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SAFE FOODS FOR EUROPEAN CONSUMERS

It is the right of European consumers to use safe and nourishing foods. The purpose of the European Union (EU) is to guarantee that the foods, which we consume, are of the same high standard for all the citizens, no difference whether they are produced in the definite country or come from a country in or outside the EU.

Since 2000 the norms for safety and quality of foods have been additionally raised. The new approach is more comprehensive and is known as "From fork-to-farm" – the fodders and foods are very carefully traced out from the farms where they are produced to the table and fork of the consumers. European authorities thoroughly estimate the risk and look for the possibly best scientific recommendation before they ban or allow the use of a particular product, ingredient, additive or genetically modified food. This refers to all foods and fodders irrespective of the fact whether they originate from a member-country or not. Safety however does not mean uniformity. EU stimulates diversity on the basis of quality, protects traditional foods and products in particular regions and guarantees the consumers clear distinguishing from imitations. EU encourages agricultural producers to concentrate on the quality – referring not only to foods but to the environment as well. EU respects the consumer's right for clear choice, encourages public discussion, requires detailed labels on the products so that consumers could be sure about the foods they consume.

The strategy of the EU on safety of foods contains four important elements:

- *Rules on safety of foods and fodders;*
- *Independent and publicly available scientific research;*
- *Actions imposing the rules and control over the processes;*
- *Acknowledgement of the consumer's right for choice on the basis of full information about the origin and contents of foods.*

When a country like Bulgaria is preparing to join the EU, it should often make supreme and costly efforts in order to meet the EU requirements and to modernize its technologies for food processing. Under exceptional circumstances after integration of the country EU can allow a transitional period to get the modernization in question over. Every year the union allots tens of millions Euros for research developments of new ways of prevention or earlier detection of epidemics of animal diseases and for promotion of work on selection of new and better grain crops. The 2002 – 2006 budget, earmarked only for research work on improvement and safety of foods, amounts to 685 million Euros.

National Topical Competition "Biological Resources, Biotechnologies and Foods– Quality, Safety" should be considered as a projection of the European Technology Platform "Food for Life", which reflects specific for our country problems and interests, namely:

- *Sustainable development and management of biological resources;*
- *Relation between food and health, healthier foodstuffs;*
- *Illnesses and allergies caused by food and contact materials;*
- *Methods for food safety risk control and analysis;*
- *More secure and safer for the environment production methods and technologies;*
- *Influence of animal feeding on human health.*

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- Providing information about national, European and trans-European research programs.
- Providing information to support the process of harmonization of the Bulgarian education and research legislation with European Union ones.
- Performing the role of institutional contact point of the Sixth Framework Program in Bulgaria.

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- *"Papers"* , Number of records: 128

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- "*Partnership for Innovation and Development"* . Information about the national research units.
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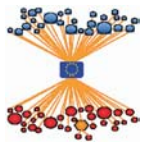

NATIONAL SCIENTIFIC PROGRAMMES WITH EUROPEAN DIMENSIONS

INVESTIGATION ON THE TECHNOLOGICAL PARAMETERS OF NEW EUROPEAN SPRING BREWING BARLEY VARIETIES, CROP 2005

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INTRODUCTION

Investigation of new European spring and winter brewing barley varieties started in 1990 in the former Institute of Brewing and Hop Industry, now Brewing department of the Institute of Cryobiology and Food Technology, Sofia. The investigations were extended after acceptance of the Union of Brewers of Bulgaria in European Brewery Convention in 1995. Every year in the South region, which includes Portugal, Spain, Italy and Bulgaria, about 30 new European brewing barley varieties (6, 7, 8) were tested. The competitive power of the Bulgarian farmers in the field of brewing barley depends strongly on the availability of the variety with good technological potential. That will be very important after accession of the country in European Union on the 1st of January 2007.

This work is continuation of our previous investigations (2, 3, 9). The varieties with good technological parameters will be suggested to be included in the Barley variety list of Bulgaria.

MATERIAL AND METHODS

At the end of March 2005 in the trial fields of the Institute of Agriculture (IA) – Karnobat and Institute of Agriculture and Seed Science "Obraztsov Chiflik" (IASS) – Rousse 14 new European spring barley varieties, distributed by the Barley and Malt Committee of the European Brewery Convention (EBC) were sown. The field trials were conducted according to the method of Latin rectangle in four replications (Enchev, 1979). The varieties Scarlett, Barke, Margret, Auriga, Tokada, Marnie and Wicket NFC were from Germany,the varieties Power,Sebastian and Christina from Denmark, the varieties Henley and Cristalia NFC – from United Kingdom, Josefin was from France and Marnie – from Switzerland. The varieties Scarlett, Barke, Margret, Auriga, Power and Sebastian were crop in 2004 to. The varieties Scarlett and Barke were used like standard ones.

The barley samples with the protein content up to 12.5 % of dry matter were malted on the pilot facility Seeger, according to the recommended procedure of the EBC (EBC Barley and Malt Committee, 2003). The technological parameters of the brewing barley and respective malts were determined according to the Analytica EBC (1998).

Investigation of technological parameters of twelve new and two standard European spring barley varieties, crop 2005 from two trial fields, was the aim of this work.

RESULTS AND DISCUSSION

Analyses and assessment of brewing barley

The moisture content of the brewing barley varieties from the IA-Karnobat fields were be-

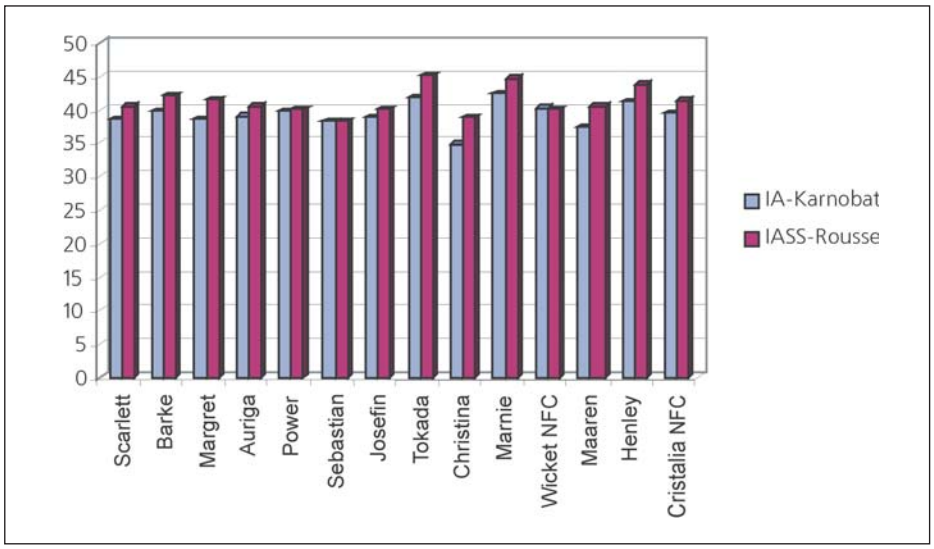

Fig.1. 1000-Kernel weight (g d.m.) of new European spring brewing barley varieties, crop 2005

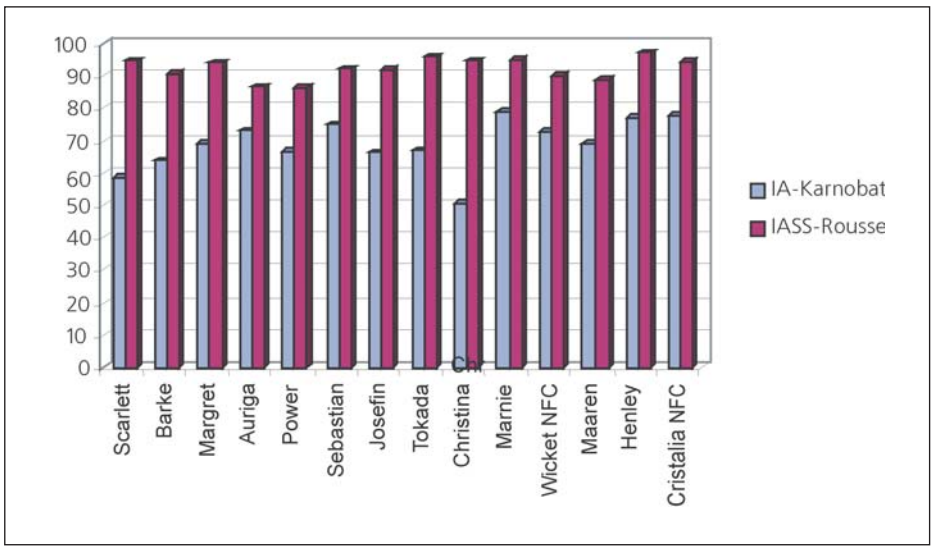

Fig.2. Grading (> 2.5 mm) of new European spring brewing barley varieties, crop 2005

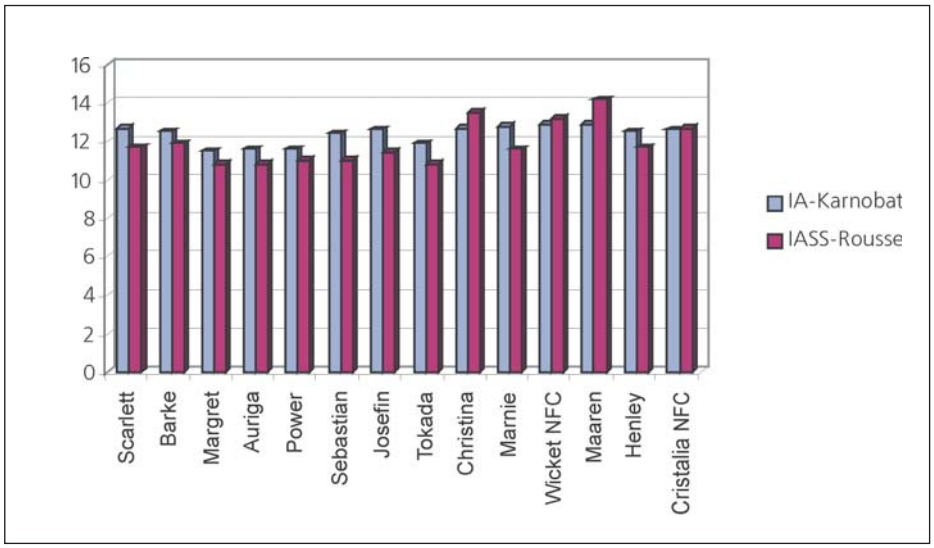

Fig.3. Protein content (% d.m.) of new European spring brewing barley varieties, crop 2005

tween 11.4% and 11.7%, and for the varieties from the IASS-Rousse between 10.3% and 11.9%. All these results are in accordance with the requirements for the brewing barley in Bulgaria.

The 1000-kernel weight of new European spring brewing barleys is shown on Fig 1. In general this parameter had lower values for the brewing barley samples from the IA-Karnobat. Only four varieties – Marnie, Tokada, Henley and Wicket – have 1000-kernel weight over 40 g. Twelve brewing barley samples from the IASS-Rousse had 1000-kernel weight between 40.0 and 45.1 g. Very high values of this parameter (45.1-43.7 g) were observed for the varieties Tokada, Marnie and Henley. The varieties Sebastian and Christina had values below 40 g. The varieties Tokada, Marnie and Henley showed the highest values of this parameter in both trial fields.

All spring brewing barley samples from IA-Karnobat trial fields had grading (part of the kernels greater than 2,5 mm) lower than 85%. The results on Fig. 2 show that only the barley variety Christina had the grading value below the results of the stand-

ard varieties Scarlett and Barke. All samples from IASS-Rousse had grading (>2.5 mm) greater than 85%. Very high values (between 97.1% and 95.2%) were observed for the varieties Henley, Tokada and Marnie. Values over 94% were shown by the varieties Scarlett, Christina, Christalia NFC, Margret and over 92% - by the varieties Sebastian and Josefin.

The principal parameter for determination of adequacy of varieties for the malting on the pilot facility is the protein content up to 12.5% d.m. Fig. 3 shows protein content of all tested varieties in both trial fields. The new spring brewing barley varieties from IA-Karnobat had the protein content between 11.5% and 12.9% d.m. Seven of all fourteen varieties had protein content below the limit of 12.5% d.m. The lowest values between 11.5% and 11.9% d.m. were observed for the varieties Margret, Power, Auriga and Tokada. The protein content results for the varieties from IASS-Rousse varied more – between 10.8% and 14.2% d.m. Ten of all fourteen varieties had values below the limit. The varieties Margret, Auriga and Tokada showed the lowest protein content 10.8% d.m. Low protein content of 11.0% d.m. was observed for the varieties Power and Sebastian. The varieties Margret,Auriga, Power and Tokada showed the lowest values of this parameter in both trial fields.

Analyses and assessment of malts

Results of the analyses of the malts are presented in Tables 1-2 and Figures 4-8.

The saccharification rates of the majority of the malt samples were between 5 and 10 min-

Table 1. Technological parameters of new European spring brewing barley malts, crop 2005 - IA-Karnobat

Variety	Moisture, %	Saccharification, min.	Extract fine grind, % d.m.	Colour, EBC u.
Barke	5.7	5-10	82.3	2.5
Margret	5.2	5-10	82.0	2.5
Auriga	4.8	5-10	82.1	2.5
Power	5.5	5-10	82.0	2.5
Sebastian	5.2	10-15	82.8	4.0
Tokada	5.2	10-15	81.5	3.0
Henley	5.6	10	83.4	2.5

Table 2. Technological parameters of new European spring brewing barley malts, crop 2005 - IASS-Rousse

Variety	Moisture, %	Saccharification, min.	Extract fine grind, % d.m.	Colour, EBC u.
Scarlett	4.6	5-10	82.2	3.0
Barke	4.6	5-10	82.0	3.0
Margret	4.4	5-10	82.5	3.5
Auriga	4.2	5-10	82.0	5.0
Power	4.8	5-10	81.8	4.0
Sebastian	4.4	10	83.0	4.5
Josefin	4.5	5-10	82.4	4.0
Tokada	5.1	10-15	81.5	3.0
Marnie	4.7	5-10	82.3	4.0
Henley	5.3	5-10	81.6	3.0

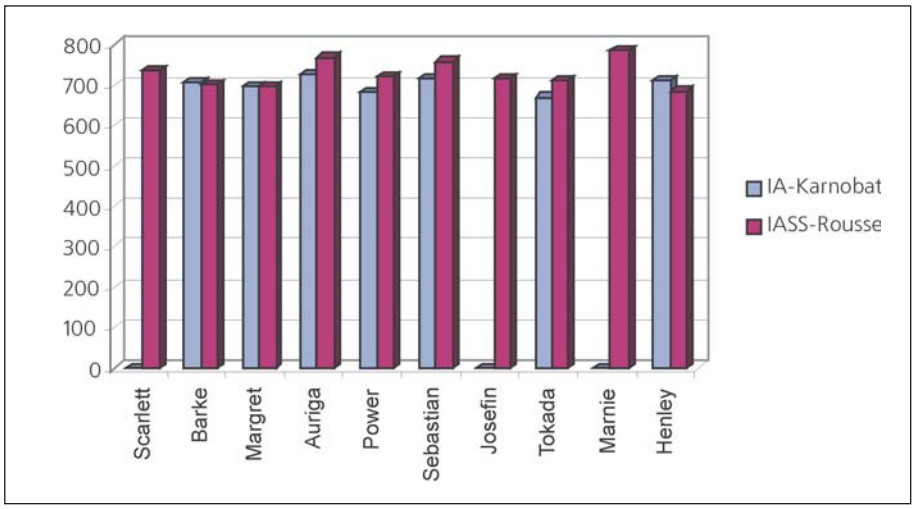

Fig.4. Soluble nitrogen of malts (mg/100 g) of new European spring brewing barley varieties, crop 2005

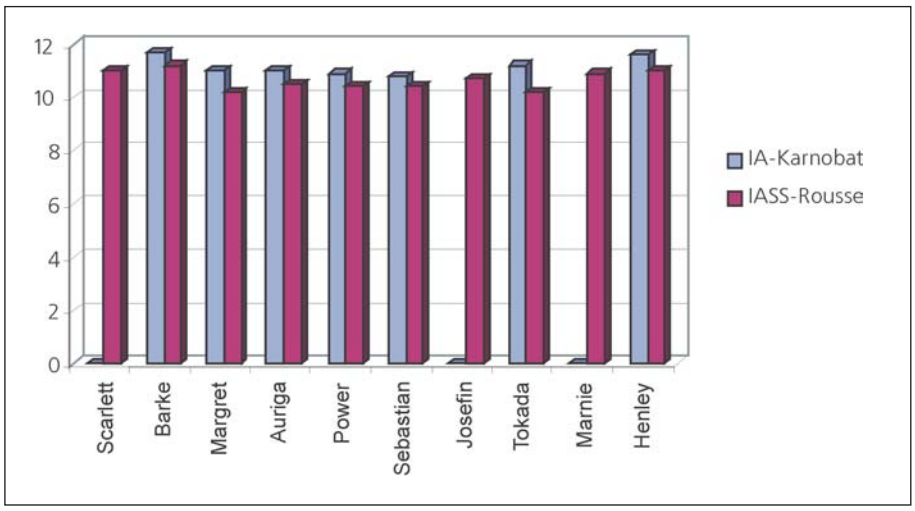

Fig.5. Protein content of malts (% d.m.) of new European spring brewing barley varieties, crop 2005

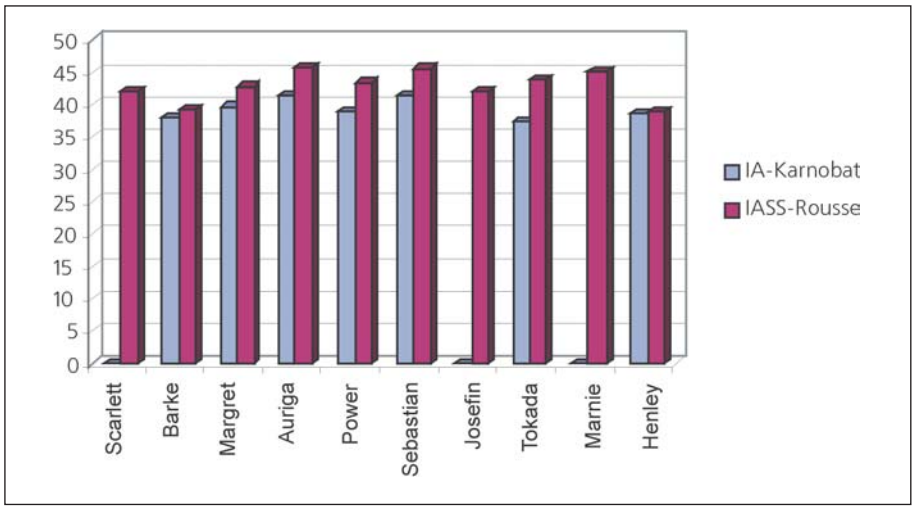

Fig.6. Kolbach index of malts (%) of new European spring brewing barley varieties, crop 2005

utes. In general the values of this parameter indicated very good amylolytic enzyme activity of malts from the both trial fields.

The extract content of fine grind is one of the most important parameter in brewing industry, because it determines the production extract yield (1, 10, 11, 12). The malt Henley from IA-Karnobat had the highest extract content of 83.4% d.m. from all tested samples. Malts Sebastian, Barke and Auriga showed extract content higher than 82.0% d.m. The rest three varieties from this field had high extract content between 81.5% and 82.0% d.m. The extract content results were too high for the malted barley varieties from IASS-Rousse. Malt Sebastian had the highest extract content of 83.0% d.m. The malts Margret, Josefin and Marnie provide extract content in the range 82.5-82.3% d.m. The malted barley variety Sebastian shows high extract content in both fields during two years of trials.

The colour of the IA-Karnobat malts was between 2.5 and 4.0 EBC units, which is appropriate for the Pilsner type malts. Rainfall during the harvest was the reason for some higher malts colour of the same barley samples from the IASS-Rousse.

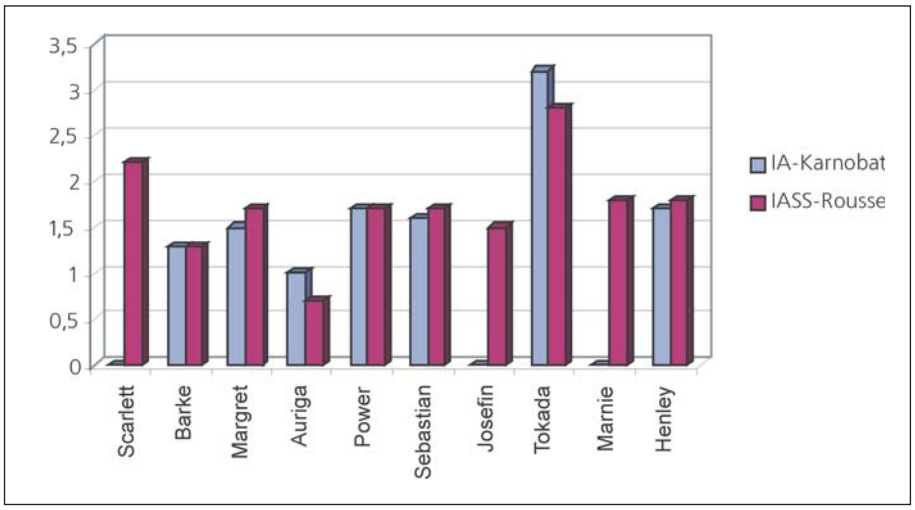

Fig.7. Extract difference of malts (%) of new European spring brewing barley varieties, crop 2005

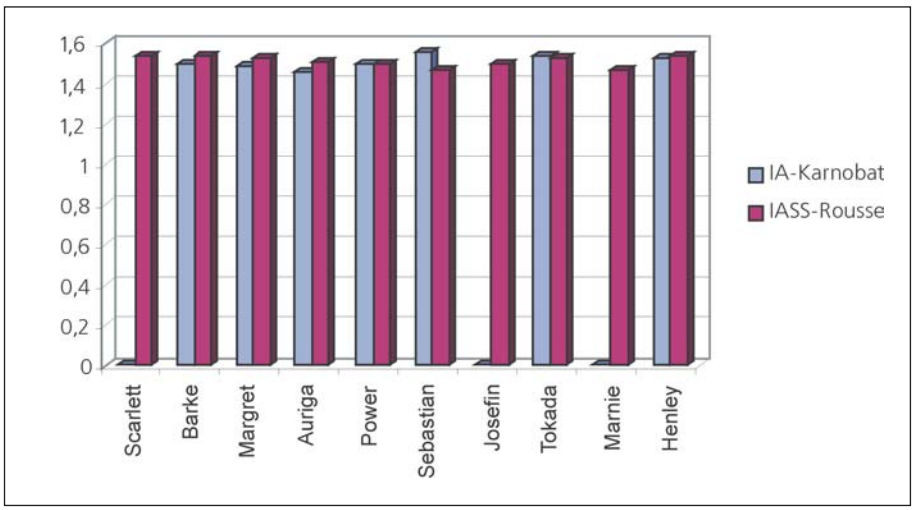

Fig.8. Viscosity of malts (cP) of new European spring brewing barley varieties, crop 2005

Information about the proteolytic modification of the malts gives the parameters soluble nitrogen, protein content and Kolbach index (the ratio soluble nitrogen : total nitrogen). The soluble nitrogen content exerts big influence on the wort fermentation, foam, colloidal and flavour stabilities. Figure 4 gives information about this parameter. The soluble nitrogen values of the IA-Karnobat malts were between 670 and 727 mg/l. Relatively higher values between 699 and 787 mg/l were determined for the soluble nitrogen content of IASS-Rousse malts.

Figure 5 indicates the protein content of malts. Protein content in the range 10.8 – 11.2% d.m. was shown by malts Sebastian, Power, Auriga, Margret and Tokada from the IA-Karnobat field. The protein content of the IASS-Rousse malts was lower between 10.2 and 11.2% d.m. Very low protein content between 10.2 and 10.4% d.m. was shown by malts Tokada, Margret, Sebastian and Power. Malted barley varieties Sebastian and Power from both trial fields provided low protein content.

Kolbach index of malts is presented on Figure 6. Five malts from IA-Karnobat field have Kolbach index between 37.4 and 39.7%. Kolbach indexes of Sebastian and Auriga were higher than 41%. All IASS-Rousse malts had higher Kolbach index in comparison with IA-Karnobat malts. Auriga, Sebastian and Marnie possessed Kolbach index higher than 45%. Very high Kolbach index was shown by malted barley samples Tokada, Power, Margret, Josefin and standard Scarlett. Only two malts had lower than 40% Kolbach index. In general malts could be characterized by good to very good proteolytic modification.

The extract difference and viscosity values (Fig. 7 and Fig. 8) give information about malts cytolytic modification. The malts distinguished significantly according to the extract differences. The malts from IA-Karnobat alter from low difference of 1.0% for malt Auriga to 3.2% for Tokada, and malts from IASS-Rousse from very low difference 0.7 for malt Auriga to 2.8 for malt Tokada.

Malted barley varieties possessed viscosity between 1.46 and 1.54 cP. The obtained results for the parameters extract difference and viscosity indicated very good cytolytic modification of malts.

CONCLUSIONS

The complex assessment of technological parameters of the investigated 14 new spring brewing barley varieties and malts obtained from them show that Auriga, Margret, Sebastian and Power have better technological parameters than the standard varieties Scarlett and Barke.

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OCHRATOXIN A IN BULGARIAN BEER AND WINE

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INTRODUCTION

Ochratoxin A (OTA) is a naturally occurring secondary fungal metabolite produced by *Penicillium verrucosum* in temperate climates and *Aspergillus ochraceus, A. carbonarius and A. niger* in hot climates (Benford 2001). These fungi differ in their species characteristics, geographical regions of occurrence and respectively in types of foods in which they develop. Occurrence of OTA in food has been broadly documented since 1965, when its first isolation is done. There is considerable information on OTA occurrence in cereals (Vrabcheva et al., 2000; Czerwiecki et al., 2002; Jorgensen et al., 2002; Lombaert et al., 2003), coffee beans (Jorgensen, 1998; Bucheli et al., 2002; Moraes et al., 2003; Romani et al., 2003; Yordanova et al., 2003), cocoa beans (Bonvehi, 2004; Amezqueta et al., 2005), spices (Thirumala-Devi et al., 2000; Gatti et al., 2003), beer (Jorgensen, 1998; Tangni et al., 2002; Visconti et al., 2000; Araguas et al., 2005; Bacaloni et al., 2005; Medina et al., 2005; Park et al., 2005), wine (Ottender et al., 2000; Belli et al., 2002; Lopez de Cerain et al., 2002; Stefanaki et al., 2003; Shephard et al., 2003; Rosa et al., 2004; Ng et al., 2004) and grape juice (Belli et al., 2002; Rosa et al., 2004; Ng et al., 2004).

The widespread occurrence of OTA has been recognized as a potential human health hazard since it is associated with Balkan Endemic Nephropathy (BEN), a kidney disease in humans and classified as "possibly carcinogenic to humans (group 2B) by the International Agency for Research on Cancer (IARC). It exhibits also immunosuppressive, teratogenic, nephrotoxic and genotoxic properties in several animal species (IARC 1993).

The main contributors to OTA exposure in humans are cereals and cereal – derived products because of its resistance to technological processes (Alldrick, 1996). It has been estimated that in Europe about 50% of the dietary intake of OTA is from cereals and cereal products (SCOOP, 2002). Several surveys show that OTA is occasionally present in barley and can partially

persist during the malting process (Gareis, 1999), brewing process (Scott and Kanhere, 1995; Scott 1996a) and finally it can be found in beer. The data about OTA in beer before 1990 are very scarce, because of lack of analytical methods with appropriate sensitivity. In recent years, the use of more accurate and sensitive analytical methods, with LOD between 0.05 and 0.1 ng/ml, led to its detection in beer in different countries: Germany (El-Dessouki, 1992; Meyer and Neugebauer, 2000), Canada (Soleas et al., 2001), Denmark (Jorgenson, 1998), Switzerland (Zimmerli and Dick, 1995), Belgium (Tangni et al., 2002), Spain (Legarda and Burdaspal, 1998; Medina et al., 2005; Araguas et al., 2005), Japan (Nakajima et al., 1999), and Italy (Visconti et al., 2000). These studies have shown that OTA can be found at high concentrations up to 1530 ng/L in a strong German beer (El-Dessouki, 1992). No limit has yet been fixed for beer; however, guidance levels are established in various European countries such as The Netherlands (0.5 µg/L), Finland (0.3 µg/L) and Italy (0.2 µg/L). There is some discussion regarding the limit that should be established for OTA in some cereal-derived foods. The appropriate level proposed for beer is 0.2 µg/L (Araguas et al., 2005).

Since the first study on the OTA in wines (Zimmerli and Dick, 1995) several studies have been carried out by different authors on a broad variety of wines (red, white, rose, etc.). The occurrence of OTA in wine is linked to the presence of moulds on grapes. Recently, black aspergilli, mainly *Aspergillus carbonarius* and members of the *Aspergillus niger* aggregate, have been described as a main possible source of OTA contamination in grapes from Argentina and Brazil (Rosa et al., 2002), France (Sage et al., 2002), Spain (Bau et al., 2005) and Italy (Battilani et al., 2003). The accumulation of OTA in wine depends on climate of the geographic region where the wine is made and grapes harvested and it has been shown that OTA is more frequently present in wines from the southern part of Europe (Zimmerli and Dick, 1995; Ottender and Majerus, 2000; Stefanaki et al., 2003).

The global results show that the median of the percentage of positive samples in red wines is around 90% (mean 71%), followed by rose wines (66%) (mean 66%) and white wines (34%) (mean 45%).

In Europe, according to the Codex Committee on Food Additives and Contaminants, wine (especially red wine) is the second major source of human exposure to OTA following cereals, giving a total dietary intake of 15% (CAC 1998). Commission Regulation (EC) № 123/2005, sets maximum level of 2.0µg/L for OTA in wine (red, white and rose).

Historic and archeological researches have shown that the territory presently occupied by the state of Bulgaria may well be the first geographical region where vines were planted and wine produced. Bulgaria's wine industry currently accounts for 30% of aggregate farm exports to the European Union. Bulgarian wines are sold in the UK, Ireland, Germany, the Netherlands, Scandinavia, the USA, Japan, Poland, and the former Soviet Republics. According to data by the national chamber of vine growing and wine making, the export of wine has increased by 20 million liters - from 90 million to 110 million liters year-on-year. EC classification based on geographical situation, soil and climate conditions of the country, rainfalls, etc. divides Bulgaria into two vine-growing areas: CII – north, east and sub-Balkan regions; CIIIa – south and southwestern regions (fig.1).

Bulgarian brewing industry is 125-years-old. The brands of beer produced in Bulgaria have increased and their quality is among the best in the world. There are 16 licensed breweries in Bulgaria. Production of beer in Bulgaria for 2005 year is 962760 hectoliters and for 2006 (January-April) is 1.119 million hectoliters. Annually the Bulgarians consume 61 liters of beer per capita. That is also the amount that is drunk in Portugal and Switzerland. In countries such as Italy and France the amount is twice lower– around 30 liters.

Regarding the possibility of OTA exposure by wine and beer and the increased production of these products in Bulgaria, the aim of the present study was to investigate OTA content in Bulgarian wine and beer.

MATERIALS AND METHODS

Materials

Samples

Beer samples: The study covered 32 commercial beers manufactured in Bulgaria, representing

10 brands (7 Bulgarian brands and 3 brands under international licences). Different lots of the brands were analyzed. Some of them were received from manufacturers and others were purchased randomly in the local retail market and analyzed during 2004 year. All samples were pale beers with alcohol content bellow 6%. The samples were stored in their original bottles at 8-10^{0}C prior to the analysis, according to technological requirements. Before analysis 500 ml. of beer samples were thoroughly degassed in ultrasonic bath for 40 min.

Wine samples: The study covered 22 samples (2 white) of bottled industrially produced Bulgarian wine differed in their year and region of production (Table 1). The samples were stored in the original bottles in fridge at 4-5 ^{0}C until analysis, according to technological requirements.

Standards, reagents and materials

Crystalline powder of Ochratoxin A was purchased from Sigma Chemical Co. A stock solution of about 20µg/ml was prepared by solving 1 mg of OTA in 50 ml. of toluene-acetic acid (99+1, V+V). The exact concentration was determined by recording the max. absorbance between a wavelength of 300 nm and 370 nm in a 1-cm. quartz cell with solvent mixture as reference (M=403g/mol; ε=544 m^2/mol) (Visconti, 2001).

Acetonitrile and methanol HPLC grade (Merck KGaA**,** Germany), glacial acetic acid 100%p.a, polyethylene glycol (PEG)-PEG 8000 (Vicam, Watertown, MA, USA).

Diluting solution-1%PEG+5%$NaHCO_3$, pH 8.3 and washing solution-2.5%NaCl +0.5%$NaHCO_3$, pH 8.1 were prepared. OchraTest immunoaffinity columns, and pump stand (Vicam, Watertown, MA, USA) were used. Glass microfiber filter (Whatman, GF/A). Nitrogen 99.5% purity.

Apparatus

The analyses were carried out by a chromatographic system consisted of a Perkin Elmer LC 200, equipped with a Rheodyne model 7125 injector valve, a 50µl loop and fluorescence detector Perkin Elmer 3000.

METHODS

Samples were analyzed for the presence of OTA by immunoaffinity column clean-up and subsequent HPLC separation with fluorescence detection based on the method of Visconti et al. (2001) with some modifications to achieve good peak shape and better sensitivity on the analytical column, which we use.

Briefly:

Sample preparation and immunoaffinity clean-up

Twenty ml. of wine or degassed beer samples were diluted with 20 ml. of diluting solution. After filtration of diluted samples through glass microfibre filter, 20ml. (equivalent to 10 ml. beer) were applied to an immunoaffinity column at a flow rate of 1-2 drops/s. The column was washed with 5ml washing solution and then with 5ml water at a flow rate of 1-2 drops/s. OTA was eluted from the column with 2ml methanol at a flow rate of 1 drop/s. This eluate was evaporated to dryness under nitrogen at ambient temperature and the residue was redissolved immediately in 250µl of HPLC mobile phase.

HPLC analysis

A reverse-phase C18 (LiChrospher 100 RP18, 250x4, 5µm, Merck) analytical column was used. Chromatographic separation was performed by isocratic elution with acetonitrile+water+glacial acetic acid (50+49+1) at a flow rate 1ml/min, at ambient temperature. From stock solution of OTA a standard solution of 1 µg/ml. was prepared by evaporating an aliquot portion under a stream of N. The dry residue was redissolved in 50 ml. HPLC mobile phase. For quantitative analysis a calibration curve was established by injection of a series of standard solutions with concentrations 0.02, 0.01, 0.008, 0.006, 0.004 and 0.002 µg/ml and the responses were checked for linearity.

Verification of the analytical method

Analytical method for OTA determination in beer and wine was verified in-house, based on the following criteria: linearity, limit of detection (LOD) and limit of quantification (LOQ), precision (within- and between-day variability), recovery and uncertainty.

In the assessment of linearity, a calibration curve was plotted in the range 0.002-0.02 µg/ml. five replicates of each calibration standard were analyzed. Criteria used to verify linearity were: correlation coefficient R^2>0.99 and relative standard deviation between responses RSD<5%. The detection and quantification limits (LOD and LOQ)

of the chromatographic procedure were calculated by the signal-to-noise ratio of 3:1 and 6:1 respectively, according to Araguss et al., (2005). Repeatability was assessed by analyzing a sample with OTA content of 0.11 μg/ml. Recovery experiments were performed with spiked samples at concentrations of 0.2 μg/L and 0.4 μg/L in triplicate. The uncertainty of the method was estimated taking into account the full analytical procedure including: the uncertainty associated with standard solutions preparation, the uncertainty associated with the volumetric material and the uncertainty associated with HPLC determination.

RESULTS AND DISCUSSION

Method validation

The results obtained for the linearity study are shown in fig.2. Within the calibration range of 0.002 μg/ml-0.02 μg/ml of OTA the relationship between the HPLC responses (peak heights) and OTA concentration showed a good linearity with coefficient of correlation R^2>0.999 and relative standard deviation (RSD) of the responses was 4.0%. The result fulfilled the criteria for linearity. The estimated limit of detection (LOD) and limit of quantification (LOQ) were 0.04μg/L and 0.10 μg/L. The achieved LOD of the analytical method for determination of OTA in beer samples compared well with the values obtained by Araguas, et al. (2005), and Visconti, et al. (2000). However these values are higher than those obtained by Tangni et al. (2002) (3 ng/L) and Nakajima et al. (1999) (1ng/L).

Recovery experiments of the full analytical procedure showed that the average recovery in the tested concentrations was 92%, which is in the reported range (87% - 95%) by Visconti et al. (2001) from an interlaboratory study of the method performance.

The experiments for within day variability showed RSDr=±5.4% (n=5) and for between day variability RSDr=±9.1% (n=10). The reported RSDr by the interlaboratory study participants vary from 4.7% to 10.6%. In addition, these results fulfilled the actual requirements established by legislation for OTA determination methods (RSD=20%, recovery 70-110%) (Commission Directive 2002/26/EC of 13 March 2002). The estimated uncertainty at 0.11 μg/L of OTA was ±0.02 μg/L, which is less than the maximum standard uncertainty of 0.03 μg/L, calculated following the recommendation of the Commission Regulation (EC) №401/2006.

Survey of OTA in beer samples

The results of the analysis of 32 beer samples are summarized in Table 2. The results showed that 20 of 32 analyzed samples were positive (62.5%). The mean level was calculated by assuming that non-detected (ND) samples contained half of LOD, while samples with trace levels of OTA contained half of LOD+LOQ. The range for positive samples was 0.04 μg/L (LOD)-0.2 μg/L. Two samples of them were with minimum OTA level at LOD, trace levels of OTA (between LOD and LOQ) were found in 3 samples. Only one sample was on the legal limit proposed (0.2 μg/L) (Araguas et al., 2005).

These results are not surprising, because there are a lot of similar data in the literature.

A survey of Jorgensen (1998) has shown that all beer samples contained traces (>0.001 μg/L) of OTA with a mean content of 0.049 μg/L and the highest value of 0.160 мg/L. OTA has been detected in 95.5% of Japanese beers and in 91.5% of non-Japanese beers at mean concentrations of 0.0125 and 0.0101 μg/L respectively (Nakajima et al., 1999). Similar results have been achieved by the study of Tangni et al. (2002) on OTA in domestic and imported beers in Belgium-97.6% of the analyzed beer samples have been contaminated. The level ranged from 0.01 to 0.185 μg/L, with a mean level of 0.033 μg/L. These studies showed a similar LOD (0.001-0.004 μg/L) but with a LOD of 0.01 μg/L, Visconti et al. (2000) found a much lower incidence (50%). OTA has been detected in 24 of 31 beer samples, with mean concentration of 0.044 μg/L (LOD 0.012 μg/L) in a study of Araguas et al. (2005) in Spain.

Quite similar to our results are the data of Bacaloni et al. (2005). Sampling was made also during 2004. The range of contamination was 0.02-0.14 μg/L with mean concentration of 0.07 μg/L. The occurrence level of this mycotoxin in Spanish beer was 83.8% with a range of 0.007-0.204 μg/L, and average concentration of OTA 0.0358 μg/L.

In the last years the highest OTA levels in

beer were reported by Je Won Park et al. (2005) in beer samples marketed in Korea with a range of 0.2-0.3 μg/L. Our results and the results of other teams show that beer is a contributor to OTA intake for humans.

Survey of OTA in wine samples

OTA content in all studied 22 samples (2 white) of bottled industrially produced Bulgarian wine was below LOD (0.04 μg/L).

The surveys concerning OTA content in wine reported that the amounts in red wines are higher than in rose wines followed by white wines. In general, OTA mean content in red wines ranged from 0.039 to 1.802 μg/L (Belli et al., 2002), very similar to that for rose wines, which ranged from 0.025 μg/L to 1.348 μg/L (Belli et al., 2002). Comparison of OTA content in Spanish wines produced in 1997 and 1998 showed differences between contaminations of samples from the two different years (Lopez de Cerain et al., 2002). Eighty five per cent of the samples from 1997 have shown OTA levels >0.05 μg/L (range 0.056-0.316 μg/L) and 15% of the samples from 1998 have been with OTA content in the range of 0.074 μg/L to 0.193 μg/L. The authors attributed this to different quality of the grapes, due to bad climate in 1997.

A survey of Stefanaki et al. (2003) on OTA content in Greek wines showed not significant difference between red and white wines. OTA content is in the ranges of <0.05 to 2.69 μg/L and < 0.05 to 1.72 μg/L for red and white wines respectively. The investigation was conducted from 1995 to 1999.

Besides the difference in contamination of red and white wines, the conclusion of the studies all over the world is that geographic region of origin is related to OTA concentration, e.g. it increases from northern to southern Greece (Stefanaki et al., 2003), from northern to southern Italy (Pietri et al., 2001) and from northern to southern Europe (Majerus and Ottender, 1996, Zimmerli and Dick, 1995, Ottender and Majerus, 2000). This can be one explanation of the absence of OTA in our study.

CONCLUSION

We have to take into account that the number of samples is very scanty. The investigations all over the world show that more extensive studies are necessary. A comprehensive survey will give a possibility to make a conclusion whether OTA in Bulgarian beer and wine is contributor to the human risk.

The method used in our lab is rapid and accurate. It can be adopted in public laboratories with national or regional responsibility for food quality control.

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Table 1. Wine, number of samples analyzed, year and region of production

Wine	Number of samples	Year of production	Region of production
Gamza	5	2000, 2002, 2003	Eastern, Southern
Broad vine from Melnic	1	2000	Southwestern
Cabernet Sauvignon	4	1996, 1999, 2000, 2001	Southern, Eastern,
Cabernet Sauvignon and Merlot	1	1999	No information
Merlot	3	1999, 2001	Southern
Manastirska izba	1	2003	Southern
Zagovor na monasite	1	2003	Southern
Mecha krav	1	2003	Southern
Red sweet wine	1	2003	Southern
Mechandgiisco	1	2003	Southern
Tarnovo	1	2003	Southern
Chardonnay/white	1	2000	Eastern
Manastirska izba/white	1	2003	Southern

Fig. 1. Bulgarian vine-growing regions

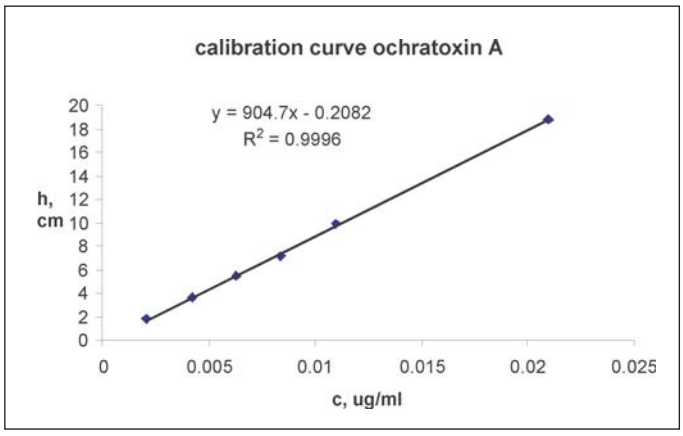

Fig. 2. Calibration curve of OTA standard solutions

Table 2. Ochratoxin A in beer samples

№ of brand	Numb. of samples positive/analyzed	min.OTA level µg/L	max.OTA level µg/L	mean OTA level µg/L
1	2/2	traces	0.12	0.10
2	4/4	traces	traces	0.09
3	9/9	0.10	0.20	0.12
4	1/3	ND	traces	0.04
5	2/3	ND	0.10	0.06
6	1/1	LOD		0.04
7	1/1	LOD		0.04
8	0/3	ND	ND	ND
9	0/3	ND	ND	ND
10	0/3	ND	ND	ND

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FOOD–BASED DIETARY GUIDELINES FOR BULGARIAN ADULTS – SCIENTIFIC BACKGROUND FOR DEVELOPMENT AND FORMULATION

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INTRODUCTION

During last decades a significant shift in the structure of population morbidity and mortality rate was observed in the world including in Bulgaria. At the same time significant unfavorable changes were observed in dietary pattern and life style, especially low physical activity, that contribute for the diet-related chronic diseases such as cardiovascular diseases (coronary heart disease, hypertension, stroke), obesity, some types of cancer, diabetes type 2, osteoporosis, dental caries [27,29, 30]. According to the WHO international ranking in the last decades Bulgaria has sustained high mortality rate related to circulatory diseases and occupies one of the first places in the world for stroke mortality [28]. With this regard a national Food and Nutrition Action Plan in Bulgaria has been developed and started to be implemented since 2005 [11]. Scientifically evidenced Food–Based Dietary Guidelines considering the current national nutrition problems are included as an important tool for positive modification of Bulgarian diet in our nutrition policy.

SCIENTIFIC BASIS FOR DEVELOPMENT OF FOOD–BASED DIETARY GUIDELINES IN BULGARIA

Scientific evidence for the association between diet and health.

It is well known that diet and nutrition are important factors in the promotion and maintenance of health throughout the entire life cycle. Malnutrition, overeating and unbalanced nutrition have negative effect on the functions of the organism and health during the ***whole life***. Mal-

nutrition and nutrient deficiencies remain among the important problems facing poor people in most of the countries. It is well known that nutritional deficiencies increase the risk of common infectious disease [23]. Given the rapidity with which traditional diets and lifestyles are changing in many countries, it was apparent that undernutrition persists in the same countries where chronic diseases related to nutrition are emerging as a major epidemic.

The role of dietary pattern and nutrient intake as determinants of chronic non-communicable diseases is now well established [29] and so represent a prominent element in prevention strategy [7, 10, 27, 30- 31].

Convincing associations [29] for reduced risk of cardiovascular disease include consumption of fruits and vegetables, fish and fish oils (eicosapentaenoic acid and docosahexaenoic acid), foods high in linoleic acid and potassium, as well as physical activity and low to moderate alcohol intake. There is convincing evidence that myristic and palmitic acids, trans-fatty acids, high sodium intake, overweight and high alcohol intake contribute to a risk increase.

There is convincing evidence that regular physical activity is protective against unhealthy weight gain, whereas sedentary lifestyles, particularly sedentary occupations and inactive recreation promote it. A high dietary intake of non-starch polysaccharides – dietary fiber promotes weight loss. Another convincing evidence for increased risk of overweight is high intake of energy-dense micronutrient-poor foods.

The association between excessive weight gain, central adiposity and development of type 2diabetes is convincing. The association has been repeatedly demonstrated in longitudinal studies in different populations, with a striking gradient of risk apparent with increasing levels of Body Mass Index, adult weight gain, waist circumference or waist-to-hip ratio. Voluntary weight loss improves insulin sensitivity and reduces the risk of progression from impaired glucose tolerance to type 2 diabetes. Increased physical activity reduces the risk of developing type 2 diabetes regardless of the degree of adiposity.

Research to day has uncovered few definitive relationships between diet and cancer risk [5,29]. Dietary factors, for which there is convincing evidence for an increase in risk, are overweight and obesity, and a high consumption of alcoholic beverages, aflatoxins and Chinese-style of salted fish. Factors, which probably increase risk, include high dietary intake of preserved meats, salt-preserved foods and salt per se, very hot drinks and foods. Probable protective factors are consumption of fruits and vegetables. After tobacco, obesity appears to be the most important known avoidable cause of cancer.

Prevalence of diet-related chronic diseases in Bulgaria

In the last decades 62-65% of all deaths in Bulgaria have been due to diseases of the **circulatory system –** ischaemic heart disease (IHD), heart attack, stroke [14]. While in West-European and Mediterranean countries in the last decade the mortality rate of circulatory diseases is sustained at a low level and decreases further, in Bulgaria it maintains high levels (Fig.1, Fig.2). In 2002 the standardized mortality rate of diseases of the circulatory system in Bulgarian men was 873/100000, the average for men in countries – members of the EU was 341/100000 [28]. The last years evidenced a certain tendency to decrease the mortality rate of IHD (Fig. 1), but Bulgaria is still one of the leaders by mortality of stroke. While the mortality rate of cerebrovascular diseases in 2002 in Bulgarian men was 204/100000, the average value for men in the EU member-states was 63/100000 (Fig.2).

Overweight and Obesity are widely prevalent in Bulgarian adults, both in men and women. The national nutrition surveys carried out in 1998 and 2004 showed that overweight and obesity prevalence among adult population increase (Fig 3). In 1998 32% of men and 22% of women aged 18 – 60 were with *overweight*, in 2004 respectively 41% of men and 34% of women. Prevalence of obesity in 1998 was 15.2% among men and 10.7% among women aged 30 - 60 years, in 2004 –it was 21% and 17% respectively in the same age group [3,26].

The studies conducted under the national CINDI Programme on lipid status show a significant prevalence of **dyslipidemias** – high total cholesterol (TC), high cholesterol in low-density lipoproteins (LDL-C),low cholesterol in high-den-

sity lipoproteins (HDL-C) and high blood triglycerides (TG) levels that elevate the risk for atherosclerosis and its consequences – coronary heart disease and stroke [25]. Dyslipidemias are associated mainly with high intake of saturated fatty acids and obesity. High TC has been detected in 27.5 – 34% of men and 23-38% of women in Bulgaria. LDL-C is increased in 49-58% of men and 43-63% of women. Low HDL-C levels are found in 16-33% of men and 6-16% of women. High prevalence of increased blood serum TG is found in 24-34% of men.

Cancer is the second leading cause for Bulgarian population mortality (13-15% of the total mortality rate) [14]. This rate has increased with 20% in the period 1990-2002. Particular increase is observed in morbidity of breast cancer in women (Fig. 4), cancer of the prostate in men and colon cancer for both men and women; the incidence of stomach cancer is also high. All these types of cancer are associated with nutrition – low consumption of fruits and vegetables, high fat intake, high use of salt, etc. [5].

The incidence of **type 2 diabetes** marks a tendency of constant increase. In the period 1980-2002 it has increased with more than 80% (Fig. 5) in parallel with increasing obesity, which is a major risk factor for this disease [14]. The low physical activity level observed for a great part of our population is an additional risk factor for diabetes, independently of obesity [10].

Osteoporosis has become a serious problem for Bulgarian women during the past 15 years with particularly great incidence in menopausal women aged 65+years [11]. In this period, dramatic decrease of consumption of milk and yogurt, best sources of calcium, and as a result low intake of this mineral with great importance for the bone health have been observed [8, 13, 19]. Women also have insufficient intake of other nutrients (zinc, magnesium, vitamin C, B_6, etc.) with a certain role in bone structure and mineralization [9, 17-18]. Low physical activity, important risk factor for osteoporosis [3], is also a significant problem with women.

Malnutrition and deficient intake of nutrients affecting the immunity, observed in population groups with low socioeconomic status [8, 9,17,18], reflects on the prevalence of a number of communicable diseases, e.g. **tuberculosis** has increasing rate in the last years [14]. The registered incidence rate of active tuberculosis in Bulgaria and in EU member-states was in 1980 almost equal; in 2002 the incidence of tuberculosis in Bulgaria is 4 times higher than the EU average rate [28] (Fig. 6).

TRENDS AND CURRENT PROBLEMS IN DIETARY AND NUTRITIONAL STATUS OF THE POPULATION.

Trends in food availability for Bulgarian population

The data obtained from the household budget surveys in Bulgaria, conducted annually by the National Institute of Statistics on a representative sample of the Bulgarian population [13], are the basis for evaluation of the food availability per capita, national dietary pattern and trends of its changes. The economic changes in the country after 1989 brought numerous positive changes, but also certain important unfavorable trends in the population food consumption pattern [16, 20, 21].

Positive trend of decreasing of added fat availability has been revealed. In 2004 the total availability of added fats was with 17% lower than that in 1989. Significant changes have occurred in the structure of availability of added fats. While the use of sunflower oil has decreased by 20%, the consumption of butter is 5 times lower, and that of lard – 2 times. On that account, the availability of margarine has increased significantly. The decreased intake of added fats is related mainly to economic problems but also to changes in the foods offered on the market. For example, margarine availability has increased because of its lower price compared to butter, but also because of the greater offer of different margarines and their advertising. The increased margarine availability cannot be accepted as a positive trend as most of the margarines on Bulgarian market are a still substantial source of trans-fatty acids.

Positive trend of increasing the availability of beans and lentils (with 30% in the period 1989 – 2003), wholegrain and other types of whole-meal bread has been noted.

The data from household budget surveys show a ***trend of decreasing the availability of***

sugar and confectionery, of soft drinks and alcoholic drinks average per capita (including beer, wine and spirits) for the period of 1990-1997, but after that there is a significant increasing in the consumption of these foods and drinks.

The availability of meat and meat products significantly decreased in the years of economic transition (Fig. 7). The availability of meat and meat products in 1990 was 100 g per day per capita for meat and 49 g for meat products. After drastic decrease in meat and meat products availability in the critical 1996-97, the availability of meat is increasing and in 2004 it reached to 68 g average daily per capita and of meat products – 35 g. This is not an unfavorable trend as the higher meat consumption is associated with increased risk of cardiovascular disease and some types of cancer. The problem lies in the great difference between meat availability in the different social groups depending on the income.

The availability of milk and dairy products has significantly decreased in the period 1989 – 2004 (Fig. 8). The availability of yogurt, a traditional Bulgarian food, has decreased particularly drastically – with 60% (175 g in 1989, 72 g in 2003 daily average per capita). The reduction of milk availability is 49% and for dairy products consumption – 10%. As this food group is the basic source of calcium with high bioavailability, this negative trend puts a substantial part of the population at risk of insufficient calcium intake revealed by the national epidemiological surveys on nutrition.

In the period after 1989, in the critical 1996-1997 significant decrease of the ***availability of fruits and vegetables*** was established, but in the last years there is a tendency to increase; in 2004 the availability of fruits and vegetables as a total has not yet reached the one in 1989 (399 g daily per capita in 2004 vs. 464 in 1989) (Fig. 9). A positive trend is also observed of reduction of seasonal differences in fruit and vegetable availability in the last few years, but nevertheless the availability in the winter-spring season in 2004 is 1.5 – 3 times less than the summer-autumn period (Fig. 10). The seasonal difference in availability of fresh fruits and vegetables (3 to 5 times less in winter-spring) is particularly pronounced, which puts the population at risk related to insufficient vitamins and minerals intake during these seasons. In winter the relative part of availability of preserved vegetables from the total vegetable quantity is high (40% in December and January 2004), which is a basis for significant increase of salt intake.

The availability of fish is low (8-10 g daily per capita at recommended at least 30 g) and this tendency has been maintained in the last two decades, although Bulgaria is a sea coastal country. The reasons are associated with both the relatively high costs of fish and the lack of fish consumption traditions.

Dietary intake, nutritional status and risk population groups in Bulgaria

Dietary and nutritional status surveys of the population provide a reliable basis for assessment of the nutritional problems of the particular population groups and identification of the risk groups.

The national representative studies on dietary and nutritional status of the entire population older than 1 year conducted during the last years by the National Center of Public Health Protection and Regional Inspectorates for Public Health Protection and Control and studies of other institutions revealed the following basic problems:

The ***energy intake*** of young women (18-30 years) and elderly people (70+ years) is below the average requirements [1]. This contributes for determined ***underweight*** in 17.3% of young women and 8% of old people [3, 26]. Most of population groups had higher energy intakes than established average requirements and this together with low physical activity results in great prevalence of overweight and obesity (see above).

Average daily protein intake has decreased during the last years with 10%-20% on the account of animal proteins, but is over the corresponding dietary reference values and is within the recommended limits of 10-15% of total energy value [2,16].

Although there is a tendency to a decreased ***fat*** intake, it is still higher than the recommended upper limit – 30% of total energy value [22, 31]. Recent surveys establish 35-40% energy intake from total fats; exceeding the upper risk limits are saturated fatty acids as well as polyunsaturated fatty acids [4]. The high intake of satu-

rated fatty acids causes increase of blood cholesterol and elevates the risk of cardiovascular disease [29]. The high intake of polyunsaturated fatty acids provides also health risks because of their oxidation and the hazardous effect of lipid peroxides on the cells [5]. It is found that the male population of Bulgaria, aged 18-60 years has an average daily intake of cholesterol above the upper recommended limit of 300 mg [3, 4], which is an additional risk factor of cardiovascular disease.

The studies showed that the average daily intake of many ***vitamins and minerals*** in all Bulgarian population groups, differentiated by age and sex, is below the corresponding dietary reference values [8, 9, 17, 18]. A significant part of our population is at risk of development of marginal micronutrient deficiencies, especially in winter and spring. Particularly low intake of folate and vitamins B_2, B_1, B_6 and of the minerals calcium, iron, zinc, magnesium is determined (Fig.11-12). The intake of vitamin C is lower in winter and spring. Young women, pregnant women, elderly people, individuals with low social status are at particular risk of vitamin and mineral deficient intake [16, 21]. The lowest dietary intake of folate is found in the female groups in childbearing age (Fig.13) that is a risk factor for folate deficiency in the first months of gestation and risk for neural tube defects. The female groups aged 18-60 years are at the greatest risk of insufficient calcium intake. This increases the risk for disturbed bone mass accumulation in growth period and risk of osteoporosis in the menopause. The average iron intake is particularly low in women in fertile age, which determines these groups at higher risk to develop iron deficiency anemia.

A serious problem is the high intake of ***sodium*** (up to 2-3 times above the upper safe limits, especially in winter), which is a risk factor for high blood pressure and stomach cancer [16]. The main source of sodium is salt, which in Bulgarian diet is mainly contributed by consumption of bread and bakery (40-50% of the total amount), followed by salt added in cooking (25-30%), meat products, preserves, brined cheese, and other salty foods.

The ***intake of dietary fiber (cellulose, hemicelluloses, pectin, etc.),*** which is contributed by vegetables and fruits, wholegrain bread, beans, lentils are also of health importance. It has been found that the high intake of dietary fiber is related to decreased risk of obesity, cardiovascular disease, diabetes type 2, colon cancer and other diseases. In the 90s our studies showed that fiber intake of Bulgarian population is below the recommended values [6, 16]. During the last years there has been some increased intake, which is contributed by the higher consumption of wholegrain bread and legumes.

The studies revealed high prevalence of ***alcohol*** consumption among men and high alcohol consumption among the individuals that drink alcoholic beverages [1, 24]. The moderate alcohol consumption is set to 16 g/day (20 ml/day) of absolute alcohol [10]. The National Dietary Survey of Bulgarian Population in 1998 showed that 49% of men and 28% of women aged 18-60 years had alcohol consumption and the average intake was 45 g absolute alcohol for men and 19 g for women [1].

The unbalanced and inadequate intake of nutrients found is associated with the established ***current negative characteristics of food consumption [16, 21]***:

- High consumption of added fat, both of animal and vegetable origin;
- High consumption of fatty meats and meat products;
- Low consumption of fish;
- Consumption mainly of high-fat milk, significantly decreased intake of yogurt – traditional healthy food for the Bulgarian population;
- Low intake of raw fruits and vegetables in winter and spring;
- Low consumption of wholegrain bread and other wholegrain products;
- Increased consumption of sugar, sugar and confectionery products, sugar-containing soft drinks;
- High salt intake (2-3 times over the recommended amounts);
- High alcohol intake among some population groups.

REFERENCE VALUES FOR ENERGY AND NUTRIENT INTAKES FOR BULGARIAN POPULATION

We used updated Recommended Dietary In-

takes (RDI) for Bulgarian population [22] to set national nutrition goal to be achieved by implementation of FBDG and other tools of the national nutrition policy [11]. The Reference Values for Energy and Nutrient Intakes for Bulgarian Population have been updated in 2004-2005 on the basis of scientific background for current RDI of WHO, USA, Canada, UK and other European countries. Current data for weight and height of Bulgarian population differentiated by age and gender have been used, data for dietary intakes and diet-related health problems and diseases obtained in the last national nutritional surveys have been considered. On the basis of RDI for Bulgarian population, considering the identified current problems in nutrition and nutritional status, morbidity and mortality rate of chronic diet-related diseases the goals to be achieved for healthy nutrition of adults have been set, presented in Table 1.

FORMULATION OF FBDG FOR BULGARIAN POPULATION

Twelve guidelines for healthy nutrition of adults in Bulgaria have been formulated on the basis of the scientific evidence for the association between diet and health, considering the prevalence of diet-related chronic diseases, trends and current problems in dietary intake and nutritional status of the population. Goals set to be achieved for healthy nutrition as well as socio-economic conditions in the country influencing food accessibility, economic possibilities of Bulgarian agriculture and food industry to reach the goals have been taken into consideration.

Food safety is very important for the health as well. Since each year many Bulgarians suffer from foodborne diseases associated with microbiological contamination of foods not following the hygienic rules for their preparation and storage [11, 21], the last recommendation has been directed to maintain food safety.

FBDG are culturally relevant and consistent with traditional dietary pattern of Bulgarians. The defined purposes are realistic and the recommendations concerned small changes, step by step on familiar and acceptable foods. The messages are conveyed in understandable way. Two variants are developed – one short version directed to the general public and second comprehensive for professionals [12, 15].

Formulated Food –Based Dietary Guidelines for healthy nutrition of adult population in Bulgaria are as follows:

1. Eat a nutritious diet with variety of

Table 1. Goals to be achieved for healthy nutrition of adult population

DIETARY factor	**Goal**
Protein (% of total energy)	10-15
Total fat (% of total energy)	20-30
Saturated fatty acids (% of total energy)	< 10
Polyunsaturated fatty acids (PUFA) (% of total energy)	6-10
Trans-fatty acids (% of total energy)	< 1
Monounsaturated fatty acids (% of total energy)	Difference*
Cholesterol (mg/day)	< 300
Total carbohydrates (% of total energy)	55-75
Total dietary fibers (g/day)**	25-35
Added sugars (% of total energy)	< 10
Sodium chloride (sodium) (g/day)	< 5 (2)
Vitamins and minerals	Dietary Reference Values
Vegetables and fruits (g/day)	≥ 400

* The difference is calculated as: total fat – (saturated fatty acids + polyunsaturated fatty acids + *trans* fatty acids)

** Depending on age and sex

foods. Do eat regularly, take enough time and enjoy your food in friendly environment.

2. Consume cereals as an important source of energy. Prefer wholegrain bread and other wholegrain products.

3. Eat a variety of vegetables and fruits more than 400 grams every day, preferably raw.

4. Prefer milk and dairy products with low fat and salt content.

5. Choose lean meat, replace meat and meat products often with fish, poultry or pulses.

6. Limit total fat intake, especially animal fat. Replace animal fats with vegetable oils when cooking.

7. Limit the consumption of sugar, sweets and confectionery, avoid sugar–containing soft drinks.

8. Reduce intake of salt and salty foods.

9. If you drink alcoholic beverages, you should consume moderate quantities.

10. Maintain a healthy body weight and be physically active every day.

11. Drink plenty of water every day.

12. Prepare and store the food in a way to ensure its quality and safety.

Determination of the portion sizes, specific for each food group and recommended portion number for average daily intake have been defined.

Pictorial presentation of Food Based Dietary Guidelines

The Healthy Diet Pyramid [Fig.14] was developed as a pictorial presentation of the main dietary recommendations for Bulgarian adults [12, 15]. The pyramid is divided into **sectors covering the food groups**. It reflects the main principle of healthy diet – variety of food intake that is achieved through daily consumption of representatives of all food groups. The size of the sectors corresponds to the proportion of recommended amounts for consumption of foods from particular groups. The food groups are distributed in three bands in the colors of the traffic light. **The green band** in the base of the pyramid contains plant foods – vegetables, fruits, cereals and potatoes that represent the main portion of the total amount of daily intake. **The yellow band** contains foods of animal origin that are important for healthy nutrition but should be consumed in smaller amounts. It also includes plant foods as pulses and nuts that are rich in protein and are the plant alternative to animal protein foods. Vegetable oils recommended as main added fats are also placed here because their consumption should be considered, as they are rich in energy. **The red band**, on top of the pyramid includes energy-dense foods with low vitamin and mineral content that should be avoided and to be consumed in limited amounts – animal fats, sugar and confectionery products. Under the pyramid the eight glasses of water represent the recommended daily water intake. The figures of walking and running people prompt the significance of physical activity to maintain healthy body weight.

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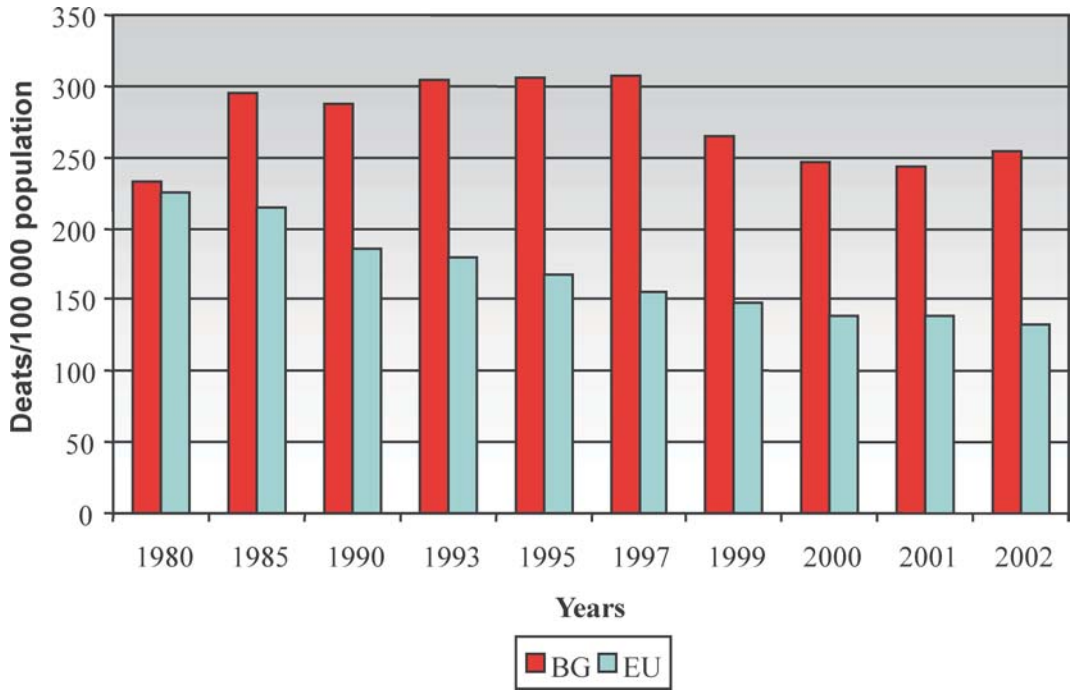

Fig. 1. Mortality Rate of Ischaemic Heart Disease for Men in Bulgaria and EU Member States
Source: WHO, 2003

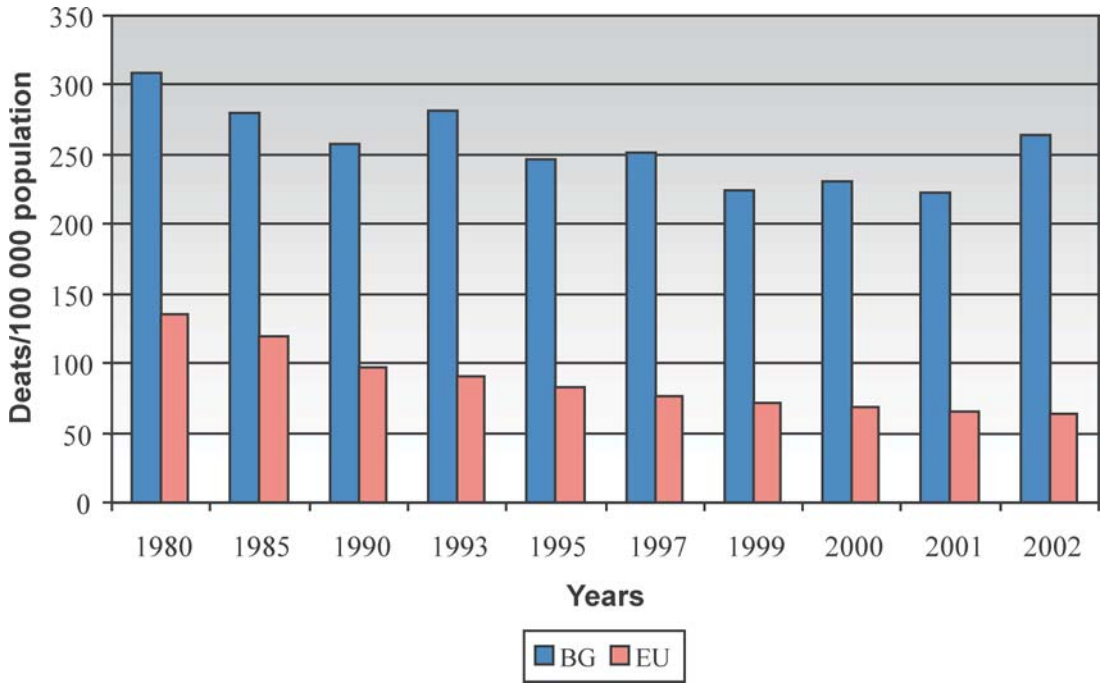

Fig. 2. Mortality Rate of Cerebrovascular Disease for Men in Bulgaria and EU Member States
Source: WHO, 2003

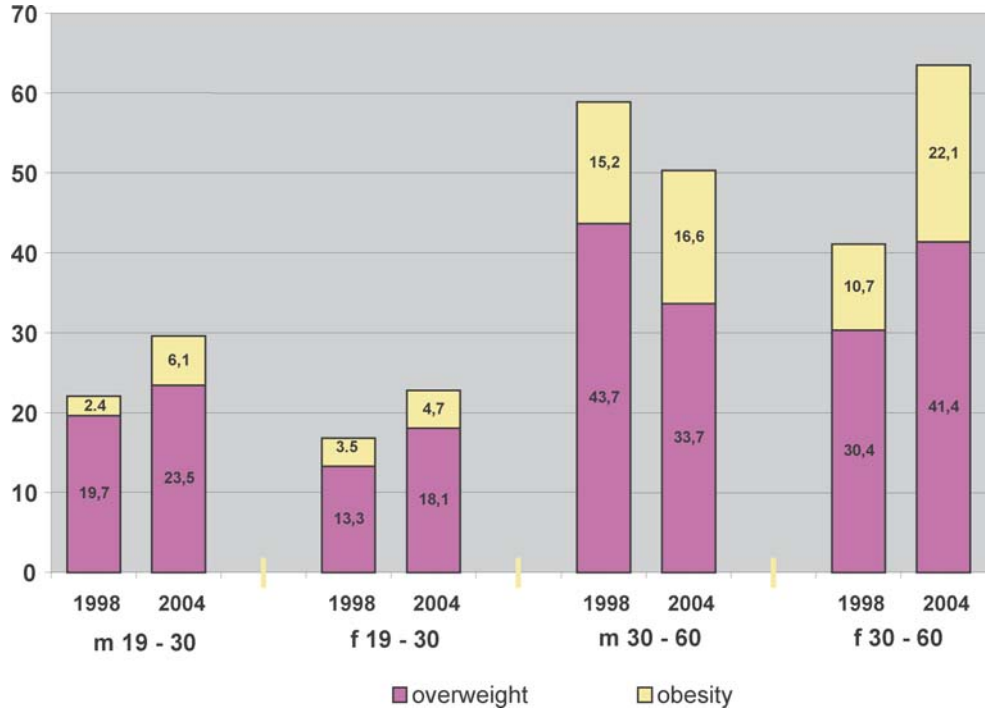

Fig. 3. Overweight and obesity prevalence among adults in Bulgaria
National nutrition surveys, 1998 and 2004

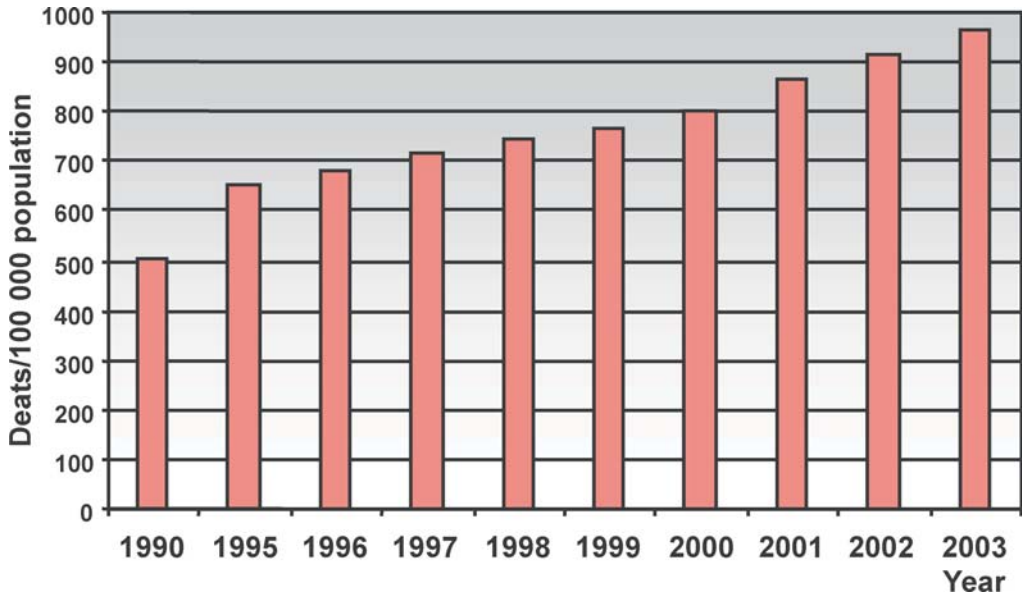

Fig. 4. Incidence of registered cases of breast cancer in Bulgaria
Source: National Center of Health Information, 2004

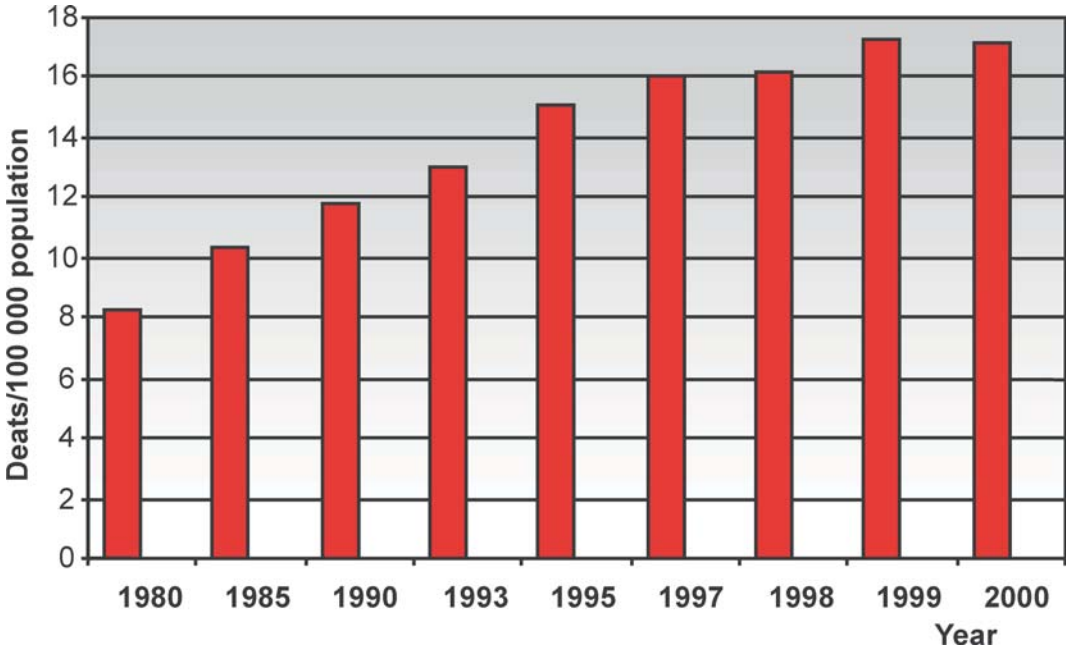

Fig. 5. Incidence of registered cases of diabetes in Bulgaria
Source: National Center of Health information, 2003

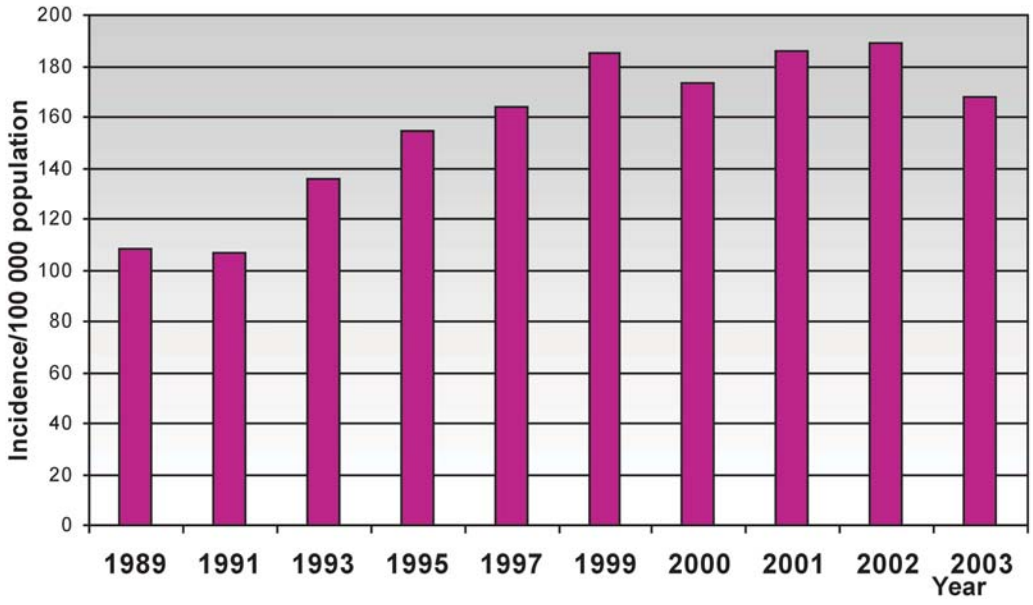

Fig. 6. Incidence of registered cases of active tuberculosis in Bulgaria
Source: National Center of Health Information, 2004

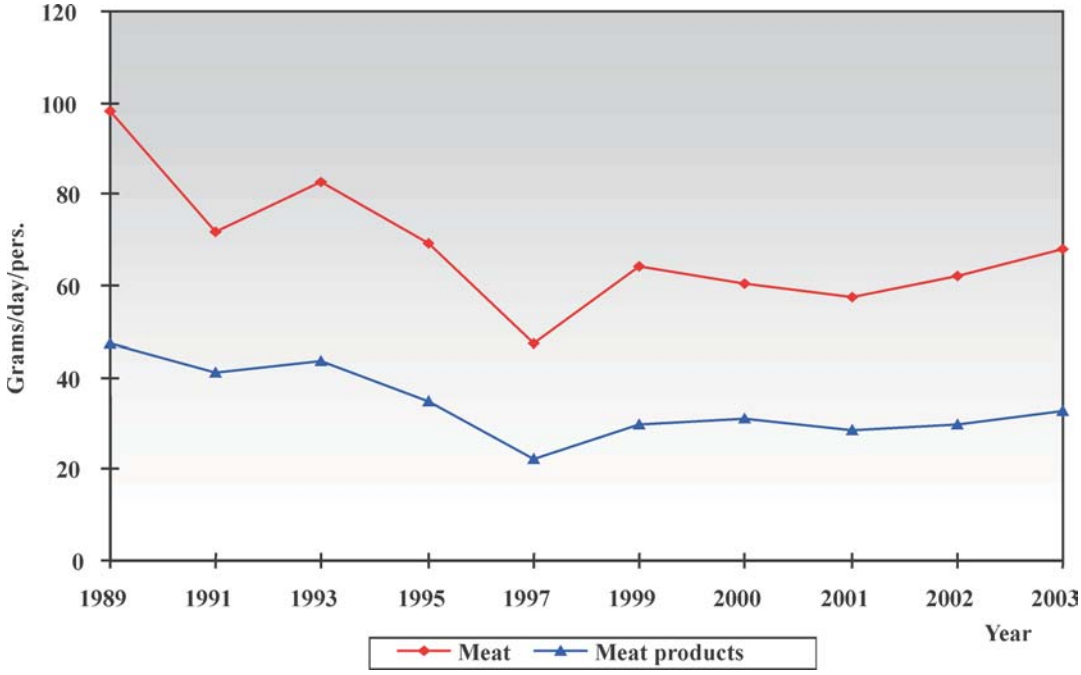

Fig. 7. Availability of meat and meat products in Bulgaria
Source: National Institute of Statistics, 2004

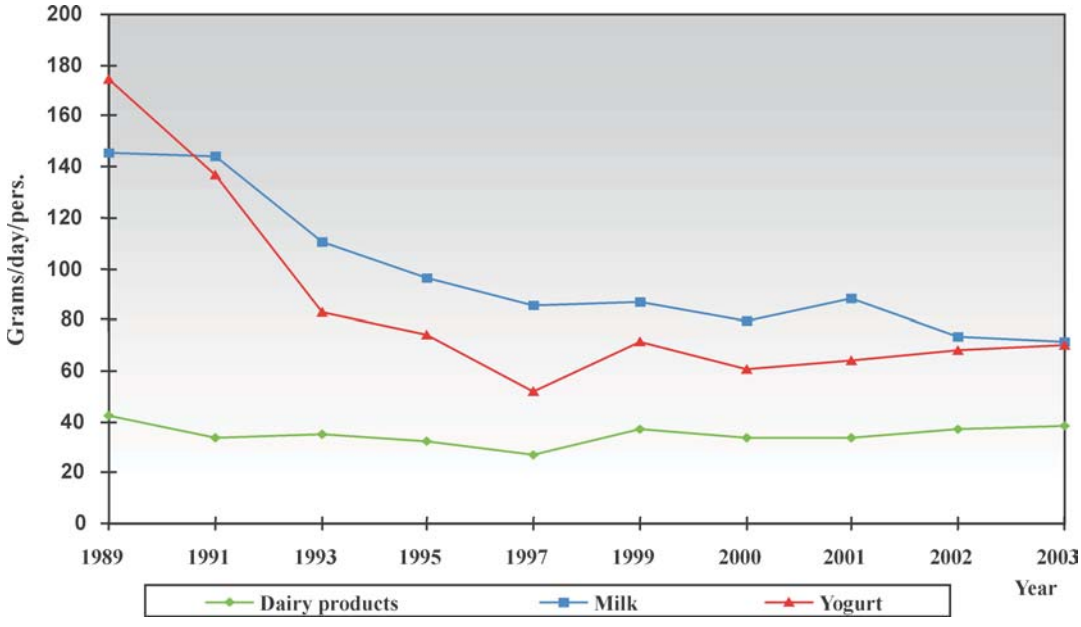

Fig. 8. Availability of milk and dairy products in Bulgaria
Source: National Institute of Statistics, 2004

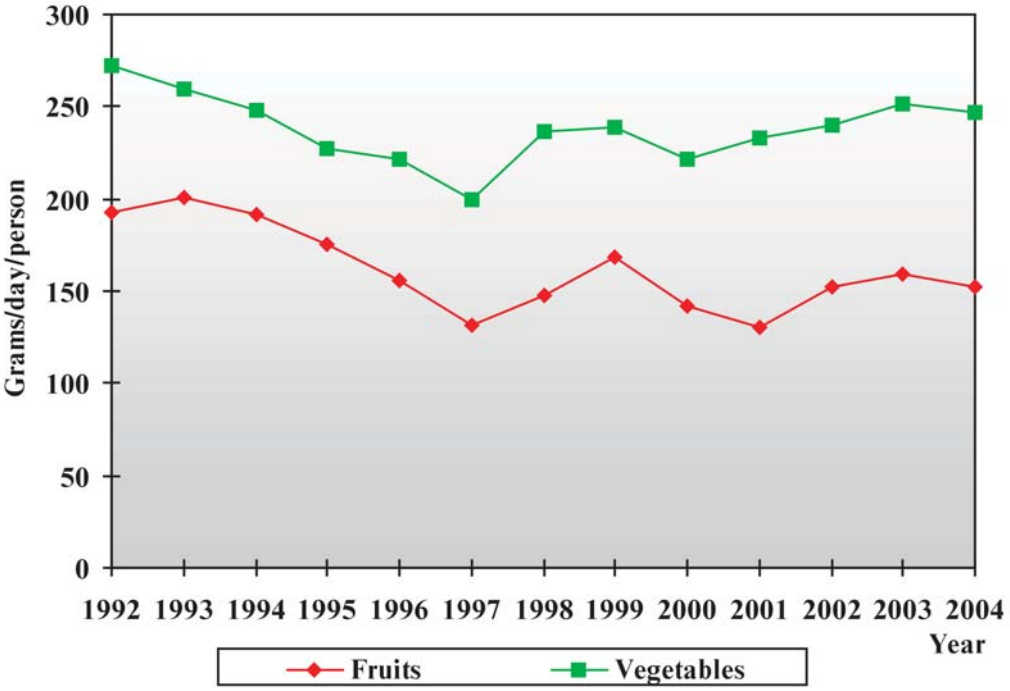

Fig. 9. Availability of fruits and vegetables in Bulgaria
Source: National Institute of Statistics, 2005

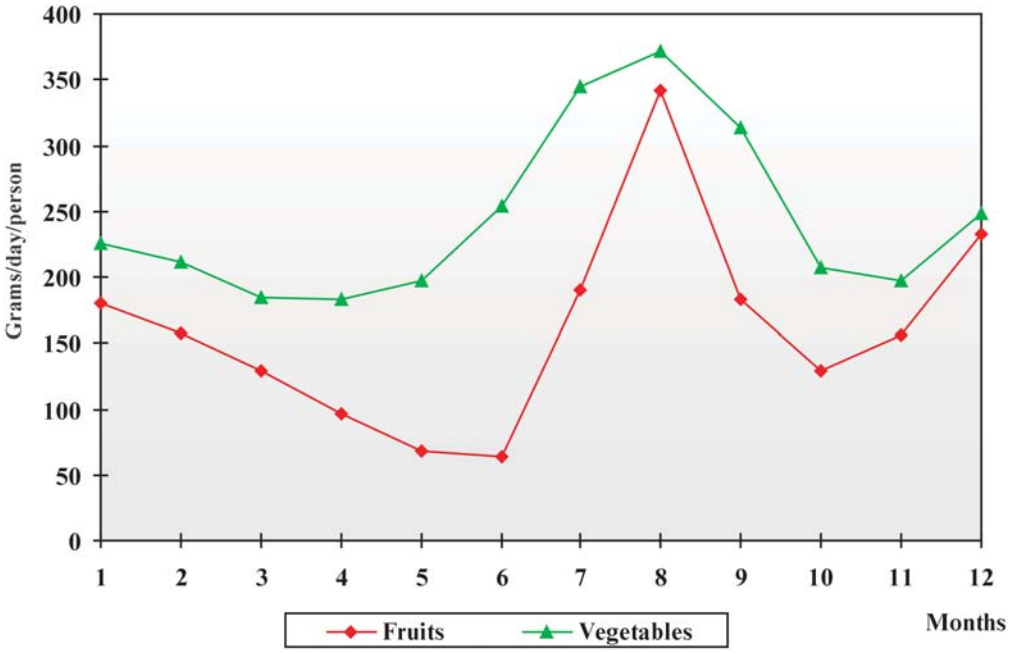

Fig. 10. Monthly changes in fruit and vegetable availability in Bulgaria, 2004
Source: National Institute of Statistics, 2005

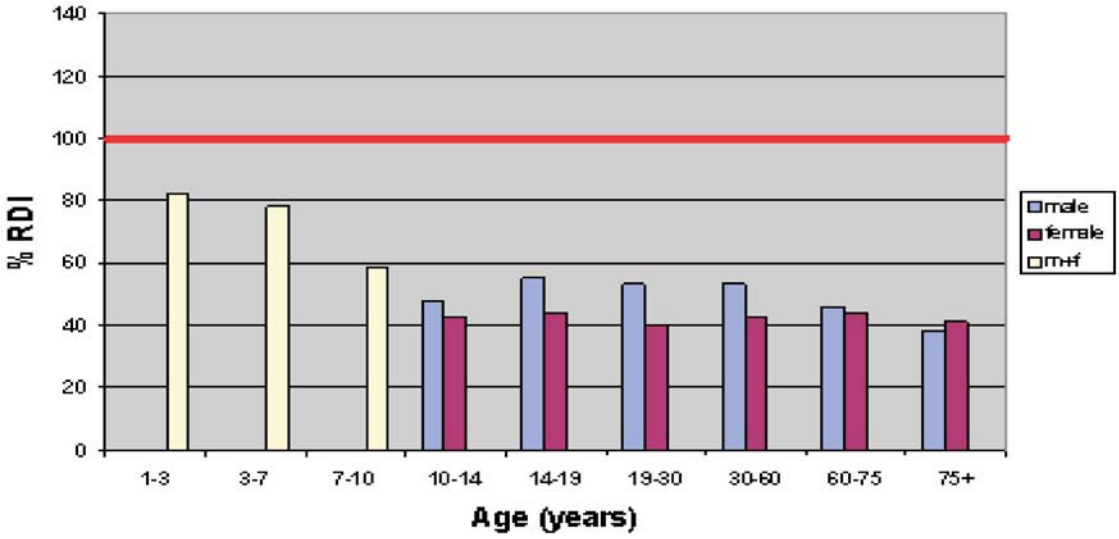

Fig. 11. Average daily intake of calcium of Bulgarian population as % of Recommended Dietary Intakes (National Nutrition Survey, 2004)

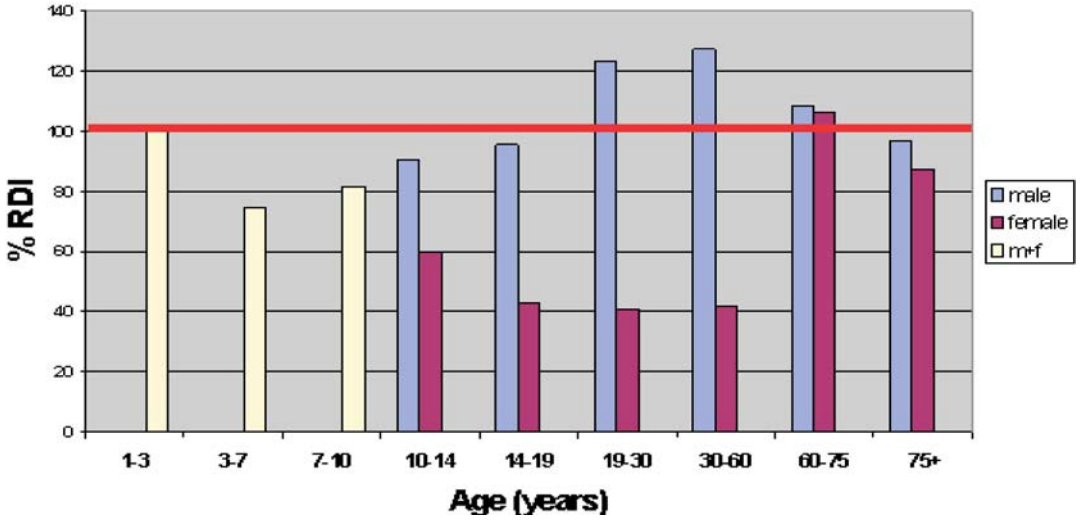

Fig. 12. Average daily intake of iron of Bulgarian population as % of Recommended Dietary Intakes (National Nutrition Survey, 2004)

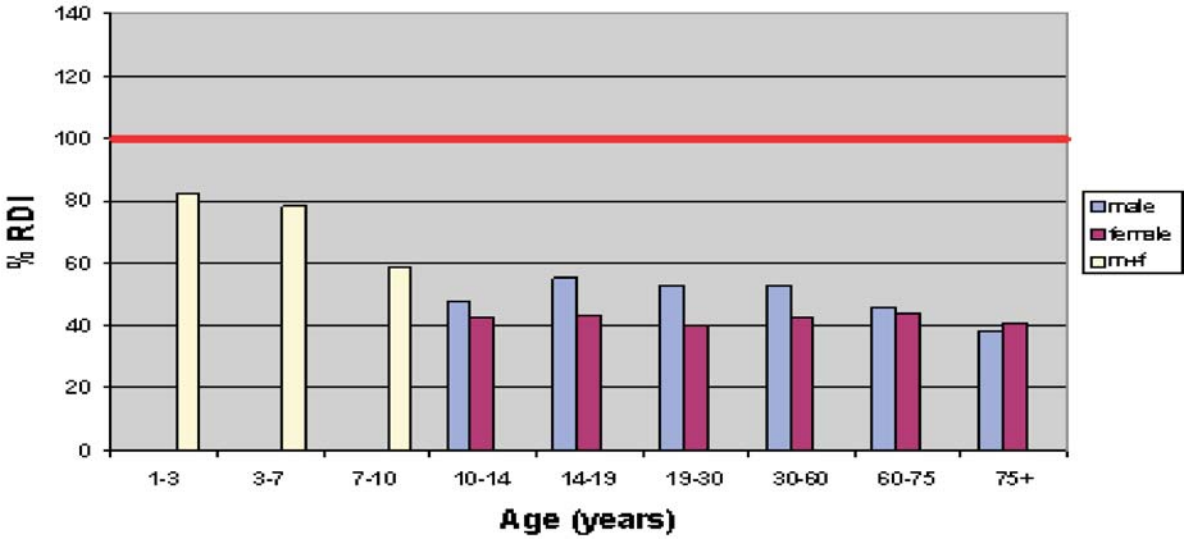

Fig. 13. Average daily intake of folate of Bulgarian population as % of Recommended Dietary Intakes (National Nutrition Survey, 2004)

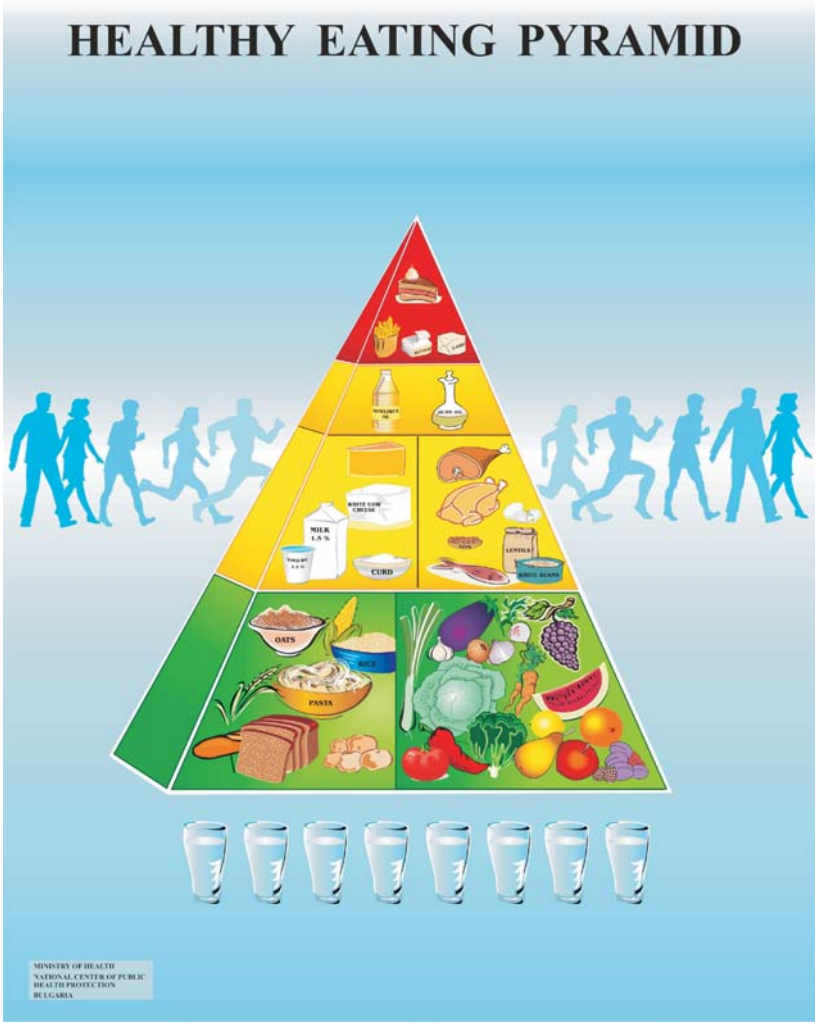

Fig. 14. Healthy Eating Pyramid for Bulgarian Adult Population, 2006

BULGARIAN NATIONAL MICROBIOLOGICAL LIMITS FOR FOODS

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Establishment of norms for microbiological indicators is a part of the complex medico-biological hygienic standardization of foods to ensure their quality, safety, harmlessness and level of production and trading culture.

The past prevention practice did not set unified international microbiological norms guaranteeing foods epidemic safety and production hygiene. This was due to the differences between the levels of national control systems, dietary traditions, particularities of national cuisines of different populations and regions, culture of production and trading in foods.

Each country had its national control system, indicators, norms and requirements [8]. At the same time advanced food producers and traders had and continue having their microbiological criteria and requirements to the quality of their production. Bulgaria has for decades been establishing a strict official system for microbiological norms for foods and those standards were part of the Bulgarian State Standardization and health hygienic requirements [11]. Nevertheless, theory and practice in this field have been undergoing significant changes during the last years.

A number of geographic, climatic and other features of the ecosystems affect food microflora. Socioeconomic prerequisites such as national traditional characteristics of the cuisine, technological level, culture of production and use create national differences between the norms. Japanese sushi, Mongolian kumis, Bulgarian yogurt, millet-ale – those are unique foods that cannot be reproduced in other regions in the world, which safety has to be guaranteed. Such particularities make national microbiological norms necessary.

The first European Regulation № 2073 covering microbiological criteria for foods was enacted in 2005 [1]. It is mandatory for the EU member states especially at international food exchange. It marked the onset of international norms of foods by microbiological indicators. Until then only recommendatory norms of Codex Alimentarius had been set for some foods – dry milk, pasteurized egg products, etc. [2,6]. The regulation, though, recommends criteria for safety and hygiene of production, storage and trading in only several food groups – mainly those of animal origin – meat, milk, egg, sea products. There are no developed and legally established microbiological requirements to cereals, confectionery, chocolate, fats and emulsion products, ready-to-serve catering production, sterilized canned products, food supplements, etc. Those foods are a source of many risks of microbiological nature and have also to be protected.

In this field the applied research in prevention in Bulgaria, joint efforts of specialists in human and veterinary medicine, and food industry in several decades have resulted in great experience and establishing a stable tradition.

The joint efforts of Bulgarian specialists in food standardization have practically begun at the end of the 60s of the last century. Based on comprehensive national studies using a unified methodology, a great amount of real data on microflora was accumulated, numerous experimental tests were conducted and the following groups of foods were microbiologically standardized:

- Bulgarian yogurts;
- Soft, semi-hard and hard cheeses – national assortments
- Traditional Bulgarian sausages;
- Confectionery creams and products;
- Pulverization-dried egg products;
- Sterilized canned preserves, including those intended for infant and child consumption;
- Infant formulas;
- Dry infant and child foods based on milk

and cereals;

• Millet-ale;

• Herbal spices, herbal drugs, teas.

Those comprehensive tests did not often evidence pathogenic organisms – causes for prevalent food toxic infections, intoxications and infections. Rather their isolation and determination in the regular production of those times was casual. This imposed the comparison of the real data for microflora by major indicators (coliforms, total number of mesophyllic aerobic microorganisms, moulds and yeast) with experimental data for survivability of pathogenic and conditionally pathogenic bacteria at classic technologies and storage conditions through experimental modeling of primary and secondary contamination. The tests covered mainly *Salmonella, Shigella, Staphylococcus aureus, Bacillus cereus, Clostridium perfringens* or *Clostridium sporogenes, Proteus, Listeria monocytogenes, Pseudomonas spp.*

The results revealed interesting findings and solved many problems concerning technology effectiveness, effect of storage conditions, assessment of the extent of risk for each product group. Thus the following facts were proven [9,10,11]:

•• In Bulgarian yogurt the pathogenic and conditionally pathogenic microorganisms – *Enterobacteriaceae (Escherichia coli, Salmonella), Listeria monocytogenes, Staphylococcus aureus* – in infectious doses up to 10^5 CfU/g are fully harmless 48 hours after fermentation start. Bulgarian yogurt is not on the list of risk foods; it is the product that has proven its doubtless "immunity" against infections – one more proof of its probiotic effect known out of Bulgaria, too. This product contains *Lactobacillus delbrueckii, ssp. bulgaricus* and *Streptococcus thermophilus* in amounts not less than 10^8 CfU/g – a prerequisite for its preventive and dietetic properties.

•• In semi-hard Bulgarian white brined cheese made of pasteurized milk *Salmonella spp., Staphylococcus aureus,* a number of conditionally pathogenic *Enterobacteriaceae* are preserved in high infectious doses by the 20-25th day after the onset of biological maturing and perish finally after the 45th day, **ergo** fresh cheese is a particularly risky product and the maturation period of the cheese should not be shorter than 45 days.

•• In fermented milk products the aerobic and anaerobic spore-forming bacteria belonging to *Bacillus* and *Clostridium* preserve their vitality over a much shorter period – 48 to 72 hours. Those microbes are not a source of microbiological risk and have no indicator importance for biologically fermented lactic products – milks and cheeses.

•• Almost similar relationships of pathogenic and conditionally pathogenic microorganisms with lactic acid microflora exist in raw-dried and raw-smoked meat delicacy products (adequately called by a French author "meat cheeses").

•• The national grain-based beverage – millet-ale is a product rich in vitamins of the B group, oligosaccharides and other substances of prebiotic character. There, however, *Salmonella spp., Shigella flexneri, Escherichia coli, Clostridium perfringens, Proteus mirabilis, Pseudomonas aeruginosa,* etc. preserve their vitality up to the natural decay of the product. In spite of the rich yeast population millet-ale does not provide conditions for microbial antagonism and can be a factor of transmission of a number of intestinal infections.

•• The development and formation of toxins of pathogenic clostridia in sterilized canned preserves with pH < 4.5 is with low probability. Bulgarian national recipes for home-made pepper mash and tomato mixes for dishes carry restricted risk for botulism.

•• Residual amounts of *Salmonella* and other pathogenic and conditionally pathogenic bacteria can be detected in pulverization-dried products (dry milks, dried egg powder). During the storage process, though, those microbes perish, most often after 2 to 3-month storage.

•• The confectionery creams, traditional for Bulgaria, are uncontrollable as media for development of pathogenic and conditionally pathogenic microorganisms, primarily or secondarily introduced. Thus the risks in confectionery are real and this food group is epidemiologically significant, requiring strict microbiological norms.

•• Herbal spices – both Bulgarian and exotic – are a powerful source of food contamination with microbes resistant to technological processing and have to be constantly controlled in food industry and catering as carriers of substantial health risk.

The outlined experience and practice of the

health control in the field of microbiological quality and safety of foods contribute to the operative resolving of the numerous complex issues emerging during the last years of restructuring of food industry and opening of Bulgaria to the world market. In conditions of liberal economy the food industry enriched and variegated the types of foods, raw materials, additives, recipes, technologies and packages. New technical means are introduced, as well as modern quality control strategies, the principles of good manufacturing practice and HACCP systems are adopted. This affects the microflora, provides unknown biological risks, sets greater requirements to the microbiological qualities of foods and to the level of control.

In the field of food microbiological control new indicator tests are introduced and the list of pathogenic microbes is constantly revised. This enables more objective assessment of the process hygiene and provides more guarantees for foods epidemic safety. As relatively new indicators implemented in practice during the last two decades *Listeria species, Listeria monocytogenes, Enterobacteriaceae, E.coli 0157, Campylobacter, Bacillus cereus, Enterobacter sakazaki,* etc. [3,4,5,7] can be listed.

The methods for sampling, preparation of the samples for microbiological tests, microbiological analyses of foods and processing and presentation of the results, adopted by the EU legislation and standardized by ISO, have been introduced and implemented in current practice.

Without such development in the field of food microbiological control it is impossible to achieve high quality and competitiveness of Bulgarian foods on both the domestic and international market.

Standardization of foods by microbiological indicators is not a single administrative or legislative act. It is a complex process where contradictory motives and interests often meet – those of medical requirements for harmlessness and safety, customers' for quality, industrial and commercial – for economic adequateness. High costs of microbiological control should not be neglected, either. Strict biological laws of introduction, development, resistance, behavior of microorganisms in foods dictate the principle of interests convergence and joint wise decision making at setting the current microbiological norms guaranteeing first human health protection, and preventing foodborne infectious diseases and toxicoses.

Regulation EC 2073 – 2005 clearly formulates two theoretical aspects of microbiological criteria and norms: one associated with limitation of direct epidemic risks from certain pathogenic microorganisms, and the other – with the indicator importance of a number of microbes groups for the hygiene of production processes. For complete assessment of those two aspects for each food group depending on the numerous ecological, hygienic, technological and epidemic factors it is necessary to combine the two categories of microbiological indicators and norms.

When assessing indicator microorganisms, the multilateral evaluation based on the following principles should be kept in mind:

• Fecal-indicator importance (in a wider context it could be addressed as anthropogenic or biogenic, etc.);

• Technological-indicator importance, i.e. the possibility to serve as indicator of the effectiveness of technological processes;

• The role of the microorganism (or group of microorganisms) in the storage processes – capacities to reproduce and release bacterial exoenzymes that catalyze the decay processes;

• Potential epidemiological risks of which it is a carrier at reproduction and accumulation of large infectious doses.

Evidently the international microbiological norms for foods and particularly Regulation EC 2073/2005 will be constantly enriched and improved depending on the comprehensive analyses of epidemic risks and on the progress in food industry and consumption in the European region and in most countries in the world. At present, though, there is a necessity to set national norms for large food groups that are not covered by this EU regulation but have their place in population nutrition and need similarly microbiological norms. Such criteria and norms have been created based on the above-mentioned national experience and are recommendable for food producers.

Naturally, when necessary, at epidemic indica-

Table 1.
Microbiological criteria and limits for fruits and vegetables for direct consumption without thermal processing

Food category	Microbiological indicators	Units	Sampling plan		Limits	Analytical	Stage of referent method
			n	c	m	M	
FRUIT AND VEGETABLE READY-TO-SERVE AND SEMI-COOKED FOODS OF WASHED, DRESSED AND CUT FRUITS AND VEGETABLES FOR DIRECT CONSUMPTION, COOLED AND FROZEN	*Enterobacteriaceae*	CfU/g ; MPN	5	2	< 1000	<10000	ISO 21528 -1,2
	Escherichia coli	CfU/g	5	2	0/0,01	0/0.001	ISO 7251
	Sulfite-reducing clostridia	CfU/g	5	2	< 100	< 1000	ISO 15213
	**Salmonella species*	in 25,0 g	5	0	0/25,0 g	-	ISO 6579
	Coagulase-positive staphylococci	CfU/g ; MPN	5	2	< 10	< 100	ISO 6888-1-3
	**Listeria monocytogenes*	in 25,0 g	5	0	0/25,0 g	-	ISO 11290-1
	*Monocellular parasites		5	0	n.a.	-	Methods of MH
	*Geohelminths		5	0	n.a.	-	Methods of MH

Stage of criteria application:
Products offered on the market by the expiry data
Actions in case of unsatisfactory results:
Improvement in the selection of raw materials and production hygiene
* Not allowed for direct consumption and drawn out of the market

Table 2.
Microbiological criteria and limits for sterilized canned preserves

Food category	Microbiological indicators	Units	Sampling plan		Limits	Analytical	Stage of referent method
			n	c	m	M	
STERILIZED CANNED PRESERVES	Mesophyllic aerobic and facultatively anaerobic microorganisms						
	· Non-spore forming microorganisms	Not found in .... g	5	0	1,0 g	-	
	· Vegetative forms of spore forming microorganisms	Not found in .... g	5	0	1,0 g	-	BSS 6916-87
	· Spores of saprophyte spore forming bacteria (counted only in canned products not subjected to thermostatic processing)	CfU/g	5	2	10	20	
	*Mesophyllic anaerobic microorganisms	Not found n .... g					
	· Vegetative forms		5	0	1,0 g	-	BSS 6916-87
	· Spore forms		5	0	1,0 g	-	
	**Thermophyllic aerobic and anaerobic microorganisms	Not found n .... g					
	· Vegetative forms		5	0	1,0 g	-	BSS 6916-87
	· Spore forms		5	0	1,0 g	-	
	***Moulds and yeasts	CfU/ g	5	0	< 10	-	ISO 7954

Stage of criteria application:
Products offered on the market by the expiry data
**Criteria applied only for canned infant and young children foods and for canned production intended for offering in tropical countries
***Criteria only for plant canned products and canned foods for infants and small children
Actions in case of unsatisfactory results
Improvement in the selection of raw materials and production hygiene
* Not allowed for direct consumption and drawn out of the market
** Not allowed for direct consumption and drawn out of the market

Table 3.
Microbiological criteria and limits for dried soups, bouillons and sauses

Food category	Microbiological indicators	Units	Sampling plan		Limits	Analytical	Stage of referent method
			n	c	m	M	
	Total count of mesophyllic aerobic and facultatively anaerobic microorganisms	CfU/g	5	2	50 000	100 000	ISO 4833
DRIED SOUPS, BOUILLONS AND SAUCES (meat, fish, vegetable, mixed), powder, granulated, cubes	*Enterobacteriaceae*	CfU/g, MPN	5	2	< 100	< 1000	ISO 21528 -1,2
	Escherichia coli	CfU/g, MPN	5	2	< 10	< 100	ISO 7251
	**Salmonella species*	Not found in ..... g	5	0	25	-	ISO 6579
	Coagulase-positive staphylococci	CfU/g, MPN	5	2	< 10	< 100	ISO 6888-1-3
	Sulfite reducing clostridia	CfU/g, MPN	5	2	< 10	< 100	ISO 15213
	Spores of microscopic mould fungi	CfU/g	5	2	500	10 000	ISO 7954
	Yeasts	CfU/g	5	2	500	10 000	ISO 7954

Stage of criteria application:
Products offered on the market by the expiry data
Actions in case of unsatisfactory results:
Improvement in the selection of raw materials and production hygiene
* Not allowed for direct consumption and drawn out of the market

Table 4.
Microbiological criteria and limits for confectionery products

Food category	Microbiological indicators	Units	Sampling plan		Limits	Analytical	Stage of referent method
			n	c	m	M	
DRY CONFECTIONARY SEMI-COOKED PRODUCTS AND BASES, WAFERS, BISCUIT, CAKES, ROLLS, CROISSANTS, EASTER CAKES, DRY DESERTS, SYRUPED ORIENTAL SWEETS	Coliforms	CfU/, MPN	5	2	< 100	< 1000	ISO 4831, ISO 4832
	Escherichia coli	CfU/, MPN	5	2	< 1	< 10	ISO 7251
	**Salmonella species*	Not found in ..... g	5	-	25,0	-	ISO 6579
	Coagulase-positive staphylococci	CfU/, MPN	5	2	< 10	< 100	ISO 6888-1-3
	Bacillus cereus	CfU/g, MPN	5	2	< 10	< 100	ISO 7932
	Spores of microscopic mould fungi	CfU/g	5	2	100	1000	ISO 7954

Stage of criteria application:
Products offered on the market by the expiry data
Actions in case of unsatisfactory results:
Improvement in the selection of raw materials and production hygiene
* Not allowed for direct consumption and drawn out of the market

Table 5.
Microbiological criteria and limits for ready-to-serve dishes for direct consumption

Food category	Microbiological indicators	Units	Sampling plan		Limits	Analytical	Stage of referent method
			n	c	m	M	
DISHES READY FOR DIRECT CONSUMPTION VEGETABLE, MEAT, FISH, POULTRY AND OTHER MEAT, MIXED	Total count of mesophyllic aerobic and facultatively anaerobic microorganisms	CfU/g	5	2	$<1,0.10^5$	$<5,0.10^5$	ISO 4833
	Coliforms	CfU/g ; MPN	5	2	< 100	< 1000	ISO 4831, ISO 4832
	Escherichia coli	CfU/g; MPN	5	2	< 10	< 100	ISO 7251
	Sulfite-reducing clostridia	CfU/g	5	2	< 10	< 100	ISO 15213
	Bacillus cereus	CfU/g	5	2	100	500	ISO 7932
	**Salmonella species*	Not found in ....g	5	0	0/25,0	-	ISO 6579
	Coagulase-positive staphylococci	CfU/g ; MPN	5	2	< 10	< 100	ISO 6888-1-3
	Pseudomonas species	CfU/g; MPN	5	2	< 10	< 100	ISO 13720
	**Listeria monocytogenes*	Not found in ....g	5	0	0/25,0	-	ISO 11290-1
	Spores of microscopic mould fungi	CfU/g	5	2	< 100	< 500	ISO 7954

Stage of criteria application:
Products offered on the market by the expiry data
Actions in case of unsatisfactory results:
Improvement in the selection of raw materials and production hygiene
* Not allowed for direct consumption and drawn out of the market

Table 6.
Microbiological criteria and limits for herbal spices

Food category	Microbiological indicators	Units	Sampling plan		Limits	Analytical	Stage of referent method
			n	c	m	M	
DRIED LEAF SPICES (savory, celery, parsley, dill, others) OTHER PLANT SPICES (black and white pepper, pimento, nutmeg, cinnamon, clove, cumin, cardamon, ginger, curry, anise, sesame, seed, other) COMBINED SPICE MIXES WITH PLANT SPICES AND FOOD ADDITIVES	Total count of mesophyllic aerobic and facultatively anaerobic microorganisms	CfU/g	5	2	$<1,0.10^6$	$<5,0.10^7$	ISO 4833
	Escherichia coli	CfU/g; MPN	5	2	< 100	< 1000	ISO 7251
	**Salmonella species*	in 25,0g	5	0	25,0	-	ISO 6579
	Coagulase-positive staphylococci	CfU/g ; MPN	5	2	< 10	< 100	ISO 6888-1-3
	Sulfite reduction clostridia	CfU/g; MPN	5	2	< 100	< 1000	ISO 15213
	Spores of microscopic moulds	CfU/g	5	2	$1,0.10^4$	$5,0.10^4$	ISO 7954
	Yeasts	CfU/g	5	2	$1,0.10^4$	$5,0.10^4$	ISO 7954

Stage of criteria application:
Products offered on the market by the expiry data
Actions in case of unsatisfactory results:
Improvement in the selection of raw materials and production hygiene
* Not allowed for direct consumption and drawn out of the market

Table 7.
Microbiological criteria and limits for the national beverage "millet-ale"

Food category	Microbiological indicators	Units	Sampling plan		Limits	Analytical	Stage of referent method
			n	c	m	M	
MILLET-ALE	Coliforms	CfU/g: MPN	5	2	<100	<1000	ISO 4833
	Escherichia coli	CfU/g; MPN	5	2	< 10	< 100	ISO 7251
	Pseudomonas aeruginosa	CfU/g	5	2	< 10	< 100	ISO 13720
	**Salmonella species*	Not found in..... g	5	0	25,0	-	ISO 6579
	Coagulase-positive staphylococci	CfU/g ; MPN	5	2	< 10	< 100	ISO 6888-1-3
	Spores of microscopic mould fungi	CfU/g	5	2	<100	<1000	ISO 7954
	*Other pathogenic organisms (*Shigella, Listeria monocytogenes*) : not admitted in 25,0 g (tested only on epidemic and other special requirements)						

Stage of criteria application:
Products offered on the market by the expiry data
Actions in case of unsatisfactory results:
Improvement in the selection of raw materials and production hygiene
* Not allowed for direct consumption and drawn out of the market

Table 8.
Microbiological criteria and limits for food supplements

Food category	Microbiological indicators	Units	Sampling plan		Limits	Analytical	Stage of referent method
			n	c	m	M	
FOOD SUPPLEMENTS	Coliforms	CfU/g: MPN	5	2	< 10	< 100	ISO 4831
	Escherichia coli	CfU/g; MPN	5	0	< 1	< 10	ISO 7251
	**Salmonella species*	Not found in..... g	5	0	25,0	-	ISO 6579
	**Listeria monocytogenes*	Not found in .....g	5	2	25,0	-	ISO 11290-1
	Coagulase-positive staphylococci	CfU/g ; MPN	5	2	< 1	<10	ISO 6888-1-3
	Pseudomonas aeruginosa	CfU/g	5	2	< 10	< 100	ISO 13720
	Spores of microscopic moulds	CfU/g	5	2	< 100	< 500	ISO 7954
	Yeasts	CfU/g	5	2	< 100	< 500	ISO 7954
	**Specific probiotic microorganisas deslared in the product formula	CfU/g; MPN	5	2	> 106	> 105	ISO 15214 ISO 7889 ISO 9232 Or other

Actions in case of unsatisfactory results:
Products offered on the market by the expiry data
** The criteria is applied only for food supplements containing probiotics
Microbiological indicators:
Improvement in the selection of raw materials and production hygiene
* Not allowed for direct consumption and drawn out of the market

Table 9.
Microbiological criteria and limits for mayonnaise and emulsion products

Food category	Microbiological indicators	Units	Sampling plan		Limits	Analytical	Stage of referent method
			n	c	m	M	
EMULSION PRODUCTS (MAYONNAISE, SAUCES, DRESSING)	Coliforms	Not found in .....g; MPN	5	3	0,1 <10	0,01 <100	ISO 4831 ISO 4832
	Escherichia coli	Not found n .....g	5	2	1,0 < 0,3	0,1 < 10	ISO 7251
	Sulfite reducing clostridia	CfU/g	5	2	< 10	< 100	ISO 15213
	Bacillus cereus	CfU/g	5	2	< 50	< 500	ISO 7932
	**Listeria monocytogenes*	Not found in .....g	5	2	25,0	-	ISO 11290-1
	**Salmonella species*	Not found in..... g	5	0	25,0	-	ISO 6579
	Coagulase-positive staphylococci	CfU/g ; MPN	5	2	0,1	-	ISO 6888-1-3
	Spores of microscopic moulds	CfU/g	5	2	<200	<500	ISO 13720

Stage of criteria application:
Products offered on the market by the expiry data
Actions in case of unsatisfactory results:
Improvement in the selection of raw materials and production hygiene
* Not allowed for direct consumption and drawn out of the market

tions or need of more comprehensive studies of particular hygienic and production problems the recommended indicators can be supported with a wider set of microorganisms characteristic to a certain extent for the particular product, with specific indicator or epidemic importance.

In conclusion we present updated recommendable microbiological norms for some large groups of Bulgarian foods with which the producers can guarantee not only epidemic safety but the hygiene of production, storage and trade processes. These are only examples and are far from covering the overall variety of foods which classification from the aspect of microbiological safety is much more difficult and variegated.

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TOXIC ELEMENT CONTAMINANTS IN FOOD ADDITIVES AND IN SOME BULGARIAN SUPPLEMENTS

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INTRODUCTION

Food supplements are concentrated sources of nutrients or other substances with a nutritional or physiological effect whose purpose is to supplement the normal diet. They are regarded as a category of food rather than as medicines and are marketed 'in dose' form i.e. as pills, tablets, capsules, liquids in measured doses, etc.

Food additive is any substance, which is not consumed as a food ingredient by itself, but which is intentionally used in the processing of raw materials, foods, or their ingredients, to fulfil a certain technological purpose during treatment or processing.

In the 1980s the policy of the US Food and Drugs Administration was to reclassify supplements as medicines and since 1994 the US has had a statute that guarantees both free availability of supplements and information about how they work. Similarly, in Canada in the 1990s, the government reclassified hundreds of herbal remedies as medicines. Following public outcry, however, they were all later declassified.

In Europe, there has been a much more varied legislative climate with regard to food (dietary) supplements, with a small number of countries such as the UK and the Netherlands regarding food supplements as a category of food.

At the moment, food supplements and food additives in the European Union are sold under food law: specific rules only on vitamins and minerals have been laid down by the EU Food Supplements Directive 2002/46/EC (FSD); the authorization and use of food additives are based on the framework Directive 89/107/EEC. On the basis of the framework Directive three specific directives were adopted: on sweeteners (94/35/EC2), colours (94/36/EC3) and on miscellaneous additives - other than colours and sweeteners (95/2/EC4).

In order to protect public health maximum levels (MLs) are set for certain contaminants in foodstuffs by EC Regulation 466/2001. Food ingredients used for the production of compound foodstuffs should comply with the maximum levels set in this Regulation prior to addition to the said compound foodstuff in order to avoid dilution. Lead, Cadmium, Tin and Mercury are the included toxic elements for which MLs are stated. For Pb and Cd the limits are set for different kinds of food; contamination with Hg may present a serious risk in fishery products; Sn migration is essential to be controlled in canned food. There are no limits for Arsenic in food stated in this regulation. Specific criteria of purity concerning sweeteners, colours and miscellaneous food additives for use in foodstuffs are laid down by Directives 95/31/EC, 95/45/EC and 96/77/EC, respectively.

In Bulgaria during the pre-accession period the new EU food safety regulations and directives were introduced into the national legislation by specific ordinances of the Ministry of Health.

After the 1st of January 2007 we have to implement the European food law.

The aim of the study was to determine the concentrations of Arsenic, Cadmium, Lead and Mercury in food additives and Bulgarian supplements of herbal origin in order to create an information database for toxic element contaminants in these foodstuffs and regarding these elements to estimate their safety for human consumption.

MATERIALS AND METHODS

For the purpose of the study different number and kinds of food additives and three groups of food supplements of herbal basis were investigated as follows:

Supplements

- Herb dry extracts (in tablets)
- Herb liquid extracts (alcohol or alcohol/water)
- Dry herbs (in tablets)

Food additives – from the sweetener and miscellaneous groups.

Sample preparation

- Microwave digestion for herb dry extracts and dry herbs using concentrated HNO_3 and HCl for determination of Pb, Cd and As.
- Microwave digestion (HNO_3 and HCl) after evaporation of the organic solvent for herb liquid extracts for determination of Pb Cd, and As.
- MIBK-APDC extraction for food additives (sodium hydrogen carbonate, ammonium hydrogen carbonate, sodium metabisulphite) for determination of Pb and Cd.
- Nitric acid extraction for food additives (emulsifiers, sodium arginate, saccharine based sweetener, Soya flour, b-carotene, potassium sorbate) for determination of Pb, Cd and As.

Trace element analysis

Apparatus:

Microwave Sample Preparation System "Multiwave", Anton Paar GmbH, Austria.

Three Perkin-Elmer atomic absorption spectrometers (Bodenseewerk Perkin-Elmer, Ueberlingen, Germany) were employed:

- A Model 4110 ZL atomic absorption spectrometer (AAS) with a transverse heated graphite atomizer and longitudinal Zeeman-effect background correction - for determination of lead and cadmium.
- A Model 3030 AAS with a Mercury/Hydride System MHS-20 and automatic deuterium background corrector - for determination of arsenic.
- A model 3110 AAS with air/acetylene flame atomizer and automatic deuterium background corrector - for determination of lead and cadmium in some samples after extraction
- Direct Mercury Analyzer DMA-80 (Milestone - Italy) - for direct analysis of mercury.

Methods

The analytical methods used for determination of the toxic elements of interest are developed and validated in the Element composition laboratory. Sample preparation and instrumental parameters were optimized for every element and kind of sample. These methods are included in the Book of methods of the accredited (ISO 17025) Test Centre "Higihrantest" of the National Centre for Public Health Protection.

RESULTS AND DISCUSSION

The detection limits (DL) for the examined elements are calculated on the basis of the sample aliquot weights, which are appropriate for the instrumental techniques used. This explains the "inconsistencies" at first sight when looking at the DLs, which differ for one and the same element between various samples.

The Pb concentrations of the analyzed food additives (Table 4) are between < 0,10 and 3,30 mg/kg; both Cd and Hg are below the DLs; As vary from <0,03 to 0,37 mg/kg. All the values are below the recommended max levels (Table 5). Regarding these elements the investigated additives are safe to be put into food following the quantitative limits for the use of a food additive, or according to the technology of production - only as much as necessary to achieve the desired effect.

The results obtained for the Pb, Cd, As, and Hg content in the investigated food supplements are presented in Tables 1, 2 and 3.

As a whole the determined concentrations of the analytes vary in a few orders. For all of the elements the highest levels are established in the tablets of dry herbs: Pb between 0,62 and 10,2 mg/kg; Cd from 0.054 to 0,86 mg/kg; As from 0,10 to 2,20 mg/kg and Hg from 0,0052 to 0,0087 mg/kg (Table 3). The lowest toxic element concentrations are in the herb liquid extracts: Pb between 0,008 and 0,30 mg/kg; Cd from 0,001 to 0,006 mg/kg; As from 0,0008 to 0,045 mg/kg and Hg from <0,0016 to 0,0052 mg/kg (Table 2).

Concentrations of the elements range between the batches of one and the same product and producers of various supplements owing to the origin of the herbs and purity of the additional ingredients used. Vegetative extracts have higher concentrations for all the analytes than the other products of this group (Table 1).

The differences of the toxic element concentrations between the three groups are due to the nature of the products. The dry herb tablets include much more dried and finely grounded

herb mass and ingredients (additives), the second group with medium concentrations of the investigated elements is a composite of dry herb extracts with additives, whereas the liquid extracts don't contain dried solid matter and are diluted in alcohol.

Are these food supplements safe for human consumption based on the results obtained for the toxic element contaminants?

Estimation is a difficult task.

First a reference to the MLs stated in Annex I of Regulation (EC) No 466/2001 has to be made. For Pb and Cd in fresh herbs they are 0,3 mg/kg and 0.2 mg/kg, resp. Further down in Article 2 it is said that in the case of products which are dried, diluted, processed or composed of more than one ingredient, the maximum level applicable shall be that laid down in Annex I, taking into account: (a) changes of the concentration of the contaminant caused by drying or dilution processes, (b) changes of the concentration of the contaminant caused by processing, and (c) the relative proportions of the ingredients in the product.

The analyzed food supplements are compound products with active substances of dried herbs. On the other hand the "more than one ingredients" are miscellaneous food additives. For them max levels of Pb, Cd, As and Hg are set in the Commission Directive 96/77/EC (Table 5) and their safety has to be assessed according to these purity criteria. The recalculation of the limits for the toxic elements in the final product is not feasible because of shortage of information about dry mass and the quantities of the herbs and additives included, the producer's 'know-how', i.e. lack of information about the technological recipes and processing.

How to overcome this complicated situation?

To provide a factual evaluation in order to assure the safety of food supplements:

• There is lack of sufficient information about the herb dry mass, processing, recipes, etc.

• No MLs are stated for these kinds of food supplements.

BUT:

• Herbs have been used for hundreds of years by humans;

• The purity criteria for toxic elements in food additives are high enough (they vary but not so much between different products), so the determined concentrations in the examined supplements remain below them;

• The daily intake is low (small quantities of the supplements are used 2-3 times daily).

So, where there is not enough information for recalculation of the limits according to the EC Regulation 466/2001, in our opinion, safety evaluation of the food supplements of herbal basis regarding Pb, Cd, Hg and As can be made according to the Commission Directive 96/77/EC, where for a lot of the food additives the requirements are no more than: 3 mg/kg for As, 5 mg/kg for Pb, 1 mg/kg for Hg and Cd and 10 mg/kg for Heavy metals (as Lead).

This assumption is helpful as a reference point, but will not resolve the problems which appeared because of the lack of stated limits for toxic element contaminants in food supplements: for instance, in Supplement 1 (Table 3) the determined Lead concentration (10,2 mg/kg) is two times higher towards the max level for that element, but compared to the limit for Heavy metals (as Lead) it remains within the limits of uncertainty of the analytical method.

Herbal food supplements have specific physiological effects on human health...How to evaluate whether they are safe?... Asking questions may continue ad infinitum.

CONCLUSION

To paraphrase Paracelsus, 'exposure isn't toxicity'. As a whole, food is comprised of 'chemicals', but concerning food additives there are no known health issues except occasional hypersensitivity – not hyperactivity (M. E. Knowles, 2004). One might say the same about food supplements. For their use and safety evaluation the key principle remains the Hippocratic requirement "primum no nocere".

References:

1. Directive 2002/46/EC of the European Parliament and of the Council on the approximation of the laws of the Member States relating to food supplements

2. Council Directive (89/107/EEC) on the approximation of the laws of the Member States concerning food additives authorized for use in foodstuffs intended for human consumption

3. Commission Directive 95/31/EC laying down specific criteria of purity concerning sweeteners for use in foodstuffs

4. Commission Directive 96/77/EC laying down specific purity criteria on food additives other than colours and sweeteners

5. Commission Regulation (EC) No 466/2001 setting maximum levels for certain contaminants in foodstuffs

6. Knowles, E., EU Legislation on Food Additives, *IUFoST Berlin Symposium*, Berlin, Germany, 25/26 May 2004.

Table 1.
Concentrations of Pb, Cd, As and Hg in food supplements based on herbal or vegetative dry extracts (in tablets)

Food supplement	Lead mg/kg	Cadmium mg/kg	Arsenic mg/kg	Mercury mg/kg
"Shlemnik Baicalski"2005 (I) (Scutellaria baicalensis Georgi).	< 0,016	0,001	0,022	< 0,002
"Shlemnik Baicalski"2005 (II)	< 0,016	0,001	0,090	< 0,0016
Vegetative extract	1,00	0,33	0,97	-
Vegetative extracts	2,07	0,86	0,036	0,0051
Tribulus Terresttris	0,80	<0,08	0,14	

N.B. "<" bellow DL of the method used

Table 2.
Concentrations of Pb, Cd and As in food supplements based on herbal and vegetative liquid extracts (alcohol or alcohol/water)

Food supplement	Lead mg/kg	Cadmium mg/kg	Arsenic mg/kg
Food supplement with honey and herb extracts	0,03	<0,005	0,0008
Echinacea purpurea	0,30	0,002	0,045
Food supplement with garlic extract (allium sativum) and herb extracts (Thimus sp. div,Mentha Piperita, Satureja Montana, Ocimum Basilicum,etc)	0,13	0,005	0,0038
Muzhik root	< 0,020	< 0,003	< 0,002
Biogenic extract from Pfaffia paniculata	0,65	0,006	-
Bio extract from Ptychopetalum olacoides	0,028	0,002	-
Ethanol extract from Eleutherococcus senticosus maxim	0,008	0,001	< 0,005

N.B. "<" bellow DL of the method used

Table 3.
Concentrations of Pb, Cd, As and Hg in food supplements based on dry herbs (in tablets)

Food supplement	Lead mg/kg	Cadmium mg/kg	Arsenic mg/kg	Mercury mg/kg
Supplement 1 (Coltsfoot, Plantain, Pine nibs, Mallow, Field Eryngo, etc.)	10,2	0,11	0,10	0,0055
Supplement 2 (Restharrow, Linseed, Lady's mantle, Dandelion, Juniper berries, etc.)	1,87	0,13	0,14	0,0087
Supplement 3 (Linseed, Garden dill, Coriander, Chycory, Elecampane, etc.)	0,84	0,12	0,18	0,0071
Supplement 4 (Plantain, Hazelebush, Christ's-thorn, Horsetail, Balm, etc.)	0,62	0,058	0,48	0,0052
Supplement 5 (Black chokeberry, St.John's wort, Vervain, Balm, Lavender, etc.)	2,05	0,41	2,20	0,0061
Supplement 6 (Small Caltrops, Thistle, Five-finger grass, Hazelbush, etc.)	0,73	0,054	0,18	0,0055
Supplement 7 (Hops, Rosemary, Peppermint, Lavender, St. John's wort, Speedwell, etc.)	0,98	0,070	0,16	0,0064
Supplement 8 (Willow, Birch, Pine nibs, Juniper berries, Meadowsweet, etc.)	1,42	0,15	0,34	0,0054
Supplement 9 (Senna, Buckthorn, Rhubarb, Marigold, Orange rind, Alfalfa, Linseed, Peppermint, etc.)	1,92	0,14	0,23	0,0057
Supplement 10 (Betony, Horsetail, Hazelbush, Bedstraw, Corn silk, Juniper berries, Field Eryngo, etc.)	1,38	0,12	0,23	0,0075

N.B. "<" bellow DL of the method used

Supplement 1 – for all types of diseases of the respiratory tract
Supplement 2 – for haemorrhoids
Supplement 3 – for all diseases of the digestive system
Supplement 4 – for varicose veins
Supplement 5 – for a difficult climax
Supplement 6 – for diseases of the prostate gland
Supplement 7 – for diseases of the nerve system
Supplement 8 – for diseases of bones
Supplement 9 – for constipation and overweight
Supplement 10 – for all types of renal diseases

Table 4.
Concentrations of Pb, Cd, Hg and As in food additives

Food additive	Lead mg/kg	Cadmium mg/kg	Arsenic mg/kg	Mercury mg/kg
Emulsifier(distilled monoglycerides)	0,40	-	<0,03	<0,008
Sodium arginate	1,10	-	0,37	< 0,008
Emulsifier (polyglycerol ester of ricinoleic acid)	<0,25	<0,25	-	< 0,008
Sweetener (saccharine based)	<0,10	<0,025	0,015	-
Soya flour	-	-	0,095	< 0,008
b-carotene	0,40	-	0,035	<0,0016
Potassium sorbate	< 0,10	-	< 0,04	-
Sodium hydrogen carbonate	From 0,10 to 1,30	-	-	-
Ammonium hydrogen carbonate	From <0,10 to 0,85	-	-	-
Sodium metabisulphite	From 0,10 to 0,60			

N.B. "<" bellow DL of the method used

Table 5.
Purity criteria for some food additives concerning Pb, Cd, Hg and As content according to the Commission Directives 96/77/EC and 95/31/EC

Product	Arsenic mg/kg	Lead mg/kg	Mercury mg/kg	Cadmium mg/kg	Heavy metals (as Lead) mg/kg
For a lot of the food additives the requirements are no more than:	3	5	1	1	10
β-carotene	3	5	1	1	
Potassium sorbate	3	5	1		10
Sodium hydrogen carbonate	3	5	1		
Ammonium hydrogen carbonate	3	5	1		
Sodium metabisulphite	3	5	1		10
Polyglycerol esters of ricinoleic acid	3	5	1	1	10
Saccharine	3	1			10

BULGARIAN ADDED VALUE TO ERA

INSTITUTE OF CRYOBIOLOGY AND FOOD TECHNOLOGY – SOFIA

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According to the Decree No. 270 of the Council of Ministers of the Republic of Bulgaria, dated December 15, 2000 the Institute of Cryobiology and Lyophylization – Sofia, Department "Chemical and Biochemical Investigation of Plant and Animal Products" and Department "Technology of Grain Storage and Bread Production" of the Institute of Grain Foods and Feed Industry – Kostinbrod, the Facility for Development and Implementation of Ionizing Emissions – Sofia, the Institute of Brewing and Hop Industry – Sofia, the Facility for Development and Implementation of Biologically Active Substances – Sofia and the Institute of Meat – Sofia shall be merged into **Institute of Cryobiology and Food Technology** with headquarters in Sofia, with the subject of activities in the field of cryobiology and food technologies.

It is evident that the Institute of Cryobiology and Food Technology (ICFT) combined the well-known scientific institutions with serious contributions to the development of Bulgarian agricultural science and practice.

The ICFT is the only scientific organization of its kind in the system of the National Centre of Agricultural Sciences and Ministry of Agriculture and Forestry that provides scientific service of different branches of the food industry – cryobiology and lyophylization, brewing technology, cereal and bread products, meat products by applying technological and methodical extension service, consultation, analyses, methods and technologies for radioecology and radioactive protection of agriculture and food industry, etc. Effective scientific service is provided also for the agricultural producers by means of contemporary bioactive substances –growth stimulators.

In the XXI century, the mass globalization on a world scale and forthcoming EU integration of Bulgaria face new challenges and priorities to ICFT. The scientific investigations and projects cover thematically both the final objectives of the European scientific programmes and those of NATO - "Food, Agriculture and Biotechnology" "Food for Life", "Food Safety and Quality", "Science for Peace" "Astronautics", etc., in which ICFT participates actively in collaboration with multinational scientific consortia.

The interdisciplinary nature of the scientific activity of ICFT is a prerequisite for implementation of many scientific ideas and concrete goals.

Cryobiology and Lyophylization Department. The Institute of Cryobiology and Lyophylization was founded 32 years ago. First, it was established as a Central Laboratory of Cryobiology and Lyophilization (CLCL) at the Institute of Meat Industry. Since then Academician Tsvetan Tsvetkov is the director of the CLCL and of the Institute of Cryobiology and Food Technology. In 1989, CLCl turned into a Scientific Research Institute of Cryobiology and Lyophilization in the structure of the Agricultural Academy (at present-National Centre of Agricultural Sciences).

ICFT has preserved its traditions and experience of many years in the field of cryobiology, on the basis of which the first Bulgarian space menu was developed in relation to the accomplishment of the mutual international project

"Shipka" and two joint Bulgarian-Russian flights. This menu, consisting of over 50 various foods, is a unique combination of Bulgarian national dishes and highly developed food cryobiotechnologies. Due to the above-mentioned space menu, Bulgaria takes a leading role (3rd place after the countries of Russia and the USA) among the producers of space foods. Moreover, new types of lyophilized products and complex food regimens for limited contingents under extreme conditions are also developed.

On the basis of already existing own hypotheses for the cold resistance of animals and plants and mechanisms of cryoprotection by low temperatures and freeze-drying conservation of biomaterials of various origin, the following technologies and regime parameters for their freeze-drying and cryogenic preservation for shelf life are developed:

- original, highly effective method for determination of the cold resistance of wood and other plant species based on dynamic and mechanical analysis and differentiated scanning caloriemetrics. The scientific results could be multiplied in the field of plant growing and ecology;

- experimental cryobank for long-term conservation of genetic plant material by applying universal technological models of cryopreservation under laboratory conditions and suitable cryoprotectors. The perspective of this highly technological approach is determined by the possibility of preservation of rare, valuable mutants, hybrid and unique cellular cultures and possibility for maximum preservation of plant material, necessary for selective investigations to be conducted;

- investigating the method for low temperatures and dehydrating changes of sample and natural biomembrane and cellular organels;

- lyophilized biopreparations for medical practice used by treatment of wounds of various nature, materials for biotransplantation (e.g. treatment of tumor defects); are considered to be completely original scientific contribution;

- functional foods and bioproducts – over 60 types, applied by prevention of many contemporary diseases and for improvement of the overall healthy status of the people;

The following awards are also worth outlining: 1987 INRA – Diploma of the established "Inventions" biopreparations and haemostatic sponge – awarded as "The best invention"; 1993 Diploma of the Federation of Astronautics in Russia; 1999 Diploma Eureca, Brussels for the bioproduct "Bodinorm"; 1999 Diploma for supporting the international expedition to Everest, Skopje; Gold medal "Archimed 2000" in Moscow for scientific achievements in the field of highly-developed food technologie; 2003 "Excellence Award"; Pensilvania; Cup and honourary diploma "Eurointellect", etc.

Acad. Tsvetan Tsvetkov, the director of ICFT, is a member of many years of the international issue "Cryobiology". He is also awarded with the prestigious title of Doctor Honoris Causa by the University of Food Technology, Plovdiv, Bulgaria.

Brewing Department. The research in the field of raw materials and brewing started in 1956 with the establishment of the Department of brewing technology at the Research Institute of Wine-making and Brewing in Sofia. The joint research activities continued till 1971, when the Centre for Research, Development and Design of Brewing and Soft Drink Industry was created. The next organization stages were transformation to the Centre for Research and Development of Brewing Industry, the Institute of Brewing and Hop Industry and Brewing department at the Institute of Cryobiology and Food Industry.

In the coarse of these 50 years the brewing scientists and specialists had a very important or decisive participation in reconstruction,innovation and new building of different breweries; agrobiological and technological investigation of new brewing barley and hop varieties; development and implementation of technologies for ordinary, original, special, luxury, alcohol-free, low-alcoholic, herbal and export beers with 12 months shelf-life; investigation and implementation of new hop products, enzymes, filter aids and stabilizing agents; investigation, assessment and support of the brewing yeast collection and delivery of pure yeast cultures to the breweries; scientific, technical and economic information for the brewing industry. Many well-known scientists in the function of directors, deputy-directors and department managers of the former Institute of Brewing and Hop Industry contributed to the devel-

opment and prosperity of the Bulgarian brewing industry.

Today the Brewing Department of the ICFT is the only specialized research unit in the country in the field of technology, physics, chemistry, biochemistry, microbiology and safety of hops, brewing barley, malt, brewing yeasts and beer, which carries out:

- Investigation on the economic and technological quality of new European and Bulgarian brewing barley varieties;
- Development and implementation of production technology for new beer brands, functional beverages and food on the malt and hops basis;
- Investigation and implementation of new malt adjuncts, hop products, filtering materials, stabilizing agents and packaging in the brewing;
- Development and implementation of new technological and technical solutions in brewing industry – pub-, small- and medium-size breweries;
- Development and implementation of technologies for malting and brewing by-products utilization;
- Development and implementation of new methods for analysis of raw materials, intermediate products, beers and other beverages;
- Physical, chemical and microbiological analysis of brewing water, hops and hop products, barley, malt adjuncts, wort, beer, enzymes, filtering materials and stabilizing agents, consultation, extension services, etc;
- Issue of import and export certificates for hops and hop products, barley, malt, and beer.
- Qualification and training courses, seminars and workshops for brewing and food industry;
- Elaboration and harmonization of standards, technical specifications and other normative documents, manuals for GMP, GHP and HACCP;
- Investigation on bioethanol and biodiesel production.

The Cereal and Bread Products Department. The research in the field of cereals and bread started in 1954 by formation of Central Laboratory for Bread Making. The outstanding activity of the unit was the reason for creation in 1967 of the Scientific and Research Institute for Grain Storage, Grain Processing and Bread. This institute was transformed in 1976 to the Institute for the Cereals and Fodder Industry.

In the coarse of 52 years the three main research lines – technology of grain storage, milling technology and bread and baked goods – investigated and developed a technology for active ventilation and grain drying at low temperature, a system of indicators and methods for establishing the functional properties of soft and trade grains, sorts and trade classifications of the plain wheat in Bulgaria, model technological schemes for milling of plain wheat, intensification of the milling process, increasing of yield and quality of different milling products; the influence of mechanical impact on the development of dough during processing by machines was clarified; technologies for production of bread, bread products, children's foods for dietary and rational nourishment, instant and extruded foods, intolerance to gluten and others were created by using of natural food additives.

At the present time the Cereal and Bread Products Department carries out:

- research on the changes in quality of cereals during storage with the aim of optimization of the storage conditions;
- research on the heat and mass-interchange processes during treatment and storage of grain;
- development of ecologically appropriate and energy-saving technological processes;
- intensification of the milling process;
- creation of model technological schemes for milling wheat;
- special milling technologies for producing flour, kernel and semolina products from corn, rye, rice, oats and soy;
- investigation of the baking quality of flours taken from different harvests of wheat grain and giving recommendations for their use in practice;
- improvement and adaptation of methods for evaluation of raw materials, semi-products and end products in bread making;
- creation of new types of bread and pasta products, children's food and extruded foods on grain basis for prophylaxis, diet and rational nutrition.

Technology of Animal Products Laboratory. The laboratory is a continuer of the tradition of

the Institute of Meet and carries out activity mainly in the field of poultry meet processing:

• delicacy stew-smoked products;

• low-perishable sausages;

• hams and ham type sausages;

• semi-manufactured products from minced meat;

• semi-manufactured products from non-minced meat;

• low-energy meat foods.

The laboratory renders scientific and technical assistance for documents preparation for poultry meet production and processing and physical and chemical analyses of meat products.

Radioecology, Radioprotection and Ionizing Emissions Department. The scientists and specialists of the department accomplish:

scientific research in the field of stimulation by radiation, sterilization by radiation and foodstuffs irradiation;

• radiosterilization of biomaterials, pharmaceutical and other products and objects;

• seeds stimulation by radiation;

• radiological, physical, chemical and microbiological analyses of foods;

• glass objects irradiation for change of crystalline bars;

Technology and Chemistry of Biologically Active Substances Department. The department carries out:

• scientific and extension service in the field of cultural plants grow regulation;

• investigation and creation of new biologically active products with guaranteed ecological purity;

• ellaboration and implementation of highly effective methods and technologies on the biologically active substances basis for agricultural cultivars yield and quality increasing.

The remarkable intellectual collection of ICFT includes over 150 patents, books, monographs, author's certificates, technological regulations, etc.

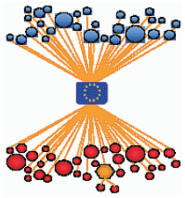
The contribution of ICFT is also proved by the numerous publications in international scientific editions, alongside with its membership as a scientific institution and scientists within the structures of lots of prominent international organizations for more than 30 years: Association of Cryobiology, International Institute of Cold, Paris, American Association of Tissue Banks – AATB, International Confederation of Thermic Analysis and Calorimetrics, Japanese Society for low temperature medicine Parenteral Society, International Organization of Yeast; International Organization of Genetics and Molecular Biology; Commission of Molecular Genetics and Cellular Biology at UNESCO, Barley and Malt Committee and Analysis Committee of European Brewery Convention.

Conductance of multidisciplinary,scientific activity on a large scale, as well as the introduction of scientific projects into practice, is possible due to the efforts of a modest scientific staff with diverse professional qualification – physisists, food technologists, biologists, biotechnologists, engineers, mathematicians, agronomists, etc. About 50% of them are scientists with scientific degrees as follows: academician, professors, associate professors and associate assistants. About 30% are doctors of science and doctors.

ICFT conducts training activity of young scholars and offers academic environment suitable for academic discussions, disposes of unique apparatuses and technological equipment for implementation of scientific research and experimental work. In the course of years, 59 Ph.D. students have successfully defended their dissertations at ICFT.

Annually lectures and seminars are held at ICFT, experiments are accomplished with the purpose of training students from the Faculty of Biology at Sofia University St. Kliment Ohridski in subjects "Biotechnology" and "Physiology of plants" and from the Faculty of Agronomy at the University of Forestry.

Scientific achievements of ICFT in the field of contemporary cryobiotechnoilogies and conventional and new food technologies are of importance for the development of our and world economy and scientific progress, as well as for the economy of the country in a series of its branches closely connected with safety and security of the population under extreme conditions as to improve the ecoprotection of natural and human resources.



MADE IN BULGARIA WITH EUROPEAN SUPPORT

ROOIBOS TEA EFFECTS ON WORKERS OCCUPATIONALLY EXPOSED TO LEAD

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INTRODUCTION

One of the most important advances in medical science of the last decade was the realization that reactive oxygen species (ROS) are the ultimate molecular events in the pathology associated with most diseases [34]. Their chemical reactivity can damage all types of cellular macromolecules,including proteins,lipids,and nucleic acids. The human body has mechanisms for defense: 1) a system of enzymes, including superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH); 2) a small-molecular-weight compound, which acts as antioxidants (glutathione, uric acid and ubiquinol produced by normal metabolism); 3) Others are provided by foods (vitamin E, vitamin C, carotenes, phenolic or polyphenolic compounds) [30].

Unfortunately, our natural defences are imperfect, they limit the harm caused by oxygen but do not eliminate it completely [30]. The imbalance of ROS and antioxidant systems results in oxidative stress that may lead to chemical modifications of biologically relevant macromolecules and provides a pathobiochemical mechanism for the initiation and development of numerous diseases [14, 21].

Lead is one of the most abundant heavy metals in the environment. According WHO [50], although many countries have incited programs to lower the level of lead in the environment, human exposure to lead remains of concern to public health officials worldwide. Main sources of this pollutant are the industries where lead is either produced or utilized. Major uses are in batteries, cables, pigments, petrol (gasoline) additives, solder and steel products. Lead produces a spectrum of adverse effects on health depending upon the level and duration of exposure. Renal, cardiovascular, reproductive and immune systems are affected. Elevated blood pressure is one of the critical effects of lead exposure in adults [1, 20]. Data obtained in some studies indicate that lead activates the processes of lipid peroxidation and that toxicological effects of lead are connected with the harmful effects of ROS [4, 26, 44]. The effects of dietary antioxidants have been extensively evaluated in epidemiological, population, and clinical studies [2, 33]. Polyphenols, particularly flavonoids are one of the hottest areas of dietary research and a se-

ries of studies have indicated their antioxidant role due to free-radical scavenging properties [38, 39].

Tea, which is one of the most widely consumed beverages across the globe, is a rich source of antioxidant flavonoids and has been linked with a reduced risk of coronary heart disease, cancers and stroke [3, 13, 28, 40]. Already there are investigations providing data for protective effect of tea catechins and other antioxidant substances against lead-induced cytotoxicity, lipid peroxidation and other indices of oxidative stress [11, 18, 19].

Rooibos tea (Aspalathus linearis) is a kind of herbal tea and it is widely used for its beneficial effects on health, linked to its high content of rich mix of polyphenols/flavonoids and their antioxidant properties. The major one is Aspalathin, found only in Rooibos and nowhere else. A number of other flavonoids have been identified including quercetin, luteolin, rutin, nothofagin, vitexin, isovitex, orientin, iso-orientin, as well as phenolic carboxylic acids [17, 37]. Rooibos is caffeine-free and has low tannin content. The tea contains also vitamin C and several minerals as calcium, potassium, magnesium, zinc as well as trace elements such as copper and manganese. Laboratory studies have shown that the antioxidant activity of Rooibos is similar to that of green tea but it is stronger than of black and oolong tea [27, 48]. It was determined that scavenger activity of Rooibos tea has been markedly higher compared to those of coffee and some vegetables [32]. It is considered that Rooibos tea possesses antioxidative effect by SOD, which is an important component of enzymatic antioxidant defence. Rooibos tea has shown that it lowers high blood pressure - one of the critical effects of lead intoxication. This is a basis to foresee possible favourable influence of the tea on workers occupationally exposed to lead and to study antioxidant and antilipidemic effects of Rooibos tea. Although tea flavonoids possess antioxidant properties in vitro, there has been little evidence to date of in vivo effects.

PURPOSE

To examine the effects of drinking of Rooibos tea upon disturbances in antioxidant status and other health injuries of workers occupationally exposed to lead and evaluate if the tea may be able to confer health benefit following its consumption.

DESIGN AND METHODS

Study design

We carried out a randomized placebo-controlled eight weeks intervention trial, performed on occupationally exposed to inorganic lead workers from a plant for battery production in Bulgaria. Rooibos tea was prepared according to the recommendations of researches from South Africa and Japan in order the substances showing antioxidant activity to be fully extracted [32]. Workers from the experimental group (EG) have taken 1 litre Rooibos tea (four filter bags of tea containing 10 grams dry leaves were brewing in 1 litre boiled water for 10 minutes in a covered stainless steel pot at 80-98^{0}C). Tea was distributed in special well-closed bottles among the investigated workers in the beginning of working time. Two bottles of tea additionally were prepared on Friday for the weekend and were given to the workers. The workers from the control group (CG) have taken 1 placebo tablet of 200 mg per day containing maltodextrin, starch, microcrystal cellulose, magnesium stearate (0.4%) and silicon dioxide and they were given 1 litre of water to drink as well in order to have the same liquid intake as the EG. Taking of the tea by the EG and the placebo tablet with water by the CG during the working time was controlled regularly by the nurse in the factory. Before and after the intervention venous blood was taken to measure main indices for biological monitoring of lead exposure, indices of antioxidants status in blood,and indices of lipid status. The blood samples immediately were driven to the laboratories and were stored at 4^0 C during the transportation.

Blood pressure values were monitored. Dietary intake has been recorded for 3 days. Weight and height were measured. Information on demographics, life style habits, work history, medical history, and medication use was obtained by examiner-administered questionnaire implementing a face-to-face interview. The information obtained from the workers has been verified with that available in the health records.

Subjects

Workers were randomly selected from the

total workforce of 150 workers regularly exposed to lead and the sample recruited represented 60% of it. 90 male workers were initially voluntary recruited on the basis of data from recorded air concentrations of lead. Lead concentration in blood was concerned as well using data from previous measurements. All participants provided written, informed consent. After the baseline investigation the workers were divided into two groups: 45 subjects in the EG and 45 individuals in the CG. Criteria used to form 2 groups with close characteristics include: blood lead concentration, age, duration of employment at working place exposed to lead, smoking habits and alcohol drinking. At the end of intervention and after the corresponding measurements the response rate of the workers decreased. The final number of workers in the EG was 37, and the number of workers in placebo CG was 38.

Methods

Blood for lead determination was collected by venepunction in vacutainers for metals (Becton-Dickinson – UK) and was stored at 4°C. Lead determination was performed on the next day in centrifugal polypropylene tubes (Elkay – USA) and were read by Perkin-Elmer 3030 Atomic Absorption Spectrometer (AAS) by flame extraction method [47]. The method of Berliner and Schaller, 1974 [7] was used to determine the level of ALA-D in erythrocytes, while the method of Piomelli, 1977 [36] was used for measuring the total porphyrins, designated as PP in erythrocytes**.** Activity of ALA-D was examined on the same day of the collection of blood and PP on the 3rd day after that, as the blood/erythrocyte samples were stored at 4^0C. The data obtained were assessed using the accepted referent values as follows: for ALA-D 17.5 – 46.5 μmol / min/l; for PP –0.2 - 2.6 nmol /g Hb. Superoxide dismutase (SOD) activity in erythrocytes was determined on the same day and by spectrophotometric method using standard kits. The normal ranges are-1102 - 1601U/g Hb or 164 - 240 U/ml. The content of reduced glutathione in blood (GSH) was measured by the spectrophotometric method of Beutler (1963) [8] on spectrophotometer SPECTRONIC 2001/Milton Roy Company, USA [32]. The values of GSH in erythrocytes were expressed in mmol/x10^9 cells or in blood (mmol/l). Lipid peroxides (LPO) in plasma and erythrocytes were analyzed by the spectrofluorimetric method of Yagi (1976) [52] on spectrofluorimeter PERKIN-ELMER, USA [33]. Methods used for analyses of studied indices of lipid status were the following: total cholesterol (TC) by enzymatic colorimetric CHOD-PAP method with lipid clearing factor [34]. Reference value: < 5.0 mmol/L; Triglycerides (TG) by enzymatic colorimetric GPO-PAP method with lipid clearing factor. Reference value: <1.7 mmol/L; Cholesterol in high-density lipoproteins (HDL-C) by enzymatic colorimetric direct homogeneous method for determination [35]. Reference value: > 1.0 mmol/L; Cholesterol in low-density lipoproteins (LDL-C) by enzymatic colorimetric direct homogeneous method. All kits were of "HUMAN", Germany [36]. Reference value: < 3.0 mmol/L. Atherogenic lipid index - calculated by formula TC:HDL-C. Reference value: < 4.5. Enzymatic colorimetric GOD-PAP method without deproteinisation for determination of serum glucose of "HUMAN", Germany was used. Reference value: 4.5-6.4 mmol/L.

Blood pressure was measured by Hg manometer – 3 times with one-minute interval in sitting posture, considering the lowest value.

Dietary data were obtained using the method of 24-hour recall for the previous day and estimated dietary records for two days (2 working days and one day off). Quantities of foods consumed were estimated using Photo album. Vitamin and mineral supplement use was also noted. Dietary data were translated into energy and nutrient intake values using computer program on the basis of Bulgarian foods and beverages composition values, recipes of national meals.

Estimation of nutritional status of workers was based on Body Mass Index. The weight of subjects was measured by electronic bathroom scales "Philips" with accuracy of 0.1 kg. The height was measured by portable statometer with accuracy of 0.5 cm.). WHO criteria [51] for assessment of BMI were used: BMI<18.5 for thinness, BMI 18.5 – 24.99 for normal weight; BMI 25.00-29.99 for overweight, BMI ≥30.0 for obesity.

Variation and alternative analyses were used for the primary statistical analysis. Differences

between the values of indices studied of the both groups were tested by t-test. Likelihood ratio test was implemented when we were looking for difference between number of cases with values in the reference range and number of workers with values below/above the referent one. Selection of the best model for describing linear relation between values of indices for antioxidant status and indicators for lead exposure, as well as indices studied of possible confounding factors was realized by stepwise forward linear regression, where p for entering was 0.05 and p for excluding was 0.10. Correlation coefficients of Pearson (for continuous variables) and Spearman (for category indices) were used to estimate direction and power between every two indicators. Values of blood lead were not normally distributed and were therefore (natural log) transformed.

RESULTS AND DISCUSSION

Subject characteristics

Demographic and employment data, as well as the potentially confounding variables as age, BMI, alcohol drinking are presented in Table 1. The number of workers included in the EG and CG, distribution of age, body mass index, average daily quantities of alcohol consumption, prevalence of current cigarette smoking, duration of occupational exposition to lead as well as the mean values of blood lead levels were close indicating that the both groups had the similar baseline influence of the possible confounding factors as well as close average start of lead exposure.

INDICES FOR LEAD EXPOSURE

Blood lead level

According to WHO data, the concentration of lead in blood in subjects without occupational exposure is within the range 10 – 20 μg/dL (0.48 – 1.20 μmol/l) [38]. The biological exposure index for lead in blood of exposed workers was estimated 50 μg% (2.4 μmol/l) [46]. Nevertheless, over the past 2 decades there has been growing awareness and concern that toxic biochemical and functional effects were occurring at lower levels of exposure than those that produced overt clinical and pathological symptoms [16].

The results obtained from the measurement of blood lead concentrations of workers from the EG and CG before and after intervention were in the range 1.73 ± 0.51 and 1.98 ± 0.58 μmol/l. The values were close ($p > 0.05$) and were not significantly changed during the intervention trial. Distribution of blood lead levels among workers from the EG and CG was similar in the baseline study before the trial: 21-24% of people from both groups had lead blood concentrations exceeding the biological exposure index (2.40 μmol/l). The percentage of subjects with blood lead values in the accepted reference range up to 1.2 μmol/l was higher in the CG compared with the EG (10.5% vs. 2.7%) indicating for some better background of the CG. Data

Table 1. Demographic data, lifestyle and exposure characteristics of subjects by group before the intervention

	EG (on Rooibos tea)	CG (on placebo)
Subjects (n)	37	38
Age, years (mean ±SD), (min ÷ max)	42.05 ± 7.9 (22 ÷ 55)	42.08 ± 8.02 (21 ÷ 55)
Gender (% males)	100%	100%
BMI (wt/ht^2, mean ±SD)	29.1±4.9	29.2± 4.2
Smoking (n, %)	25 (67.6%)	27 (71%)
Alcohol drinking (g ethanol/day, mean ±SD)	26.08±21.36	26.78 ±23.56
Duration of employment exposed to lead		
< 10 years (n, %)	12 (32.4%)	12 (32%)
> 10 years (n, %)	25 (67.6%)	26 (68%)
Blood lead level, μmol/l	1.88 ± 0.49	1.73 ± 0.51

obtained for blood lead concentrations of both studied groups demonstrated stable levels of internal exposure to lead of workers studied for the period of time of 8 weeks during the intervention trial performed.

ALA-D activity in blood and PP

Lead inhibits the activity of certain enzymes involved in heme biosynthesis, namely ALA-D, ferrochelatase, etc. The end results of these changes in enzyme activities are increased urinary porphyrins, increased erythrocyte protopophyrin, etc. [46]. ALA-D is the earliest and most sensitive biomarker of lead exposure. The level of PP in blood is an indicator of the chronic effect of lead, reflecting the lead body burden [50].

In our study strong inverse correlation was found between blood lead and the level of ALA-D (R= - 0.68,p=0,001) and a direct relationship was observed between PP and the blood lead (R= 0.53 p=0,001). PP levels before the trial in both groups significantly exceeded the referent value of 2.6 nmol/g Hb (before the trial EG 35.5 ± 31.0; after the trial 28.7 ± 27.0, p >0.05). No significant differences of mean PP levels have been estimated when compared with PP values after the experimental study. For the reason that erythrocyte PP in blood is an index of the chronic effect of lead, reflecting the lead body burden and because of the half-life of erythrocyte population is shorter than the experimental period, no significant effect on this indicator can be expected. High correlation between PP and lead levels confirms one of the conditions of the trial - constant internal lead exposure of the studied persons.

Data obtained for ALA-D for both groups in the baseline study were significantly below the lower referent value of 17.5 μmol/min/l erythrocytes. After the trial it was determined significant increasing ($p<0.05$) of ALA-D activities among the workers drinking Rooibos tea as well as among subjects who have taken placebo without significant difference of the changes in both groups. This positive dynamics of ALA-D activity was more clearly observed at low lead level up to 1.92 μmol/l where ALA-D activity reached almost normal values. The changes in ALA-D occurring without a change in the internal exposure could be explained by possible antioxidant effect on workers. The estimated increasing of ALA-D activity of the CG could be linked with determined increasing in the consumption of foods rich in antioxidants during the trial related to seasonal changes in dietary pattern. After the intervention trial there were determined direct correlations between ALA-D activity and intakes of vitamin E ($r= 324$, $p<0.05$). The weak antioxidant effect of Rooibos tea on ALA-D activity in workers with chronic lead exposure may be related to significant damage of the antioxidant capacity of erythrocytes.

INDICES OF ANTIOXIDANT STATUS

Superoxide dismutase

Since SOD is present in all aerobic organisms and most (if not all) subcellular compartments that generate activated oxygen, it has been assumed that SOD has a central role in the defence against oxidative stress [10, 45]. Workers chronically exposed to lead in the baseline study showed elevated activity of SOD in erythrocytes. Mean values in both experimental and control group were close and statistically insignificant.

There is evidence on the leading role of metal-bound reactive oxygen forms and production of hydroxyl radicals in the organism at exposure to heavy metals. The data obtained in regard to deviations in the oxidant/antioxidant status at lead exposure are somewhat contradictory. A number of authors report decreased amount of reduced glutathion [23] on the background of significant increase of malonic dialdehyde and superoxide dismutase values at lead exposure. Some researchers [53] report stimulated SOD activity at exposure to lead and manganese. Other authors establish decreased SOD activity [25]. Differences in response of SOD activity are also established at mercury exposure [6, 35]. Data obtained in our study show that the balance between oxidants and antioxidants was disturbed in workers exposed to lead. In the baseline study a direct correlation was evidenced between SOD and PP ($r=0.250$, $P=0.033$*), as well as with levels of LPO ($r=0.265$, $p=0.02$*) and GSH per Er count and in Er. The significantly elevated SOD activity suggests great tension in the natural antioxidant protection of the organism. The lack of correlation between increased SOD activity and other factors as age, alcohol con-

sumption and smoking prove the prior role of lead exposure in regard to the revealed deviations.

Drinking of Rooibos tea, which is rich in antioxidant substances, significantly decreased the compensatory elevated SOD activity in the experimental group (Table 2). As a result, mean value of SOD of the group reached the referent limits of the index. The revealed decrease is statistically significant vs. the control group ($p<0.0001$) as well as vs. the first study before the intervention trial ($p_1<0.0001$).

It was established that Rooibos tea possesses antioxidant activity likely by SOD mimetic substances [17]. It could be assumed that the intake of these flavonoids with SOD mimetic effect by Rooibos tea have decreased favourably the compensatory elevated values of SOD activity.

Reduced glutathione and lipid peroxides in plasma and erythrocytes

A decrease of GSH in the subgroups with increased blood lead (PbB) above 1,93 μmol /l was estimated. GSH in blood was significantly increased by 47.8% after the intervention trial in the workers that have taken Rooibos tea but there was not a significant difference with the changes of control group. The weak effect of Rooibos tea on GSH levels of workers with chronic lead exposure may be related to a significant damage of the antioxidant capacity of erythrocytes. The consumption of Rooibos tea during a period of 8 weeks seems to be insufficient to exert a more pronounced effect.

The mean plasma value of LPO in the total exposed group was significantly higher than estimated referent values. [8, 12, 43, 53]. LPO levels in plasma decreased significantly after the intake of Rooibos tea ($p < 0,006$), but not in the control group (Table 3). The decreasing effect on plasma LPO content was more pronounced in the subgroups with low exposure to lead. The intervention trial proved statistically significant beneficial effect of Rooibos tea on increased LPO levels in plasma of workers exposed to lead.

A correlation of plasma and erythrocyte LPO with SOD before the intake of Rooibos tea was determined, suggesting that the increase of LPO leads to a compensatory increase in the erythrocyte antioxidant defence capacity. A correlation between LPO content in RBC and the parameters of lipid status -TC and TG was revealed also.

LPO in plasma and RBC were significantly correlated with the level of alcohol consumption ($p < 0,05$).

LIPID STATUS

There are literature data indicating that citrus flavonoids supplementation decreases total cholesterol (TC) levels in vivo,inhibits apolipoprotein B secretion, and increases in LDL-receptors expression, changes that may explain flavonoid hypolipidaemic effects [5]. Until recently, there were no randomized clinical trials of the effect of antioxidant supplementation on lipid status. The evidence rested on clinical observations and prospective cohort data [42]. Some researchers reported that total flavonoid intake correlated inversely with LDL-C and TC after adjustment for age, sex, BMI and energy intake [3]. SOD bound to LDL and HDL could exert a protective role against oxidative damage of these lipoprotein classes that carry out an important role in the cholesterol transport [31]. Higher levels of serum lipid peroxides and HDL-C were found in lead exposure workers [25].

Table 2. Superoxide dismutase activity in erythrocytes of workers

Group	Before intervention trial		After intervention trial	
	Experimental group n = 37 Mean ± SD	Control group n = 38 Mean ± SD	Experimental group n = 37 Mean ± SD	Control group n =38 Mean ± SD
SOD U/g Hb	**1708.0** ± 104.22	1695.0 ± 88.68	**1548.0** ± 128.8 $p_1 < 0.0001$	**1675.0** ± 104.8 $p_2 > 0.05$

p_1 – experimental group – comparing status before and after tea administration

p_2 – control group – comparing status before and after placebo administration

Table 3. "Concentration – effect" relationships between lead blood level (PbB) and lipid peroxides in plasma (LPO)

Indices / Groups	Experimental group/subgroups LPO (Mean ± SD)		Placebo group/subgroups LPO (Mean ± SD)	
	Before trial	After trial	Before trial	After trial
Total group	6.28±2.19	5.31±2.04*	5.57±1.12	5.51±1.91
Subgroups PbB (µmol/l l)				
<1.2 µmol/l	5.69±2.91	3.91±0.79*	5.87± 1.49	5.39±1.34
1.21 – 1,92 µmol/l	6.77±2.82	5.69±2.31*	5.39±0.79	5.62±1.39
1.93 - 2,40 µmol/l	6.09±1.80	5.29±1.64*	5.39±1.25	5.22±1.91
> 2.40 µmol/l	5.66±0.62	4.39±1.77*	6.21±1.19	5.76±3.00

* Significant difference between values before and after intervention trail

In our study lead exposed workers showed abnormal levels of serum lipids - TC,LDL-C,HDL-C,TG and TC / HDL-C index. The most of lipid indices and blood glucose were not significantly shifted by 8 week drinking of Rooibos tea as well as taking placebo. Drinking of Rooibos tea significantly decreased the mean value of TG in workers ($p<0.001$), but beneficial effect of the tea was not significantly different compared to the changes in the CG. E. Some authors have found also increased TC and LDL-C in lead exposed workers compared to office employees and their values have depended on the level of lead exposure [29]. In the current trial the nonparametric analysis (χ^2) for eventual associations between lipid indices and different lead exposure in workers distributed in four groups found statistical significance only for TG (likelihood ratio 0.023). The levels of TG did not correlate with dietary intakes and BMI of the workers indicating for the primary effect of lead on this lipid index. The weak effect of Rooibos tea on TG is likely due to the strong disturbances in this index of lipid status related to chronic exposure of lead.

Additionally subgroups were formed from the EG and CG consisting of individuals with levels of indices of lipid status over/under the reference values that were determined in the baseline study. Presence and magnitude of the changes in the lipid indices after the trial in every subgroup and between them were estimated by t - test (Table 4).

It was determined that HDL-C was significantly increased by 27.8% after the intervention trial in the workers that have drunk Rooibos tea. The changes in the levels of HDL-C were similar in the persons from the control group, but the difference between mean values before and after the trial was much smaller (13.4%). The results of the formed subgroups concerning the LDL-C showed a tendency for decreasing as well, but the differences were not significant. The decrease of the mean value of this lipid index was more pronounced for the EG compared with the CG. The same was revealed concerning the TC/HDL-C ratio. Individual comparison between all persons with positively shifted lipid indices from both groups before and after the intervention trial indicated significantly higher levels of HDL-C in the group taking Rooibos tea vs. placebo group ($p<0.09$).

Lipid indices before the trial significantly correlated with dietary intakes of some antioxidant nutrients, anthropometric and blood pressure parameters. After the trial in workers drinking Rooibos tea this correlation was lost likely as a result of the antioxidant effect of the tea.

BLOOD PRESSURE

Equal number of subjects with controlled essential arterial hypertension was included in both studied groups. Subjects with symptomatic hypertension, showing extreme blood pressure (BP) values, have been excluded. BP values were

Table 4. Magnitude of changes after the intervention trial in some indices of lipid status of individuals with determined disturbances in baseline study

Indices	Experimental Group Mean (n)		Magnitude of changes in %, (p value)	Control group Mean (n)		Magnitude of changes in %, (p value)
	Before the trial	After the trial		Before the trial	After the trial	
HDL-C	0.83 (n=8)	1.06 (n=8)	+27.8 % (0.007)*	0.88 (n=11)	1.00 (n=11)	+13.4 % (0.055)*
LDL-C	4.32 (n=17)	3.82 (n=17)	-11.5% (0.161)	3.99 (n=22)	3.82 (n=22)	- 4.4% (0.454)
TC/HDL-C	5.99 (n=23)	5.32 (n=23)	-11.2 % (0.113)	-11.2 % (n=25)	5.26 (n=25)	- 6.2 % (0.250)

* Statistically significant difference between mean values before and after the trial

monitored before and after 8 weeks consumption of Rooibos and placebo (Table 5). The second BP measurement was carried out on the same days, at the same time and using the same method. Systolic BP > 140 mm Hg and diastolic BP > 90 mm Hg have been considered as normal according the criteria of WHO [49].

Statistically significant decrease of mean values of systolic and diastolic BP was found after the treatment only in the EG drinking Rooibos tea ($p < 0.01$) on the background of the almost equal mean baseline levels of arterial pressure of both groups before the trial.

Additionally subgroups were formed from the EG and CG consisting of individuals with levels of systolic and diastolic BP over the reference values of 140 resp. 90 mm Hg that were determined in the baseline study. Presence and magnitude of the changes in the BP values after the trial in every subgroup and between them were estimated by t - test (Table 6).

Reducing effect of Rooibos tea on both systolic and diastolic BP of workers from the subgroup with hypertension determined in the baseline study was considerable (by 18%) and statistically significant ($p= 0.001$) in contrast to the weak changes in elevated values of BP of the workers from control group after the trial.

No correlations between BP levels with alcohol consumption and current smoking habit have been found, but a correlation was revealed with the BMI – $r=0.443$, $p= 0.007$ as well as with TC levels in blood ($r=0.292$, $p<0.01$).

Data obtained in this study as well as some literature data about the changes of lipid peroxidation in lead exposed persons show an activation of peroxidation process [25, 44]. During the recent years several reports have been published, confirming the relationship between the oxidative stress and the vasoconstriction or

Table 5. Arterial pressure values [mm Hg] by groups

Index (Mean± SD)	Before the trail		After the trail	
	Experimental group (n=37)	Control group (n=38)	Experimental group (n=37)	Control group (n=38)
Systolic BP	128.6 ±15.57 m = 2.59	129.46 ±15.45 m=2.54	117.7 ±14.02* m=2.32	125.7 ±16.45 2.76
Diastolic BP	87.64±12.04 m=2.01	86.68±11.87 m= 1.95	78.51 ±9.04* m=1.49	86.58 ±10.79 m=1.75

*Significance of the difference after the intervention compared with the values before the trial, $p < 0.01$

Table 6. Magnitude of changes after the intervention trial of blood pressure values of individuals with determined hypertension in the baseline study

Indices	Individuals from Experimental group Mean (n)		Magnitude of changes in %, (p value)	Individuals from Control group Mean (n)		Magnitude of changes in %, (p value)
	Before the trial	After the trial		Before the trial	After the trial	
Systolic BP	149.3 (n=7)	122.1 (n=7)	-18.2 % (0.0001)*	155 (n=11)	146.7 (n=11)	-5.4 % (0.29)
Diastolic BP	102.5 (n=10)	84 (n=10)	-18. 0% (0.001)*	102.4 (n=9)	98.9 (n=9)	- 3.5% (0.534)

* Statistically significant difference between mean values before and after the trial

hypertension [22, 41]. Great prevalence of overweight and obesity among the workers studied could contribute to the increasing of BP as well.

Our findings about the effect of Rooibos on BP are consistent with the results of studies carried out by a number of authors, who estimated beneficial effect exerted by flavonoids and other antioxidants on BP [9, 15]. The lower BP can be due to the beneficial effect of Rooibos on the antioxidative stress or to some vasodilatation mechanisms involved in.

LIFE STYLE FACTORS

Dietary intake

Diets of workers from the experimental and control group were close in regards with the content of energy and macronutrients expressed as E%, and their changes were in parallel during the trial indicating similar effects of these components of the diet on the biochemical indices studied.

Alcohol consumption

The mean and medium intakes of alcohol expressed as ethanol of the both investigated groups were close and the values were in the range of the estimated non-injurious levels for healthy men – 30-40 grams/day [24]. There were persons however from the EG and CG with much higher alcohol consumption – up to 82-100 grams daily. Using Pearson correlation analysis a significant positive correlation was determined between alcohol intakes and levels of LPO in plasma and erythrocytes ($r= 0.277$, $p< 0.02$). This means that the alcohol consumption exerted negative influence on antioxidant status of workers from both groups.

Anthropometric indices of nutritional status

The mean BMI of workers from the experimental group on Rooibos tea before the treatment was 29.1±4.9 and 28.8±5.2 after the trial. The mean BMI determined for workers on Placebo were similar: 29.2± 4.2 in the first investigation and 28.5±4.4 in the second one. There were no significant differences between mean BMI of both groups before and after the intervention trial.

In the baseline study the estimated prevalence of overweight among workers was 43.8% and those of the obesity - 41.1%. The distribution of workers with overweight and obesity was similar in both groups. The high prevalence of overweight and obesity likely has contributed to the high frequency of determined dislipidemia among workers studied.

CONCLUSIONS

Drinking of one litre daily of Rooibos tea prepared from 10 grams of dry leaves for a period of two months by male workers chronically exposed to lead exerted positive health effects on the individuals studied, including: improvement of the antioxidant status especially concerning the superoxide dismutase activity and lipid peroxides in plasma; decreasing of elevated systolic and diastolic blood pressure; improvement of high-density lipoprotein cholesterol level in blood. Duration of taking of Rooibos tea likely needs to be longer than 8 weeks to reveal fully its beneficial health effects on occupationally exposed

to lead subjects.

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CIRCADIAN TEMPORAL SYSTEM, PATTERN OF FOOD INTAKE AND HEALTH RISK

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INTRODUCTION

The circadian temporal system, genetically determined (27) and hierarchically structured in the brain and periphery, controls the temporal homeostasis (31) - the coherent dynamics of the molecular, metabolic, physiological, hormonal and behavioral processes in organism (10, 22, 33), temporal regulation and synchronization towards geophysical 24-hour environmental light-dark cycle through photo-induced phase shift of the endogenous oscillator and central pacemaker — *Nucleus suprachiasmaticus* (SCN), (1, 40, 41). Circadian rhythms are generated by cyclic genetic expression of cellular biological clocks and feedback regulation (34). The central pacemaker induces through neural and humoral transmission in multisynaptic pathways and intercellular network the oscillations of biological clocks in periphery (16,38,53).

The night shift work is associated with dyschronization or desynchrony of circadian system (4) and eating pattern (30, 48) and is re-

lated to increased risk of cardiovascular disease, metabolic disturbances, gastrointestinal disorders and ulcerogenesis (relative risk, RR range 2 ÷ 8), sleep deterioration, traumatic injuries incidence (9). Night shift work is an attributable risk factor for major nuclear and chemical plants disasters and accidents (Three Mile Island, Chernobyl, Exxon Valdez, Bhopal), (4) because circadian rhythms of performance, vigilance, alertness, attention are in bathyphase as well as the inverted eating cycle induces parasympathetic effect on cognitive performance (11, 12, 20).

CIRCADIAN SYSTEM AND FOOD INTAKE CYCLE

In environment with absence of time cues, human physiological processes (body temperature cycle, hormonal cycles) and behaviour (sleep- wakefulness, eating cycle) express oscillations with period different but close to 24 hours and thus the term *circa dies*. Circadian rhythms are controlled by endogenous "clocks", which are synchronized to 24-h light-dark cycle by dominant synchronizer – light through photoinduction of central pacemaker SCN (41) via retinohypothalamic tract with specific photoreceptors. Retinal stimulation for circadian synchronization is different from visual perception and is determined by novel class retinal ganglion cells with expression of novel opsin-like protein melanopsin (7, 14) - (precursor vitamin A or retinol and retinoic acid), and with mediation of flavoprotein FAD (precursor vitamin B2 or riboflavin) related to cryptochromes, spectral sensitivity of which is different from that of the visual photoreceptors. In humans, their highest sensitivity is towards spectral waves 470 nm (blue), 497 nm (blue-green) and 525 nm (green) light (35). The light suppresses melatonin – phase marker of circadian system and hormone of pineal gland (25), coupled by feedback with melatonin receptors on SCN, MT1 (29). The activity of SCN is related with neuropeptides arginine-vasopressin (AVP), vasoactive-intestinal-polypeptide (VIP), neurotransmitters Glutamate and GABA. In the dark phase, the synthesis in *raphe* of serotonin (5-hydroxy-tryptamine, 5HT), neurotransmitter of slow-wave sleep (SWS, NREM) is activated and 5HT is the precursor of the synthesis of melatonin (N-acetyl-5-methoxy-tryptamine), with highest concentration during the dark phase. The melatonin is associated with the synchronization of cycle sleep-wakefulness (8) as well as with the putative brain oscillator, synchronized with feeding cycle – the so called Food-Entrainable-Oscillator (FEO), (46). In connection with the C.N.S. control of food intake (42), an association with GABA mediation is discovered between SCN and hypothalamic satiety center PVN with stimulation of melatonin by PVN. A suggestion is made for a relation between FEO and peripheral circadian oscillator in liver – the molecular biological clock gene *per 1*, that regulates the protein and enzyme synthesis, metabolic rhythms, energy homeostasis of cellular redox-potential and mitotic cycles of hepatocytes (19, 47). Nutrient-synchronized-oscillator or FEO is associated with other "clock" genes of peripheral organs, e.g. pancreas and kidney (17, 18). Cellular mitotic cycles of enterocytes are synchronized with acrophase during sleep phase. The discovery of molecular basis of circadian clock shows that circadian rhythms are generated by periodical activation of transcription of population of the so-called "clock genes" and their respective transcripts – regulatory protein factors with parallel terminology – Per (Period), Clock, Cycle, Cry (Cryptochrome), Dbt (Double time), (1, 5). The "clock" genes are expressed at the level of transcription and translation of genetic information and the oscillations are generated by feedback mechanisms. The metabolic rhythms are timely integrated in a coherent system and are coordinated by SCN with mediation of neural and humoral signals to periphery clock genes with expression of clock transcriptional factors. The association between clock protein transcriptional factors and clock genes in the cell nucleus depends on the fluctuation of cellular redox potential, i.e. the relation NAD^+/NADH and $NADP^+$/NADPH or respiratory enzyme chain. NADH stimulates and NAD^+ inhibits the binding of Cryptochrome-transcriptional protein repressor with DNA of clock gene (1). The precursors of NADH are essential aminoacid tryptophan and the vitamin niacin. The liver clock genes are expressed even at starvation, but timely restrictive food intake could phase shift the liver metabolic rhythms, which persist in phase at the sequenti-

ally imposed starvation (13). The restrictive feeding cycle has influence on the rhythm of cortisol/corticosterone (6), which is superimposed on circadian regulation by SCN. It is suggested that the model of biological clock comprises two opposed oscillators determining the temporal pattern of human chronotypes – M (morning) and E (evening), but about 50% of individuals are denoted as intermediate or I-types (24, 36).

Health risk at dyschronization of circadian system and cycle of food intake

Night shift work is associated with dyschronization of circadian system and disturbances of eating pattern (3, 11, 12, 28, 51). The pattern of food intake at night shift work is characterized with irregularity in relation to energy density distribution, scattered ingestive periods, inversion of eating cycle in nyctohemeral period (52). Food intake during the night shift is imposed on the stomach secretion phase of minimal activity (39, 45). Food intake with high energy density during the night shift is associated with risk for disturbances in lipid metabolism (21), increased parasympathetic activity in postingestive/postprandial period with decreased cognitive performance and attention. The increased cardiovascular risk associated with night shift work, RR~1,5 times higher incidence (26), is related with unfavourable changes of serum cholesterol (21), higher postprandial triacylglycerol (TAG) levels (2, 26), blood pressure (15), psychosocial stress (50).

Our studies of subjects working on night shifts (women – operators in Telefon company) with mean period 15,4 ± 9,2 years of exposure, compared with control group, show development of unfavourable trends in all serum biomarkers of the lipid status – increased level of serum total cholesterol, LDL-cholesterol, decreased HDL-cholesterol, increased levels of triacylglycerols and cholesterol in VLDL lipoproteins,**Table 1**. The findings show prevalence of 66% dyslipoproteinaemia and the evaluation of health risk by Prevalence Ratio (PR) is PR = 1.3 with 95% Confidence Interval (CI) CI = 0.95 ÷ 1.8 (P=0,129), and Odds Ratio (OR) is statistically very high OR=5.03 (2.02 ÷ 12.57), P < 0.001. The increased risk for development of dyslipoproteinaemia could be associated with unfavourable long interingestive periods, high energy density of food intake of ensuing eating episode (our results show coefficient of correlation r = 0,854) and induction by insulin secretion of the key enzyme of cholesterol synthesis Hydroxymethylglutaryl-CoA-reductase (12, 44). Our results are supported by the findings that postprandial lipaemia and especially the levels of serum triacylglycerols and VLDL are higher and are increased for longer periods at food intake during the night hours (2, 37). The acrophase of circadian rhythm of cholesterol synthesis is during the night and early hours in the morning.

Our studies of male subjects working at night shifts 15-17 years (operators in Transport Company) show that parameters of blood pressure are increased SBP = 127,8 ± 8,8 mm Hg and DBP = 87,5 ± 5,9 mm Hg during the night shift in comparison with the day shifts – SBP = 125,1 ± 9,6 mm Hg and DBP = 81,6 ± 8,5 mm Hg, with statistical significance for DBP (P < 0,05), **Table 2**. A reversed 24-h circadian rhythm of arterial blood pressure (49) as well as unfavourable stress markers and stress correlates at night shift are found and this could be linked to the increased cardiovascular risk at night shift workers. We also studied the fluctuations of cognitive performance, attention and memory during the night shift and the results show that sleep deprivation induces reduction in cognitive performance with minimal levels of the correlates at 02:00 ÷ 05:00 hour. The perception of stress exposure in constellation with food intake during the phase of minimal stomach secretion are probably connected with increased risk of ulcerogenesis – our results showed that the prevalence of ulcer (with hospitalization) was 19,3% - this represents a higher prevalence than the mean values for males in the control group.

The intake of melatonin as food supplement, the so-called "chronobiotic" (8) for reducing the disturbances of sleep/wakefulness cycle in night shift workers (43) and regulation of human circadian rhythms and sleep, is not recommended by some authors because of hormonal and antigonadal action as well as the unknown remote effect of its application (23). The preferred intervention is the intake of foods rich in melatonin, discovered in plant foods (32) such as ce-

reals and some vegetables and fruits.

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Table 1. Lipid status, lipoprotein profiles and cardiovascular risk (PR and OR) of female night shift workers vs control group

Serum Markers of lipid status	Night shift		Controls		Criteria for law cardiovascular risk
	Mean (SD)	CV (%)	Mean (SD)	CV (%)	
Total Cholesterol (mmol/L)	5.42 (1.02)	18.7	5.33 (1.07)	20.0	5.2
LDL-C (mmol/L)	3.24 (0.98)	30.4	3.17 (0.99)	31.5	2.6
HDL-C (mmol/L)	1.49 (0.40)	26.9	1.59 (0.33)	20.7	> 1.0
VLDL-C (mmol/L)	0.68 (0.39)	58.6	0.59 (0.76)	129.5	< 0.8
TAG (mmol/L)	1.49 (0.87)	58.4	1.29 (1.68)	129.9	< 1.7
Cholesterol/ HDL-C	3.89 (1.30)	33.5	3.56 (1.33)	37.4	< 4.5
Prevalence Ratio (PR)	1.3 (0.95 – 1.80) P=0.129				
Odds Ratio (OR)	5.03 (2.02 – 12.57) P < 0.001				

Table 2. Parameters of arterial blood pressure, systolic (SBP), diastolic (DBP) and heart rate in male night shift workers vs control group

Parameters of blood pressure	Night shift	Control group	Statistical significance
SBP (mm Hg) Mean	127.8	125.0	> 0.05
SD	8.8	9.6	
CV (%)	6.9	7.7	
DBP (mm Hg) Mean	87.5	81.6	< 0.05
SD	5.9	8.5	
CV (%)	6.7	10.4	
HR (b/min) Mean	86.5	87.9	> 0.05
SD	7.2	4.9	
CV (%)	8.3	5.6	

A METHOD AND EQUIPMENT FOR PRODUCTION OF SPARKLING (NATURALLY OR ARTIFICIALLY SPARKLING) ALCOHOLIC BEVERAGES

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A Bulgarian company (winery) has developed a method and equipment for production of naturally or artificially sparkling (Champagne process) alcoholic beverages (wines, beer, etc.). The process of production of sparkling alcoholic beverages is based on natural primary and secondary fermentation. Artificial carbonation is not needed. The new method allows the equipment to be utilized to be very simple, based on conventional elements and new methods for distribution of the final product. The equipment can be very compact and may be mounted even in restaurants and the product to be sold directly. A very simple principle and equipment were developed, allowing maximum organoleptic (taste) qualities and expiry period to be reached. In fact there is no need for pasteurization, especially for production of beer. The equipment designed allows natural stabilization (colloid and microbiological).

Innovative Aspects: A new principle for production of alcoholic beverages is used, allowing natural conservation of the product - all the bio-ingredients are active (vitamins etc.). The new method allows utilization of very simple and cheap equipment. A wide range of beverages may be produced – sparkling wines, sprit wines, Champagne-like wines, any type of fruit wines, beer, etc.

Main Advantages: Using the method all the range of known and new sparkling alcoholic beverages (with very low alcohol content) may be produced. The proposed method could be a base for new beverage industry - it is very cheap to be implemented. It saves time, energy and place for long-term storage of the produced beverages. There is no need of additional stabilization of the product - no pasteurization, no additives. The installation may be designed for any scale of production. It could be used in restaurants as well as in big production companies. The end products have excellent organoleptic qualities and all the bio-ingredients are active due to the lack of thermal treatment; the production cycle is dramatically shortened.

SMALL ROBOTIZED COMPLEX FOR ECOLOGICAL MANUFACTURING OF UP TO 132 000 SQUARE METERS OPEN AREA OF FRESH STRAWBERRIES

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A small Bulgarian team of research experts has developed a small Robotized Complex for ecological manufacturing of up to 132 000 square meters open area of fresh strawberries. Each project is based on robotized fruits and vegetables harvesting, which covers full automation of the complete production cycle from the preparation of the manufacturing area to the automated selling of end production, including mobile business and e-commerce. The necessary power is generally processed by photovoltaic systems. The plants' leaves and stems after the fruits' harvesting are also gathered as a raw material for bio-production. Each project for creation of Robotized Complex is specific for every different sort of cultivated plants. It is determined, considering the growing area, geographical position and the slope of the terrain. The characteristic of the soil is also an important issue. The water source also should be considered - integration system, etc. The Robotized Complex body structure is specific for each type. That is because it is based on plant characteristics - height, crown diameter, the number of fruits, period of vegetation, how many times you do harvest in the harvesting period. The fruit characteristics are also considered - sizes, weight, etc. The harvesting is based on the working principle of industrial robots with linear degree of freedom, when they are working jointly with assembly lines (flow production). The main difference is that the Robotized Complexes are able to move, while the assembly line is not. It is separated by sectors with same sizes - the rows and the beds.

Innovative Aspects: Innovative aspects are the new production processes and devices, food quality and food safety. It ensures stable production and decreases global changes in the ecosystem. If these technologies are in use, the humus of eroded agricultural areas restores itself by active work in growing of cultivated plants. There are possibilities to minimize the damage caused by hails. Additional stationary modules should be used for this purpose. Using them the whole harvesting period per year in geographical zones where necessary could increase.

Main Advantages: This initiative is the only really effective variant for integrating the infrastructure of the production processes with coordinating and specific supporting procedures. The technologies ensure effective connections between different high-science attainments and the technologies, practically operating in this or in similar scopes. Basic materials for creation of the technologies are polymers and that minimizes energy consumption. Other advantages are low operating energy and work expenses.

EQUAL IN EUROPEAN RESEARCH AREA

BULGARIAN VIPs

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Professor in the field of alcoholic and non-alcoholic beverages with over 110 publications and scientific papers, more than 110 research and development projects, and 20 registered Bulgarian Patents.

Mr. Valentin Batchvarov defends his MSc Engineering degree in 1974 on Fermentation Chemistry and Technology in the Prague Institute of Chemical Technology, Czechoslovakia. Shortly afterwards, in1977 he defends his PhD dissertation at the same institute and starts his professional career as a technologist, production manager at the Sofiisko pivo Brewery, Sofia, Bulgaria.

The work experience of Mr. Batchvarov includes the following consecutive positions:

• Research Associate, Associate Professor, Deputy Director, Head of Brewing Technology Department at the Institute of Brewing & Hop Industry, Sofia in the period 1980-1999, Director of the institute between 1999-2001.

• Brewing Department Manager at the Institute of Cryobiology & Food Technology, Sofia, 2001-2002.

• Scientific Director at the National Wine and Spirituous Beverages Research Institute, Sofia, 2002.

• Brewing Department Manager, Professor in the field of alcoholic and non-alcoholic beverages at the Institute of Cryobiology & Food Technology, Sofia since 2002.

Mr. Batchvarov has been a member of a number of programme, managing and editorial boards, including:

• Member of the EBC Analysis Committee and Chairman of two Sub-Committees at the European Brewery Convention, 1995-2003.

• Member of the Editorial Board of the Food Processing Industry Magazine, (Bulgarian Journal), 2001.

• Member of the Managing Board of the National Centre for Agricultural Science, 2003-2004.

• Member of the Managing Board of the Union of Food Industry, 2005.

• National Contact Point for Priority 5 Food Quality and Safety of the Sixth Framework Programme for research, technological development and demonstration of European Union, 2003-2005.

• Member of the Food Quality and Safety Programme Committee of the Sixth Framework Programme, 2003-2006.

The key scientific and professional interests of Mr. Batchvarov include the following fields: technology of malt and beer, technology of wine and spirituous beverages, food and beverage technology, functional foods and beverages; food quality and safety, food additives, food contaminants and residues; food hygiene; HACCP, GMP, GHP, food labeling.

The main contribution to science and industry of Mr. Valentin Batchvarov can be summarized in the following way: Introduction of new hops and brewing barley varieties, wort production, enzyme application, kieselghur and membrane filtration, beer colloidal stability and methods for

prediction, methods for analysis and control, HACCP, GMP, HGP in food industry, food and beverages quality and safety.

During his scientific and professional career Mr. Batchvarov has over 110 publications and scientific papers as well as more than 110 research and development projects. He has invented and registered 20 Bulgarian Patents.

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Expert in the fields of toxicology and nutrition with over 100 articles, chapters of books etc., reported on more than 60 national and international congresses, conferences and symposia.

Assoc. Prof. Dr. Stefka Petrova is a physician; she has graduated the Higher Medical Institute in Sofia. Dr. Petrova has two medical specialties: "Toxicology" and "Nutrition and Dietetics". Her PhD thesis is in the field of nutritional toxicology, but most of her research and practical work is in the field of nutritional epidemiology and nutrition policy. She has specialized in Nutrition Information Management at Emory University, Atlanta, USA; Nutritional Epidemiology in the Institute of Public Health & Dunn Nutrition Centre at Cambridge University,UK. Dr. Petrova has participated in training courses on Epidemiological Methods in Public Health, Nutrition Education, Development of Food Based Dietary Guidelines, Development of Food and Nutrition Policy, etc., organized by WHO and FAO.

Dr. Stefka Petrova is a Senior Research Associate, Professor on nutrition since 1991. Since 2005 Prof. Petrova is a head of the newly formed Department "Foods and Nutrition" at the National Center of Public Health Protection. The department includes four laboratories: "Nutrition and Public Health", "Food Microbiology", "Chemical Contaminants and Additives in Foods" and "Food Composition". Previous posts held by Dr. Petrova include: Head of Laboratory "Nutrition", Head of Laboratory "Preventive Nutrition", researcher in Laboratory "Preventive Nutrition" and post-graduate student at Laboratory "Nutritional Toxicology".

Research in the field of Nutrition

On national level

• Epidemiological studies on dietary intake and nutritional status of different population groups – infants and young children, schoolchildren, pregnant women, women in fertile age, elderly people, different professional groups, and of the whole Bulgarian population on national level; risk assessment related to dietary intakes and nutritional status in deferent populations.

• Development of Recommended Dietary Intakes for Bulgarian Population, Head of the National Task Force, author of RDA for vitamins, 1994 and 2005.

• Development of Food-Based Dietary Guidelines for Bulgarian Adults, development of Dietary Guidelines for different risk population groups – elderly people, pregnant women, schoolchildren, children aged 3-7 years, infants and young children 1-3 years old.

• Development of software programs for assessment of dietary intakes;

• Studies on the effects of nutrients and foods on pollutant toxicity;

• Toxicological assessment of food pollutants.

On international level

• Project "Varna stroke study" (2000-2003) – in collaboration with Medical University, Varna, University Hospital, Varna and Department of Public Health and Primary Care, Institute of Public Health, Cambridge, UK

• Project "Identification of N-3 fatty acid deficiency in Bulgaria" (2006-2007) – in collaboration with Harvard School of Public Health, USA.

Collaboration with International Organizations

WHO:

• Project "Development of Food-Based Dietary

Guidelines for Bulgarian Adults" (2005-2006);

• Project "Strengthening Community Based Interventions for Scaling up Fruit and Vegetable Intake" (2007)

UNICEF:

• Project "National Survey on Nutrition of Infants and Children Under 5 and Family Childrearing Practices in Bulgaria" (2006-2007)

Results of the scientific research are published in more than 100 articles, chapters of books are reported on more than 60 national and international congresses, conferences and symposia.

Nutrition Policy

On national level

• Food and Nutrition Action Plan of Bulgaria, 2005-2010 – principal author and national coordinator for its implementation;

• National Program for Counteracting Osteoporosis, 2006-2010 – participation in its development and implementation;

• Nutrition Surveillance Program of Bulgaria, 1995 - author;

• National Program for Elimination of Iodine Deficiency Disorders in Bulgaria, 1993, co-author;

• Food and Nutrition Policy of Bulgaria, 1992 - principal author;

• Participation in the development of Bulgarian food and nutrition related legislation, as well as in harmonization of Bulgarian food legislation with EC directives, 1991-present.

On international level

• As Temporary advisor at WHO Regional Office for Europe – Participation in the Consultations for Development of the First Food and Nutrition Action Plan for WHO European Region, 2000-2005; development of Nutrition Action Plans in South East Europe; development of a Global Strategy on Infant and Young Child Feeding (2003); Global Strategy on Diet, Physical Activity and Health (2004); development of the Second Food and Nutrition Action Plan for WHO European Region: 1999- present

Expert and Consulting Activities

On national level

• National Consultant at the Ministry of Health on Nutrition, 1992 – present: consultant on all aspects of the National Nutrition Policy; preparation of the national comments/ positions on nutrition-related national and international documents on behalf of the Bulgarian Ministry of Health.

• Member of National Committee for Promotion of Breastfeeding, 2005-present

• Deputy chief of the Expert Committee on Genetically Modified Foods at the Ministry of Health, 2006-present and Member of Intersectoral Expert Committee on Genetically Modified Organisms at the Ministry of Environment and Waters, 2006-present

On international level

• Bulgarian counterpart on Nutrition and Nutrition Policy at WHO, Regional Office for Europe: 1992 – present.

• National Representative in the European Network on Nutrition and Physical Activity at the EC: 2004-present.

• EFSA Food Consumption Database Managers' Network

Training in Nutrition

On national level

• Development and updating of the Curricula for training in medical specialty "Nutrition and Dietetics, 1996 – present;

• Member of the Examination Commission for the medical specialty Nutrition and Dietetics, 1991 – present;

• Principal author of the Curricula for the long-term and short-term training courses in Nutrition for health professionals;

• Lecturer on Nutrition, Nutrition Epidemiology and Nutrition Policy in all training courses for health professionals, 1985 – present;

• Professor on Nutrition, International University, Sofia, Course on nutrition and food hygiene, 1999 – present.

On international level

• Lecturer in WHO/UNICEF Training Courses: Healthy Food and Nutrition for Women and Their Families; Feeding and Nutrition of Infants and Young Children

Membership of Professional Societies

• Member of European Academy of Nutritional Sciences, 1988 – present;

• Member of International Association for the Study of Obesity (IASO), 1996 – present;

• Member of Bulgarian Scientific Association on Nutrition and Dietetics at the Bulgarian Union of Scientific Medical Associations, 1980 – present.

AWARDS

FOR DISTINGUISHED CONTRIBUTION TO SCIENCE AND BASIC CONTRIBUTION TO SCIENCE FOR YOUNG SCIENTIST FOR 2006

The award of the Ministry of Education for distinguished contribution to science has been bestowed for 5 years and is a complex assessment, which includes basic criteria such as scientific achievements in the country and abroad during the recent 5 years, scientific works, monographs, prestigious international awards, creation of science schools, personal contribution of the successfully defended doctorands, participation and work in international research programs and collective bodies.

On December 27, 2006 at a special ceremony the Vice Prime-minister and Minister of Education and Science Daniel Valchev **bestowed awards** for distinguished contribution to science and basic contribution to science for young scientist. "Bulgaria joins the EU not with mentality of people awaiting social aids, but with awareness of people who have intellectual and spiritual potential to participate in solving the problems of the modern society", he said in his speech.

Prof. Dr. Vanyo Ivanov Mitev, doctor of biochemical sciences from Medical University in Sofia, became **the bearer of the award for distinguished contribution to science for 2006.**

During recent 5 years Prof. V. Mitev has over 50 publications in our and foreign periodicals in the field of biochemical science. He is an authoritative consultant in a number of foreign scientific organizations and companies in the sphere of medicine and pharmacy and an invited lecturer in France and Belgium, editor-in-chief of "Medicine and Sport" journal, a member of editorial boards of Bulgarian and foreign periodicals. Prof. Mitev has created a school in the field of cell signaling, which is **the only one of its kind on the Balkans**. The Department of Chemistry and Biochemistry at the Medical University headed by Prof. Mitev has introduced the only in Bulgaria for the time-being electronic education in biochemistry. Prof. Vanyo Mitev has awards for high scientific achievements, and in 2006 he gets the award **Signum laudis with ribbon** of the Medical University in Sofia. At present his team works over examination of molecular mechanisms of cancer. They investigate which molecules help for transformation of a normal cell into a cancer one and how this process can be stopped.

The award for particular contribution to science of young scientist for 2006, which was set up in 2004, was delivered to **Research Associate I degree Dr. Eng. Vesela Tzvetanova Krasteva** from Central Laboratory of Biomedical Engineering at the Bulgarian Academy of Sciences

Dr. Krasteva is a graduate of the Technical University in Sofia and up to now besides working in the Academy of Sciences she works for her former university. She is the tutor of diploma projects of seven students from the University. In the recent years the young woman has over 45 scientific publications. The contribution of Dr. Krasteva is not only in her wide scientific activity, but also in her participation in the international team that created **a new kind of defibrillator** for first aid, which was honoured by the European Organization for Standardization with a European certificate "CE" for high efficiency and for two years has been in serial production for the markets in Europe and USA. Dr. Krasteva is a bearer of the award of the Bulgarian Academy of Sciences for 2006.

At present Dr. Vesela Krasteva and her colleagues from the Central Laboratory of Biomedical Engineering are developing a new method for diagnostics and treatment of cardiovascular diseases. The new equipment the specialists are working upon will localize myocardial infraction. It will prognosticate whether and how long the patient will live after the heart attack. The scientists from BAS are still carrying out experiments and collecting information about the new method.

ARTICLES

RECENT PUBLICATIONS OF BULGARIAN SCIENTISTS

Title: **The Importance of Aeromonas Hydrophila in Food Safety.**
Authors: Daskalov, Hristo[1] *hdaskal@uni-sz.bg*
Source: Food Control; Vol. 17, 6, (2006 Jun.), 474-483
Document Type: Article
Author Affiliations: [1]Department of Food Hygiene, Technology and Control of Foods and Food stuffs, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria
ISSN: 0956-7135

Title: **Survival and Growth of Listeria Monocytogenes on Sausage Formulated with Inoculated and Stored Rework Product**
Authors: Daskalov, Hristo[1], *hdaskal@uni-sz.bg,*
Momfre, Joe[2], Sofos, John N.[2]
Source: Food Control, Vol. 17, 12, (2006 Dec.), 981-986
Document Type: Article
Author Affiliations: [1]Department of Hygiene, Technology and Control of Food and Foodstuffs, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria;
[2]Department of Animal Sciences, Colorado State University, Fort Collins, Colorado 80523-1171, USA
ISSN: 0956-7135

Title: **Conversion of Sucrose into Palatinose in a Batch and Continuous Processes by Immobilized Serratia Plymuthica Cells.**
Authors: Krastanov, Albert[1], Blazheva, Denica[1], *dblazheva@gmail.com,* Yanakieva, Irina[2], Kratchanova, Maria[2]
Source: Enzyme & Microbial Technology; Vol. 39, 6, (2006 Oct.), 1306-1312
Document Type: Article
Author Affiliations: [1]University of Food Technologies, Department of Biotechnology, 26 Maritza Blvd., 4002 Plovdiv, Bulgaria;
[2]Laboratory of Biologically Active Substances, Institute of Organic Chemistry, Bulgarian Academy of Sciences, Plovdiv, Bulgaria
ISSN: 0141-0229

Title: **Effect of Furfural on Carbon Metabolism Key Enzymes of Lactose–assimilating Yeasts.**
Authors: Hristozova, Ts.[1] Angelov, A.[2] *angelov@uft-bio.com,* Tzvetkova, B.[1], Paskaleva, D.[1], Gotcheva, V.[2], Gargova, S.[2], Pavlova, K.[1]
Source: Enzyme & Microbial Technology; Vol. 39,5,(2006 Sep.),1108-1112
Document Type: Article
Author Affiliations: [1]Institute of Microbiology, Bulgarian Academy of Sciences, Sofia 1113, 26 Akad. Bonchev Street, Bulgaria;
[2]Department of Biotechnology, University of Food Technologies, 26 Maritza Blvd., 4002 Plovdiv, Bulgaria
ISSN: 0141-0229

Title: **Stable Isotopic Evidence of Diet in a Greek Colonial Population from the Black Sea.**
Authors: Keenleyside, Anne[1], *akeenleyside@trentu.ca,* Schwarcz, Henry[2], Panayotova, Kristina[3]
Source: Journal of Archaeological Science; Vol. 33,9,(2006 Sep.),1205-1215
Document Type: Article
Author Affiliations: [1]Department of Anthropology, Trent University, Peterborough, Ontario K9J 7B8, Canada;
[2]School of Geography and Geology, McMaster University, Hamilton, Ontario L8S 4K1, Canada;
[3]Institute of Archaeology, Sofia, Bulgaria
ISSN: 0305-4403

Title: **Effects of Processing on Pesticide Residues in Peaches Intended for Baby *Food*.**
Authors: Balinova, Anna M.[1], *abalinova@abv.bg,* Mladenova, Rositsa I.[1], Shtereva, Deyana D.[1]
Source: Food Additives & Contaminants; Vol. 23,9,(2006 Sep),895-901,3 charts, 1 diagram, 1 graph
Document Type: Article
Author Affiliations: [1]Department of Toxicology, Plant Protection Institute, Kostinbrod 2230, Bulgaria
ISSN: 0265-203X

Title: **Multifrequency EPR Study on Freeze–dried Fruits before and after X–ray Irradiation.**
Authors: Yordanov, N.D.[1] *ndyepr@ic.bas.bg,* Aleksieva, K.[1], Dimitrova, A.[1], Georgieva, L.[2], Tzvetkova, E.[2]
Source: Radiation Physics & Chemistry; Vol. 75,9,(2006 Sep.),1069-1074
Document Type: Article
Author Affiliations: [1]Laboratory EPR, Institute of Catalysis, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria;
[2]Institute of Cryobiology and Food Technologies, 1162 Sofia, Bulgaria
ISSN: 0969-806X

Title: **Involvement of Ethylene and Lipid Signalling in Cadmium–induced Programmed Cell Death in Tomato Suspension Cells**.
Authors: Yakimova, E.T.[1], Kapchina-Toteva, V.M.[2], Laarhoven, L.-J.[3], Harren, F.M.[3], Woltering, E.J.[4] *ernst.woltering@wur.nl*
Source: Plant Physiology & Biochemistry; Vol. 44, 10, (2006 Oct.) 581-589
Document Type: Article
Author Affiliations: [1]Regional Research Centre and Extension Service of Floriculture and Agriculture (RCNPO), 1222 Negovan, Sofia, Bulgaria;
[2]Department of Plant Physiology, Faculty of Biology, University of Sofia, 8 Dragan Tsankov Blvd., 1164 Sofia, Bulgaria;
[3]Department of Molecular and Laser Physics, Institute for Molecules and Materials, Radboud University Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands;
[4]Agrotechnology and Food Sciences Group (AFSG), Wageningen University and Research Centre, P.O. Box 17, 6700 AA Wageningen, The Netherlands
ISSN: 0981-9428

Title:	**Coalescence Stability of Emulsions Containing Globular Milk Proteins.**
Authors:	Tcholakova, Slavka[1], Denkov, Nikolai D.[1], Ivanov, Ivan B.[1], *II@lcpe.uni-sofia.bg,* Campbell, Bruce[2]
Source:	Advances in Colloid & Interface Science; Vol. 123-126, (2006 Nov.) 259-293
Document Type:	Article
Author Affiliations:	[1]Laboratory of Chemical Physics and Engineering, Faculty of Chemistry, Sofia University, 1 James Boucher Blvd., 1164 Sofia, Bulgaria; [2]Kraft Foods Global Inc., 801 Waukegan Road, Glenview, Illinois 60025, USA
ISSN:	0001-8686

Title:	**Biodegradation of High Amounts of Phenol, Catechol, 2,4–Dichlorophenol and 2,6–Dimethoxyphenol by Aspergillus Awamori Cells**
Authors:	Stoilova, Ivanka[1], Krastanov, Albert[1] *abt-kr@rocketmail.com,* Stanchev, Veselin[2], Daniel, David[3], Gerginova, Maria[4], Alexieva, Zlatka[4]
Source:	Enzyme & Microbial Technology; Vol. 39,5,(2006 Sep.),1036-1041
Document Type:	Article
Author Affiliations:	[1]Department of Biotechnology, University of Food Technologies, 4002 Plovdiv**,** Bulgaria; [2]Department of Automatics, Information and Control Systems, University of Food Technologies, 4002 Plovdiv, Bulgaria; [3]Department of Chemical Engineering, Vellore Institute of Technology, Vellore 632014, India; [4]Institute of Microbiology, Bulgarian Academy of Sciences, 26 Acad. G. Bontchev Str., 1113 Sofia**,** Bulgaria
ISSN:	0141-0229

Title:	**Liquid/Liquid Extraction and Column Solid Phase Extraction Procedures for Iron Species Determination in Wines**
Authors:	Tašev, Krste[1], Karadjova, Irina[2], Arpadjan, Sonja[2], Svetkovič, Julijana[3], Stafilov, Trajče[1] *trajcest@iunona.pmf.ukim.edu.mk*
Source:	Food Control, Vol. 17, 6, (2006 Jun.) 484-488
Document Type:	Article
Author Affiliations:	[1]Institute of Chemistry, Faculty of Science, St. Cyril and Methodius University, P.O. Box 162, 1000 Skopje, Republic of Macedonia; [2]Faculty of Chemistry, University of Sofia, 1126 Sofia, Bulgaria; [3]Institute of Agriculture, Bull. A. Makedonski bb, 1000 Skopje, Republic of Macedonia
ISSN:	0956-7135

DEFENDED DISSERTATIONS ON THE SUBJECT: "FOODS, QUALITY AND SAFETY OF FOODS" "SIRENA" Database– NACID

Author Kuzelov, Atso Apostol
Degree PhD
Title Investigation on the Opportunities for Improving the Quality of Meat Products from Big Ruminants by Enzyme Treatment
Affiliated Organization University of Food Technologies, 26 Maritsa Str., 4000 Plovdiv
Abstract The opportunities have been studied for application of bacterial enzyme preparation with collagenase action in the production of meat products with a view to intensification of the processes of ripening in meat raw materials from big ruminants /BR/, improving of the technological properties of low functional meat raw materials and the quality of the final meat products. For this purpose it has been established the influence of bacterial enzyme preparation on the state and properties of the meat raw materials used for production of some characteristic assortments of meat products as well as on the state, properties, yield and quality of the meat products. The optimum quantity of the enzyme preparation used in the production of the assortments under study has also been established and it has been made an evaluation concerning the influence of the enzyme preparation on the nutritive value and the yield of the meat products produced with it.
Depositary Library Central Research and Technical Library

Author Vachkova-Petrova, Rumiana Bojanova
Degree DSc
Title Toxilogical Problems Releated to Chemical Food Safety
Affiliated Organization National Centre of Hygiene, Medical Ecology and Feeding, 15, D. Nestorov Str., 1431 Sofia
Abstract The dissertation is aimed at elaborating the toxicological criteria of the system of Food Safety assurance and Risk assessment. Different toxicological problems related to chemical food safety are discussed: pesticides residues; food additives of natural origin and biotechnology products; modificators of toxic effects with special reference to mutagenesis and carcinogenesis; exposure assessment and risk assessment of exposure to food additives permitted for use and of high exposure to nitrate in food and drinking water in Bulgarian rural population. A large scale of toxicological studies and methods are used: Genotoxic activity in vitro /sos chromotest, ames test, a. nidulans, chromosomal aberration and sister chromatid exchanges/ and in vivo /chromosomal aberrations, micronucleus test in bone marrow, dominant lethal test/; biochemical; histopathological; ultramicroscopic; epidemiological; statistical. In most cases the acceptable daily intake and/or maximum permissible levels are estimated. The work includes evaluation of genotoxic activity and/or full toxicological assessment of pesticides: organophosphorous - phosalon, pyrazophos, chloracetophone, benomyl, kilakar, alaclbor, dithiocarbamates /basfungin, propyneb, endodan/. Methodological approach to the safety assessment of natural food additives, novel foods and biotechnology products constitutes main part of this work. Two Bulgarian natural additives are discussed: the red colour derived from s. ebulus, sweetener from svetia rebaudiana as well as microbial single-cell protein and the mycelium of the higher fungus p. squmosus.
Depositary Library Central Medical Library

Author Gotcheva, Velitchka Gotcheva
Degree PhD
Title Studies on the Production of Fermented Cereal-based Functional Drinks
Affiliated Organization University of Food Technologies, 26, Maritsa Str., 4000 Plovdiv
Abstract New trends in food biotechnology are directed towards improving the nutritional profile and adding functional ingredients to traditional products, as well as developing and introducing new functional products. An intriguing approach to achieve this is fermentation of oats - a cereal with valuable functional properties by probiotic strains lactic acid bacteria and yeasts for the development of new symbiotic drinks. The present work was initially focused on studies of the fermentation process and the microflora of the traditional cereal-based drink boza. From this product lactic acid bacteria and yeast strains with probiotic properties were then selected and their application for oat base fermentation was explored in order to develop new functional drinks. As a result, a principal flowchart for the production of new drinks is proposed, combining functional properties of oats as the raw material and of a probiotic starter culture isolated from the traditional product. The probiotic lactic acid bacteria and yeast strains and the appropriate conditions selected for carrying out the fermentation process can be a suitable base for the production of different oat-based functional products.
Depositary Library Central Research and Technical Library

Author Batchvarov, Valentin Hristov
Degree DSc
Title Investigation on the Beer Colloidal Stability. Methods for Prediction and Stabilizing Agents
Affiliated Organization University of Food Technologies, 26, Maritsa Str., 4000 Plovdiv
Abstract The survey of literature deals with the role of beer colloidal stability, turbidity, mechanism of beer haze formation, the impact of proteins, polyphenols and technology on the beer colloidal stability, different stabilizing agents and methods for prediction. Special apparatus for investigation on beer colloidal stability and for methods for prediction is developed. Nephelometric methods were elaborated for determination of beer proteins with picric acid, $CC_{l3}COOH$, $MgSO_4$ and $(NH_4)2SO_4$. The Chapon's alcohol chill test and test sensitive proteins were modified. Methods for prediction of colloidal stability of beers produced by different and same breweries were established. The colloidal stability changes during the bottled beer storage were studied. 23 laboratory and 10 full production scale series for colloidal stabilization of beer were carried out. The stabilizing effect of different enzymes, silicagels, tannic acid, PVPP, carageenan, isinglass, antioxidants, PVPV-sheets was investigated. The Bulgarian silicagel agents for protein stabilization and for combined polyphenols and proteins stabilization were developed. These agents have the same or better stabilizing effect than the imported ones. On the basis of the obtained results presented on 149 tables and 89 figures the scientific and scientific-application contributions were formulated.
Depositary Library Central Research and Technical Library

EVENTS

19 – 21 April, 2007
Workshop on Ecomaterials and Processes: Characterization and Metrology
MetEcoMat
St. Kirik, Plovdiv, Bulgaria
Address:
Centre of Competence on Multifunctional Materials and New Processes with Environmental Impact (MISSION), Institute of General and Inorganic Chemistry, BAS, Acad. G. Bonchev Str., Bldg. 11,1113 Sofia, Bulgaria
Fax: + 359 2 870 50 24
E-mail: *metecomat@svr.igic.bas.bg*

31 May – 4 June, 2007
9-th International Symposium
TECHNOMAT 2007
Materials, Methods and Technology
Sunny Beach resort, Bulgaria
Address:
Phone: +359 56 846075,
Mob. phone: +359 87 8844410
E-Mail: *office@sciencebg.net*

7 – 8 June, 2007
International Science Conference
"Challenges for Bulgarian Science in This Country's EU Membership"
Stara Zagora, Bulgaria
Address:
Conference secretariat
140 Tzar Simeon Veliki Str., P.O. Box 304
6000 Stara Zagora,
E-mail: *chiefcoordinator@sub.stz.orbitel.bg*

5 – 9 June, 2007
6-th International Conference on
"Large-Scale Scientific Computations"
Sozopol, Bulgaria
Address:
Institute for Parallel Processing, BAS
Acad. G. Bonchev Str., Bl. 25A,
1113 Sofia, Bulgaria;
FAX: +359-2-8722-349;
E-mail: *scicom07@parallel.bas.bg*

4 – 8 June, 2007
16-th International Symposium
ECOLOGY 2007
Sunny Beach resort, Bulgaria
Address:
Phone: +359 56 846075,
Mob. phone**:** +359 87 8844410
E-mail: *office@sciencebg.net*

17 – 25 June, 2007
Thirteenth International Conference
Knowledge – Dialogue – Solution (KDS)
Varna, Bulgaria
Address:
Association of Developers and Users of Intelligent Systems (ADUIS)
P.O. Box 775, Sofia-1090, Bulgaria;
E-mail: *markov@foibg.com*

16 – 22 June, 2007
Fifth International Workshop on
OPTIMAL CODES AND RELATED TOPICS
Balchik, Bulgaria
Address:
IMI / MFI
8, Acad. G. Bonchev, 1113 Sofia, Bulgaria
Phone: +359 2 979 28 21, Fax: +359 2 971 36 49
E-mail: *oc2007@moi.math.bas.bg.*

17 – 25 June, 2007
Workshop "General Information Theory"
G I T 2007
Varna, Bulgaria

Address:
P.O. Box 775, 1090 Sofia, Bulgaria
Phone /fax: (++ 359 2) 920 19 69
E-mail: *info@foibg.com*

18 – 20 June, 2007
International Scientific Conference
"TOURISM IN THE NEW MILLENIUM"
Blagoevgrad, Bulgaria
Address:
Tourism Department, Faculty of Economics in the SWU "Neofit Rilski"
2, Krali Marko Str., 2700 Blagoevgrad, Bulgaria
Phone: + 359 73 859 52,
Fax: + 359 73 308 35
E-mail: *Tourism_ Conference@mail.bg*; *stopfak@aix.swu.bg*

25 June – 01 July, 2007
Fourth International Conference
i.TECH 2007
Information Research and Applications
Varna, Bulgaria
Address:
P.O. Box 775, 1090 Sofia, Bulgaria
Phone/fax: (++ 359 2) 920 19 69
E-mail**:** *info@foibg.com*

1 – 7July, 2007
International Conference
MeL 2007
MODERN (e-) LEARNING
Address:
P.O. Box 775, 1090 Sofia, Bulgaria
Phone /fax: (++ 359 2) 920 19 69
E-mail: *info@foibg.com*