel pot in 200 ml sunflower oil. The roasting of 30 g of nuts and seeds were performed for 10 minutes at 180°C without salt. Also 30 g of food sample were boiled for 20 minutes in 200 ml in distillated water. The resulted products were pressed to remove de excess oil/water and dried in oven at 105°C up to constant weight and ashes 8 hours at 450°C.

2.3. Dissolution of food samples

An exactly amount of food ash was dissolved in concentrate HNO_3 (30%), the filtered solution was put in a 25 cm^3 flask and brought to sign with distillated water.

2.4. Total iron content analysis

Analysis of total iron content in above mentioned solutions were performed by atomic absorption spectrometry by using an AAS Perkin Elmer 800 at 248.3 nm wavelength. In Figure 1 is presented the calibration curve.

2.5. Statistical analysis

All the determinations were done in three replicates, and the average values are reported. Statistical analysis of analytical data was done, employing the T test, by using software Origin 6.0.

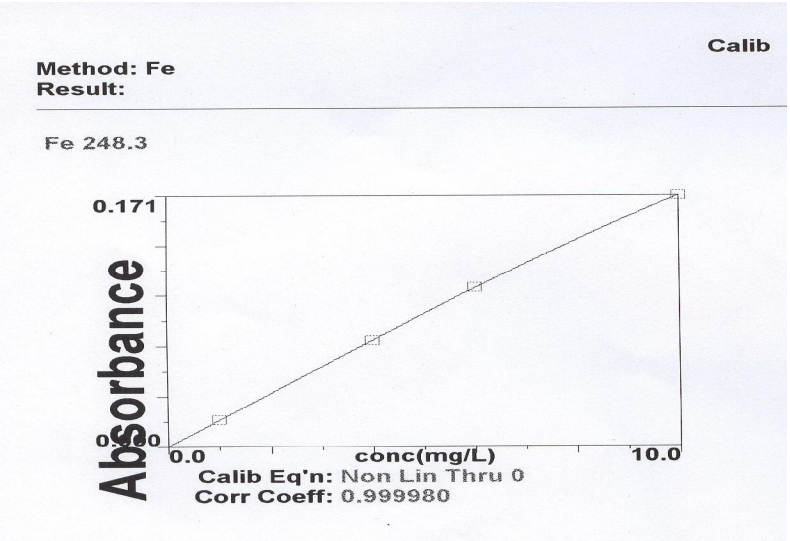


Figure 1. The calibration curve for total iron analysis

3. Results and discussions

In Figure 2 are presented the total iron contents in different liver types.

The comparative analysis indicates that the maxim total iron content is in raw cow liver (350.04 mg/Kg), 2.42 times higher than the content in raw pork liver (144.59 mg/Kg), 6.06 times higher than the iron content in raw chicken liver (89.53 mg/Kg) and by 4.99 times higher than the content of raw weanling (70.10 mg/Kg). Thermal treatment of liver decreases the total iron content as follow: • 1.77 times in boiled cow liver (196.80 mg/Kg) and 3.34 times in fried cow liver (104.75 mg/Kg); • 1.24 times in boiled pork liver (115.70 mg/Kg) and 2.26 times in fried pork liver (64.94 mg/Kg); •1.55 times in boiled chicken liver (57.70 mg/Kg) and 2.39 times in fried chicken liver (37.41 mg/Kg); • 2.15 times in boiled weanling liver (32.46 mg/Kg) and 2.31 times in fried weanling liver (30.24 mg/Kg).

As previous values indicate, the most agressive thermal treatment seems to be the frying. From impact of thermal treatment point of view, the weanling liver is most affected under boiling treatment and the cow liver under the frying treatment. The less afected is pork liver under the boiling processing and the weanling liver under frying processing (Figure 3).

The pate liver has a total iron content near to raw pork liver (155.24 mg/Kg), but beeing a products obtained after some technological processing steps the iron content can vary in wide limits.

Considering the meat, the total iron contents are presented in Figure 4.

The highest content of iron is recorded in raw weanling meat (45.34 mg/Kg), 1.74 times higher than the raw pork meat (25.99 mg/Kg). During the frying, the pork meat is less affected in terms of iron lossing, by 1.03 times comparing with 1.36 in case of weanling meat. The trout meat has a small total iron concentration (1.51 mg/Kg).

As Figure 5 indicates, the total iron content in Pleurotus mushrooms (41.80 mg/Kg) is 5.69 times higher than in white Champignon mushrooms (7.34 mg/Kg) and 6.96 times higher than in brown Champignon mushrooms (6.00 mg/Kg).

The levels of total iron content in some vegetables are presented in Figures 6 a, b.

The highest total iron concentration was determined in olives (160.13 mg/Kg), 1.68 times higher than in hot peppers (95.09 mg/Kg), 1.83 times higher than in white cabbage (87.48 mg/Kg). High content in iron was obtained for green pepper (75.15 mg/Kg), red radish (69.52 mg/Kg) and spinach (61.01 mg/Kg).The lowest total iron content is in horseradish (5.30 mg/kg), followed by parsnip (8.89 mg/Kg) and parsley roots (8.98 mg/Kg).

The impact of thermal treatment on preservation of total iron content in vegetables is indicated in Figure 7.

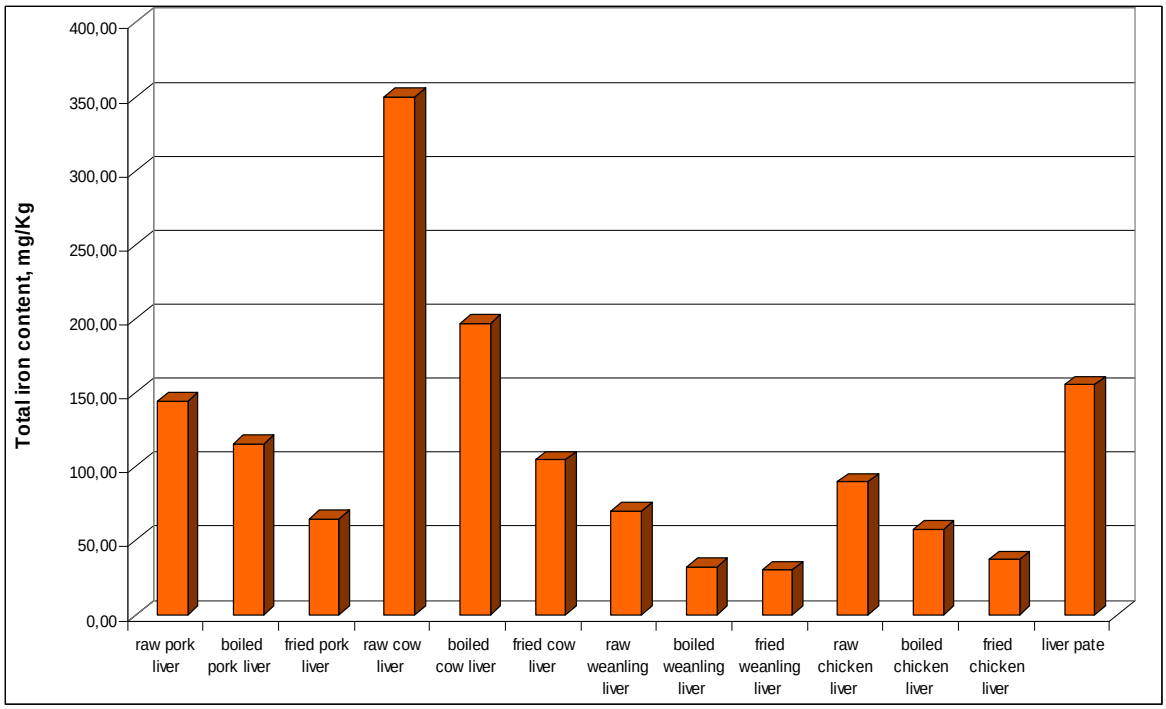


Figure 2. The total iron content in different raw and thermal treated liver types

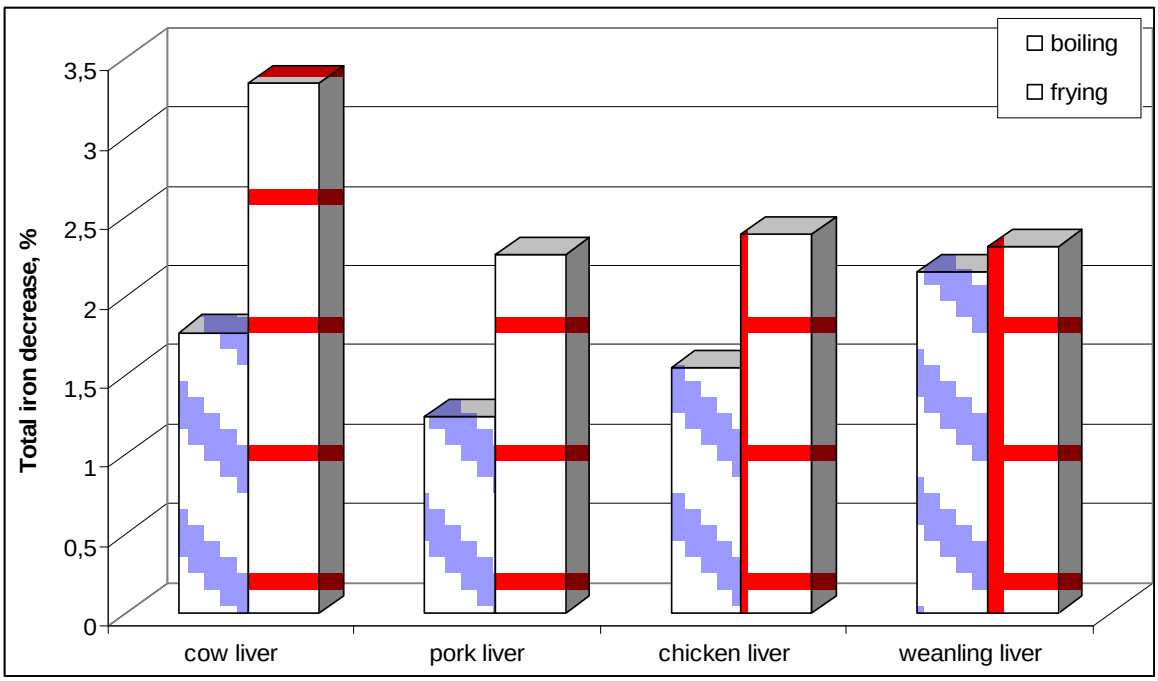


Figure 3. The decrease rate of total iron content in liver under the thermal treatment

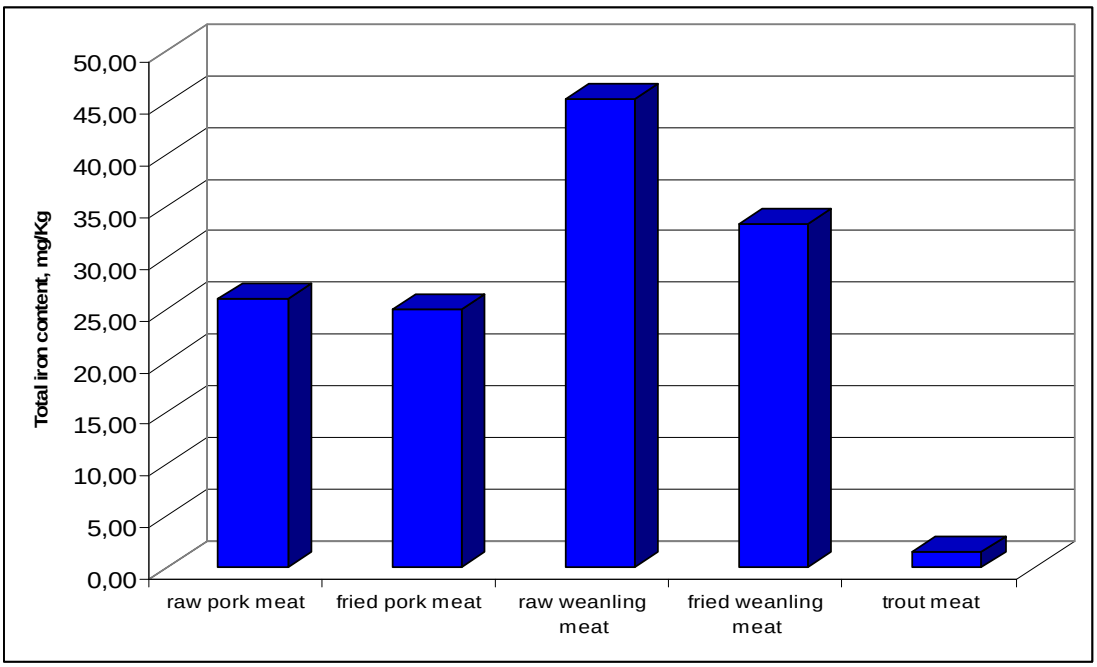


Figure 4. The total iron content in meat

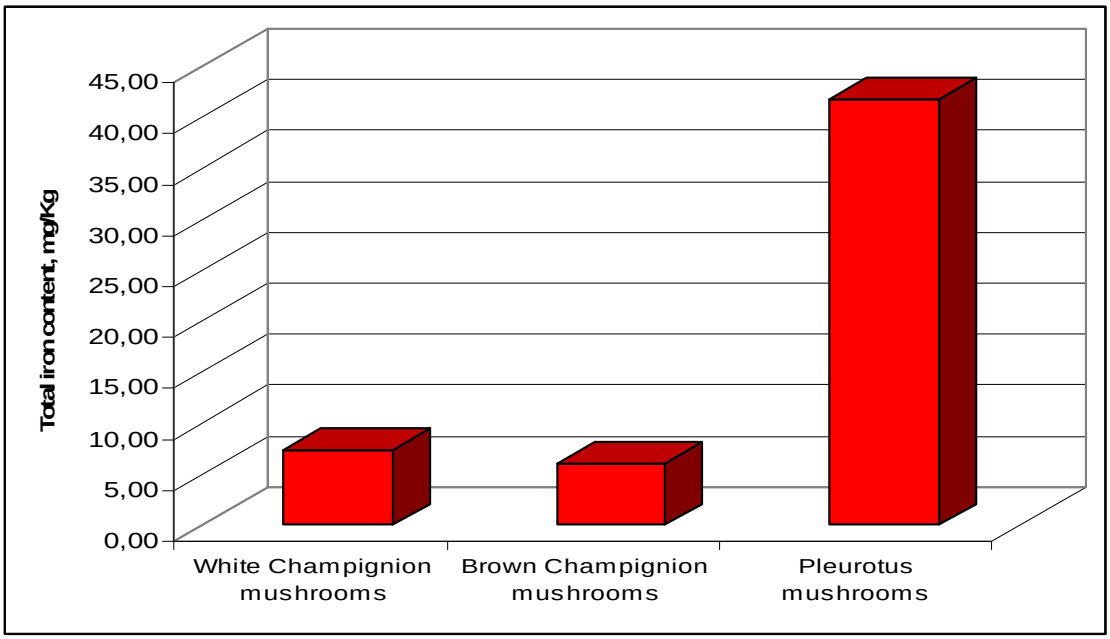
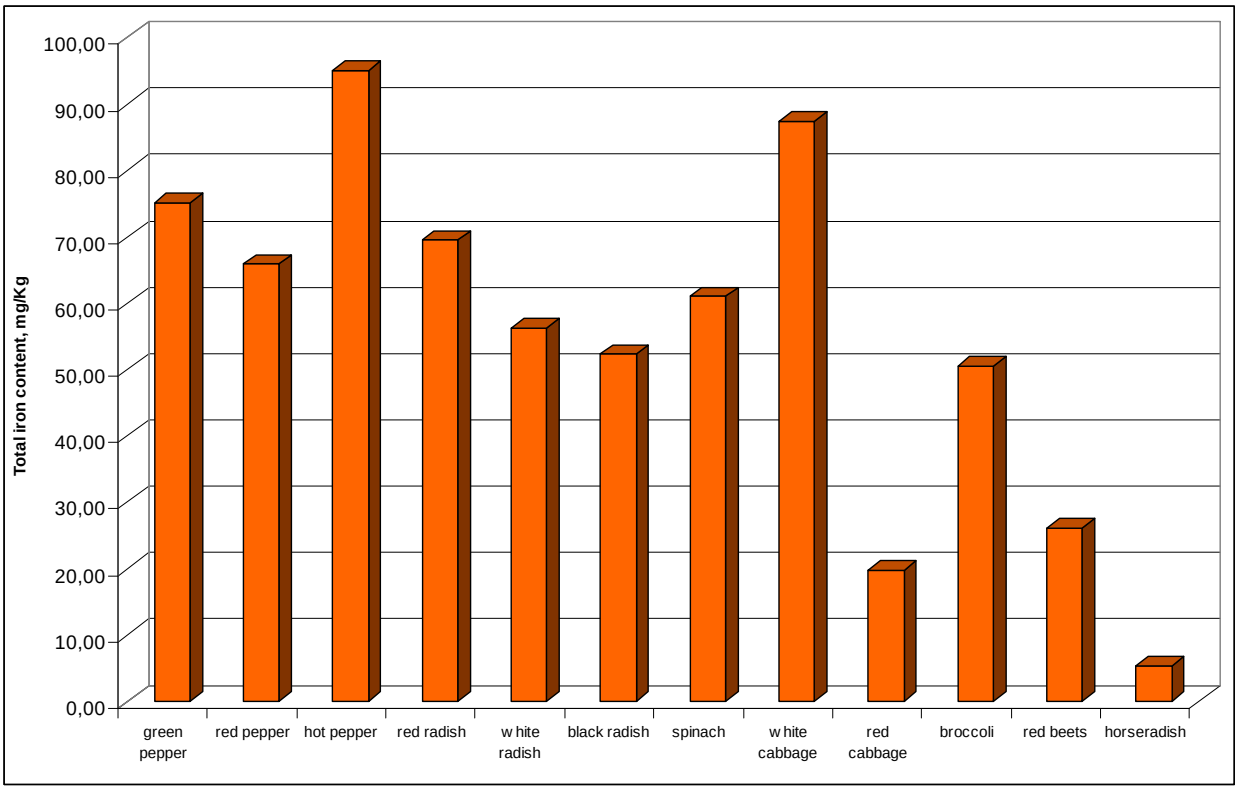
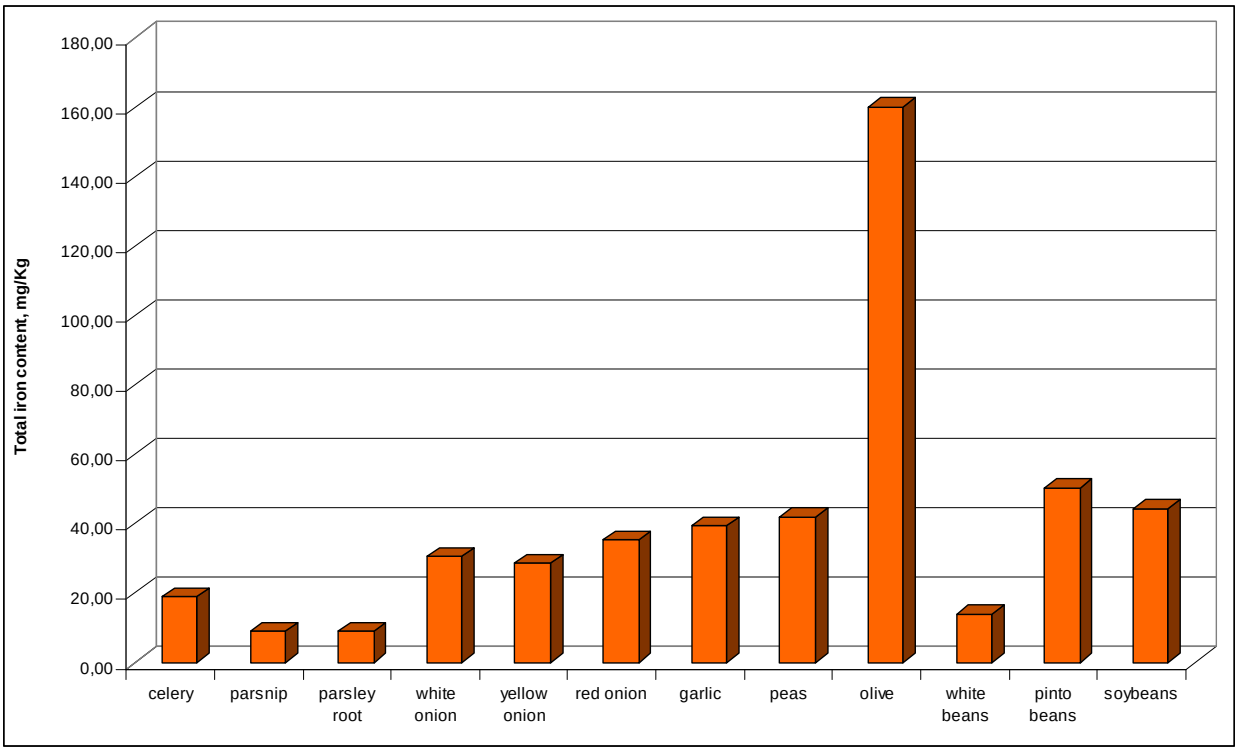


Figure 5. Total iron content in mushrooms



a



b

Figure 6. The toral iron content in studied vegetables

The raw sweet potatoes contain 1.23 times total iron (56.06 mg/Kg) than raw carrots (45.44 mg/Kg), by 2.06 times than raw potatoes (27.17 mg/Kg) and by 1.83 times comparing with raw red potatoes (30.59 mg/Kg). Boiling reduces the total iron content by 17.81 times in case of carrots (2.55 mg/Kg), by 1.05 times in case of potatoes (25.86 mg/Kg), by 2.44 times in case of red potatoes (12.49 mg/kg) and by 2.21 times in case of sweet potatoes (25.36 mg/Kg). The impact of frying is more destructive on iron content: 1.26 times in case of potatoes, 3.94 in case of red potatoes and 13.91 in case of sweet potatoes. In the literature, the iron content in boiled sweet potatoes was found to 7 mg/Kg (5).

He have to notice the carrots due the fact that above the high content of total iron, the presence of beta-carotene has a significant positive effect on the bioaccessibility of iron and zinc (6) which make the carrot a very valuable food for anemia reducing. The total iron contents in some fruits are presented in Figure 8.

The highest content of total iron is presented in dates (43 mg/Kg), followed by raisins (35.65 mg/Kg) and figs (26.64 mg/Kg). High content of iron can be noticed in apples (28.42 mg/Kg in case of Golden apples and 19.98 mg/Kg in case of Ionatan apples) and no iron content (or under the analysis equipment sensibility) in case of oranges and grapefruits. Even they have no iron content, the C vitamine from oranges and grapefruit improve the iron bioasimilation in human body.

In case of chicken eggs, the analysis indicates the iron presence only in yolk (39.50 mg/Kg), which decreases after boiling to 21.99 mg/Kg.

In the bread and cereals category, the highest iron content is in wheat bran (88.81 mg/Kg), by 17.11 times higher than in the white bread (5.19 mg/Kg) (Figure 9). We can aspected that a large part of total iron that exist in bran to be easily absorbed in human body, due the the presence of carotenoids [7].

Nuts and seeds are among food with high iron content (Figure 10). Poppy seeds contains the highest iron level (89.86 mg/Kg) followed by raw hazelnut (55.10 mg/Kg). Roasting of seeds decreases the iron level.Thus, in case of roaste peanuts, the decrease is by 1.24 times (from 27.29 mg/Kg to 21.87 mg/Kg).

The total iron content in raw peanut is lower (27.29 mg/Kg) than the]value indicated by the literature (68 mg/Kg) [8).

The bee's products has no high content of iron. The analysis indicates 26.29 mg/Kg in floral pollen and 3.59 mg/Kg in floral honey.

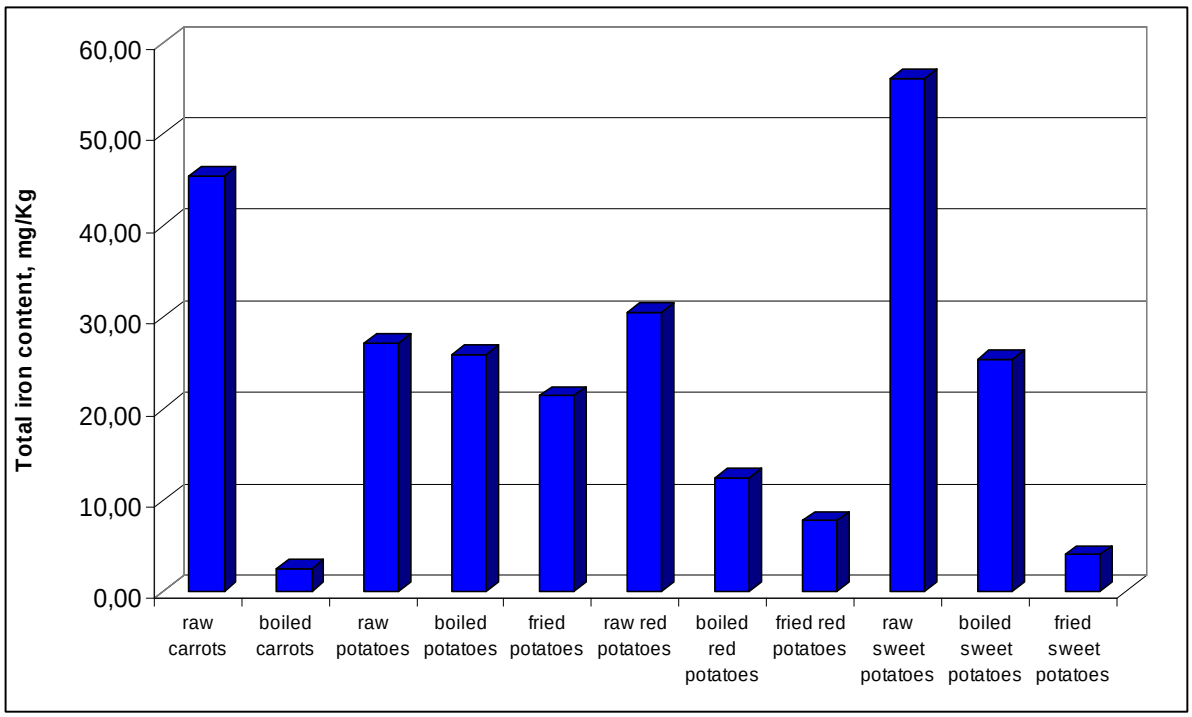


Figure 7. The impact of thermal treatment on carrots and potatoes

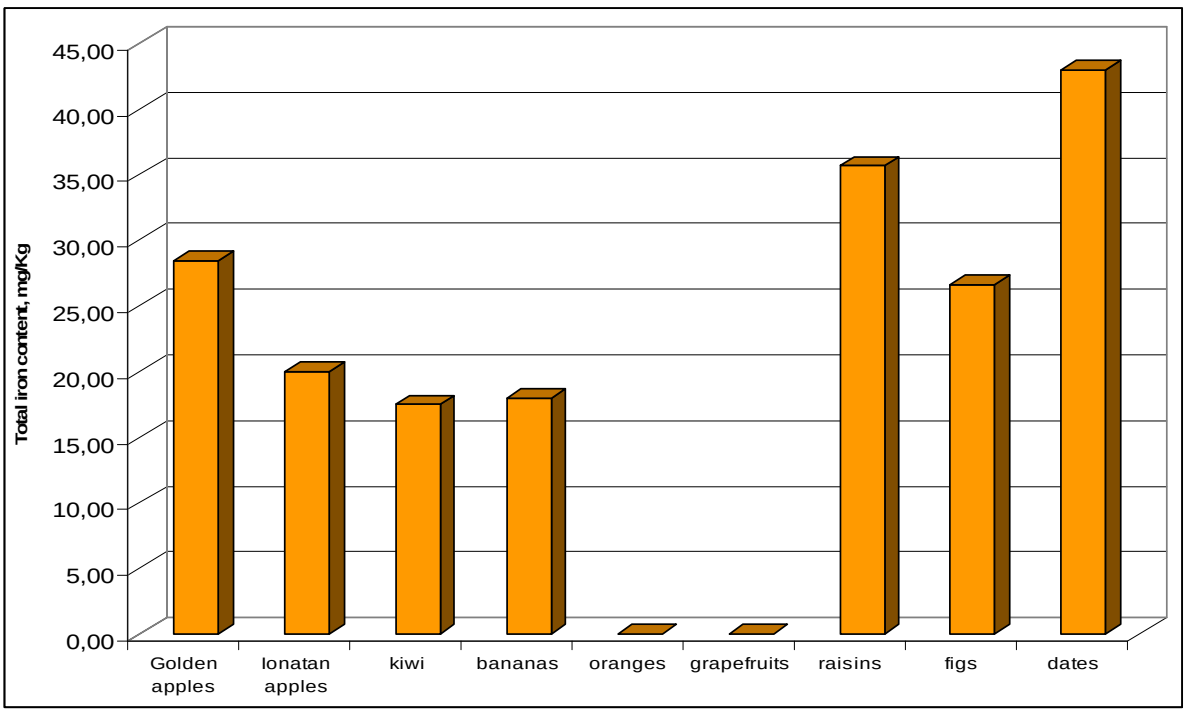


Figure 8. The total iron content in studied fruits Figure 5. The impact of honey's processing conditions on HMF content

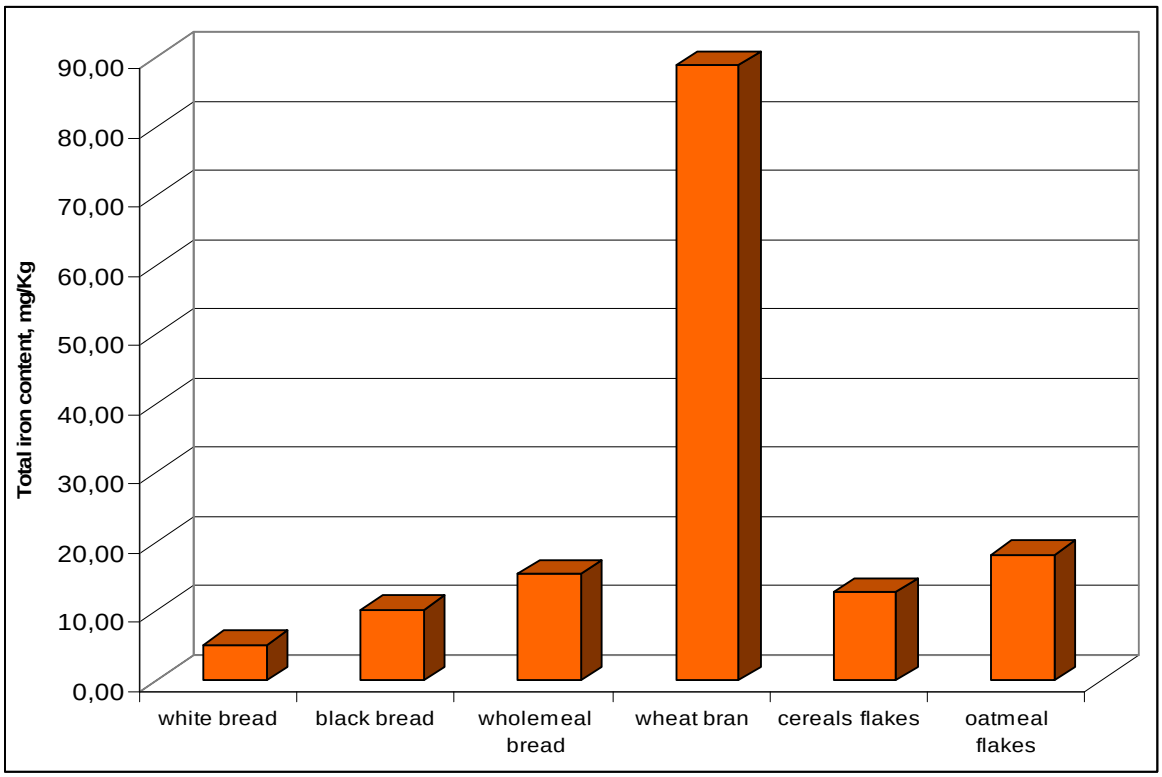


Figure 9. The total iron content in bread and cereals

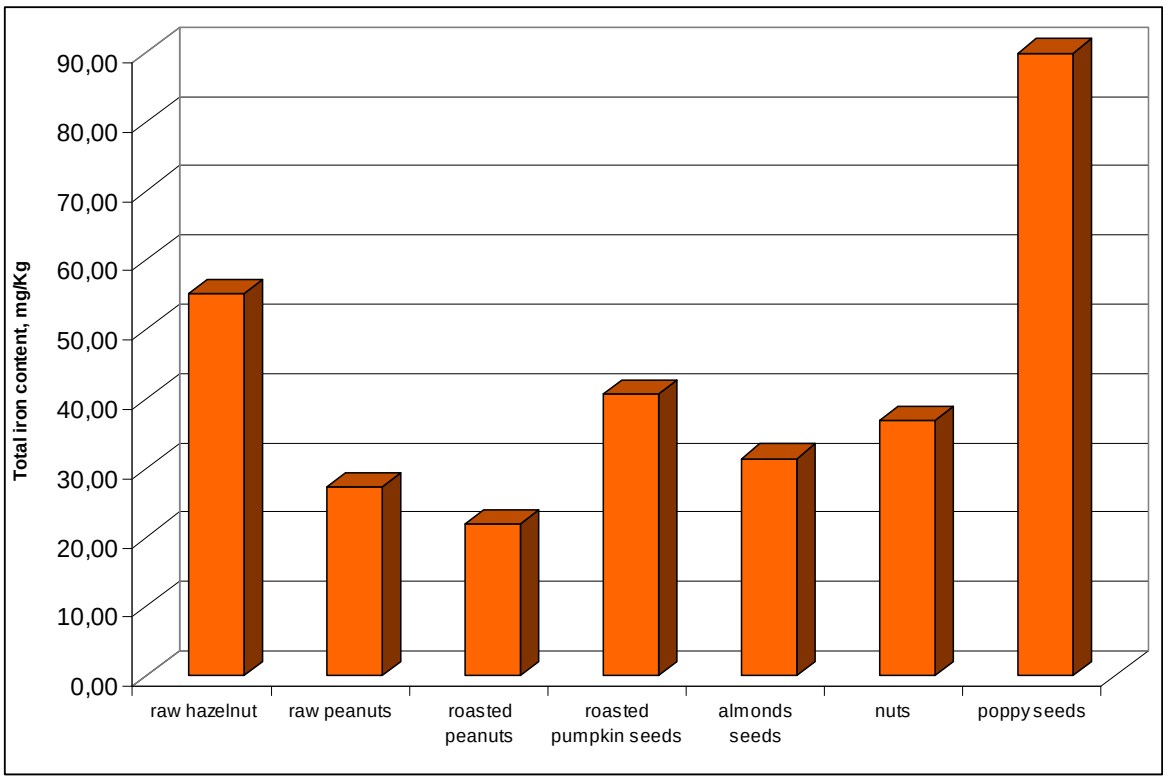


Figure 10. The total iron content in nuts and seeds

4. Conclusions

Considering the experimental data presented above, the most total iron concentrated food are: raw cow liver (350.04 mg/Kg), olive (160.13 mg/Kg), raw pork liver (144.59 mg/Kg). The lowest iron content was found in: trout meat (1.51 mg/Kg), floral honey (3.59 mg/Kg), white bread (5.19 mg/Kg), horseradish (5.30 mg/Kg), brown Champignon mushrooms (6 mg/Kg) and parsley roots (8.98 mg/Kg). Thermal treatment decreases the total iron content, the highest impact being presented by the frying/roasting comparing with boiling.

The present study was developed considering the total iron content in different food categories. Important for human body is the content of iron bioavailability in food, the presence of compounds that increase its bioavailability (beta-carotenoides or decrease it (phytic acid, ascorbic acid).

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THE MOULDS MICROFLORA DEVELOPED ON FRUITS

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Abstract

The fruits have a specific epiphyte microflora, which grows as it can find good conditions for development. In this study, the moulds collected from the fruits were inoculated on nutritional media to identify them and to isolate pure strains from them. Analyzing these moulds at macroscopic and microscopic level we could identify species of genera: *Penicillium sp.*; *Rhizopus sp.; Alternaria sp.; Botrytis sp.; Fusarium sp.; Cladosporium sp.* and *Torula sp.* These genera species can release a series of secondary metabolites which have negative effects on humans health, like the mycotoxines.

Keywords: *mycotoxines, strains, fungal colonies, Penicillium sp.*

1. Introduction

On the fruits surface, there is always a variable number of microorganisms whose development depends on the natural conditions and on a series of factors which can interfere during harvest, transportation, storage, packaging and commercialization. The microorganisms come from the environment, most of them from the air, brought by dust, insects and animals, but also from the ground or from the pluvial water or from the irrigation water.

Generraly, the fruits are invaded by mouls which locally destroy the pericarp, allowing the surface germs to penetrate the fruits. They can produce alterations of the fruits during their growth, maturisation and storage [1]. The most developing genera on fruits are: *Monilia, Phoma, Rhizopus, Penicillium, Oospora, Fusarium, Trichothecium, Aspergillus, Alternaria* etc [2].

The moulds produce in fruits 2 types of degradation: the wet rottenness and the dry rottenness, with differences concerning colour and location [1].

Fungal growth occurs under favorable environmental conditions and is associated with the production of a wide range of secondary metabolites, collectively called mycotoxins [3]. The amount of nutrients available, the ambient temperature, water activity and oxygen are the most important factors governing the growth and mycotoxin production of fungi [4]. The most important groups of mycotoxins that occur quite often in food are: aflatoxins, ochratoxins, trichothecenes (deoxynivalenol, nivalenol), zearalenone and fumonisins [5].

The mycotoxins act especially on the neuraxis, on the vital organs (heart, liver, kidneys) and on the blood, but they have also an estrogenic effect, also act on the cellular respiration by inhibiting some metal ions and on the genetic information transcription by modifying of the nucleic acids. Aflatoxins are most potent carcinogens in animal and human populations [6]. They are produced by *Aspergillus sp.* Patulin, first isolated from *Penicillium patulum* (later called *Penicillium urticae* now *Penicillium griseofulvum*). Zearalenone is a non-steroidal estrogenic mycotoxin produced by several *Fusarium sp.* [5]. Fumonisins are produced by a number of *Fusarium* species, notably *Fusarium verticillioides* (formerly *Fusarium moniliforme* = *Gibberella fujikuroi*), *Fusarium*

proliferatum and *Fusarium nygamai* as well as *Alternaria alternata f.sp. lycopersici* [7, 8]. Ochratoxin A and B are produced by *Aspergillus* species, ochratoxin A was discovered as a metabolite of *Aspergillus ochraceus* [5].

It is important to identify fungal contaminants in fresh fruits because some moulds can cause infections or allergies.

The fruits present immunity to the microorganisms through their structure and composition when they are undamaged due to their cellulosic cover and to their cerous layer, which bolcks the microorganisms to attach in a great number. If the fruits soften, the protective layer reduces its mechanical protection and so the mouls development is encouraged.

2. Materials and methods

To isolate pure moulds strains from the fruits we used the following nutritional media: liquid medium of glucose peptone water and solid medium of tetracicline – glucose – yeast extract agar.

The moulds were sampled from the fruits using a steril ansa and inoculated on the liquid medium of glucose peptone water. After 24-48 hours of incubation, we made inoculations on the solid medium of tetracicline – glucose – yeast extract agar in Petri dishes. The inoculated media were incubated for 72-120 hours at room temperature (21-22 ^{o}C). These procedures were repeated until we obtained pure strains for all the mould species. The pure strains were reinoculated on the solid medium in dishes to obtain the colonies and to take pictures at macroscopic and microscopic level, in vivo between the slide and the lamella. By direct observations and from the pictures we could identify the mould genera.

To take the pictures at macroscopic and microscopic level we used the following equipments: MLB 2100 A. Kruss Optronic biological microscope with 3 field glasses, Nikon Coolpix P5000 digital camera, Fujifilm FinePix S 9600 digital camera.

The fruits used in this experiment were bought from different markets. They were transported and stored in such a mater to avoid the infestation from other sources.

For this study we analysed the following fruits: apple, red pears, Nashi pears, orange peel candied, orange, lemon, banana, nectarine, avocado, mango, pomegranate, pomelos, strawberry, nuts and lychees.

3. Results and discussion

After the microbiological analyses there were isolated and identified 28 pure strains from the following 7 moulds genera: *Penicillium, Rhizopus, Alternaria, Botrytis, Cladosporium, Fusarium* and *Torula.*

The *Penicillium* genus was isolated from all the analysed fruits, alone or together with other 1-3 genera. Two genera were identified on each 3 fruits: *Alternaria* on orange, avocado and nuts and *Fusarium* genus on orange, banana and nectarine. Two genera were identified on each 2 fruits: *Cladosporium* genus was identified on apple and orange and *Rhizopus* genus on apple and mango. The *Botrytis* genus was isolated only on grapes and *Torula* genus only on nuts.

In Table 1, we presented the mould genera identified on all the analysed fruits.

Considering all these fruits, the orange presented 4 moulds genera; from the apple and also from the nuts there have been isolated 3 genera, from banana, nectarine, avocado, mango and grapes a number of 2 genera for each of them The other analysed fruits (red pears, Nashi pears, orange peel, lemon, pomegranate,

pomelo, strawberry and lychees) have been infected with only one genus, the *Penicillium*.

In figures 1-7, we presented the infected fruits, the mycelial colonies developed on the solid medium and the microscopic images of the moulds isolated from the fruits.

Table 1
Genera of fungi found on the fruits

No.	Fruits analysis	Genus
1	Apple	*Cladosporium sp.*
		Penicillium sp.
		Rhizopus sp.
2	Red pears	*Penicillium sp.*
3	Nashi pears	*Penicillium sp.*
4	Orange peel candied	*Penicillium sp.*
5	Orange	*Alternaria sp.*
		Cladosporium sp.
		Fusarium sp.
		Penicillium sp.
6	Lemon	*Penicillium sp.*
7	Banana	*Fusarium sp.*
		Penicillium sp.
8	Nectarina	*Fusarium sp.*
		Penicillium sp.
9	Avocado	*Alternaria sp.*
		Penicillium sp.
10	Mango	*Penicillium sp.*
		Rhizopus sp.
11	Pomegranate	*Penicillium sp.*
12	Pomelos	*Penicillium sp.*
13	Grapes	*Botrytis sp.*
		Penicillium sp.
14	Strawberry	*Penicillium sp.*
15	Nuts	*Alternaria sp.*
		Torula sp.
		Penicillium sp.
16	Lychees	*Penicillium sp.*

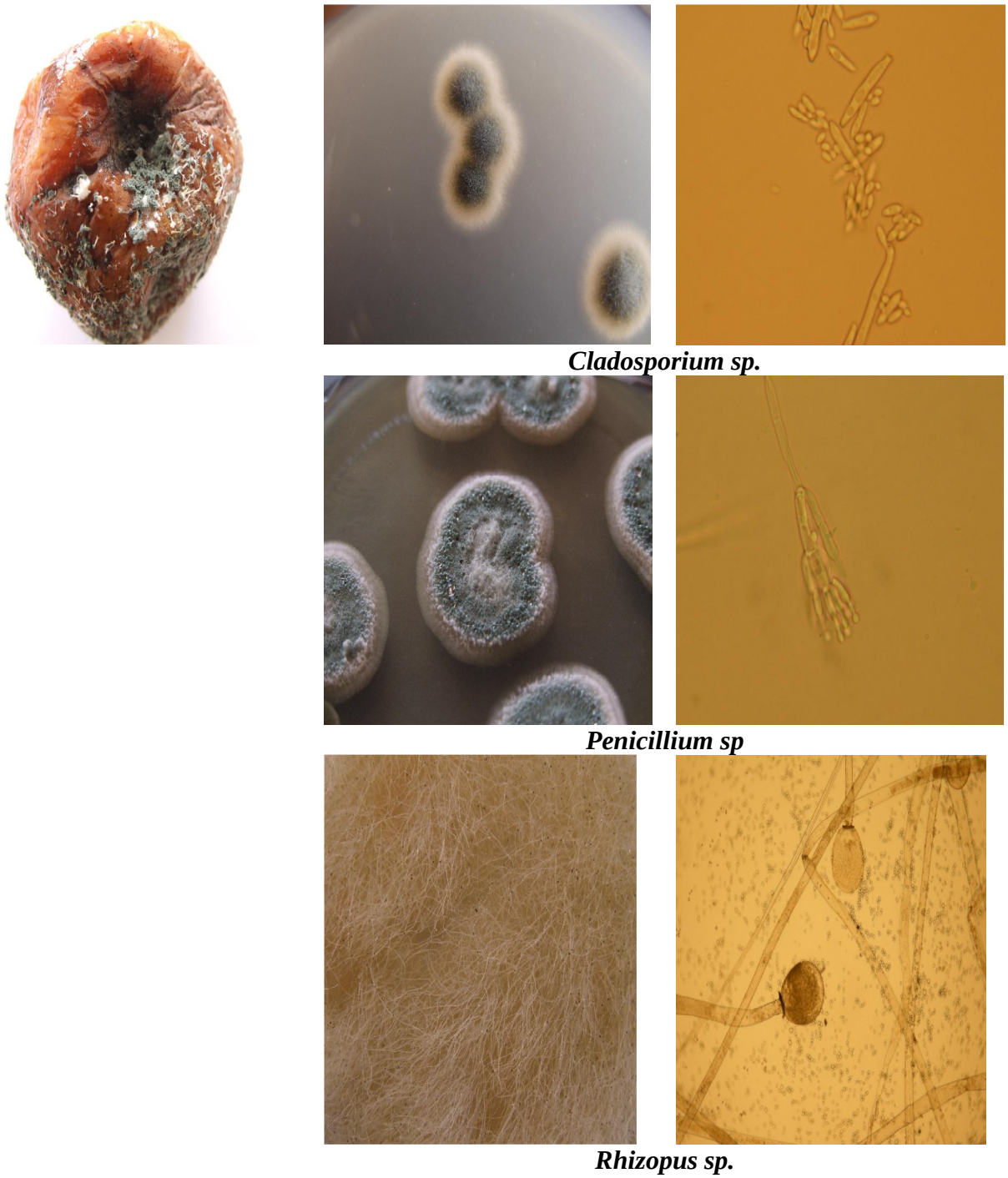
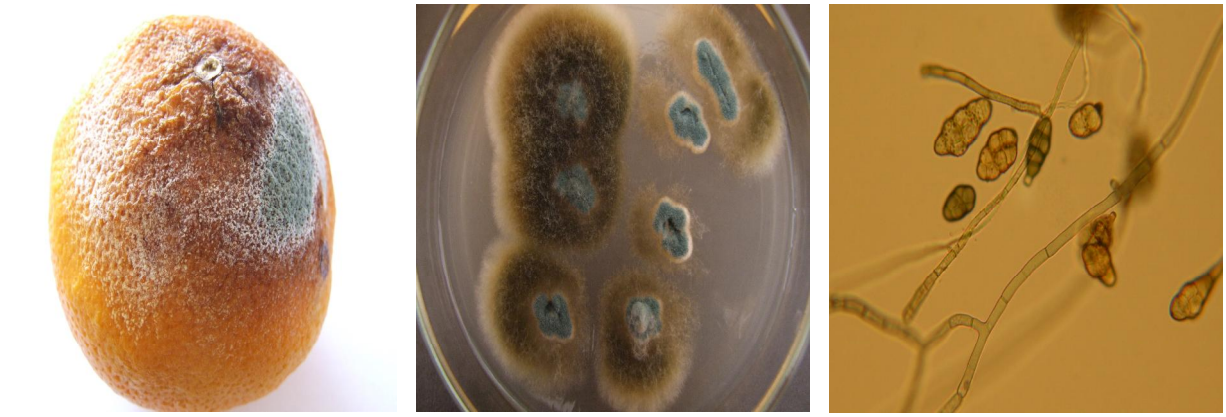
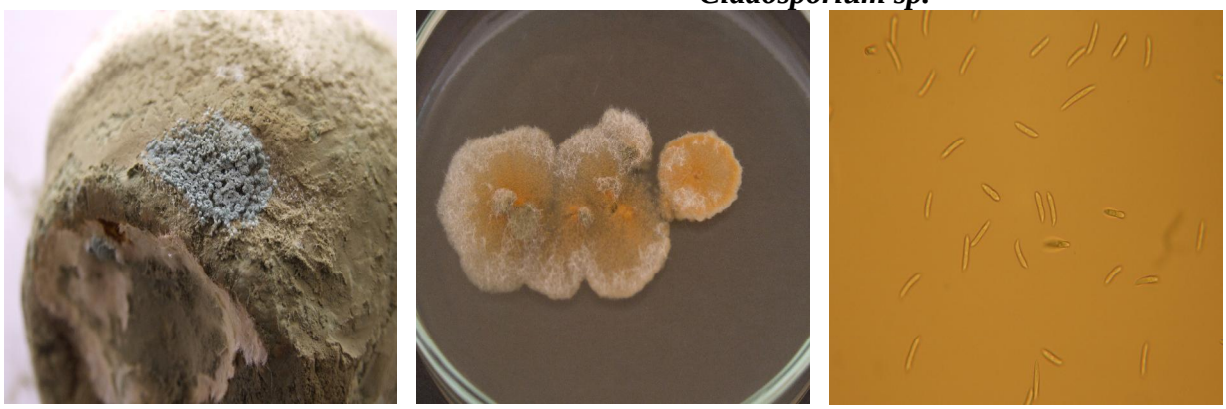


Figure 1. Genera of fungi found on apple

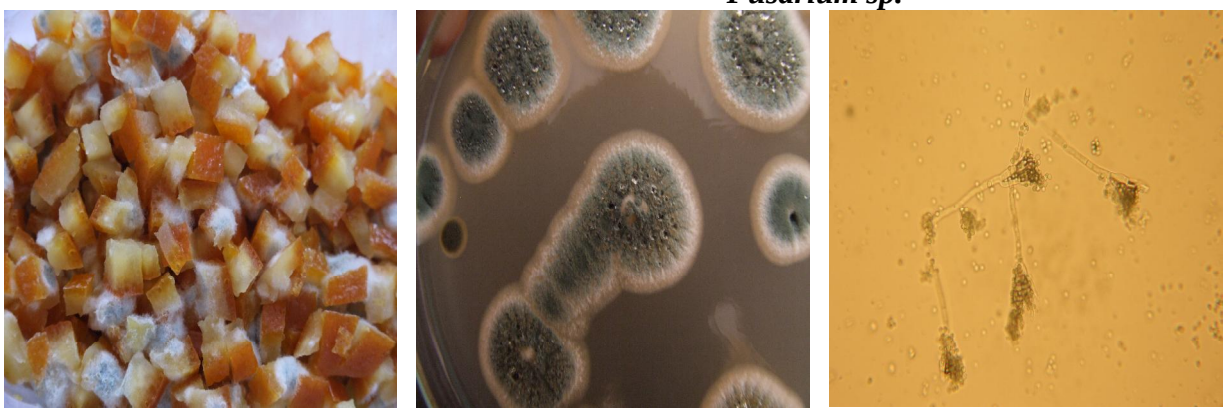


Alternaria sp.

Cladosporium sp.



Fusarium sp.

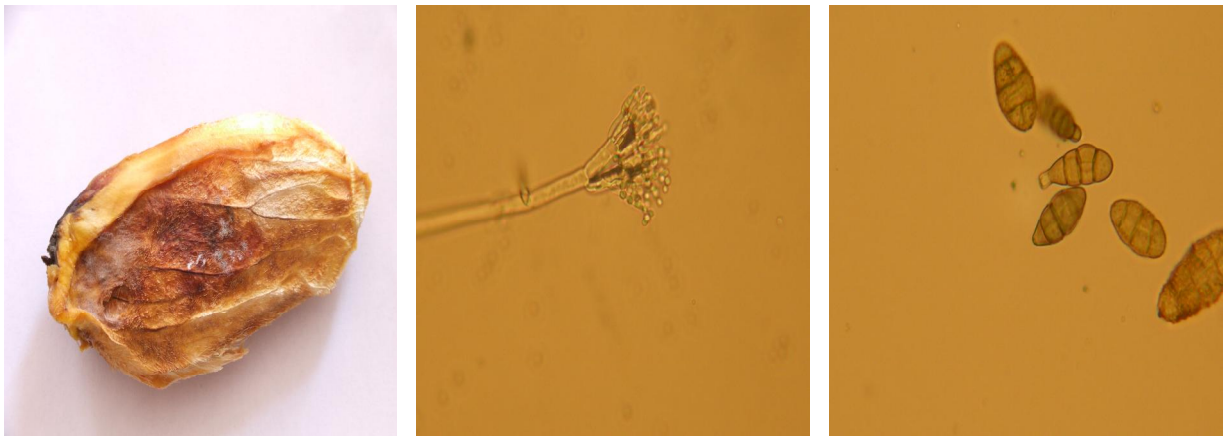


Penicillium sp.

Figure 2. Genera of fungi found on orange

Fusarium sp.

Figure 3. Genera of fungi found on banana

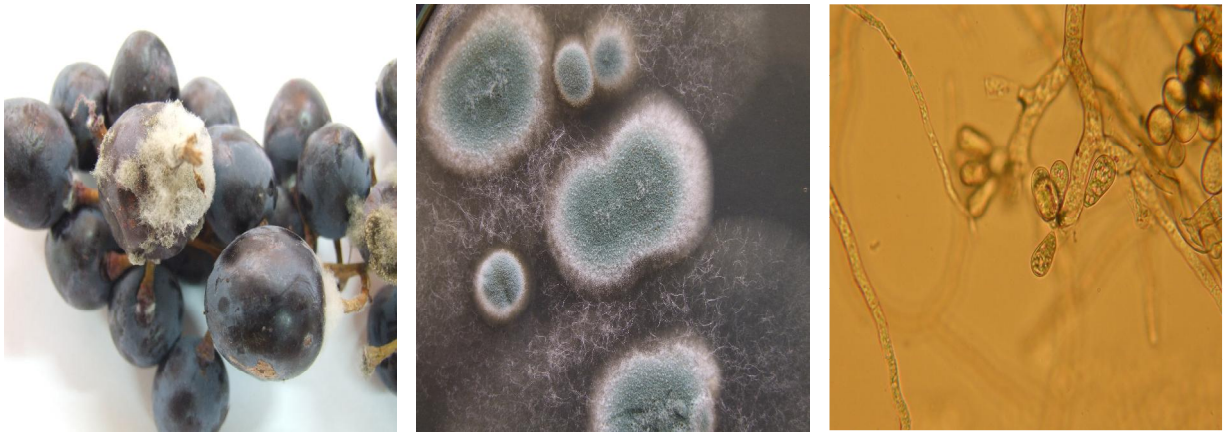


Penicillium sp ***Alternaria sp.***

Figure 4. Genera of fungi found on avocado

Rhizopus sp.

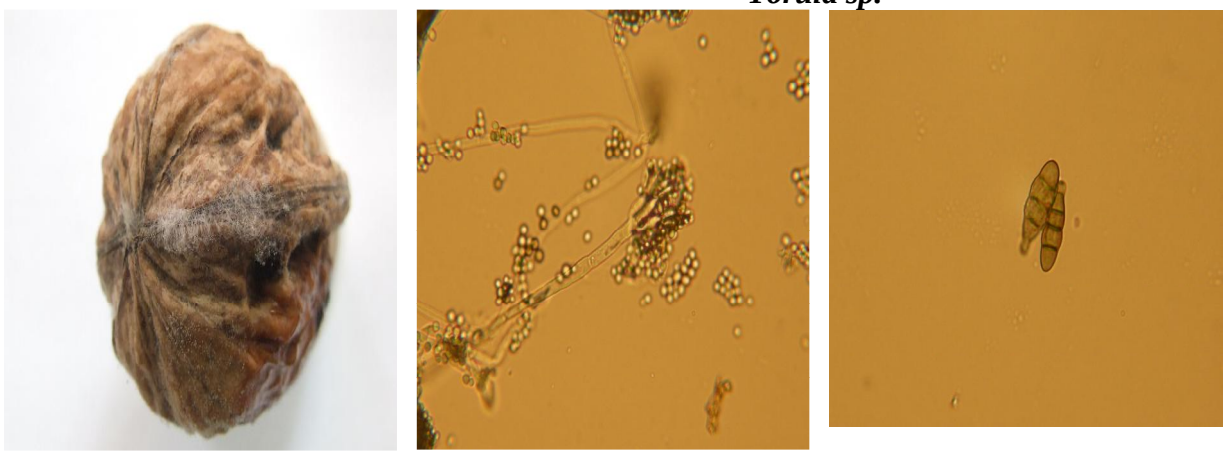
Figure 5. Genera of fungi found on mango



Penicillium sp.* and *Botrytis sp. ***Botrytis sp.***

Figure 6. Genera of fungi found on grapes

Torula sp.



Penicillium sp. ***Alternaria sp.***

Figure 7. Genera of fungi found on nuts

4. Conclusions

Regarding this study, we detected the presence of 7 moulds genera on the fruits sold in these markets. The most frequent genus isolated from the fruits was *Penicillium*, present on all the studied fruits, followed by *Fusarium*, *Alternaria* (each on 3 fruits), *Cladosporium*, *Rizopus* (each on 2 fruits) *Botrytis* and *Torula* (each on 1 fruit).

Using microscopic analyses, we could observe that there is a great variety of mould types which can prejudice human health if the hygienic conditions and the corresponding standards are not accomplished.

In order to reduce the microbiological contamination degree an essential role is played by the way in which the harvest is made and by the conditions of transportation and storage of the fruits. Thus, we can reduce the loss risks, loss of financial nature when the products depreciate and of human nature by prejudicing the humans health.

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THE MICROBIOLOGICAL QUALITY OF FLOUR AND PASTRY IN MARAMURES AREA

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Abstract

The forms of organization for the quality of pastry products have exceeded the point of investigation, of checking the conformity with the specifications (standards, norms).The microbial factors that can influence the quality of pastry products can be generated by microorganisms (bacteria, mould, yeasts, viruses) and pest. This paper aims to analyse comparatively the number of microorganisms starting from flour, which is the raw material, to the finite product, which are bread and pastry. From the analysis of the data, we observe an extremely important decrease of mezophil aerobe germs in the finite samples – bread, bread sticks, comparatively with the number of these germs in the flour.

Keywords: *flour, germs, pathogen, bread, quality*

1. Introduction

The requests of the consumers regarding the microbiological quality of the finite products of pastry represent persistent mercantile challenges in the industry of pastry. The important characteristic in the management of food safety consists in the imposition of some microbiological specifications to ensure the microbiological quality and food safety not only for flour, but also for pastry.

The microbiological factors that can influence the quality of pastry can be generated by microorganisms (bacteria, mould, yeasts, viruses) and pest.

An important approach in the management of quality is represented by the operating with microbiological risks through the deployment of a H.A.C.C.P (the analyses of the critical points of control) starting with the harvesting and processing of grains for the prevention of potential risks. A plan of measures of management for the analyses and control of microbiological risks was made for an efficient control of these risks of microbiological nature [1, 2].

Among the dangers that can be encountered most often in flour there are those connected to the germs of Bacillus, coliforms and mould. The literature of specialty describes the Bacillus as very resistant to heat, it also being able to survive naturally in the interior of the bread even to temperatures of baking [3, 4]. Correlations between the germs of Bacillus from flour and the viscosity of the dough did not show any significant correlation, suggesting the fact that different types of germs can influence the potential of impairment [5, 6].

In the case of soft bread, the flaw is caused by the presence, in a great number, of the Bacillus mesentericus germs, as well as by

the manufacturing and keeping of bread in favourable conditions for the development of the vegetative forms and propagation of this germ. Risks associated with the increasing number of mould in pastry are in general prevented by the subjunction of food additives like: sorbic acid, acetic acid and their salts [7, 8].

This present paper aims to identify the microbiological risks through the comparative analyses of the number of microorganisms starting from flour, which is a raw material, to the finite product, represented by bread and pastry.

2. Materials and methods

2.1 Preparation of the samples

The preparation of the samples for the microbiological analyses was made in conformity with SR ISO 6887-1:2002. For the determinations, the technique of work mentioned in the international standards was followed, for which the imposed microbiological parameters were determined.

The samples of flour were numbered with the letter F followed by indices corresponding to the number of order, thus: F1, F2, F3, F4, F5, F6. The samples of flour designed for pastry were taken from different private companies from the Maramures county.

2.2 The analyses of the pathogens

The identification and analyses of the pathogens for flour was done according to the following methods:

- SR ISO 4831-2006 for the detection and counting of the coliform bacteria
- SR ISO 16649-2 :2007 for the enumeration *Escherichia coli*
- ISO 21527-1:2008 and ISO 21527-2:2008 – enumeration of yeast and mould
- SR EN ISO 6579/2003 and SR EN ISO 6579/AC/2006 - for the detection of the bacteria of *Salmonell*a gender;
- SR EN ISO 4833 :2003 – for the enumeration of microorganisms (N.T.G.)
- SR EN ISO 7932 – for the counting of the putative *Bacillus cereus*

SR EN ISO 7218:2007 – for calculating and expressing the results.

3. Results and discussions

For the determinations, the technique mentioned above was followed, technique for which the microbiological parameters imposed at six samples of flour designed for pastry, taken from different private companies from the Maramures county.

3.1. N.T.G. Determination

The way in which the value of this parameter suffers modifications along the technological process of obtaining bread was followed.

Table 1. *The variation of the total number of aerobe mezophil*

Flour sample	NTG Value UFC/g ± RSD*		
	Flour	Bread	Crescent
F_1	$9{,}54\cdot10^4 \pm 0{,}2$	$1{,}3\cdot10^3 \pm 1{,}6$	$4{,}8\cdot10^2 \pm 0{,}7$
F_2	$9{,}22\cdot10^4 \pm 1{,}2$	$1{,}75\cdot10^3 \pm 0{,}1$	$3{,}25\cdot10^2 \pm 0{,}1$
F_3	$7{,}62\cdot10^4 \pm 0{,}4$	$1{,}04\cdot10^3 \pm 2.1$	$3{,}21\cdot10^2 \pm 1.4$
F_4	$9{,}21\cdot10^4 \pm 0.7$	$1{,}73\cdot10^3 \pm 0{,}3$	$3{,}71\cdot10^2 \pm 2$
F_5	$7{,}72\cdot10^4 \pm 0.7$	$8{,}9\cdot10^2 \pm 0{,}6$	$7{,}82\cdot10 \pm 0{,}1$
F_6	$6{,}31\cdot10^4 \pm 1{,}2$	$7{,}7\cdot10^2 \pm 0{,}3$	$5{,}2\cdot10 \pm 0{,}2$

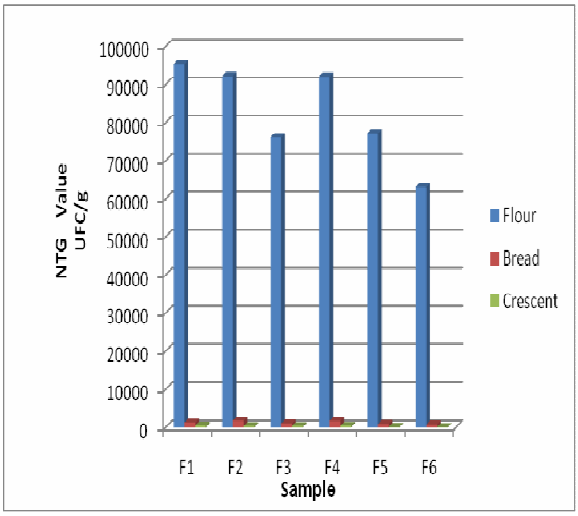

Figure 1. The comparative aspect regarding the NTG value

From the analysis of the data, an extremely important decrease of mezophil aerobe germs can be seen in the finite products – bread and crescents, respectively – comparatively with the number of these germs registered in flour. An explanation of this phenomenon can be based on the high temperatures existent in the ovens for pastry.

The germs that go to bread and crescent are represented by the vegetative forms of bacterial germs that have germinated after the bread was taken out of the oven.

3.2.The determination of coliform bacteria

In the case of bread and crescent, the number of coliform germs is very low, 1-2 germens/g, and in the case of samples from flour numbered F_3, F_5 and F_6, the number of coliform germs is 0. The presence of these germs on this kind of products was due to the ulterior contamination, as coliform germs, being asporulated and acapsulated, did not resist to so high temperatures and they were not totally destroyed while baking.

Table 2. *The variation of the number of coliform germs*

Flour sample	The value of the number of coliform germs UFC/g ± RSD*		
	Flour	Bread	Crescents
F_1	5,3·10 ± 0.1	4 ± 0.1	2± 0.5
F_2	4,4·10 ± 0.2	2± 0.2	1± 0.3
F_3	4,7·10 ± 0.1	4± 0.1	2± 0.1
F_4	7,2·10 ± 0.1	1± 0.2	1± 0.7
F_5	1,0·10 ± 0.7	7± 0.1	1± 0.1
F_6	3,5·10 ± 0.1	0± 0.1	0± 0.1

RSD standard relative deviation*

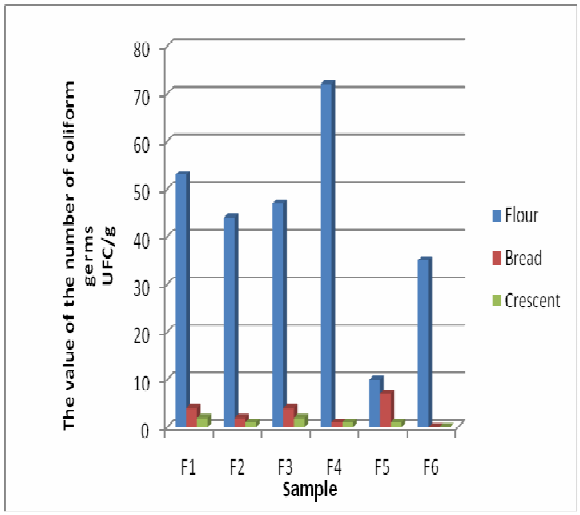

Figure 2. The comparative aspect regarding the number of coliform germs

Through the application of biochemical tests for the coliform germs, the species they belong to could be determined. In this regard, the T.S.I. (triple sugar iron) tests were made, the ureaza test, Simmons citrate and the SIM test (the production of H_2S, indol, mobility).

After making these tests, the following conclusions were reached: the contaminants belong to the *Escherichia coli*, *Proteus mirabilis* şi *Enterobacter aerogenes* species. These parameters have

represented microbiological and salubrity indicators of the products, suggesting the existence of a recent contamination.

3.3 The determination of the number of germs from the Bacillus Mesentericus species

Bacillus mesentericus synthesises proteolytic and amilolytic enzymes, which degrade the proteins and starch.

Table 3. *The variation of the number of germs from the Bacillus mesentericus species*

Flour sample	The value of germs from the Bacillus mesentericus species UFC/g ± RSD*		
	Flour	Bread	Crescents
F_1	9,7·10 ± 0.2	9 ± 0.1	6± 0.4
F_2	8,5·10 ± 0.2	7± 0.2	7± 0.1
F_3	8,2·10 ± 0.8	8 ± 0.1	6± 0.1
F_4	9,2·10 ± 0.2	9 ± 0.2	7± 0.4
F_5	7,6·10 ± 0.1	6± 0.1	5± 0.5
F_6	7,1·10 ± 3,2	7± 0.3	4± 0.6

RSD standard relative deviation*

The degrading of starch can be put into light in Petri plates, through the dissemination on the surface of the plate in which the development of colonies supposed to be of *Bacillus mesentericus* can be noticed in a solution of potassium iodine. In the presence of starch, the culture environment acquires a blue colour, and after the hydrolysis of starch by *Bacillus subtilis*, the disappearance of the blue colour can be noticed, phenomenon that starts to be obvious in the second colony from the Petri plate in the right.

The proteolysis is obvious through the appearance of the clear halo around the colonies of *Bacillus mesentericus*.

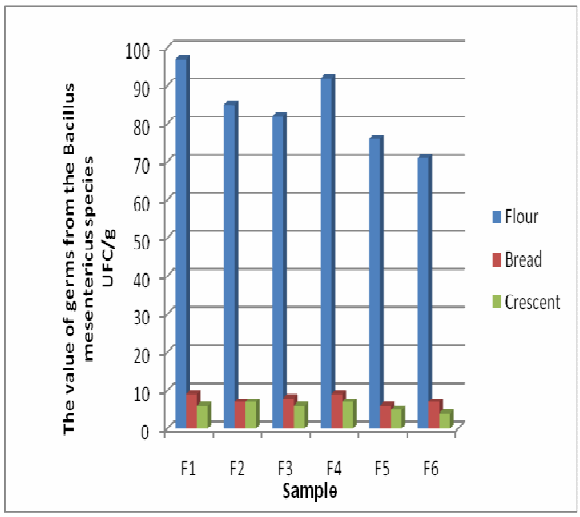

Figure 3. The comparative aspect regarding the number of germs belonging to Bacillus mesentericus species

3.4. *The determination of the number of germs from the Bacillus cereus species*

The current legislation (H.G. 975/1998) mentions the possibility of the existence of a number of maximum 100 de germs/ flour gram designed for pastry and does not norm this parameter for bread and other pastry.

Bacillus cereus is not a pathogen germ, only in concentrations of 10^6 in food. Being a sporulated germ, the germs have lived under the temperatures of baking and therefore their number did not reduce significantly in the finite products.

Table 4. *The variation of the number of germs from the Bacillus Cereus species*

Flour sample	The value of the number of the germs belonging to Bacillus Cereus species UFC/g ± RSD*		
	Flour	Bread	Crescents
F_1	$9,9 \cdot 10 \pm 0.5$	10 ± 2.1	6 ± 2.4
F_2	$9,2 \cdot 10 \pm 0.1$	8 ± 0.5	9 ± 0.7
F_3	$6,7 \cdot 10 \pm 0.7$	9 ± 2.5	7 ± 0.8
F_4	$7,8 \cdot 10 \pm 0.3$	10 ± 2.2	8 ± 0.4
F_5	$5,3 \cdot 10 \pm 0.3$	10 ± 0.1	7 ± 7.1
F_6	$4,6 \cdot 10 \pm 0.1$	6 ± 0.3	4 ± 0.3

Table 5. *Variation of the number of yeast and mould*

Flour sample	Value of the total number of yeast and mould UFC/g ± RSD*		
	Flour	Bread	Flour
F_1	$9,5 \cdot 10^2 \pm 0.3$	$8,8 \cdot 10 \pm 0.6$	$7,5 \cdot 10 \pm 2.1$
F_2	$9,2 \cdot 10^2 \pm 0.2$	$8,1 \cdot 10 \pm 0.5$	$6,3 \cdot 10 \pm 0.3$
F_3	$7,7 \cdot 10^2 \pm 0.7$	$6,3 \cdot 10 \pm 0.8$	$5,2 \cdot 10 \pm 0.7$
F_4	$7,2 \cdot 10^2 \pm 0.3$	$7,2 \cdot 10 \pm 2.7$	$5,5 \cdot 10 \pm 0.2$
F_5	$5,55 \cdot 10^2 \pm 0.4$	$5,7 \cdot 10 \pm 0.3$	$4,7 \cdot 10 \pm 1.3$
F_6	$4,6 \cdot 10^2 \pm 0.2$	$5,8 \cdot 10 \pm 5.4$	$3,1 \cdot 10 \pm 0.4$

RSD standard relative deviation*

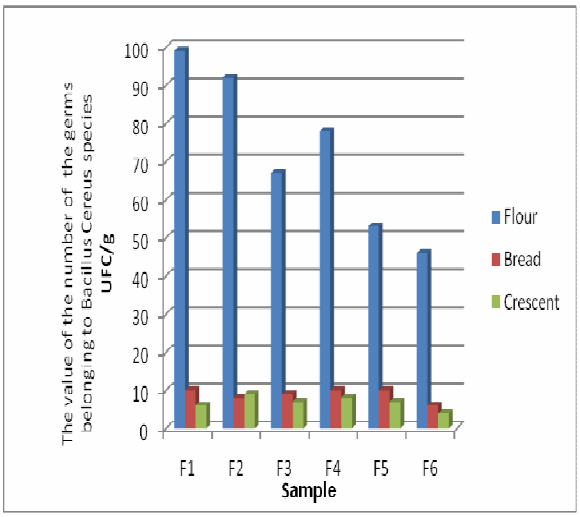

Figure 4. The comparative aspect regarding the number of germs belonging to Bacillus cereus species

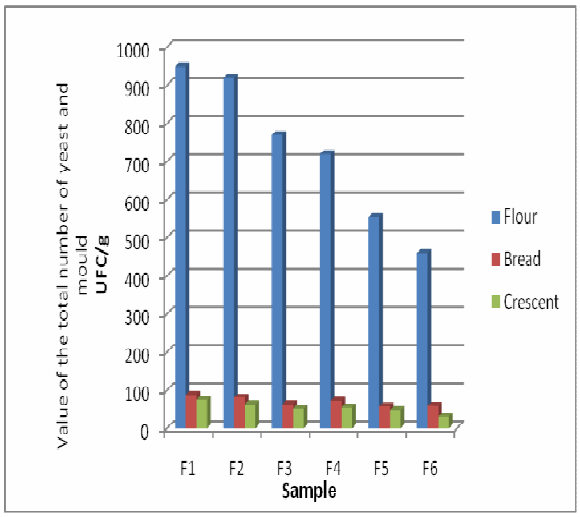

Figure 5. The comparative aspect regarding the number of yeast and mould

3.5. The determination of the number of mould and yeast

The current legislation (H.G. 975/1998) mentions for this parameter an admitted superior limit of 1000 germs/flour gram and 100 germs/bread and crescent gram.

After macroscopic observations – general characteristics of the colonies (speed of development, the colour of the colonies in relation to the time of harvesting, the colour of the base of the colony and the changing of colour of the environment, the possible presence of drops of "sweat" on the aerial mycelium, the smell of the colonies) and microscopic, after colouring in blue – cotton – lactefenol and the observation with the 40X lens, the main species of fungi present in the analysed products were determined. In this

way, the following species were identified: *Alternaria,Helminthosporium, Aspergillus, Penicillium, Mucor, Rhizopus, Fusarium*.

4. Conclusions

From the analyses, we have seenthat the number of microorganisms decreases progressively from the raw material to the finite products, as a direct consequence of the diverse phases of the technological process, that ends with the baking, which means the exposure to high temperatures.

The number of microorganisms of each type decreases for crescents comparatively with their number registered in the obtained bread, even if the raw material and the conditions of cooking are the same, phenomenon that can be explained through the fact that the dimensions of the samples are considerably lower, and the temperature from the interior of the crescent-crumb is higher than in the interior of the bread-crumb.

In each case analysed, the samples of flour, bread and pastry were situated, from a microbiologic point of view, in the limits of the current legislation.

The contamination of the finite products (bread, crescents) with coliform germs was consecutive to the baking process, and the *E coli* germs and *Proteus mirabilis* have human origin. Their presence indicates a recent contamination.

In the case of the technological process, we observe that the finite products have a micrologic charge a lot inferior to the raw materials.

In each bread exposed to the experiment, the phenomenon of finace could be noticed, meaning the presence of *B. subtilis-mesentericus.*

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INFLUENCE OF HEAVY METAL IONS CONCENTRATION ON GERMINATION AND PLANT GROWTH

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Abstract

Bioaccumulation of heavy metals is a process which depends among other resistance/tolerance of plants against chemical stress, provided the mechanisms of metal influences and features of the species of organism. This study focuses on the influence of the amount of heavy metals in germination and plant growth. For this study used seeds of beans, maize and cucumbers from polluted and unpolluted soils of Maramues. We followed these seed germination and growth on media enriched with heavy metal ions as Fe, Cu, Zn, Pb. Germination experiments in the presence of metal were performed on seeds of *Phaseolus vulgaris*, *Zea mays* and *Cucumis sativus* harvested from polluted and unpolluted areas, shows, since the state of imbibition [1-3] as the harvested land metal, have developed adaptive mechanisms. Extending the study and the stage of plant growth, under certain conditions, even the seeds harvested from unpolluted land manifest behavior toward metallic ions, which can be translated as "accommodating" to chemical stress.

Keywords: *heavy metals, germination, bean, maize, cucumbers*

1. Introduction

Plants have been shown to accumulate, over time, large amounts of heavy metals without visible changes in their appearance or yield. In many plants, the level of metal accumulation can exceed even several hundred times the maximum level permissible for human beings, without having any negative effect on their growth [4]

The large amount of heavy metals in soil is a problem that mankind faces for many years and has influence on quantities of metals in the human body can achieve. Many studies have been done to see what effect on plant growth have polluted/unpolluted soil, and tried to explain the results. The plants growing in polluted habitats probably have the ability to inactivate the heavy metal by binding the excess metal ions and/or by changing the chemical composition and physical organization of their cell membranes [5].

Determining routes of adaptation and learning mechanisms for obtaining resistance to unfavorable factors of living organisms shows importance. Being open systems, which are in permanent exchange of matter and energy with the environment, plants respond adequately to adverse actions. The plant body, the pollutants generated so-called stress, which consist of modifications of growth and development, photosynthesis, respiration, hormonal activity and other processes at the molecular level set. Pb is the most dangerous metal because of its elevated level in the environment in certain areas. These areas include urban regions polluted by wastes that are beginning to reach thresholds able to evoke the first signs of toxicity in humans [6].

Lane and Martin [7] showed that seed coats of *Raphanus sativus* were strong barrier to lead, and helped prevent contamination of embryos until the seed coat was torn apart by the germinating embryonic root. On other hand, there are reports on the inhibitory effect of lead on the germination of seeds of the following species: *Lupinis luteus* [8], *Oryza sativa* [9] and *Sinapsis alba* [10]. Stefanov et al. [5], which studied the accumulation of Pb, Zn and Cd in different plants seeds, showed that plants accumulate selectively heavy metal ions in their seeds. Peanut and corn seeds accumulate mainly Pb, pea seeds accumulate mainly Cd and wheat seeds accumulate mainly Zn. Another study reveals that the seeds originating in non metalliferous areas display a higher adsorption capacity of metal ions (except Fe) than seeds from metalliferous areas.

The bean is the most used test organisms for environmental assessment of air pollution effects [11].

This study was conducted to determinate the influence of heavy metal ions on the germination and growth of cucumber, maize and beans plants. We do this study for Fe, Cu, Zn and Pb ions using different concentration of ions.

2. Materials and methods

Satu Nou de Jos is a village in the N-E neighborhood of Baia Mare (see upper circle on the map in Figure 4, in the the Maramures County (N-W of Romania). It is known as heavily polluted area due to intensive mining and ore processing activities in the past. Oarta de Jos is a village located about 50 km S-W of Baia Mare (see lower circle on the same map), in an area that is rather clean, just because of being exempted from any sort of mining and industrial activity. Even more, the village is about 10 km far of any significant road traffic, which is by itself a source of pollution [12].

We have used cucumber, maize and beans seeds for each area. The number of samples was 30 for each sample. We have disinfected the seeds with alcohol, washed them three times with distillated water and have introduced them to stay during germination in solutions with different concentrations of ions of heavy metals. The solutions containing the metal ions were prepared using $FeSO_4$, $(CH_3COO)_2Pb$, $CuSO_4$, $ZnSO_4$ salts. We have prepared solutions with the following concentrations: 10, 50 and 250 mg/L for Fe^{2+}; 10, 20 and 25 mg/L for Cu^{2+}; 5, 10 and 15 mg/L for Zn^{2+}; 100, 1000 and 10000 mg/L for Pb^{2+}. As blanks (witness samples) were used each type of seed (polluted and unpolluted bean, polluted and unpolluted maize) introduced in pots for germination containing distillated water. The germination time was 6 days.Germinations conditions were 22ºC, light 16h/day and dark 8h/day.

After germinating the seeds were transferred to the broth tubes Knopp. In tubes were added solutions with different concentrations of ions. We used the same heavy metal ions at the same concentrations as germination. As blanks (witness samples) were used each type of seed introduced for 7 day in pots for germination containing distillated water and then in tubes containing Knopp nutrient solution. The growth time was 14 days.

3. Results and discussion

The results obtained are representing graphically in Figures. 1-3. Figure 1 shows the results of the germination of cucumber, maize and bean seeds, polluted and unpolluted after 2, 3 and 6 days, at different concentrations of ions of Fe, Cu, Zn and Pb. Figure 1(a) and (b) shows that the polluted cucumber seeds germinate since day 2 for all metal ions concentration, except Fe 250 and Pb 1000 where germination starts since the day 3. The unpolluted cucumber seeds germinate for all metal ions concentration except Fe 250, Cu 25, Zn 15, and Pb 1000 where germination starts since day 3. The polluted maize seeds germinate since second days, except Fe and Cu 10 where the germination starts since day 3 and unpolluted maize seeds germinate since day 3 except Cu 20, Zn 5, Zn 15, Pb 100 and Pb 1000 where the germination can be observe in the day 6. The polluted bean seeds germinate since the day 3 except Zn 10 where germination starts in day 6, the unpolluted bean seeds germinate since the day 3 for Fe 250, Cu 10, Cu 20, Zn 5, Zn 10, Zn 15 and for the remaining ions the germination can be observe in the day 6, except Pb 1000 where germination is inhibited. The images also reveal that polluted seeds germination level was higher than in unpolluted seeds which could indicate that polluted seeds develop, in time, a mechanism for adaptation to harsh environmental conditions.

We also see that for Zn and Cu are greater degree of sprouting seeds so the polluted and unpolluted which could be explained by the fact that Zn and Cu are elements which have a role in seed germination and plant growth.

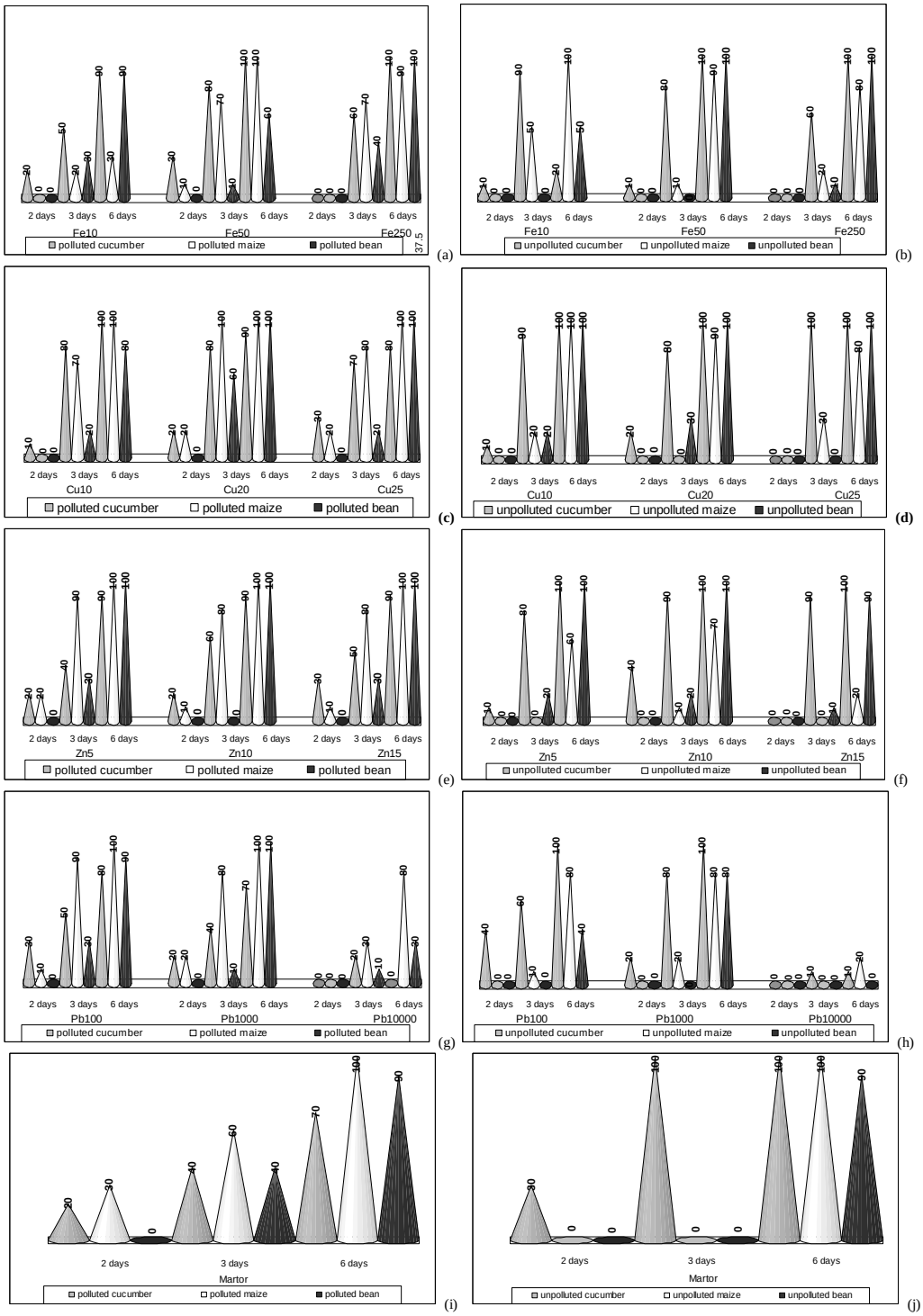


Figure 1. The germination percentage of polluted and unpolluted seeds in 2, 3 and 6 days

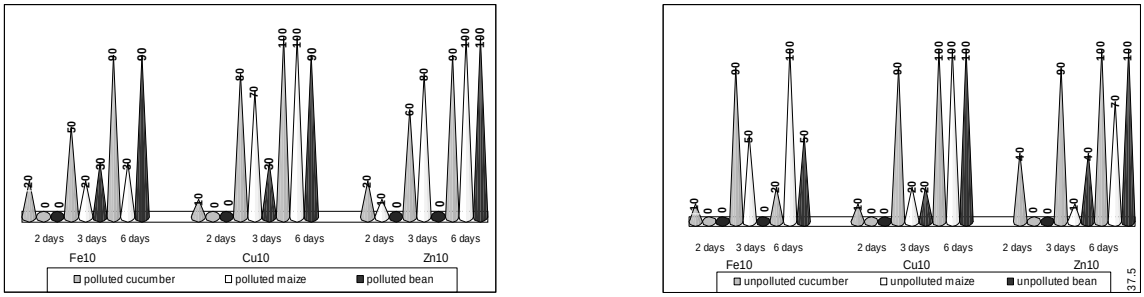


Figure 2. Germination rate at the same concentration of Fe, Cu, and Pb (10 mg/l)

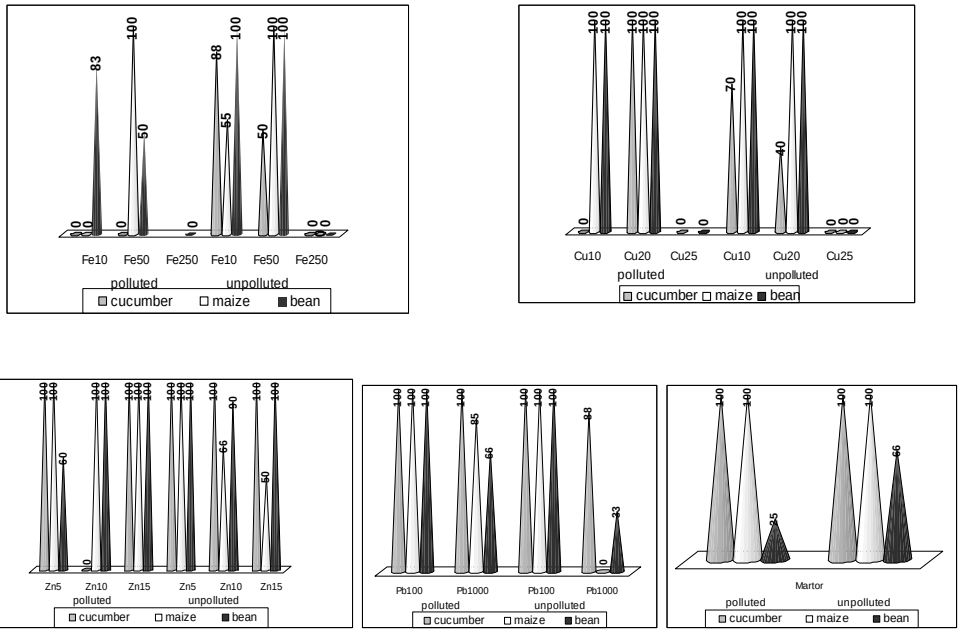
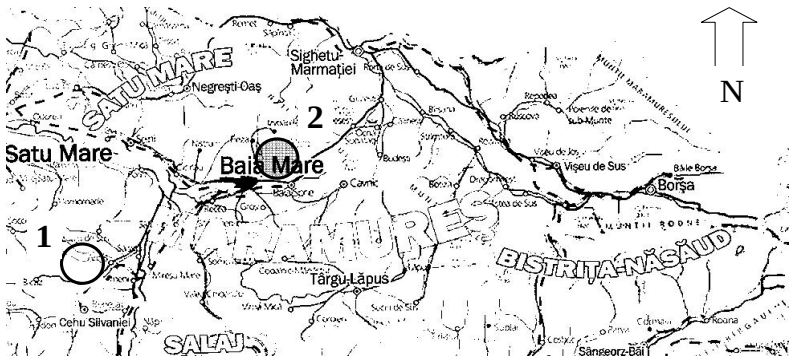


Figure 3. The growth rate of polluted and unpolluted seedlings



1. Area free of pollution with ions of heavy metals
2. Area heavily polluted with ions of heavy metals

Source : part of a map edited and posted on the Internet by RestRom SRL

Figure 4. Maramures map

Number of germinated seeds increases with increasing concentrations in both the polluted and the unpolluted except to Pb where we can see that the concentration of 10000 is a noticeable decrease. By the end of germination could be seen as seedlings grown at lower concentrations were higher than those grown at higher concentrations. For Zn15 polluted seeds formed seedlings higher than unpolluted seeds. The reference is seen as polluted seeds germinate faster than unpolluted.

A comparison between different types of ions such as Fe, Cu and Zn at the same concentration 10 mg/L (figure 2) shows that the germination rate is the same for all the three types of ions, which may indicate that low concentrations of ions do not have a negative influence on germination. Also we can see that the germination rate is the same for both polluted and unpolluted seeds.

Figure 3 shows the degree of growth of plants for cucumber, maize and beans, 7 days after inoculation. We can observe that the number of plants from polluted seeds are almost the same with number of plants from unpolluted seeds which shows us that even plants from unpolluted seeds probably have the ability to inactivate the heavy metal by binding the excess metal ions and/or by changing the chemical composition and physical organization of their cell membranes [2].

4. Conclusions

- Polluted seeds germination level was higher than in unpolluted seeds
- Number of germinated seeds increases with increasing concentrations in both the polluted and the unpolluted except to lead where we can see that the concentration of 10000 is a noticeable decrease.
- Low concentrations of ions do not have a negative influence on germination.
- Even plants from unpolluted seeds probably have the ability to inactivate the heavy metal by binding the excess metal ions and/or by changing the chemical composition and physical organization of their cell membranes.

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EVOLUTION OF PHYSICO-CHEMICAL PROPERTIES OF IODINE FORTIFIED LIPIDS

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Abstract

Experimental research were effectuated to establish the modalities of incorporation of the molecular iodine in refined and deodorized sunflower oil. To investigate the influence of iodine administration in sunflower oil were evaluated the quality indices of oil (acid value, peroxide value, iodine value, refractive index), which were reported to the standard. Iodized oil manufacture and of food products fortified with iodine by additive administration in the form of iodized oil would constitute a considerable iodine supplement, which associated to iodine intake of kitchen salt, would contribute to the eradication of iodine deficiency. Incorporation of exogenous iodine in a complex system, such as food, requires elucidation of physicochemical transformations, which may intervene in manufacturing process and storage of the product. It was established that the iodization of sunflower oil may be considered as an admissible method, which allows the incorporation of a considerable amount of iodine (0,1; 1 g/L) without modifying the physicochemical properties of the product. Research of quality indices variation of iodized oil during product storage (5 weeks), demonstrated that only in the case of maximum concentration of iodine (1 g/L) it was manifested a slight overcome of the maximum permitted limit caused by the free iodine presence.

Keywords: *fortification with iodine, sunflower oil, quality indices, oxidative stability, storage*

1. Introduction

Currently the number of persons in the world, which are under conditions of insufficient intake of iodine is estimated at 1.5 billion, of which about 800 millions are suffering from iodine lacking diseases [1]. Iodine is an essential mineral for the body, for thyroid hormone synthesis. It is complex physiological and depends on the available intake of the body, on mechanism of its organification by the thyroid gland and on fluid adjusting intra- and extrathyroid. Clinical expression translates through the appearance of thyroid dysfunction, usual biological exploration not always allow accurate identification of the dysfunction causes. Deficiency in iodine during prenatal and to children of early age decreases the intellectual coefficient with 5-13 points (IQ) [2, 3, 4, 5], causes mental retardation which affects 9-9.6% of children between 5-12 years [6, 7, 8, 9, 10, 14]. Medicinal supplement requires a significant period of time, at least 12 weeks [11, 13], which involves some complications, especially in countries with an insufficiently developed infrastructure. In many cases, medicinal supplements administration leads to the occurrence of side effects, which determine carente subjects to stop receiving the medicine [18, 19]. In particular case, iron and calcium supplements, orally administered, may cause vomiting, constipation, diarrhea [15, 17, 21, 24]. The correction of iodine intake with iodized salt represents a method used at international scale, but which can not meet the needs of all categories of population [26].

Iodized salt using is the traditional method most widely applied in practice, due to accessible price and general consumption by the population. According to the study [27-30], iodine intake of kitchen salt is still insufficient. This depends on several factors: salt consumption varies from 2 g/day to 10-12 g/day, being lower to children and older people. Iodized salt technology (iodides,

iodates), iodine concentration (10-15 mg/kg salt), as the full or partial use of iodized salt, causes considerable fluctuation in iodine intake coming this way. An inconvenience of the method is iodine instability in salt [31, 32]. Iodine being a liposoluble element, its administration in lipid products has a special interest. This allows easy incorporation of iodine in food and daily consumption of fat being limited, iodine intake can be easily adjusted. The advantage of this method of regulating the iodine intake consists in the fact, that iodine uptake from lipid food is gradually, depending on the body needs [16]. Sunflower oil is a product of current consumption. Iodized oil manufacture and of food products fortified with iodine by additive administration in the form of iodized oil would constitute a considerable iodine supplement (40-50 μg/day), which associated to iodine intake of kitchen salt, would contribute to the eradication of iodine carente. Incorporation of exogenous iodine in a complex system, such as food, requires elucidation of physicochemical transformations, which may intervene in manufacturing process and storage of the product.

The objective of the paper consists in research chemical transformations occurring during the incorporation of iodine in lipids of plant origin- sunflower oil and tracking physicochemical transformations, caused by iodine incorporation. Achieving of this goal requires studying the evolution of composition and physicochemical indices of iodized oil during thermal processing and storage of the product.

2. Materials and methods

Samples

The raw material used - sunflower oil has the ability to retrieve, by dissolving, representative quantities of iodine. For research was used double refined and deodorized oil. To obtain iodized oil, in a liter of product was added 1 g of crystalline iodine (I_2), chemically pure. Iodized oil has intense brown color, caused by the free iodine. The oil obtained with a total iodine content of 1 g/L, was diluted, obtaining sample, where the iodine content was 0,1 g/L.

Physicochemical examination

The values of refractive index are conditioned by the nature and proportion of fatty acids, unsaturated fatty acids increase the value, and saturated fatty acids decrease it. To determine the refractive index we used the PAL-RI (Tokyo, Japan) with the following technical characteristics: field: 1,3306-1,5284; resolution: 0.0001; accuracy: ± 0.0003; measuring temperature: 5-45^oC (resolution 1^oC); measuring time: 3s; in accordance with the requirements of EMC Directive 93/68/EEC.

Iodine value was determined using Hanus method. Approximately, 0.5 g sample (dissolved in 15 mL CCl_4) was mixed with 25 mL Hanus solution (IBr) to halogenate the double bonds. After storing the mixture in dark for 30 min., excess IBr was reduced to free I_2 in the presence of 20 mL of KI (100 g/L) and 100 ml distilled water. Free I_2 was measured by titration with 24.9 g/L $Na_2S_2O_3 \cdot 5H_2O$ using starch (1.0 g/100 mL) as an indicator. IV was calculated as g I_2/100 g sample, [35]. Saponification index value is conditioned by the number of fatty acids (the number of carboxylic groups), existing in a given quantity of fat, and this number is conditioned by the molecular weight of fatty acids in question. Were weighed, 2 g, with precision, which were brought to 25 mL alcoholic solution of potassium hydroxide (40 g KOH dissolved in 1 L 96% alcohol), in a boiling balloon with air refrigerant, boiling moderate and uniform. After saponification, the refrigerant was washed with few mL of hot distilled water, and the cooled sample was titrated with

0.5 N hydrochloric acid in the presence of phenolphthalein (1 ‰ alcoholic solution), until the disappearance of red color. In parallel, was executed a control sample, in the same conditions but without oil. Saponification index was expressed as the amount of potassium hydroxide, in mg, required for saponification of a gram of oil, in working conditions, [34]. Determination of acidity is the basic criterion for assessing the installation and intensity of hydrolysis. The method consists in neutralizing acidity with sodium hydroxide 0.1 N, using phenophtaleine, as an indicator. Acidity was expressed in oleic acid grams to 100 grams sample [34, 35].

Peroxide value was determined using UV-VIS T60U spectrophotometer (London, England): operating temperature 5 – 45^{o}C; field wavelength 190 - 1100 nm; wave length accuracy 0.1 nm. This protocol was based on the spectrophotometric determination of ferric ions (Fe^{3+}) derived from the oxidation of ferrous ions (Fe^{2+}) by hydroperoxides, in the presence of ammonium thiocyanate (NH_4SCN). Thiocyanate ions (SCN^-) react with Fe^{3+} ions to give a red-violet chromogen that can be determined spectrophotometrically, the absorbance of each solution was read at 500 nm. To quantify PV, a calibration curve (absorbance at 500 nm vs. Fe^{3+} expressed in μg) was constructed and peroxide value was expressed as meq O_2/kg sample [33].

3. Results and discussion

To investigate the influence of iodine administration in sunflower oil were evaluated the quality indices of oil, which were reported to the standard. Physicochemical indices of iodized oil are presented in Table 1. Refractive index is used to assess the unsaturation degree of oils, it decreases with the double bonds saturation and iodine value decreases in parallel, too. This indicates that the oil processing can be controlled by these parameters measurement. Iodine value is commonly used in oil classification, depending on the field of use and the hydrogenation process control. Saponification index is used to assess the average length of fatty acids chain from triglycerides composition. Acidity index and free fatty acid content is an indicator of hydrolytic rancidity and peroxide value indicates the degree of oxidative rancidity.

Saturated, monounsaturated and polyunsaturated fatty acids content appear on various food label, which requires analysis of commercial fat in terms of fatty acids content. This analysis also establish the source of the fat material and certainly determine its degree of purity.

It was found that iodine value varieed a little, even in the sample with a maximum content of iodine (1 g/L), its values did not exceed permitted limit, iodine being added to the double bounds. Refractive index varied insignificantly, that contests the free iodine presence in samples with a content of 0,1 g/L. Only in samples with a content of 1 g/L was shown the free iodine presence. According to experimental data, were established the following: incorporation of molecular iodine in sunflower oil does not lead to double bonds breaking, this has been established by fatty acid composition determining in a range of iodine concentrations of 0,1-1 g/L, the verification of the unsaturated degree of the product (iodine value) confirm the invariableness of double bonds number of triglyceride molecules. It was established, that iodization of sunflower oil can be considered an acceptable method, which allows incorporation of a considerable amount of iodine (0,1; 1 g/L), without noticeably modifying physicochemical properties of the product.

It was found that physico-chemical parameters of iodized oil did not differ significantly from the blank and fits the maximum allowable limits for sunflower oil, assumption that iodized oil can be used in nutrition to treat iodine deficiency.

Table 1
Physicochemical indices of iodized sunflower oil

Physico chemical indices	**Control sample**	**Iodized oil (g I_2/L)**		**Max. allowed**
		1	**0.1**	
Acidity % (g oleic acid)	0.28	0.24	0.26	1.0
Peroxide value, (meq O_2/kg)	6.0	8.0	7.0	12
Iodine value, (g I_2/100 g)	58.37	64.74	59.96	70-80
Refractive index, (20°C)	1.4693	1.4697	1.4695	1.4800 – 1.4872

Table 2
Evolution of physico-chemical parameters of iodized oil during product storage

Physico chemical indices	**Iodized oil (1 g I_2/L)**					**Iodized oil (0,1 g I_2/L)**				
	Control sample	**Time (weeks)**				**Control sample**	**Time (weeks)**			
		2	**3**	**4**	**5**		**2**	**3**	**4**	**5**
Acidity % (g oleic acid)	0.24	0.27	0.29	0.30	0.32	0.26	0.28	0.29	0.31	0.33
Peroxide value, (meq O_2/kg)	8.0	7.8	8.1	8.5	9.3	7.0	7.3	7.7	8.2	9.2
Iodine value, (g I_2/100 g)	64.74	62.25	60.48	59.35	58.21	59.96	58.21	57.69	55.74	52.12
Refractive index, (20°C)	1.4697	1.4700	1.4694	1.4688	1.4679	1.4695	1.4687	1.4681	1.4674	1.4667

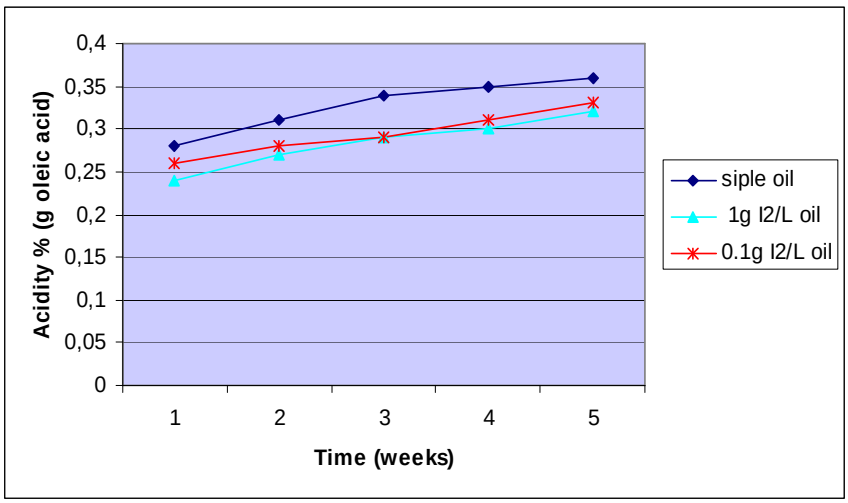

Figure 1. Acidity variation for oil samples

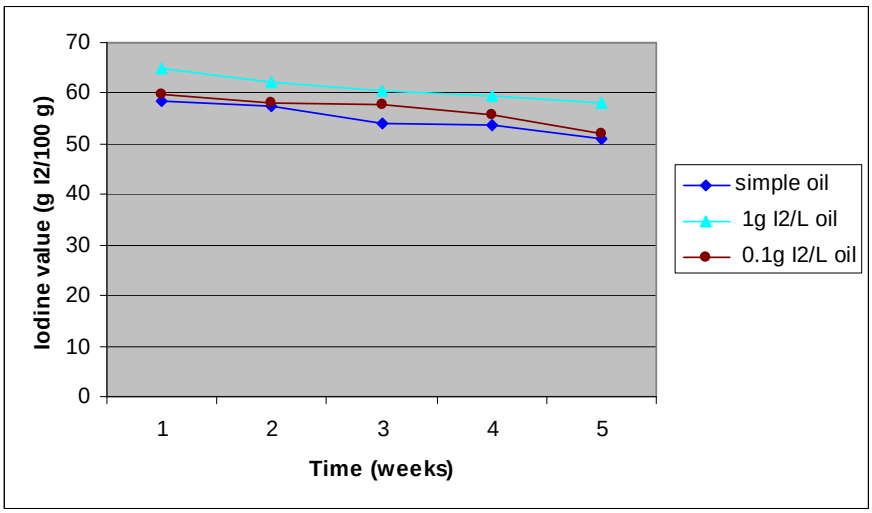

Figure 2. Iodine index variation for oil samples

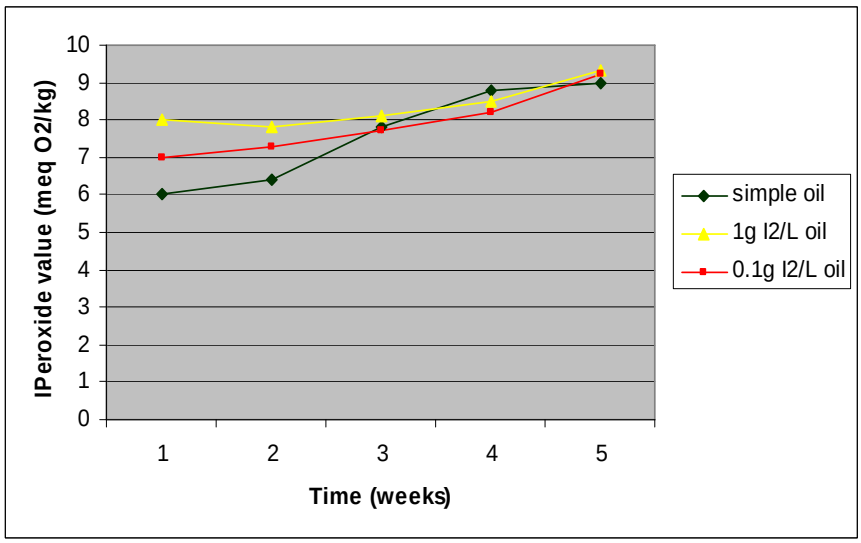

Figure 3. Peroxide index variation for oil samples

Lipids represent a easily perishable fraction of food, so duration and conditions of storage, depend by their nature and concentration. The stability of fatty material in storage raise a number of problems in front of producers and network marketing of these products. For this reason it is absolutely necessary the research of physicochemical properties evolution of iodized oil in terms of oil storage. It should be noted, that the main quality indices of iodized oil varied inessential during product storage (5 weeks).

Iodine value and refractive index varied inessential for each concentration of iodine in examined oil. In the case of maximum concentration of iodine (1 g/L), where was noted free iodine presence, refractive index remained indefinitely during product storage, this behavior was confirmed by the intense color of the product. In the case of maximum concentration of administered iodine was noted an inessential decrease of peroxide value, to the product characteristic immediately after iodization.

Acidity shows an upward trend for the three types of oil (Fig.1), but do not exceed the maximum allowable limit, and iodine value shows an descending evolution during storage (Fig.2). Acidity and iodine varied inessential for each concentration of iodine in examined oil. The peroxide value varied slightly for samples with an iodine content of 0.1 and 1 g/L and remains in allowable limits (Fig.3).

4. Conclusions

Based on experimental data it should be noted that the iodization of sunflower oil can be considered an acceptable method, which allows the incorporation of a considerable amounts of iodine (0,1; 1 g/L) without sensitive modifying the physicochemical properties of the product. Physicochemical indices of iodized oil are within acceptable norms for this product. Research of quality indices variation of iodized oil during product storage (5 weeks), demonstrated that only in the case of maximum concentration of iodine (1 g/L) it was manifested a slight overcome of the maximum permitted limit caused by the free iodine presence.

The conducted research showed that lipids represent an important vehicle for food fortification with iodine.

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ASSESSMENT OF HEAVY METAL CONTAMINATION IN MILK COMING FROM BAIA MARE AREA

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Abstract

The presence of lead in animal products is a consequence of the environmental pollution in all its segments, accidental introduction of this element in animal products, the addition of spices and adjuvants contaminated with lead or lead concentration due to storage in various containers containing lead and under the influence of certain factors,as a result being transferred to the food product. The average of the milk samples from the cows in the polluted area exceeded the maximum limit allowed by 2.42 times, the statistic difference between those two lots of milk samples being very significant. In milk samples collected from the polluted area the results obtained exceeded the maximum limit allowed by 0.1 mg/kg as established by the Order of the Ministry of Health 975/1998 for 24 samples. Although the concentration of lead and cadmium far exceeds the maximum allowes limits set by the Ministry of Health Order 975/1998, the toxic deficiencies events are not of clear clinical reason because of the many interrealations which can be established with other metals.

Keywords: *milk, heavy metals, lead, cadmium, manganese, iron, zinc, cooper*

1. Introduction

Lead, mercury, cadmium are, together with arsenic, the most common and spread harmful metals in the environment [1, 2]. They are also among the metallic contaminants with the most encountered problems in the industry of food processing. Each of them is responsible for high scale incidents of contamination and, despite the steps that were taken by the governmental authorities, they continue to be possible threats to the safety of consumers, in certain circumstances. They are included especially in the official regulations regarding the food safety in many countries and among them are responsible for many official documentation, more than other food contaminants. For many consumers, these three metals are elements that come for the first time in mind when contamination with heavy metals is mentioned [3, 4].

According to Friberg et al. [5], there are six major categories of sources of environment contamination with heavy metals: natural sources, products used in agriculture, polluters resulted from mining activities, industrial emissions, thermal power station emissions and vehicle emission [6, 7, 8]. Because of the connection between soil and the metals in our bodies, through the food products, we have to give a certain attention to the nature of the soil and to the way in which this contributes to the metallic content of the food products.

The soil is not only the main source of all the metals that get accumulated in a

natural way in the plants and animals. In a world where the spreading in the environment of the elements could harm as a result of the human activity is a growing problem, it is important that those that are preoccupied with the food safety to consider the metals in the soil as an integrated part of their processes of planning and management [9]. The absorption of heavy metals from the soil in plants has a major role in the contamination of plants with heavy metals [10].

A number of 25 samples of cow's milk from the area of industrial pollution in Baia Mare have been analysed and distributed as follows: Baia Sprie - Satu Nou de Sus - 7 samples; Groşi - 6 samples; Baia Mare - Ferneziu - 8 samples; Tăuții Măgherăuș - 2 samples; Tăuții Măgherăuș - Bozânta Mare - 2 samples. As an sample area, we have chosen Sighetu Marmatiei, an area located 60 km from major pollution sources and which is divided by the same mountain chain Gutai. From this area there have been taken a number of 25 samples of milk. These samples of milk were mineralized. The mineralization was achieved through wet cleaning.

2. Materials and methods

The mineralisation takes place in order to eliminate the organic substances that could interfere through the absorption of wavelength specific to metals. For the mineralization of the samples in order to determine the metals we have used the wet mineralization. For this determination, it was used a spectrophotometer of atomic absorption type SpectrAA 220 VARIAN.

All the determinations were done in three replicates, and the average values are reported. Statistical analysis of analytical data was done, employing the T test, by using software Origin 6.0.

3. Results and discussions

The results which have been obtained are presented in tables 1-2.

The lead's concentration (mg/ kg)in the milk samples from the polluted area was on average of 0242 ± 0,1% mg / kg (between 0097 - 0617 mg / kg of MAL exceeding 0.97 times respectively 6.17 times) compared to milk samples from the unpolluted area which was 0052 ± 0,1% mg / kg. The average of the milk samples from the cows in the polluted area exceeded the maximum limit allowed by 2.42 times, the statistic difference between those two lots of milk samples being very significant.

In milk samples collected from the polluted area the results obtained exceeded the maximum limit allowed by 0.1 mg / kg as established by the Order of the Ministry of Health 975/1998 for 24 samples.

The lead's elimination through milk is made, especialy, by a protein fraction in the proportion of 90 - 96%. The cows' grazing in the polluted areas near the industrial pollutants units can cause the contamination of milk with lead and cadmium The lead transfer in milk is not made immediately, it is realised in time [11].

The cadmium concentration in the milk sample from the polluted area was never situated under the MAL of 0.01 mg / kg, set by the Order of the Ministry of Health [12]. All samples exceeding this worth, the average being of 0022 ± 0008 mg / kg (between 0012 - 0042 mg / kg exceeding the MAL 1.2 times or 4.2 times respectively), compared to a witness sample who was 0002 ± 0002 mg / kg (between 0-0.0069), the statistic difference between those two lots of milk samples being very significant.

Manganese concentration in the samples of milk collected from the polluted area was 0185 ± 0016 mg / kg (between 0148 - 0216 mg / kg) compared to samples of milk collected from the

witness area in the area that was 0058 ± 0090 mg / kg (with values in the 0011 - 0048 mg / kg). Although for manganese, The Ministry of Health does not stipulate MAL compared to the values considered normal 0.02 mg/kg [13], average level of the evidence that the milk in the unpolutted area is exceeded on average by 2.9 times the milk samples from the polluted area the environment of the normal concentration exceeds by 9.25 times the statistical difference between the two lots of samples of milk, thus being highly significant.

Concentration of iron in the lot of samples taken from the polluted area was 1321 ± 0149 mg / kg (between 1112 - 1651 mg / kg) versus a lot of samples taken from the witnesses who were 0694 ± 0145 mg/kg (worth between 0478-0971). The Ministry of Health Order does not provide MAL for this item.

Cow's milk is a food poor in iron, he found the regular cow's milk in quantities of 0450 - 0650 mg/kg [14]. Compared with these,the values considered as a normal amount of iron in cow's milk collected from the polluted area was higher by 1.71 - 2.54 times in the unpolutted area and this was exceeded by 1.49 times, the statistical difference between the two groups was very significant .

Zinc concentration has not presented any evidence from the milk lot of samples collected from the area regarded as polluted and the unpolutted values over MAL provided by the The Ministry of Health Order [11]. Non-milk samples from polluted area was 1970 ± 0449 mg / kg (between 1287 - 2981 mg/kg) versus batch of samples of milk collected from the clean area which was 1107 ± 0276 mg / kg (between 0689 - 1628 mg / kg), the statistical difference between the two lots of samples is very significant (***).

Zinc concentration in the cow milk reflects the amount of zinc in serum and daily intake. The maximum quantity of zinc in milk is the protein fraction of milk [14] and it differs depending on the stage of lactation and breed of cows being higher in the first days postpartum.

Copper concentration in the lot of samples from polluted area was 0194 ± 0035 mg / kg (between 0141 - 0257 mg/kg) versus lot of samples of milk collected from the unpolluted area which was 0144 ± 0031 mg / kg (the limit values was between 0111 - 0268 mg / kg). Neither in this case the copper, was not value over the maximum allowed limit (MAL) provided by the Ministry of Health Order of 0.5 mg / kg, both samples taken from the area and considered polluted area between the statistical unpolluted. Statistical difference between two groups is very significant.

4. Conclusions

From the analysis of data submitted the following conclusions can be made:

- feeding cows with excessive vegetation polluted with heavy metals cause the pollution of the milk with these metals
- major pollutants milk in the area of industrial pollution in Baia Mare are represented by lead, cadmium and manganese
- the average lead concentration in the milk reaches values of 0.242 mg / kg exceeding MLA of 2.42 times
- the average concentration of cadmium in the milk reaches values of 0022 mg / kg exceeding MLA by 2.2 times
- the average concentration of manganese reaches values of 0185 mg / kg, exceeding the amounts allowed by technical literature by 9.25 times.
- although the concentration of lead and cadmium far exceeds the maximum allowes limits set by the Ministry of Health Order 975/1998, the toxic deficiencies events are not of clear clinical reason because of the many interrealations which can be established with other metals.

Table 1

The concentration of heavy metals in cow's milk raw material in the area polluted

Sample no.	The concentration of heavy metals in polluted area mg/kg					
	Pb	**Cd**	**Mn**	**Fe**	**Zn**	**Cu**
1	0,157	0,031	0,216	1,358	1,941	0,243
2	0,206	0,019	0,206	1,254	1,847	0,211
3	0,149	0,017	0,183	1,247	1,347	0,197
4	0,274	0,022	0,201	1,357	1,975	0,214
5	0,159	0,017	0,148	1,651	1,875	0,235
6	0,147	0,021	0,194	1,541	1,752	0,197
7	0,241	0,019	0,171	1,314	1,687	0,168
8	0,114	0,034	0,182	1,112	1,287	0,215
9	0,167	0,034	0,172	1,487	1,978	0,247
10	0,104	0,042	0,189	1,297	1,642	0,214
11	0,135	0,036	0,211	1,262	1,952	0,211
12	0,417	0,032	0,167	1,249	2,358	0,211
13	0,216	0,031	0,187	1,357	2,674	0,192
14	0,147	0,015	0,177	1,364	2,981	0,159
15	0,157	0,014	0,184	1,249	2,198	0,148
16	0,354	0,017	0,198	1,547	2,458	0,257
17	0,145	0,021	0,208	1,213	2,354	0,149
18	0,487	0,014	0,178	1,351	2,221	0,247
19	0,617	0,021	0,184	1,162	2,354	0,157
20	0,248	0,019	0,168	1,647	2,354	0,168
21	0,543	0,016	0,197	1,246	2,101	0,157

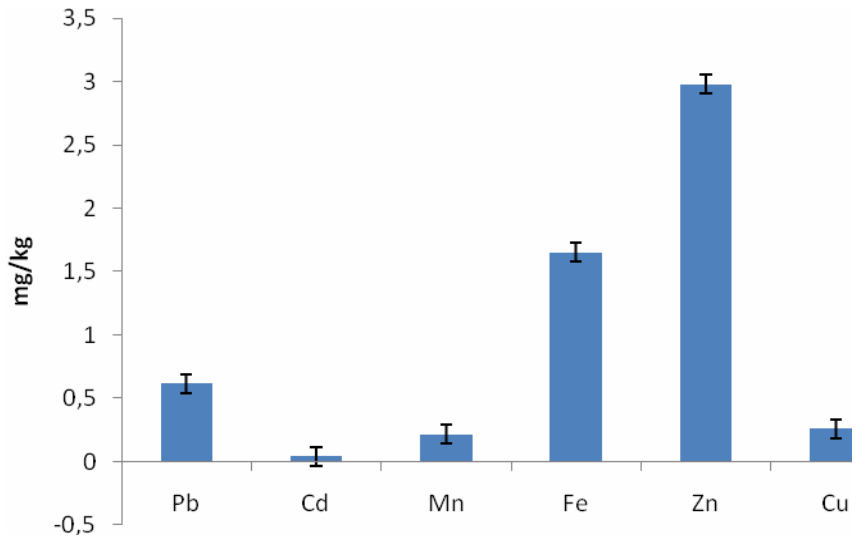

Figure 1. The concentration of heavy metals in cow's milk raw material in the area polluted

Table 2

The concentration of heavy metals in cow's milk raw material in the unpolluted area

Sample no.	**The concentration of heavy metals in polluted area mg/kg**					
	Pb	**Cd**	**Mn**	**Fe**	**Zn**	**Cu**
1	0,081	0,0081	0,055	0,652	1,250	0,243
2	0,078	0,0033	0,0458	0,918	0,894	0,211
3	0,067	0,0041	0,0587	0,875	1,035	0,197
4	0,058	0	0,0257	0,478	1,614	0,214
5	0,068	0	0,0354	0,592	0,957	0,235
6	0,051	0	0,0478	0,749	0,875	0,197
7	0,068	0,0048	0,0579	0,687	0,689	0,168
8	0,054	0,0052	0,0457	0,955	0,759	0,215
9	0,047	0	0,0547	0,825	1,358	0,247
10	0,035	0	0,0748	0,694	1,241	0,214
11	0,049	0	0,0498	0,487	0,971	0,211
12	0,061	0,0035	0,0428	0,679	0,873	0,211
13	0,031	0,0031	0,0415	0,681	1,498	0,192
14	0,024	0,0036	0,0294	0,543	1,574	0,159
15	0,061	0	0,0485	0,679	1,035	0,148
16	0,064	0	0,0628	0,971	1,211	0,257
17	0,035	0	0,0483	0,612	0,976	0,149
18	0,042	0	0,0241	0,654	0,958	0,247
19	0,043	0,0069	0,0151	0,687	0,962	0,157
20	0,051	0,0046	0,0254	0,729	0,753	0,168
21	0,061	0,0037	0,0347	0,519	1,113	0,157

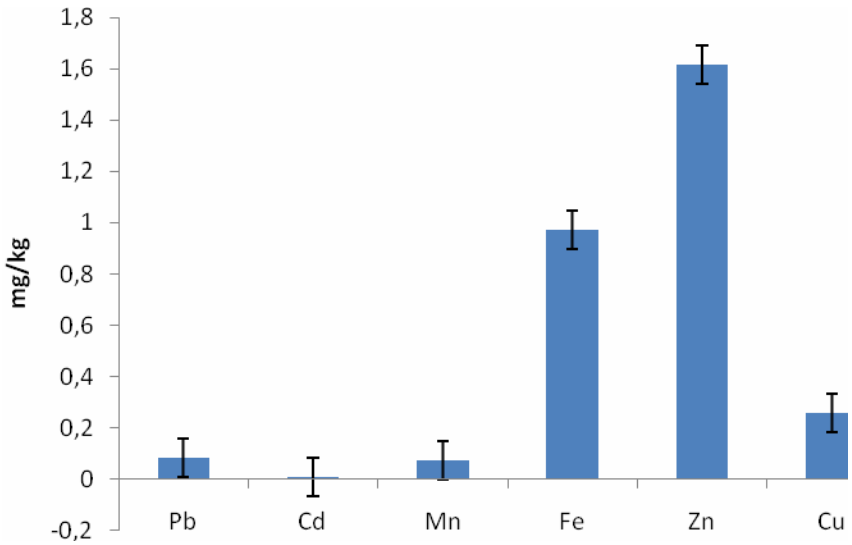

Figure 2. The concentration of heavy metals in cow's milk raw material in the unpolluted area

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