



# Influence of the microenvironment of thiol groups in low molecular mass thiols and serum albumin on the reaction with methylglyoxal

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## ARTICLE INFO

### Article history:

Received 13 April 2010

Received in revised form 23 June 2010

Accepted 12 July 2010

Available online 18 July 2010

### Keywords:

Methylglyoxal

Low molecular mass thiols

HSA

Thiol group reactivity and

microenvironment

Protein glycation

## ABSTRACT

Methylglyoxal (MG), a reactive  $\alpha$ -oxoaldehyde that is produced in higher quantities in diabetes, uremia, oxidative stress, aging and inflammation, reacts with the thiol groups (in addition to the amino and guanidino groups) of proteins. This causes protein modification, formation of advanced glycation end products (AGEs) and cross-linking. Low molecular mass thiols can be used as competitive targets for MG, preventing the reactions mentioned above. Therefore, this paper investigated how the microenvironment of the thiol group in low molecular mass thiols (cysteine, N-acetylcysteine (NACys), carboxymethylcysteine (CMC) and glutathione (GSH)) and human serum albumin (HSA) affected the thiol reaction with MG. The SH group reaction course was monitored by <sup>1</sup>H-NMR spectroscopy and spectrophotometric quantification. Changes in the HSA molecules were monitored by SDS-PAGE. The microenvironment of the SH group had a major effect on its reactivity and on the product yield. The reactivity of SH groups decreased in the order Cys > GSH > NACys. CMC did not react. The percentages of the reacted SH groups in the equilibrium state were almost equal regardless of the ratio of thiol compound/MG (1:1, 1:2, 1:5): 38.1 ± 0.9%; 38.2 ± 0.7% and 39.0 ± 0.8% for Cys; 26.5 ± 0.6%; 26.6 ± 2.6% and 27.4 ± 2.3% for GSH; 10.8 ± 0.9%; and 11.2 ± 0.7% and 12.2 ± 0.9% for NACys, respectively. Our results explain why substances containing  $\alpha$ -amino- $\beta$ -mercapto-ethane as a pharmacophore are successful scavengers of MG. In equilibrium, HSA SH reacted in high percentages both with an insufficient amount and with an excess of MG (55% and 65%, respectively). An analysis of the hydrophobicity of the microenvironment of the SH group on the HSA surface showed that it could contribute to high levels of SH modification, leading to an increase in the scavenging activity of the albumin thiol.

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## 1. Introduction

Methylglyoxal (MG) is a naturally occurring  $\alpha$ -oxoaldehyde that is produced by non-enzymatic pathways from the spontaneous decomposition of triose phosphates, the autooxidation of carbohydrates, and glucose degradation, as well as by several minor metabolic pathways including the Maillard reaction and lipid peroxidation. The rate of MG formation in normal systems is on the order of 120  $\mu$ mol/day or 0.1–0.4% of the glycolytic flux [1]. MG is increased in diabetes [2,3] (by 5- to 6-fold), oxidative stress, uremia [4], during the aging process and in inflammation. As a highly

reactive compound ( $\alpha$ -dicarbonyls are 20,000-fold more reactive than glucose in glycation reactions [5]), MG is a potent modifying agent of proteins [6,7] and nucleic acids [8]. Protein modification occurs readily under physiological conditions at steady-state concentrations of dicarbonyls of high nanomolar to low micromolar and leads to formation of advanced glycation end products (AGEs) [9]. Molecules modified with MG and their derivatives can affect cellular functionality via gene expression [10], lead to micro- and macro-vascular complications in diabetes [11] and contribute to the upregulation of inflammatory and tissue injury-provoking molecules through the interaction of AGEs and receptors for advanced glycation end products (RAGE) [12,13], protein cross-linking and apoptosis [14].

N-terminal and Lys side chain amino groups, the guanidine group of Arg [11] and the sulfhydryl group of Cys [14] present on protein surfaces participate in protein modification by MG. The thiol group of Cys is a powerful nucleophile at physiological pH values. However, its abundance on the protein surface is much lower than amino and guanidine groups. Therefore, the role of the thiol group in protein modifications with MG has not

Abbreviations: MG, methylglyoxal; AGEs, advanced glycation end products; RAGE, receptor for advanced glycation end products; HTA, hemithioacetal; CMC, S-(carboxymethyl)cysteine; CEC, S-(2-carboxyethyl)cysteine; HSA, human serum albumin; ASA, accessible surface area.

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## The possibility of determining *N*-acetyl- $\beta$ -D-glucosaminidase isoenzymes under alkaline conditions

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Received 26 November 2003; received in revised form 21 December 2004; accepted 10 January 2005

### Abstract

**Objectives:** To have a reliable diagnostic test, the influence of urine pH on the determination of the total activity of *N*-acetyl- $\beta$ -D-glucosaminidase (NAG) and NAG isoenzyme activities was studied.

**Design and methods:** After ultrafiltration and dialysis of the acidic and alkaline urines, the B, A, and A<sub>2</sub> forms of NAG were separated by ion-exchange chromatography on DEAE cellulose.

**Results:** A significant decrease in the total activity of NAG in alkaline urines (pH around 8 or higher) was found, which makes this determination unreliable. Analysis of the isoenzymic profiles obtained for weakly acidic and alkaline urines (in the pH range from 5.5 to 10.0) showed that the percent fractions of the individual isoenzyme activities in the total NAG activity and their ratios changed only at pH values above 9.5.

**Conclusions:** The determination of the denoted isoenzymes of urinary NAG after ultrafiltration, dialysis, and chromatographic separation on DEAE cellulose is reliable in a wide range of alkaline pH values of urine.

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**Keywords:** *N*-Acetyl- $\beta$ -D-glucosaminidase (NAG); Isoenzymes A, B, and A<sub>2</sub>; Isoenzyme stabilities; Alkaline conditions; Determination

### Introduction

*N*-Acetyl- $\beta$ -D-glucosaminidase (NAG; EC 3.2.1.30) and the isoenzymes of NAG in human urine represent a very important parameter in clinical practice. Thus, urinary NAG is an indicator of the anatomic decomposition or functional damage of renal tubular cells [1,2] and a good parameter for determining the beginning of rejection of transplanted kidneys [3]. Changes in the activity of urinary NAG are indicators of the nephrotoxic effects of various substances: organic solvents, medications [4], metals such as lead and manganese [5], cadmium [6] and mercury [7]. Urinary NAG is also a parameter for the early detection of renal changes in IDDM [8,9] and NIDDM [10] patients, a marker of tubular dysfunction in diabetes [11].

Under some pathological conditions, beside the total activity of urinary NAG, some relevant changes in the isoenzymic profiles [12–16] have also been detected. There are two main NAG isoenzymes in human kidneys. Isoenzyme A is a part of the soluble intralysosomal compartment and is normally secreted in urine by exocytosis [17]. Isoenzyme B is linked to the lysosomal membrane and excreted in the urine during tubular damage. The fact that the activities of the isoenzymes of NAG change under some pathological conditions enables clarification of the mechanism leading to increased urinary NAG by investigations of the isoenzyme profiles.

The investigation of NAG stability at various pH values has shown that NAG is inactivated at pH values around 8 [18]. It was also determined that the loss of activity under alkaline conditions was the consequence of the inactivation of the predominant A form [19]. The urine of patients with pyelonephritis and of persons undergoing drug (methotrex-

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## The influence of fatty acids on determination of human serum albumin thiol group



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### ARTICLE INFO

#### Article history:

Received 18 September 2013

Received in revised form 13 November 2013

Accepted 25 November 2013

Available online 4 December 2013

#### Keywords:

HSA-thiol group determination

Fatty acid

Albumin isolation

Affinity chromatography

Diabetes

### ABSTRACT

During investigation of the changes of the Cys34 thiol group of human serum albumin (HSA) (isolated by affinity chromatography with Cibacron Blue (CB)) in diabetes, we found that the HSA-SH content was higher (11–33%) than the total serum thiol content. The influence of fatty acids (FA) binding to HSA on this discrepancy was investigated *in vitro* (using fluorescence and CD spectroscopy and GC) and with HSA samples from diabetic ( $n=20$ ) and control groups ( $n=17$ ). HSA-bound FA determine the selection of HSA molecules by CB and enhance reactivity and/or accessibility of the SH group. A high content of polyunsaturated FA (35.6%) leads to weaker binding of HSA molecules to CB. Rate constants of DTNB reaction with the SH group of HSA applied to a CB column, bound-HSA and unbound-HSA fractions, were  $4.8 \times 10^{-3}$ ,  $21.6 \times 10^{-3}$ , and  $11.2 \times 10^{-3} \text{ s}^{-1}$ , respectively. The HSA-SH group of diabetics is more reactive compared with control individuals (rate constants  $20.9 \times 10^{-3} \pm 4.4 \times 10^{-3}$  vs  $12.9 \times 10^{-3} \pm 2.6 \times 10^{-3} \text{ s}^{-1}$ ,  $P < 0.05$ ). Recovery values of the SH group obtained after chromatography of HSA with bound stearic acid ranged from 110 to 140%, while those for defatted HSA were from 98.5 to 101.7%. Thus, HSA-bound FA leads to an increase of HSA-SH content and a contribution to total serum thiols, which make the determination of the thiol group unreliable.

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Human serum albumin (HSA)<sup>1</sup> is the most abundant plasma protein (~0.6 mM). It is a 66.5-kDa protein organized into three homologous domains (labeled I–III) and each domain comprises two subdomains (A and B) that share common structural elements [1,2]. A total of 17 disulfide bridges located exclusively within subdomains contribute toward HSA's stability [3].

HSA transports many endogenous ligands such as long chain (C13–C21) fatty acids (FA), hemin, bilirubin, and thyroxine, all of which bind HSA with high affinity [3]. Although most ligands for HSA are hydrophobic anions, heavy metals are also known to bind to this protein [1,3–5]. Moreover, HSA has the ability to bind a wide variety of drug molecules and alter their pharmacokinetic parameters [6]. This binding occurs via hydrophobic cavities in subdomains IIa and IIIa, known as Sudlow I and Sudlow II, respectively

[7,8], with the sole tryptophan residue in HSA located in Sudlow I (Trp-214) [9].

HSA is the primary transporter for delivering FA to the tissues and possesses at least seven binding sites of varying affinities for this ligand (Fig. 1) [10–12]. Although none of the FA-binding sites are completely identical, each comprises a hydrophobic pocket that interacts with the hydrocarbon chain, while five binding sites cap FA at one end with basic or polar residues that interact closely with the carboxyl group of bound FA [13]. Under normal physiological conditions, between 0.1 and 2 mol of FA are bound to HSA, but the molar ratio of FA/HSA can rise above 6:1 in the peripheral vasculature during fasting or extreme exercise [5,14] or under pathological conditions such as diabetes, liver disease, and cardiovascular disease [3,15]. In order to completely understand the role of HSA *in vivo*, it is crucial to obtain detailed information about the variety of ligands that HSA binds, as well as how these ligands interact and influence each other during binding to HSA.

Besides its role in transport, HSA is one of the most important extracellular antioxidants [3]. The one free cysteine-derived thiol (–SH) group (Cys34) (located in subdomain I), which can exist in both reduced and oxidized forms, provides a part of the antioxidant property of HSA. As HSA is the most abundant plasma protein

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<sup>1</sup> Abbreviations used: BCG, bromocresol green; CB, Cibacron Blue; CD, circular dichroism; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DTNB, 5,5'-dithiobis(2-nitrobenzoic acid); FA, fatty acids; GC, gas chromatography; HSA, human serum albumin; HSA-SH, human serum albumin thiol content; PUFA, polyunsaturated fatty acids; RSD, relative standard deviation; TG, triglyceride; UV, ultraviolet.

## The efficiency of compounds with $\alpha$ -amino- $\beta$ -mercapto-ethane group in protection of human serum albumin carbonylation and cross-linking with methylglyoxal

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Cite this: DOI: 10.1039/C4MB00217B

$\alpha$ -Oxoaldehydes, which are produced in higher quantities in diabetes, uremia, oxidative stress, inflammation and aging, react with the amino, guanidine and thiol groups of proteins and cause the formation of advanced glycated end-products and protein cross-linking. To prevent these reactions, the efficiency of low molecular mass thiols with an  $\alpha$ -amino- $\beta$ -mercapto-ethane group (Cys, penicillamine and *N*-acetylcysteine (NACys, with a blocked amino group)) as scavengers of methylglyoxal, compared with glutathione (GSH) and the biguanidine derivative metformin, was investigated. The time courses of the reactions of the aforementioned compounds with methylglyoxal were assayed. The reactivity of their thiol and amino groups decreased in the order of Cys > penicillamine > GSH > NACys and penicillamine > Cys > GSH, respectively. Human serum albumin (HSA) carbonylation in the absence or presence of methylglyoxal scavengers were monitored by the determination of the amino, guanidine and thiol groups' contents, as well as by spectrofluorimetry, CD and native and SDS PAGE. Cys and penicillamine were highly efficient in the prevention of the carbonylation of the HSA-amino (for 80%) and guanidine (for 84% and 55%, respectively) groups and the formation of fluorescent AGEs. GSH and metformin exhibited medium efficiency (reduction of amino group's carbonylation for 60% and guanidine for about 30%); the least efficient was NACys. The presence of Cys, penicillamine and NACys led to an almost complete protection of the HSA-thiol group's carbonylation, whereas metformin was inefficient. The efficiency in the prevention of protein cross-linking increased in the order of metformin, NACys < GSH < penicillamine < Cys. Thus, the substances with an  $\alpha$ -amino- $\beta$ -mercapto-ethane group as a pharmacophore exhibit great potential as an efficient methylglyoxal scavengers, and are thus promising compounds for medicinal chemistry. In addition, they protect the HSA-SH group and preserve its antioxidative potential, which is very important for the HSA's function *in vivo*.

Received 3rd April 2014,  
Accepted 13th May 2014

DOI: 10.1039/C4MB00217B

www.rsc.org/molecularbiosystems

### 1. Introduction

The reactions of non-enzymatic glycation lead to the development of numerous pathological conditions such as micro- and macrovascular complications in diabetes, Alzheimer's disease, cirrhosis, uremia, arthritis and changes in the aging process.<sup>1–3</sup>  $\alpha$ -Dicarbonyls such as methylglyoxal (MG) are 20 000-fold more reactive than glucose in glycation reactions.<sup>4</sup> They react with thiol, amino and guanidine groups of proteins, causing protein modification, formation of advanced glycated end-products (AGEs) and protein cross-linking.<sup>5–7</sup> The factors considered most closely linked with the development of micro- and

macrovascular complications in diabetes are AGEs, their biochemical nature and their mode of action.<sup>8,9</sup> Because it is difficult to achieve and maintain normal glycemia in diabetes,<sup>10</sup> the inhibition of the formation of AGEs is very important in the prevention and treatment of complications in diabetes and other pathological conditions where carbonyl stress is present.

To prevent the aforementioned reactions, numerous natural and synthetic inhibitors of glycation and AGE formation were developed, and a few of them are in their final phases of clinical trials. Aspirin, diclofenac, inositol, pioglitazone, metformin and pentoxifylline are listed as inhibitors of early glycation product formation. They interfere with the initial reaction between reducing sugars and amino groups, thus inhibiting the formation of a Schiff base.<sup>11</sup> The substances, which bind reactive carbonyl compounds and radicals that are generated in

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# Influence of Pigments and pH of Urine on the Determination of *N*-Acetyl- $\beta$ -D-Glucosaminidase Activity With 2-Methoxy-4-(2'-Nitrovinyl)-Phenyl-*N*-Acetyl- $\beta$ -D-Glucosaminide

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The influence of urinary pigments and urine pH on the spectrophotometric determination of *N*-acetyl- $\beta$ -D-glucosaminidase (NAG; EC 3.2.1.30) activity with 2-methoxy-4-(2'-nitrovinyl)-phenyl-*N*-acetyl- $\beta$ -D-glucosaminide as a substrate was studied. The investigation was performed with human and rabbit urine samples. It was found that alkaline urine pH values influenced NAG activity in two ways: 1) NAG activity decreased due to enzyme instability with pH increase, and 2) NAG activity increased because of the contribution of urinary pigments to absorbance of 2-methoxy-4-(2'-nitrovinyl)-phenol (MNP) at 505 nm. It was shown that besides the maximum (I) in the range of 350–360 nm of the absorption

spectra of alkaline urine, there was a maximum (II) in the range of 380–460 nm. With the increase of pH, maximum II was shifted toward higher wavelengths and contributed to MNP absorption (5–90%). On the other hand, the maximum of MNP absorption was shifted toward lower wavelengths (495–400 nm) with increasing pH. Two procedures to eliminate the influence of urinary pigments are presented. The justification of applying a correction to the values of NAG activity in human and rabbit urine (a model system for studying the toxic effects of cadmium) was discussed. *J. Clin. Lab. Anal.* 19:260–266, 2005. © 2005 Wiley-Liss, Inc.

**Key words:** alkaline urine; urinary pigments; *N*-acetyl- $\beta$ -D-glucosaminidase (NAG) spectrophotometric determination

## INTRODUCTION

The activity of the lysosomal enzyme *N*-acetyl- $\beta$ -D-glucosaminidase (NAG) in urine is a sensitive indicator of early damage to the renal proximal tubules (1,2). Increased urinary NAG activity originates in pathological states (e.g., diabetes (3–5)) and renal damage during treatment with medications (6), organic solvents (7), metals (cadmium (8), mercury (9), and lead and manganese (10)), and during the rejection of kidney transplants (11). In experiments investigating the nephrotoxicity of various substances with animal models (rabbits (12,13), rats (14), and hamsters (15)), NAG activity was determined as an indicator of damage to the renal tubules.

The most common substrates applied for the spectrophotometric determination of NAG activity in clinical and toxicological laboratories are *p*-nitrophenyl-*N*-acetyl-beta-D-glucosaminide (pNP-NAG) (16), 2-chloro-4-nitrophenyl-*N*-acetyl-beta-D-glucosaminide

(CNP-NAG) (17), 2-methoxy-4-(2'-nitrovinyl)-phenyl-*N*-acetyl- $\beta$ -D-glucosaminide (MNP-NAG) (18), and ammonium 5-[4-(2-acetamido-2-deoxy-beta-D-glucopyranosyloxy)-3-methoxyphenylmethylene]-2-thioxothiazolidin-4-one-3-ethanoate (VRA-NAG) (19). The influence of the yellow color of urine samples on the determination of NAG activity decreases by

**Abbreviations:** MNP-NAG, 2-methoxy-4-(2'-nitrovinyl)-phenyl-*N*-acetyl- $\beta$ -D-glucosaminide; MNP, 2-methoxy-4-(2'-nitrovinyl)-phenol; NAG, *N*-acetyl- $\beta$ -D-glucosaminidase

Grant sponsor: Ministry for Science, Technology and Development of the Republic of Serbia; Grant number: 1802.

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Received 5 April 2005; Accepted 22 July 2005

DOI 10.1002/jcla.20088

Published online in Wiley InterScience (www.interscience.wiley.com).



J. Serb. Chem. Soc. 79 (12) 1491–1503 (2014)  
JSCS-4682

Journal of  
the Serbian  
Chemical Society

JSCS-info@shd.org.rs • www.shd.org.rs/JSCS

UDC 547.455.623:292:54.05+

577.15:616.379-008.64

Original scientific paper

## How the sialylation level of serum *N*-acetyl- $\beta$ -D-glucosaminidase A form in Type 1 diabetes mellitus influences their activity?

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(Received 30 April, revised 9 July, accepted 17 July 2014)

**Abstract:** It was verified that the serum *N*-acetyl- $\beta$ -D-glucosaminidase (NAG) activity is elevated in diabetes, but there are no reports about changes in the sialic acid (SA) content in the carbohydrate parts of the NAG A form and its influence on the total changes in NAG activity in type 1 diabetes mellitus patients with and without secondary complications. The NAG A form was isolated from the serum of 81 insulin-dependent diabetes mellitus (IDDM) patients with and without secondary complications (retinopathy, polyneuropathy and nephropathy) and 25 healthy persons, and purified and characterised. The content of  $\alpha$ -2,6-bound SA, the isoenzyme patterns of the purified A form, and the total NAG and A form activities were determined. In all diabetic groups, the sialylation levels of the A form were 2–3.5 times lower compared to control, while their acidities (fractions with *pI* 4.25–5.1) increased, particularly with progression of secondary complications. Total serum NAG activities and percentages of A form were significantly higher ( $P < 0.001$ ) in all diabetic groups and negatively correlated with the  $\alpha$ -2,6-bound SA content of the A form. In addition, they decreased as secondary diabetic complications became more complex. The observed changes could be the consequence of structural changes in the A form due to significant increases in its acidity, i.e., negative charge, which originated from groups other than SA.

**Keywords:** *N*-acetyl- $\beta$ -D-glucosaminidase; A isoenzyme isolation and characterization; sialylation level; diabetes mellitus type 1; secondary complications.

### INTRODUCTION

The serum *N*-acetyl- $\beta$ -D-glucosaminidase (NAG, EC 3.2.1.52) activity and sialic acid (SA) are elevated in individuals diagnosed with diabetes mellitus<sup>1–3</sup>

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doi: 10.2298/JSC140430076J

## Serum N-Acetyl- $\beta$ -D-Glucosaminidase Profiles in Type 1 Diabetes Secondary Complications: Causes of Changes and Significance of Determination

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The connection between changes in the activity of serum N-acetyl- $\beta$ -D-glucosaminidase (NAG, E.C.3.2.1.30) and iso-enzymes and degree of secondary complications was analyzed in four groups of type 1 diabetic patients ( $n = 69$ ): without complications ( $n = 22$ ); with retinopathy ( $n = 16$ ); with retinopathy and polyneuropathy ( $n = 13$ ), and with retinopathy, neuropathy, and nephropathy ( $n = 18$ ). In all groups statistically significant higher ( $P < 0.001$ ) percent fraction of A form ( $83.84 \pm 6.09$ ,  $84.37 \pm 5.74$ ,  $81.76 \pm 6.02$ ,  $76.37 \pm 7.38\%$ , resp.) and lower ( $P < 0.001$ ,  $P < 0.01$ ) fraction of B form ( $15.87 \pm 5.65$ ,  $15.66 \pm 5.74$ ,  $18.33 \pm 5.98$ ,  $23.63 \pm 7.38$ , resp.) in total NAG compared with the control ( $A = 69.38 \pm 4.79\%$ ,  $B = 30.61 \pm 4.78\%$ ) were found. The differences in A as well as B forms between diabetic groups were not statistically significant. Significant

strong positive correlations between total NAG and glycemia ( $0.494$ – $0.623$ ), total NAG and A form ( $0.934$ – $0.966$ ), and A form and glycemia ( $0.512$ – $0.638$ ) were found in all groups. No correlation was found between the fractions of B and A forms, except in the fourth group. The A form of diabetic patients in the fourth group was more acidic compared with the control and other diabetic groups. It was concluded that the changes in serum NAG and iso-enzymic profiles in diabetes are the consequence of its increased exocytosis, especially of the A form, in hyperglycemia and posttranslational modifications of iso-enzymes. The total activity of serum NAG and iso-enzymic profiles cannot be used for monitoring the development and distinction of type 1 diabetes secondary complications. *J. Clin. Lab. Anal.* 22:307–313, 2008. ©2008 Wiley-Liss, Inc.

**Key words:** serum N-acetyl- $\beta$ -D-glucosaminidase; iso-enzymes A and B; type 1 diabetes; diabetic complications; retinopathy; neuropathy; nephropathy

### INTRODUCTION

Increased activity of N-acetyl- $\beta$ -D-glucosaminidase (NAG, EC 3.2.1.30) in type 1 diabetic patients has been described by many authors (1–3). Poor glycemia control (4), long-term disruption of metabolic equilibrium in diabetes conditions with alleviated release of lysosomal enzymes in the extracellular liquid, which interfere with the mechanisms controlling the half-life of enzymes (5,6), the activation of NAG by glycomaterial accumulated on the walls of blood vessels, i.e., the induction of NAG owing to the degradation of mucopolysaccharides (7,8), were proposed as possible causes of changes in the activity of serum NAG. The proposed causes of the NAG activity changes have not yet been fully explained and proven.

The pathogenesis of diabetic microangiopathy (retinopathy—R, neuropathy—P, and nephropathy—N) is highly complex and not elucidated so far. Diabetic

**Abbreviations:** NAG, N-acetyl- $\beta$ -D-glucosaminidase; R, retinopathy; P, neuropathy; N, nephropathy; DBP, diastolic blood pressure; SBP, systolic blood pressure; DEAE, diethylaminoethyl.

Grant sponsor: Ministry for Science, Technology, and Development of the Republic of Serbia; Grant number: 142020.

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Received 24 May 2007; Accepted 16 April 2008

DOI 10.1002/jcla.20262

Published online in Wiley InterScience (www.interscience.wiley.com).

## ISOENZYMIC FORMS OF N-ACETYL- $\beta$ -D-GLUCOSAMINIDASE IN THE URINE OF THE INDIVIDUALS EXPOSED TO MERCURY

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### ABSTRACT

Mercury compounds are toxic environmental pollutants and cumulative poisons concentrating in the kidney. In order to find a parameter appropriate for early discovery of an injured kidney in individuals exposed to mercury effect, the activities of isoenzymic forms of urinary N-acetyl- $\beta$ -D-glucosaminidase (NAG, EC 3.2.1.30) were being determined in this work. For the separation and determination of the activities of isoenzymic forms a simple, fast and reproducible method was developed, suitable for toxicological and clinical practice. The analysis of the obtained isoenzymic urinary NAG profiles indicated that the B form contribution to the total NAG activity is statistically considerably increased under mercury effect ( $p < 0.05$ ) compared to the control group. The increase in total NAG activity is correlated with the B form activity ( $r = 0.583$ ). Since the NAG B form is bound with lysosomal membrane its increase in urine is an early parameter indicating the injuries of lysosomes. The contributions of the A ( $64.13 \pm 16.51\%$ ) and M ( $21.32 \pm 6.32$ ) forms activity to the total NAG activity do not change considerably compared to the controls.

**Key words:** N-acetyl- $\beta$ -D-glucosaminidase, Isoenzymes, Mercury, Nephrotoxicity.

### INTRODUCTION

Although very toxic, inorganic mercury compounds have various applications and dealing with them is inevitable. Mercury is easily absorbed by skin, and gastrointestinal and respiratory tracts and is selectively concentrated in kidneys, which are particularly sensitive to its toxic effect (Bomhard et al., 1985; Rosenman et al., 1986; Eli et al., 1995). The assessment of renal injury based upon serum creatinine or blood urea nitrogen levels is insensitive, since these tests show abnormal findings only when major impairment of renal excretory function has developed. Other measures of renal function such as creatinine clearance, p-aminohippuric acid clearance, urinary concentrating ability, proteinuria, glycosuria may be somewhat more sensitive but are technically not satisfactory for use of screening tests (Meyer et al., 1984). In contrast, urinary enzyme analysis is an extremely sensitive indicator of renal injury. N-acetyl- $\beta$ -D-glucosaminidase (NAG) is a lysosomal enzyme present in renal tubular cells. NAG can be assayed easily and reproducibly and has been shown to be a sensitive indicator of early renal injury (Mandic et al., 1995). Some results (Boogaard et al., 1996) suggest that after exposure to mercury at levels below the biological exposure index, a transient increase in NAG can be observed, but is not an early indicator of developing renal dysfunction.



# Serbian Biochemical Society Seventh Conference

with international participation

Faculty of Chemistry, University of Belgrade  
10.11.2017. Belgrade, Serbia

***“Biochemistry of Control in Life and Technology”***

## Serum redox-homeostasis in half-marathons

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Strenuous physical exercise is known to result in the formation of Reactive Oxygen Species (ROS) in the body<sup>1</sup>. Mitochondrial electron transport chain, hemoglobin and myoglobin, NADPH oxidase and catecholamine autooxidation are the main sources of ROS<sup>2</sup>. When the formation of ROS is higher than antioxidant defense, oxidative stress occurs which leads to damage of macromolecules such as proteins, lipids and nucleic acids. During the evolution, two main antioxidant systems (enzyme and non-enzyme system) were developed. Beside the high activities of antioxidant enzymes, glutathione is the most important intracellular antioxidant. Extracellular fluids contain only small amounts of antioxidant enzymes, so human serum albumin (HSA) is proposed as a major extracellular (intravascular and extravascular compartment) antioxidant<sup>3</sup>. Because HSA is the most abundant extracellular protein which has one free Cys34 thiol group in 75 % reduced form, it contributes 80% to the total thiol serum content. The property of molecule HSA to bind different endogenous (free fatty acids, hormones, bilirubin and hem) and exogenous (polyphenols, drugs and metal anions) substances, also contributes to the antioxidative role of HSA.

The aim of this study was to examine how oxidative stress caused by intense physical activity, affects serum redox-homeostasis. Total serum thiol content, and content and reactivity of HSA-SH groups were determined before the start of the race (control) and 15 min, 60 min and 24 h after the end of the race. Participants were amateur half-marathon racers ( $n = 10$ ), ages between 20 and 30 years old, who ran the half-marathon (21.1 km). The total thiol content in serum was statistically significantly ( $p < 0.05$ ) higher after 15 min from the end of the race ( $0.425 \pm 0.019$  mM) compared to control ( $0.400 \pm 0.029$  mM). After 60 min it gradually decreases ( $0.416 \pm 0.037$  mM) and after 24h it is almost equal to the value obtained before the start of the race ( $0.401 \pm 0.025$  mM). Mean value of the content of HSA-SH groups in HSA preparations (isolated from the serum by precipitation with saturated ammonium sulfate solution in two steps) was  $0.564 \pm 0.025$  mol SH/mol HSA, which is in agreement with published claims that about 60 % of HSA-SH groups in healthy people are in a reduced state<sup>4</sup>. A statistically significant ( $p < 0.05$ ) decrease in the HSA-SH group content was found 15 min after the end of the race ( $0.519 \pm 0.039$  mol SH/mol HSA) compared to control. After 60 min the content of HSA-SH groups ( $0.562 \pm$

13<sup>th</sup> CONGRESS OF NUTRITION  
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# BOOK OF ABSTRACTS

Belgrade, Serbia  
26<sup>th</sup>-28<sup>th</sup> October 2016.

## BINDING OF GALLIC ACID TO HUMAN SERUM ALBUMIN: INCREASE OF CYSTEINE-34 THIOL GROUP REACTIVITY

Tamara Uzelac<sup>1\*</sup>, Vesna Jovanovic<sup>1</sup>, Marija Takic<sup>2</sup>, Ivan Pavicevic<sup>1</sup>, Jelena Acimovic<sup>1</sup>, Danijela-Ristic Medic<sup>2</sup>, Marija Glibetic<sup>2</sup>, Ljuba Mandic<sup>1</sup>

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Human serum albumin (HSA) is the most abundant plasma protein and with free Cys34 thiol represents a significant pool of the plasma anti-oxidative thiols. It transports many endogenous and exogenous ligands (metals, fatty acids, cholesterol, hormones, polyphenols and drugs). Gallic acid (GA), a natural plant phenolic acid, and its derivatives have been extensively evaluated for their therapeutic properties as they are potent antioxidant. Several studies investigated GA binding to HSA, but the changes of HSA-SH reactivity that can occur upon GA binding (possible way of expressing its antioxidant properties) are not considered yet. The influence of GA on HSA-SH reactivity was investigated after 30 minutes of pre-incubation of stearic acid/HSA (molar ratio 2:1) without (control) and with GA (molar ratio 1:1) by determination of HSA-SH reaction with 5, 5'-dithiobis-(2-nitrobenzoic acid) and pseudo first order rate constant ( $k'$ ) of this reaction. The obtained  $k'$  value for S/HSA 2:1 complex with GA was 26.2% higher compared to the control ( $12.0 \pm 0.4 \times 10^{-3} \text{ s}^{-1}$ ), while the content of HSA-SH groups was 25.2 % lower compared to the control. The obtained results suggest that upon GA binding to HSA, induced conformation changes of HSA molecule may cause an increase in reactivity of Cys34 group, as well as an increase in its antioxidant role.

**Keywords:** gallic acid, human serum albumin, Cys34 thiol group, phenolic acid

# Serbian Biochemical Society

## Sixth Conference

with international participation

Faculty of Chemistry, University of Belgrade,  
18.11.2016. Belgrade, Serbia

***“Biochemistry and Interdisciplinarity: Transcending the Limits  
of Field”***

# Carbonylation of HSA with methylglyoxal leads to release of copper(II) ions and changes in its antioxidant capacity

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Human serum albumin (HSA), has important physiological functions like regulation of oncotic pressure and also serves as a transporter of metal ions, fatty acids, cholesterol, hormones. With normal serum concentration between 35 and 50 gL<sup>-1</sup>, and 70–80% of its Cys34 in sulfhydryl form, HSA is the predominant serum protein and a major plasma antioxidant. It contains two binding sites for copper ions, one with high affinity, located at the N-terminus (NT).

The goal of this study was to decipher the effect of HSA modification with methylglyoxal (MG) on its ability to bind and sequester Cu<sup>2+</sup>, which could prove to be useful in better understanding and possible treating of pathological states with prominent oxidative/carbonyl stress such as diabetes.

In this study, we monitored the changes in Cu<sup>2+</sup> ion and Cys34-thiol group content, as well as their ratios, upon modification of HSA with MG. Also, the conformational changes in HSA molecule were monitored by recording fluorescent spectra. Electrophoretic properties of samples were monitored by performing SDS and native PAGE. Samples from diabetic patients and controls were also analyzed.

The ability of HSA to coordinate Cu<sup>2+</sup> decreases upon carbonylation of the Cys34-SH group. Carbonylation of Cu-HSA complexes caused a decrease in Cys34-SH content and leakage of Cu<sup>2+</sup> from Cu-HSA complexes. Conformational changes in samples modified with MG were also observed. The ratio between the percentage of reduction in the Cys34-SH group content and the percentage of release of Cu<sup>2+</sup> from complexes is  $2.12 \pm 0.28$ . The same ratio ( $1.96 \pm 0.36$ ) was obtained upon oxidation of the Cys34-SH group (with no changes in HSA conformation), leading to conclusion that the binding/release of Cu<sup>2+</sup> by HSA depends mainly on the redox state of the Cys34-SH group. Samples from diabetic patients had significantly lower contents of Cys34-SH and HSA-bound Cu<sup>2+</sup> ( $0.457 \pm 0.081$  mol SH per mol HSA,  $10.7 \pm 0.01$  mmol per mol HSA, resp.) ( $p < 0.01$ ) ( $0.609 \pm 0.027$  mol SH per mol HSA;  $13.4 \pm 0.01$  mmol per mol HSA, resp.). Strong correlations between the values for HSA-SH and glycated hemoglobin, HbA1c, ( $R = -0.803$ ,  $p < 0.01$ ), and between the values for the HSA-bound Cu<sup>2+</sup> content and HSA-SH content ( $R = 0.841$ ,  $p < 0.002$ ) were found in the diabetic group.

Overall, the reaction of HSA carbonylation *in vitro* (with MG) and *in vivo* (diabetes), leads to release of Cu<sup>2+</sup> ions from copper-HSA complexes in an extent which depends mainly on the redox state of the Cys34 free thiol group.



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## **11<sup>th</sup> Meeting of the Slovenian Biochemical Society**

September 16-19, 2015  
Portorož, Slovenia

# **Book of Abstracts**

S37

### **Carbonylation of HSA with methylglyoxal affects its copper(II) binding affinity**

Ana Penezic<sup>1,2</sup>, Ivan Pavicevic<sup>1</sup>, Vesna Jovanovic<sup>1</sup>, Jelena Acimovic<sup>1</sup>, Ljuba Mandic<sup>1</sup>

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Carbonylation of human serum albumin (HSA) with methylglyoxal affects its copper(II) binding affinity

The effects of carbonylation with methylglyoxal on HSA's copper(II) binding affinity and copper(II) release from copper-HSA complexes were studied. Carbonylation causes conformational changes in HSA molecule, Cys34-SH content decrease, copper binding affinity decrease and release of copper from copper-HSA complexes. The percent reduction ratio (Cys34-SH group content/HSA bound copper) upon HSA carbonylation and oxidation was the same, indicating that binding/release of copper(II) ions depends mainly on the redox state of Cys34-SH. Observed changes were tested in diabetic group. Cys34-SH and HSA-bound copper(II) ions contents are significantly lower (  $0.457 \pm 0.081$  mol SH/mol HSA,  $1.07 \pm 0.01$   $\mu$ mol/mol HSA, resp.) compared to the control group ( $0.609 \pm 0.027$  mol SH/mol HSA,  $1.34 \pm 0.01$   $\mu$ mol/mol HSA, resp.). Strong correlations between HSA-SH content and HbA1c ( $R = -0.803$ ), and between HSA-bound copper(II) and the HSA-SH content ( $R = 0.841$ ) were found. Thus, HSA carbonylation lead to decrease of HSA-SH content and to increase of free copper(II) ion level in serum, contributing to further enhancement of oxidative and carbonyl stress in diabetes.



# ICOSECS 8

University of Belgrade  
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Belgrade, Serbia, June 27-29, 2013



8<sup>th</sup> International Conference  
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## BOOK OF ABSTRACTS

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Supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia



# Kinetic characterization of Cys34 thiol group of human serum albumin loaded with different long chain free fatty acids

**Ivan D. Pavšević, Ana Z. Petrović Romanjuk, Vesna B. Jovanović, Jelena M. Aćimović, Ljiljana M. Mandić**  
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Human serum albumin (HSA) is the most important transporter of free fatty acids in sera, and about six molecules of the short and long chain fatty acids (C10 - C18) are bind to one molecule of serum albumin. HSA has one free thiol group located at cysteine-34 (Cys34) amino acid residue, and that is very important source of reductive (antioxidant) capacity according to HSA abundance in the plasma. Crystallographic studies showed that accessibility of HSA Cys34 residue to oxidation was significantly changed when free fatty acids were attached to HSA [1]. Therefore, the aim of this study was investigation of the impact of stearic acid (C18:0), oleic acid (C18:1), and fatty omega-3 polyunsaturated fatty acids from fish oil diet supplement EPA (C20:5) [(5Z,8Z,11Z,14Z,17Z)-5,8,11,14,17-icosapentaenoic acid] and DHA (C22:6) [(4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoic acid] on reactivity of the Cys34 free thiol group. Amount of the free fatty acids bound to HSA was measured with utilized quantitative thin layer chromatography (qTLC) method preceded by Folch chloroform/methanol (2:1) extraction of fatty acids from HSA. Free thiol groups were assayed spectrophotometrically according to the modified Ellman's method (with 5,5'-dithiobis-(2-nitrobenzoic acid as reagent)). Reaction kinetics of free thiol and 2 mM Ellman's reagent were observed during 30 minutes from reaction start [2]. The time course of reaction shows statistically significant difference between reactivity of Cys34 in the presence of stearic acid (with adjusted pseudo-first order reaction constant  $k' = 0.0175$ ) compared to reactivity of Cys34 of fatty acids free HSA which was control sample ( $k' = 0.0048$ ). Also, reactivity in the presence of oleic acid ( $k' = 0.0196$ ), EPA ( $k' = 0.0253$ ), and DHA ( $k' = 0.0158$ ) was greater than control, but surprisingly it was statistically significantly greater than in experiment with stearic acid, thus making possible connection between degree of unsaturation and reactivity of Cys34 thiol group.

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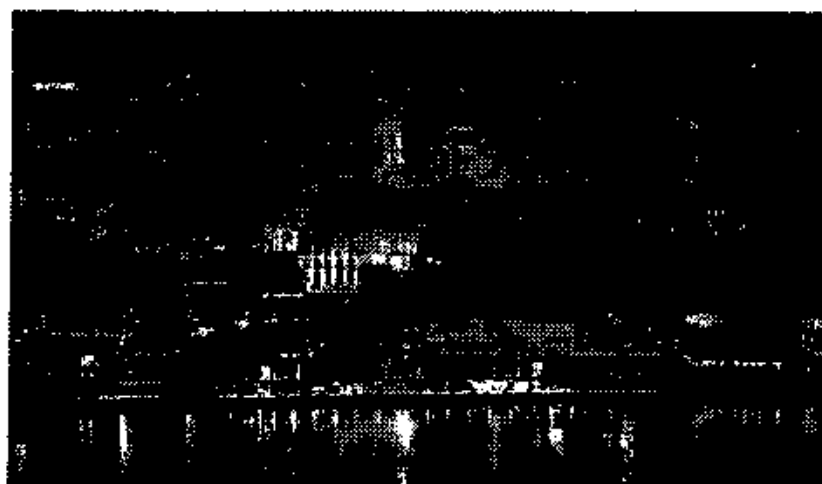
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## **Belgrade Food International Conference**

**Food, health and well being**

**Belgrade, 26<sup>th</sup> to 28<sup>th</sup> November 2012.**



### P 1.23. Determination of Arg guanidine group changes as marker of food protein carbonilation in Maillard reaction

Jelena M. Aćimović, Vesna B. Jovanović, Ivan D. Pavićević, Ana Z. Penezić Romanjuk, Ljuba M. Mandić

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During food processing, Maillard reaction (i.e. the reaction of free amino acids and protein amino acid side chain with reducing carbohydrates) takes place, which leads to loss of amino acids availability. This is of importance for the essential amino acid lysine which is also the limiting amino acid in many proteins and conditionally essential amino acid arginine. The methylglyoxal and other highly reactive  $\alpha,\beta$ -dicarbonyls (produced during these reactions) act as propagators which lead to the formation of advanced glycation end products (AGEs). AGEs of Lys side chains (furosine, N- $\epsilon$ -carboxymethyllysine, pyralline, pentosidine and pronyl-lysine) are most widely used as markers of the nutritional quality of foods [1]. As the guanidine groups are very abundant on protein surface and reactive toward the dicarbonyls, they are susceptible to modifications. The Arg derived AGEs (e.g. N- $\epsilon$ -carboxymethylarginine, [2]) are unstable in the acid hydrolysis of proteins, but they could be detected in the protein hydrolyzate obtained by enzymatic digestion. That was a limiting factor for development of methods for the monitoring of arginine side-chain modification. Therefore, the aim of this study was to develop a simple spectrophotometric method for monitoring of the protein guanidine group changes during Maillard reactions, without previously performed digestion.

The method was based on the formation of colored adduct between guanidine group of protein and thymol-sodium hypobromite reagent in the alkaline media [3]. The curve slopes of absorption vs. concentration plot of substances containing guanidine groups [arginine, human (HSA), caseine and bovine serum albumin (BSA)] were substance dependent. The results obtained were in agreement with the Beer's law for the guanidine group concentrations of 1–40 mM. Precision of the method (RSD) was in the range of 0.9–2%. Accuracy was examined by standard addition method (recovery about 100%). It was found that quantification of guanidine groups during protein carbonylation *in vitro* enables examination of the kinetics of these reactions, competitiveness of guanidine against thiol and amino groups. As the determination of protein guanidine groups with thymol-sodium hypobromite is simple and fast, accurate and precise, it could be useful for monitoring of the protein modification in Maillard reaction during food processing, i.e. for nutritional evaluation of food protein.

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#### Acknowledgment

The authors acknowledge support of the FP7 RegPot project FCUB ERA GA No. 256716. The EC does not share responsibility for the content of the article.



FCUB ERA



## **2<sup>nd</sup> FCUB ERA Workshop**

### **Food Chemistry and Biotechnology**

**Belgrade, 18<sup>th</sup> and 19<sup>th</sup> October 2011.**



**P 13. Impact of fatty acids binding to human serum albumin on the reaction of free thiol group of albumin**

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Supplementation of omega-3 fatty acids reduces the risk of heart attack and coronary heart disease, and helps in hypertriglyceridemia. In etiology of these diseases oxidative stress is implicated. Human serum albumin (HSA) is the most important transporter of free fatty acids in circulation. Also, it contains one free thiol group (Cys34, noted as hydroxyl radical scavenger), which determine antioxidant property and carbonyl scavenger capacity of albumin. Crystallographic studies showed that accessibility of HSA Cys34 residue to oxidation was significantly changed when free fatty acids were attached to HSA. Therefore, the aim of this study was investigation of the impact of unsaturated fatty acids binding to HSA on the reaction of HSA- free thiol group with methylglyoxal.

The incubation of HSA (0.5 mM) with MG(41 mM) in 0.1 M sodium phosphate buffer (pH 7.4) at 37°C was performed without and with unsaturated fatty acids (oleic acid, EPA and DHA) and fish oil during the six hours. The percent contents of the most abundant omega-3 fatty acids in fish oil (eicosapentaenoic acid, 20:5, Δ5,8,11,14,17, and docosahexaenoic acid, 22:6, Δ4,7,10,13,16,19) were analyzed with GC-FID. Free thiol groups were assayed spectrophotometrically according to the modified Ellman's method (with 5,5'-dithiobis-(2-nitrobenzoic acid as reagent)).

Changes in the reactivity of free HSA thiol group with methylglyoxal in the function of unsaturation of the fatty acid attached to HSA were observed. The percent of reacted thiol group of HSA incubated with MG in the absence or presence of oleic acid, increased from 21% to 33%. Further increasing of reactivity of HSA thiol group was detected when HSA was incubated with more unsaturated fatty acids, EPA and DHA.

These results showed that reactivity, i.e. accessibility of thiol group of Cys34 residue to carbonylation increased with increasing unsaturation of fatty acids attached to albumin.

**Keywords:** HSA thiol group reaction with methylglyoxal; HSA-fatty acids binding, fish oil dietary supplement

**P 28. The influence of methylglyoxal reaction with human serum albumin on its copper(II) binding properties**

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Processing, cooking and prolonged storage of food leads to the formation of methylglyoxal (MG). MG is also produced in some pathological states (diabetes, uremia, oxidative stress, aging and inflammation). As a highly reactive compound ( $\alpha$ -dicarbonyls are 20,000-fold more reactive than glucose in glycation reactions) it is a potent protein and nucleic acid modifying agent. Besides ceruloplasmin, human serum albumin (HSA) represents a relatively large pool of bound copper in human plasma. HSA has one high affinity site for copper(II), located near N-terminus (Asp-Ala-His tripeptide). Redox activity of protein bound copper is strictly regulated and its uncontrolled release can make it extremely dangerous, since it can catalyze production of free radical intermediates from molecular oxygen. The aim of this study was the investigation of influence of MG reaction with human serum albumin on its copper(II) binding properties.

HSA was preincubated with different copper(II) concentrations to obtain HSA-copper complex. Any unbound copper was removed by ultrafiltration (10kDa cutoff). These HSA-copper complexes were then incubated with MG during six hours. Following incubation, HSA bound copper concentration was determined spectrophotometrically with bathocuproine reagent. Also, conformational changes in HSA molecule were monitored by recording fluorescence spectra ( $\lambda_{ex}$  294 nm,  $\lambda_{em}$  300-450 nm). All samples were analysed by native electrophoresis.

The content of bounded copper to HSA rises with increasing concentration of added copper. Concentration of bound HSA copper decreases during the six hours incubation with MG (up to 40% of starting value). It was observed that MG had less influence on HSA bound copper release when higher copper concentration was initially preincubated with HSA. The fluorescence spectra indicate conformational changes in HSA after reaction with MG. These conformational changes could be the reason of copper leakage.

Based on these findings, it was concluded that the modifications of HSA molecules with MG can lead to uncontrolled release of HSA bound copper and consequently could contribute to the production of free radical intermediates from molecular oxygen.

**Keywords:** methylglyoxal; HSA carbonylation; HSA-copper(II) binding



*1<sup>st</sup> FCUB ERA Workshop*

*Food Safety and Health Effects of Food*

*Belgrade, January 31-February 1, 2011*



FCUB ERA





**P.11. Influence of the microenvironment of thiol groups in low molecular mass thiols and protein on the reaction with methylglyoxal**

Jelena M. Aćimović<sup>#</sup>, Bojana D. Stanimirović<sup>#</sup>, Nina Todorović<sup>§</sup>, Vesna B. Jovanović<sup>#</sup>  
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The reaction between cysteine thiol group and  $\alpha$ -oxoaldehydes (glyoxal, methylglyoxal (MG)) occurs during food production and also it takes place in some pathological states in which  $\alpha$ -oxoaldehydes generated in high extent. The products of reactions between  $\alpha$ -dicarbonyls, (byproducts of alcoholic and malolactic fermentations) and cysteine (Cys) have characteristic odors that significantly influence wine quality and character. On the other hand, reactive  $\alpha$ -oxoaldehydes that are produced in higher quantities in diabetes, uremia, oxidative stress, aging and inflammation, react with the thiol groups of proteins. This causes protein modification, formation of advanced glycated end-products (AGEs) and cross-linking. Low molecular mass thiols can be used as competitive targets for MG, preventing the reactions mentioned above. Therefore, this paper investigated how the microenvironment of the thiol group in low molecular mass thiols (cysteine, N-acetylcysteine (NACys), carboxymethylcysteine (CMC) and glutathione (GSH)) and human serum albumin (HSA) affected the thiol reaction with MG. The SH group reaction course was monitored by <sup>1</sup>H-NMR spectroscopy and spectrophotometric quantification. Changes in the HSA molecules were monitored by SDS-PAGE. The microenvironment of the SH group had a major effect on its reactivity and on the product yield. The reactivity of SH groups decreased in the order Cys > GSH > NACys. CMC did not react. The percentages of the reacted SH groups in the equilibrium state were almost equal, regardless of the ratio of thiol compound/MG (1:1, 1:2, 1:5): 38.1  $\pm$  0.9%; 38.2  $\pm$  0.7% and 39.0  $\pm$  0.8% for Cys; 26.5  $\pm$  0.6%; 26.6  $\pm$  2.6% and 27.4  $\pm$  2.5% for GSH; 10.8  $\pm$  0.9%; and 11.2  $\pm$  0.7% and 12.2  $\pm$  0.9% for NACys, respectively. Our results explain why substances containing  $\alpha$ -amino- $\beta$ -mercapto-ethane are successful scavengers of MG. In equilibrium, HSA SH reacted in high percentages both with an insufficient amount and with an excess of MG (55% and 65%, respectively). An analysis of the hydrophobicity of the microenvironment of the SH group on the HSA surface showed that it could contribute to high levels of SH modification, leading to an increase in the scavenging activity of the albumin thiol.



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2011

16<sup>th</sup> European Conference on Analytical Chemistry

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11-15 September 2011 - Congress Center SAVA, Belgrade, Serbia

### ABSTRACTS

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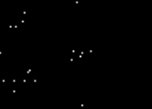
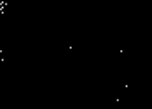
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**DETERMINATION OF HUMAN SERUM ALBUMIN THIOL GROUP**

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Oxidative stress is increased in many pathological processes. Serum albumin (HSA) is the most abundant protein in plasma and a major extracellular antioxidant. The free thiol group of Cys 34 contributes to HSA antioxidant capacity. There is no simple, fast and cheap method for determination of the free HSA thiol group content for clinical purposes. Precipitation of serum proteins (HSA isolation) by ammonium sulfate as the first step in thiol determination was tested. The different factors such as various concentrations of AS, the method of precipitation (solid or saturated AS) and centrifugation (time and relative centrifugal force) and removal of AS on the content of HSA and globulin in all precipitates and supernatants were examined. In order to study the effects of various treatments on the composition of these precipitates and supernatants all of them were analyzed by determination of HSA, total protein and free thiol group concentrations (with bromocresol green, Biuret method and 5,5'-dithiobis-(2-nitrobenzoic acid), as well as by electrophoresis and immunoblotting (with antibodies against HSA). Also, the purity of HSA obtained by AS precipitation was compared to HSA isolated by affinity chromatography on Cibacron Blue F3G Sepharose. The 50% to 65% saturated AS fraction obtained after centrifugation 10 minutes at 3000 g has almost the same HSA purity as HSA isolated by affinity chromatography. The method is simple, fast, and accurate (recovery about 100% of total serum thiol groups). It was applied to isolation of serum HSA from 15 healthy persons and 15 patients with diabetes. The content of thiol groups in HSA of diabetics was significantly lower ( $p < 0.01$ ) in comparison with the healthy persons. Therefore, the method developed is suitable for monitoring of HSA thiol groups in clinical practice.

# THE SPECTROPHOTOMETRIC METHOD FOR MONITORING OF PROTEIN GUANIDINE GROUP CHANGES DURING CARBONYLATION

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The accumulation of methylglyoxal (MG) and other  $\alpha$ -oxoaldehydes from both glycoxidation and lipoxidation sources lead to carbonyl stress. They are produced in higher quantities in diabetes, uremia, oxidative stress, aging and inflammation. Carbonylation of the protein amino, guanidino and thiol groups is one of the important causes of vascular complications in diabetes. As the guanidine groups are very abundant on protein surface and the most reactive toward carbonyls, the changes in their levels could be the measure of carbonyl stress. Therefore, the aim of this study was to develop a simple spectrophotometric method for monitoring of the protein guanidine group changes during carbonylation. It was based on the formation of coloured adduct between thymol and product of reaction of guanidine group with hypobromite under alkaline conditions. The curve slopes of absorption vs. concentration plot of substances containing guanidine groups [arginine, human (HSA) and bovine serum albumin (BSA)] were substance dependent. The results obtained were in agreement with the Beer's law for the guanidine group concentrations of 1–36 mM. The method is simple, fast, precise (RSD in the range of 0.9-2% ), accurate (recovery about 100%) It was applied for *in vitro* experiments of incubation of HSA with MG, as well as for monitoring of the changes of HSA molecule isolated by affinity chromatography from serum of patients with diabetes type 2. It was found that quantification of guanidine groups during protein carbonylation *in vitro* enables examination of the kinetics of these reactions, competitiveness of guanidine against thiol and amino groups. On the other hand it is suitable for monitoring of the HSA modification in carbonyl stress in clinical practice.



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## **ABSTRACTS**



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location. ABQS have been demonstrated to have mutagenic and DNA binding capacity on A431 cells. A431 and TK6 cells in culture where exposed for 48 hours to determine the  $IC_{50}$  dose, mitochondrial membrane permeability, ROS generation and fluorescent microscopy analysis of intracellular binding.  $IC_{50}$  was determined by trypan blue and mitochondrial membrane permeability by the JC-1 assay. For ROS determination the fluorescent dye 2,7-dichlorofluorescein diacetate was applied. Intracellular binding was performed by confocal microscopy. In the TK6 normal lymphoblast cells the observed  $IC_{50}$  were as follow: ABQ38=310 $\mu$ M, ABQ95=270 $\mu$ M, NBQ38 400 $\mu$ M and NBQ95 33 $\mu$ M. On A431  $IC_{50}$ s were: ABQ38= 32  $\mu$ M, ABQ95= 36  $\mu$ M, NBQ38 36 $\mu$ M and NBQ95 28 $\mu$ M. Overall, TK6 demonstrated higher tolerance to the tested ABQS than A 431. Preliminary results on ROS generation indicated stronger ROS release on A431 than TK6 lymphoblast. The effects caused by ABQS on mitochondrial permeability where consistent with the cytotoxicity, as these were higher on treated tumor cells. Microscopy of ABQS treated cells revealed location of these fluorescent compounds in cellular organelles. DNA fragmentation revealed activity of BQ 38 on A431 cells. This study presents preliminary evidence of an overall higher tolerance of normal TK6 lymphoblast to the tested fluorescent drugs in comparison to A431.

**603. Chemical analysis of the cytotoxic plant *Croton discolor*.** *Karla M. Santos-Ocasio*<sup>(1)</sup>, *mayra.pagan@upr.edu*, 205 Antonio R. Barceló Avenue, Cayey PR 00736, United States ; *Isamar Ortiz-Rivera*<sup>(1)</sup>; *Mayra Pagán-Ortiz*<sup>(1)</sup>; *Claudia Ospina*<sup>(1)</sup>. (1) Department of Chemistry, University of Puerto Rico at Cayey, Cayey PR 00736, United States

Some plants of Puerto Rico have been used in traditional medicine to treat diseases including colds, coughs, diarrhea, respiratory infections, and skin lesions among others. Unfortunately, only a few endemic and native plants with potential medical applications have been studied. Our research efforts have been focused on the study of endemic and native plants from Puerto Rico and the Caribbean. *Croton discolor* is a native plant of the Antilles that belongs to the Euphorbiaceae family. The objective of this study is to identify and discover new metabolites from the medicinal plant *Croton discolor* and evaluate its biological activity against breast cancer cell lines. In a preliminary screening using brine shrimp lethality test, we found that the crude extract showed significant cytotoxicity with a  $LC_{50}$  of <150  $\mu$ M. Based on this result, different extracts will be examined and their active constituents identified and evaluated. The results will be presented and discussed.

**604. Does the sialic acid content in A form of N-acetyl- $\beta$ -D-glucosaminidase influence the changes of its activities in diabetic secondary complications?** *Ljuba M. Mandić*<sup>(1)</sup>, *ljmandic@chem.bg.ac.rs*, Studentski trg 16, Belgrade Serbia 11158, Serbia and Montenegro; *Vesna B. Jovanović*<sup>(1)</sup>; *Jelena M. Acimović*<sup>(1)</sup>; *Radmila Maksimović*<sup>(2)</sup>. (1) Department of Biochemistry, Faculty of Chemistry, Belgrade Serbia 11158, Serbia and Montenegro (2) Clinical center, Krusevac, Serbia and Montenegro

N-acetyl- $\beta$ -D-glucosaminidase (EC 3.2.1.52, NAG) is a lysosomal glycohydrolases which activity increased in diabetes. Connection between the changes of NAG activity in different secondary complications and carbohydrate parts of A form was investigated with 69 type 1 diabetics (divided into four groups: without complications, with retinopathy(R) and polyneuropathy(R+P) and nephropathy(R+P+N)). In all diabetic groups a statistically significant increase ( $p < 0.001$ ) of total serum(s) and urine(u) NAG, as well as A(s) form increase and B(s) form decrease, compared to the control group ( $n=25$ ) were found. High positive correlations between total NAG(s) and A form, and A form and hyperglycaemia were obtained. PAGE mobility of A forms and binding strength to DEAE cellulose indicated that A(u) forms were more acidic than A(s) forms. In R+P+N group the A(s) and A(u) were more acidic then in other diabetic groups. Sialic acid content (determined by lectin ELBA test) in isolated and purified A(s) (180 to 220 fold) and A(u) (324 and 351 fold) of diabetics was 2-3.5 times less compared with the control. The lowest degree of sialyzations in group with R and the highest in R+P+N group indicated different rate of A(s) clearance from circulation. Therefore, the increase of total NAG(s) and the percent fractions of A(s) form were consequence of balance between exocytosis in hyperglycaemia and degree of A(s) desialization. The changes of A(s) and A(u) forms acidities in diabetics compared to control could be also consequence of the other negative charge groups presence (sulphate and/or phosphate) or the charge changes during glycosylation.

**605. Structural determinants for the formation of sulfheme complexes.** *Eddie Marie Román-Morales*<sup>(1)</sup>, *elddie1marie@gmail.com*, La Palma 1E, Peral #14, Mayagüez Puerto Rico 00680, Puerto Rico ; *Ruth Pietri*<sup>(1)</sup>; *Brenda Ramon-Santana*<sup>(1)</sup>; *Serge N Vinogradov*<sup>(2)</sup>; *Ariel Lewis-Ballester*<sup>(3)</sup>; *Juan López-Garriga*<sup>(1)</sup>. (1) Chemistry, University of Puerto Rico at Mayagüez, Mayagüez Puerto Rico 00680, Puerto Rico (2) Department of Biochemistry and Molecular Biology, Wayne State University School of Medicine, Detroit Michiga 48201, United States (3) Physiology and Biophysics, Albert Einstein College of Medicine, Bronx New York 10461, United States

Historically, hydrogen sulfide ( $H_2S$ ) has been regarded as a dangerous and poisonous gas with a wide spectrum of cytotoxic effects. However, a new controversial role is emerging for  $H_2S$  in the chemistry of biological systems as a neuromodulator, a smooth muscle relaxant, and a transmitter of informational signaling between cells. Nonetheless, reactions of  $H_2S$  with myoglobin and hemoglobin, in the presence of  $O_2$  and/or  $H_2O_2$ , result in covalent modification of the pyrrole "B" of the heme, generating the so-called sulfheme protein derivatives. These derivatives have lower  $O_2$  affinity, eventually leading to a detrimental blood disease called sulfhemoglobinemia. On the other hand, the hemoglobins from the clam *Lucina pectinata* (HbI, HbII and HbIII), also form these ferryl species in the presence of  $H_2S$  without generating the sulfheme derivatives. Therefore, a hypothesis that the distal site environment may play a crucial role in sulfheme production emerged. Horse heart



ISSN 1644-0732

2013, Vol. 53, Suppl. 1

Translating food chemistry  
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Gdansk 6th-8th July 2013

# Polish Journal of Food and Nutrition Sciences



made fish product were used as blanks for the preparation of fortified samples. They were spiked by adding the appropriate volumes of standard solutions of individual sweetener at one concentration level i.e. 50% of maximum usable dose value specified for each food product. The final products were packed in jars and stored at three temperatures: +20°C, +4°C and -20°C for the following time periods i.e. 3 days, 1, 2, 3 and 4 weeks. A reference test samples were kept at temperature -80°C. To determine the effect of storage on the sweeteners stability, final analyses of the target compounds were performed by HPLC-MS, whereas sample preparation included an SPE step. The obtained data gave opportunity to evaluate behaviour of sweeteners in different storage conditions. Such data could then be used during storage of food samples with different matrices containing sweeteners.

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## P209

### Influence of the Microenvironment of Thiol groups in Low Molecular Mass Thiols and Protein on the Reaction with Methylglyoxal

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Key words: methylglyoxal, cysteine, N-acetylcysteine, carboxymethylcysteine, glutathione, protein thiol groups

Processing, cooking and prolonged storage of food leads to the formation of methylglyoxal (MG). Intake of low doses of MG over prolonged period of time and increased endogenous MG in some pathological states (diabetes, uremia, oxidative stress, aging and inflammation) causes protein modification, formation of advanced glycation end products (AGEs) and cross-linking. N-terminal and Lys side chain amino groups, the guanidine group of Arg and the sulfhydryl group of Cys present on protein surfaces participate in protein modification by MG. This paper investigated how the microenvironment of the thiol group in low molecular mass thiols (cysteine, N-acetylcysteine (NACys), carboxymethylcysteine (CMC) and glutathione (GSH)) and proteins affected the thiol reaction with MG. The SH group reaction course was monitored by <sup>1</sup>H-NMR spectroscopy and spectrophotometric quantification. The microenvironment of the SH group had a major effect on its reactivity and on the product yield. The reactivity of SH groups decreased in the order Cys > GSH > NACys. CMC did not react. The percentages of the reacted SH groups in the equilibrium state were almost equal, regardless of the ratio of thiol compound/MG (1:1, 1:2, 1:5): 38.1±0.9%; 38.2±0.7% and 39.0±0.8%

for Cys; 26.3±0.6%; 26.6±2.6% and 27.4±2.5% for 10.8±0.9%; and 11.2±0.7% and 12.2±0.9% for NACys, respectively. Despite very low levels of thiol groups on the surface of protein molecule (in BSA, approx. 80 times lower than those of amino and guanidine groups), a very high portion of it reacts (25-85%). On the basis of all results the pattern of thiol group reaction with methylglyoxal was determined.

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## P210

### Formation of Hydrogen Peroxide in Model Solutions Enriched in Green Tea Extract

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Key words: hydrogen peroxide, polyphenols, antioxidants, reactive species

Polyphenols are a wide group of antioxidants occurring in fruits, vegetables, and some beverages like tea, wine or coffee. Antioxidants are very important for man health, since the production of reactive oxygen species is thought to be a significant cause of aging and cancer. It is proved that polyphenols can act as free radical scavengers, quenching hydroxyl radicals or superoxide anions and may chelate metal ions etc.

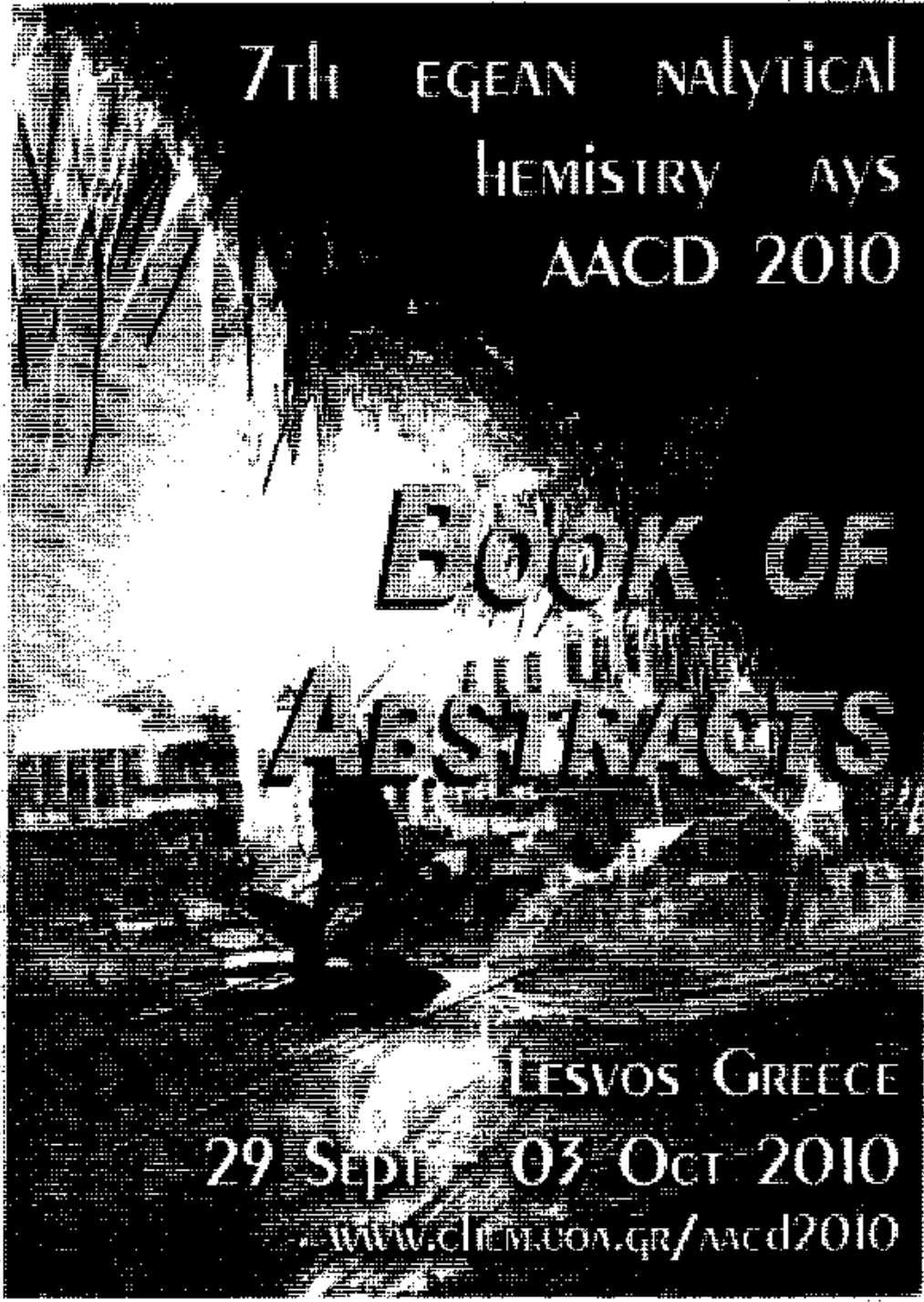
Contrary to their beneficial effects, it has been reported that some polyphenols promote oxidative damage to DNA, lipids and deoxyribose in the presence of metal under certain conditions *in vitro*. Presumably, it is due to pro-oxidant action of polyphenols which result in generating reactive oxygen species during the autooxidation process. Of great importance is the generation of toxic hydrogen peroxide that can be transformed into reactive hydroxyl radicals via Fenton reaction. There are some data in the literature concerning the hydrogen peroxide formation in polyphenol beverages, e.g. green tea, black tea, coffee.

The aim of the study was to determine the ability of hydrogen peroxide formation in model solutions enriched in green tea extract. The influence of pH, extract concentration and incubation was examined. 0.2% and 0.5% green tea extract solutions were diluted in phosphate buffer (pH from 4 to 7). The concentration of hydrogen peroxide was measured by a colorimetric method after 0, 1, 3, 4.5, 6, 24 and 48 hours of storage in room temperature.

In solutions with lower pH (under 6) formation of hydrogen peroxide was not observed. In solutions with pH over 7 the amount of hydrogen peroxide simultaneously increased with storage time and incubation time (over 6 hours). The highest amount of H<sub>2</sub>O<sub>2</sub> was detected after 24-hour storage and was 1.2 mM in solution with pH 10.

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# MONITORING OF THE PROTEIN AMINO GROUP CHANGES DURING CARBONYLATION

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Carbonyl stress is one of the important mechanisms of tissue damage in vascular complications.  $\alpha$ -oxoaldehydes are produced in higher quantities in diabetes, uremia, oxidative stress, aging and inflammation. As a highly reactive they are the potent protein (1) and nucleic acid modifying agent. In protein modification with reactive  $\alpha$ -oxoaldehydes N-terminal and Lys side chain amino groups, the guanidine group of Arg (2) and sulfhydryl group of Cys (3) present on protein surface participate. Some of the products of these reactions are well-characterized conjugates (N-(carboxymethyl)lysine, N-(carboxyethyl)lysine, N-(carboxymethyl)arginine, S-(carboxymethyl)cysteine and S-(carboxyethyl)cysteine) which are used as markers of glycation, i.e. of secondary complications development in diabetes.

The aim of this study was to develop the method that allows easily monitoring of proteins changes in carbonyl stress. As amino groups are highly abundant on the surface of proteins the changes in their levels could be the measure of carbonyl stress.

The spectrophotometric monitoring of decrease in the content of protein amino-groups, as a consequence of the carbonylation, was based on their reaction with p-benzoquinone (PBQ) (4) in weak alkaline conditions. The product of reaction between protein amino group and PBQ in potassium phosphate buffer pH 7.2 has absorption maximum at 480 nm. The kinetic of the reaction was investigated and the optimal time of determination was found. Different slopes of curves of the dependence between absorption and concentrations of substance containing amino groups (alanine, lysine, HSA) were obtained. Beer's law is followed in the range 5-60 mmol/L amino groups of Lys, Ala and HSA. The potential influence of thiol group was minimized using weak alkaline conditions of reaction mixture. The method was applied during *in vitro* experiments of incubation of human serum albumin (HSA) with methylglyoxal as well as for monitoring changes of HSA molecules *in vivo* in diabetic patients type I with different secondary complications. For the later HSA was isolated from dialyzed serum using affinity chromatography. In both cases the usefulness of this method was found.

**KEYWORDS:** carbonylation, determination of protein amino group, p-benzoquinone

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ISSN 1365-3059

# FEBS Journal

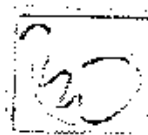
Volume 272 Supplement 1 July 2005



## Abstracts

30th FEBS Congress  
and 9th IUBMB Conference

The Protein World



Budapest, Hungary  
2-7 July 2005

  
Blackwell  
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## L1-019P

**Developmental expression of neogenin protein in human brain**

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Neogenin (NGN) protein was first identified in a chicken as developmentally highly regulated protein in neuronal tissue, suggesting a role in the generation of the fully functional nervous system. To address possible role of NGN in a critical period for human brain development we used an affinity-purified antibody raised against NGN of human origin in Western blot, immunoperoxidase histochemistry and immunofluorescent multilabeling analyzing NGN cell-origin and its spatio-temporal expression, on postmortem human fetal brains, staged from 10th week of gestation (w.g.) to newborn. The most prominent feature was perinuclear and extracellular expression in subventricular subcallosal zone below anterior extent of corpus callosum (cc) in cells with low neuron-specific nuclei protein (NeuN) expression or activated caspase-3 and on the surfaces of growing fibers in ventral cc at midgestation. Furthermore, strong cellular NGN expression was displayed where fibers of fornix diverge from cc, as well as in the fornix fibers on the insertion of plexus chorioideus. At about 30 w.g. the most prominent was expression confined to bushy astroglial like cell-population in the developing putamen. From 35 w.g. to newborn we could only observe a very low level of NGN expression in cells in subventricular zone laterally to and in cc.

In conclusion, NGN is expressed during important gestational developmental window showing topographically very specific localization confined to a small number of differentiating cells or cells undergoing apoptosis and to some midline growing fibers. In developing human brain NGN expression decreases and disappears during latest fetal stage.

## L1-020P

**The investigation of serum N-acetyl- $\beta$ -D-glucosaminidase and its isoenzymes as markers of the progression of diabetic complications in IDDM**V. B. Jovanovic<sup>1</sup>, J. M. Acimovic<sup>1</sup>, V. S. Dimitrijevic-Sreckovic<sup>2</sup> and L. M. Mandic<sup>1</sup>*<sup>1</sup>Faculty of Chemistry, University of Belgrade, Belgrade, Serbia and Montenegro, <sup>2</sup>Faculty of Medicine, University of Belgrade, Belgrade, Serbia and Montenegro. E-mail: vjovanov@chem.bg.ac.yu*

Significantly increased of serum N-acetyl- $\beta$ -D-glucosaminidase (NAG, EC 3.2.1.30) activity in diabetic patients, especially in diabetics with secondary complications was found. However, the results obtained for total NAG and its relationship with development of the secondary diabetic complications are often contradictory and unexplained. Consequently, we have attempted to establish whether total NAG and/or NAG isoenzymes can provide additional diagnostic information regarding diabetic status and the complications of diabetes. The serum NAG isoenzymes in control ( $n = 18$ ) and in four groups of IDDM patients (1st - without complications,  $n = 20$ ; 2nd - with retinopathy,  $n = 6$ ; 3rd - with retinopathy and neuropathy,  $n = 11$ ; 4th - with retinopathy, neuropathy and nephropathy,  $n = 12$ ) were separated by ion-exchange chromatography on DEAE cellulose. In all diabetic groups there were a statistically significantly increase ( $P < 0.001$ ;  $P < 0.01$ ) of total NAG activity compared to the control. Analysis of isoenzyme profiles in all diabetic groups showed significantly decreased ( $P < 0.001$ ) contribution of the B form to total NAG activity ( $15.1 \pm 4.5\%$ ;  $16.3 \pm 3.4\%$ ;  $18.3 \pm 6.0\%$ ;



# **4<sup>th</sup> Aegean Analytical Chemistry Days**

*29 September-3 October 2004, Kuşadası / Aydın - TURKEY*

## **ABSTRACTS**

adjust parameters of ozone generator and the reactor for water treatment in laboratory conditions. The ozonisation time and water flow rate were optimised for the treatment of waste water with high concentration of organic compounds.

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### **SPECTROPHOTOMETRIC KINETIC METHOD FOR GOLD (III) DETERMINATION**

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The spectrophotometric kinetic method for Au(III) determination was developed and validated. It is based on the catalytic effect of gold on the oxidation of methylene blue B (3,7 di-(dimethyl amino)-10-dehydro-phenotiazin chloride) by ammonium peroxy-disulfate in citric buffer solution. Beers law was obeyed in the range of 0.09 to 2.90 µg/ml and relative standard deviation was found to be 2.50%. The detection limit of the method was 1.1 ng/ml. The selectivity was tested on the basis of influence of known amounts of different ions in the reaction mixture, upon the reaction rate. Kinetic and thermodynamic parameters are reported for both catalytic, and non-catalytic, reaction. The method is verified by Au(III) determination in anti rheumatic drug "Tauredon" and in human urine samples, using ICP-AES as the comparative method.

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### **THE INFLUENCE OF PIGMENTS AND pH OF URINE ON THE DETERMINATION OF N-ACETYL-β-GLUCOSAMINIDASE ACTIVITY**

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Activity of the urinary N-acetyl-D-glucosaminidase (NAG, EC 3.2.1.30) is very useful marker in clinical and toxicological practice for detection of renal proximal tubular damage. It was shown earlier that NAG is unstable in alkaline urine, which makes its determination unreliable. So in this paper the influence of pH on the activity of urinary NAG as well as the contribution of urinary pigments to the absorbance of the enzyme reaction product absorption was investigated. The investigations were made with human and rabbit urine samples. On the basis of the obtained results the possibilities for the corrections of NAG activities were given.

**4<sup>th</sup> International Conference of the  
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## The Investigation of the Conditions for the Determination of N-Acetyl- $\beta$ -D-glucosaminidase Isoenzymes in Neutrophils

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Recognition of the leukemic cell type is crucial for the diagnosis, management and prognosis of different types of the disease. In the leukemias not only cell type but stage of differentiation must be assessed. Classification of the leukemic cell has generally rested on its morphological, histochemical and immunological characteristics. Such studies may be complemented by the recognition of certain biochemical markers. The studies on the lysosomal hydrolases in blood leukocyte types showed that the activities and properties of various lysosomal hydrolases from different leukocyte types are not the same. The changes of N-acetyl- $\beta$ -D-glucosaminidase (E.C.3.2.1.30, NAG) activity in different types of leukemia suggest that NAG may be a useful biochemical marker for the differentiation of subtypes of acute lymphocytic and myelo(mono)genous leukemias. It is not known whether the enzymatic abnormality is derived from abnormal hematopoiesis in leukemia or whether the alterations reflect an intrinsic variability in disturbance of the control mechanisms of these enzymes and in altered cellular metabolism. The analysis of isoenzymic profiles of NAG partially clarify the significance of lysosomal enzymes in malignant cells. Therefore, the aim of this paper is the investigation of conditions for separation and determination of NAG isoenzyme activities in neutrophils.

Ion-exchange chromatography on DEAE cellulose was applied to separate the isoenzyme forms of NAG from neutrophils of healthy persons. Neutrophils were separated from peripheral blood cells using three steps. Most of the red blood cells were removed by sedimentation with 6% dextran. Leukocytes and lymphocytes rested suspended in solution. Remaining red blood cells and platelets were removed by hypotonic lysis. Mononuclear cells were separated from neutrophils using Ficoll-Hypaque sedimentation. The neutrophils sink to the bottom of the Ficoll, mononuclear cells remain at the Ficoll/isolate interface. Total NAG activity and protein concentration were measured in sonicated cell extracts. The cell extract was applied to DEAE cellulose column (1 x 13 cm) previously equilibrated with 0.01 mol/l phosphate buffer of pH 7.0. The NAG activity in the fractions was determined by 2-methoxy-4-(2'-nitrovinyl)-phenyl-N-acetyl- $\beta$ -D-glucosaminide as the substrate (calibration curve:  $y=0.854x + 0.00499$ ,  $r=0.999$ ).

Analysis of the elution profiles of NAG showed that good separation of the major A and B isoenzyme forms of urinary NAG using this method was achieved. The B form was eluted with 0.01 mol/l phosphate buffer of pH 7.0, while a linear concentration gradient (0-0.3 mol/l) of sodium chloride in the same buffer was used for the A form. The fractions of the dominant A form activity in the total activity of healthy persons were from 49 to 57%. The fractions of the B form activity were from 7 to 15%. Beside the major A and B isoenzymes of NAG, the A<sub>2</sub> form was also isolated. It was eluted with 0.4 mol/l of sodium chloride in a phosphate buffer of pH 7.0. Its percent fractions in total NAG activity of healthy person were from 35 to 45%. It was concluded that the applied method is appropriate for the separation and determination of NAG isoenzymes in neutrophils.





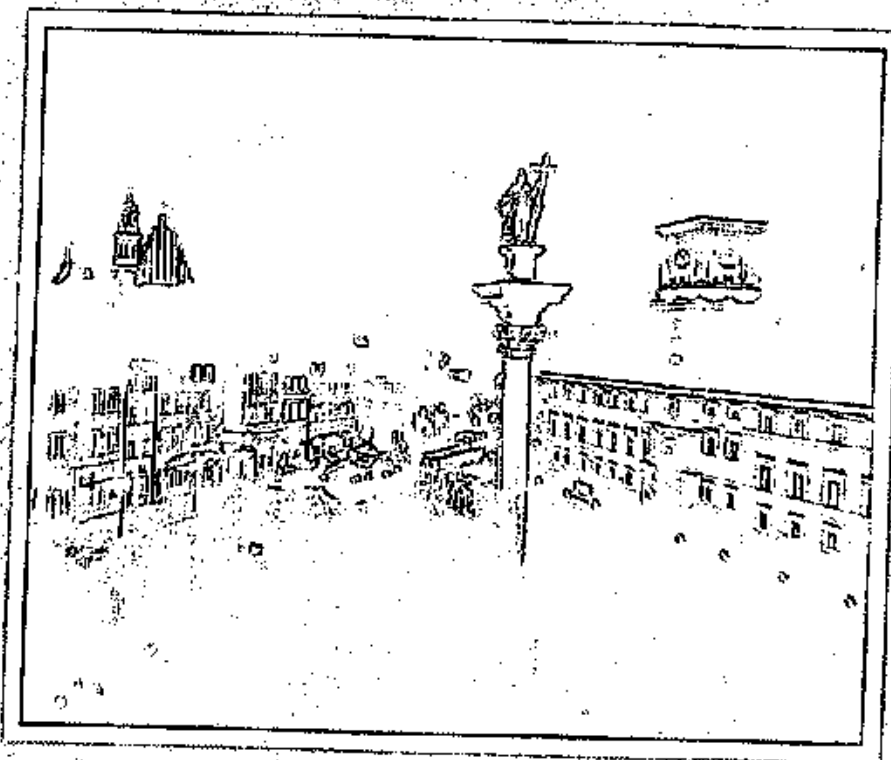
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29th Meeting of the  
Federation of the  
European Biochemical  
Societies

Organized by the  
Polish Biochemical Society

Warsaw, Poland  
26 June - 1 July 2004



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Publishing

tumor markers CEA, CA 19-9, and CA 72-4. In conclusion, two consecutive decreases of the postoperative serum levels of the glycoprotein CD26 may predict tumor recurrence, and a recovery of higher levels of CD26 in postoperative sera, a good evolution of colorectal cancer treated patients.

#### P4.3-15

##### Association between total sialic acid plasma level and markers of apoptosis in patients with coronary heart disease

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It was previously reported that total sialic acid (SA) plasma concentration is elevated in patients undergoing cardiac surgery. An elevated concentration of plasma total SA may result from shedding or secreting of SA from the cell membrane surface or releasing intracellular SA. The alterations within cell membranes may induce apoptosis - biochemical mechanism that allows cell to die in a controlled manner. Loss of terminal SA residues from carbohydrate moieties facilitates recognition and removal of apoptotic cells by phagocytes. The purpose of the present study was to investigate the association between total SA plasma level and apoptosis. In 17 patients undergoing coronary artery bypass grafting (CABG) surgery, plasma total SA concentration was measured and percentage of apoptotic lymphocytes was determined before operation, after aortic clamping, after the end of operation and at 6, 18, 30, and 48 h after operation. Preoperative plasma total SA concentration decreases after aortic clamping and then increases gradually in subsequent samples. Percentage of apoptotic cells significantly increases during and after surgery with the exception of sample taken at 18 hours after operation, when it is significantly reduced. Correlation between total SA plasma level and rate of apoptosis was found at all postoperative intervals with the exception of 18 h after operation. Our findings indicate the bimodal character of apoptosis and dynamic increase in total SA plasma level, which may be considered as a result of mechanical damage taken place during operation or inflammatory response on surgical trauma. The reason of observed data should be investigated.

#### P4.3-16

##### The characterization of glyco-parts of N-acetyl- $\beta$ -D-glucosaminidase isoenzymes in diabetes

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Increased urinary and serum N-acetyl- $\beta$ -D-glucosaminidase (NAG, EC 3.2.1.30) activities were found in diabetics. However, the results obtained in the secondary diabetic complications are often contradictory and unexplained, especially for urinary NAG. For better understanding the cause of urinary NAG changes in complications, the serum and urinary isoenzymes were separated by ionexchange chromatography on DEAE cellulose in control ( $n = 16$ ) and in IDDM patients without complications ( $n = 19$ ), with retinopathy and neuropathy ( $n = 15$ ) and with retinopathy, neuropathy, and nephropathy ( $n = 11$ ). After the separation and purification of serum and urinary A isoforms of NAG the analysis of their carbohydrate parts by lectin assays on solid phase were performed. Analysis of isoenzyme profiles in all groups of diabetics showed significantly increased ( $P < 0.001$ ,  $P < 0.05$ ) contribution of the B form ( $14.9 \pm 4.7$ ;  $13.2 \pm 5.5$ ;  $18.0 \pm 5.6\%$ , respectively) to total NAG activity, significantly decreased ( $P < 0.001$ ) of the A form ( $75.5 \pm 4.2$ ;  $76.0 \pm 5.3$ ;  $74.2 \pm 5.4\%$ , respectively) and increased of the A2 form ( $9.6 \pm 2.9$ ;  $10.8 \pm 3.2$ ;  $7.8 \pm 2.9\%$ , respectively) compared with the control group ( $A = 9.7 \pm 3.5\%$ ;  $A = 81.8 \pm 5.5\%$ ;  $A2 = 8.5 \pm 4.6\%$ ). A significant differentiation in the enzyme profiles was not found between diabetes groups. The most abundant A isoenzyme of NAG in serum diabetics without and with complications was eluted by  $0.064 \pm 0.006$ ;  $0.065 \pm 0.006$ ;  $0.068 \pm 0.010$  mol/l NaCl, respectively. However, the urinary A isoenzyme in diabetics was eluted by significantly ( $P < 0.001$ ) higher concentrations of NaCl ( $0.087 \pm 0.006$ ;  $0.084 \pm 0.009$ ;  $0.095 \pm 0.016$  mol/l NaCl, respectively), e.g. it is more acid compared with the serum form. The carbohydrate moieties of serum and urinary A form are composed of the same monosaccharides (mannose, galactose, N-acetylglucosamine, sialic acid and fucose) but they are different in the sialic acid content. On the basis of all results it was concluded that an increase in urinary NAG activity in diabetics is a consequence of renal proximal tubular injury in poor metabolic control and it is not result of glomerular damage (of passing serum A form).



# JUGOSLOVENSKA MEDICINSKA BIOHEMIJA

Časopis Društva medicinskih biohemičara Srbije i Crne Gore  
Official Journal of the Society of Medical Biochemists of Serbia and Montenegro

Godište: 22

Beograd, septembar 2003

Specijalno izdanje: 2

**XI MEETING OF THE  
BALKAN CLINICAL  
LABORATORY FEDERATION  
BELGRADE  
SERBIA AND MONTENEGRO  
24-27 SEPTEMBER 2003**

**YUGOSLAV MEDICAL BIOCHEMISTRY**

Volume: 22

Belgrade, September 2003

Supplement: N° 2

Jugoslov Med Biohem 22 (Suppl 2): 61-238, 2003

## F119

## GAMMA-GLUTAMYLTRANSFERASE ACTIVITY IN BLOOD OF NEWBORN CHILDREN WITH INDIRECT HYPERBILIRUBINEMIA

M. Ilić<sup>1</sup>, G. Bjelaković<sup>2</sup>, G. Kostić<sup>1</sup>, V. Bogičević<sup>1</sup>, G. Kocić<sup>2</sup>, I. Stojanović<sup>2</sup><sup>1</sup>Department of Pediatrics, Clinical Centre Niš and Institute of Biochemistry, Niš<sup>2</sup>University School of Medicine, Niš, Serbia and Montenegro

Gamma-glutamyltransferase (GGT; EC 2.3.2.2) is a microsomal enzyme that is widely distributed in human tissues involved in secretory processes, particularly the bile canaliculi. It is also present in the heart, pancreas, lungs and seminal vesicles. It is located in cell membranes, where its role is transmembrane amino acid transport in the form of gamma-glutamyl amino acids by the action of the gamma-glutamyl cycle. The most of the activity in blood appears to originate primarily from the hepatobiliary system, and GGT is elevated in any type of liver diseases accompanying by hyperbilirubinemia. The aim of our examination was to elucidate the activity of this enzyme, as a

specific membrane enzyme, in sera of children with high levels of total and unconjugated bilirubin (levels of total bilirubin ranged from 114.54 to 303.0  $\mu\text{mol/L}$ ). The determination of bilirubine levels was performed by the spectrophotometric Ewelin Melloy method. The examination of gamma-GT activity was done on the biochemical analyzer ILAB 300 using commercial «Eli-tech» test. In all specimens with high amount of bilirubine gamma-GT activity was high. These results point to the significant importance of examination of gamma-GT activity as one of specific membrane enzymes of unconjugated hyperbilirubinemia in childhood.

## F120

IZOENZYMES OF URINARY N-ACETYL- $\beta$ -D-GLUCOSAMINIDASE IN INSULINE-DEPENDENT DIABETICS WITH OR WITHOUT COMPLICATIONSV. Jovanović<sup>1</sup>, Lj. Mandić<sup>1</sup>, V. Dimitrijević-Srećković<sup>2</sup><sup>1</sup>University School of Chemistry, Belgrade<sup>2</sup>University School of Medicine, Belgrade, Serbia and Montenegro

Increased urinary and serum N-acetyl- $\beta$ -D-glucosaminidase (NAG, EC 3.2.1.30) activities were found in diabetics. However, the results obtained during the secondary diabetic complications are often contradictory and unexplained, especially for urinary NAG. The aim of this examination is to study the cause of urinary NAG changes in complications using analysis of isoenzyme profiles. The activities of NAG isoenzymes in IDDM patients without complications ( $n=13$ ), with retinopathy and neuropathy ( $n=21$ ), with retinopathy, neuropathy and nephropathy ( $n=12$ ) and control group ( $n=7$ ), were determined. For the determination of NAG activity a synthetic substrate 2-MNP-GlcNAc was used. The urinary isoenzymes were separated by DEAE cellulose chromatography. The total activity of urinary NAG in diabetic patients of all

groups was higher compared to the control group ( $p < 0.001$ ). Analysis of isoenzyme profiles showed significantly increased contribution of the B form ( $12.5 \pm 3.8\%$ ) to total NAG activity in all groups of diabetics ( $p < 0.05$ ), significantly decreased ( $p < 0.001$ ) of the A form ( $77.4 \pm 4.1\%$ ) and increased of the A<sub>2</sub> form ( $10.1 \pm 2.4\%$ ) compared to the control group ( $8.7 \pm 2.5\%$ ;  $83.9 \pm 2.6\%$ ;  $7.2 \pm 2.2\%$ , resp.). A significant differentiation in the enzyme profiles was not found among diabetics groups. As the contributions of all isoenzyme forms are almost equal in all groups, it was concluded that the increase of total urinary NAG activity is the consequence of changes in kidney excretion of NAG isoenzymes in diabetic complications.

SCIENCE

# CLINICAL CHEMISTRY AND LABORATORY MEDICINE

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## T-70

## DIABETES MELLITUS: ASSESSMENT OF MAGNESIUM STATUS

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**DIABETES MELLITUS: ASSESSMENT OF MAGNESIUM STATUS** D.Pap, M. Bakić (1), B. Obradović Clinical-biochemical Laboratory, Medical Center Sremska Mitrovica, Yugoslavia (1) Medical Center, Poreč, Yugoslavia. There is emerging evidence that low intake of Mg or abnormal Mg metabolism are associated with etiologic factors in various metabolic diseases as well as diabetes mellitus. Mg<sup>2+</sup> is a co-factor to more than 300 enzymes systems in cells and is the second most abundant intracellular cation. Decrements in the enzymatic activities of several metabolic pathways are seen in diabetes mellitus (DM) as a result of magnesium deficiency. We decided to test changes of total Mg<sup>2+</sup> concentration in plasma of 40 healthy subjects and 40 patients with DM. Patients were divided in two groups: NIDDM on oral hypoglycemic therapy and NIDDM on insulin. Glucose levels were measured with standard enzymatic methods. Mg<sup>2+</sup> in plasma was measured by calmagite test. Statistically significant differences in Mg plasma levels and glucose concentrations were detected between controls and patients, with significantly lower in patients than controls ( $p < 0.01$ ). The obtained results demonstrated an inverse relationship between plasma Mg levels and fasting blood glucose levels in both DM patients regardless to their therapy. There was no correlation between Mg and glucose concentrations in both groups. Neither age nor sex influenced these results in both groups. The results suggest potential mechanism whereby low Mg status may contribute to the pathogenesis of DM while Mg may beneficially alter outcomes in DM patients and interest in Mg supplementation is in the hopes of preventing long term complications of diabetes.

## T-71

## CALCIUM CHANNEL BLOCKERS CAN CONTROL HYPERGLYCEMIA AND PREVENT WEIGHT LOSS IN EXPERIMENTAL DIABETIC RATS

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**Introduction:** Diabetes mellitus is a condition that imposes a tremendous health and societal burden worldwide. Calcium channel blockers i.e. nifedipine and verapamil can prevent calcium influx into the cells and restore vasculopathy caused by diabetes. The aim of this study was to investigate whether these drugs can control hyperglycemia and weight loss in diabetic rats in addition to their effect on vasculopathy.

**Materials and Methods:** This was an experimental interventional study that was carried out in rats that divided into six (ten in each) groups including: normal (I), diabetic (II), normal with nifedipine and verapamil (III and V), and diabetic with nifedipine and verapamil (IV and VI). To induce type I diabetes the rats received 50mg/kg Streptozotocin by intraperitoneal injection. Animals consumed daily, 40mg/kg verapamil and 20mg/kg nifedipine orally for forty days. Blood was collected on day 1 and 40, and the biochemical factors including: glucose, cholesterol (C), HDL-C, LDL-C, triglycerides, urea, creatinine, and HbA1c were measured. Body weight also recorded on day 1 and 40. Results: Blood glucose decreased significantly in diabetic animals treated with nifedipine, compare to non-treated animals ( $P < 0.001$ ). Diabetic animals lost weight during study significantly ( $P < 0.05$ ), while in animals treated with nifedipine and verapamil, weight loss was prevented. There were no significant differences in other biochemical factors. Conclusion: The results of this study indicated that calcium channel blockers in addition to restoration of vasculopathy can control blood glucose and weight loss in type I diabetic rats.

## T-72

## ISOENZYMES OF URINARY N-ACETYL-BETA-D-GLUCOSAMINIDASE-A MARKER OF TUBULAR OR GLOMERULAR DAMAGE IN DIABETES?

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(2) Faculty of Medicine, University of Belgrade, Serbia and Montenegro.

In diabetic patients with nephropathy an increased activity of urinary N-acetyl-beta-D-glucosaminidase (EC 3.2.1.30; NAG) was found. Although urinary NAG activity is an indicator of renal glomerular damage, it might reflect increased lysosomal activity in renal tubular cells. For better understanding the cause of increase in the activity of urinary NAG, the serum and urinary isoenzymes of NAG were separated by ionexchange chromatography on DEAE cellulose in control (n=10) and in IDDM patients without (n=20) and with complications (n=30). As shown earlier the total serum and urinary NAG activities increase from the group without complications (1st group) through the group with retinopathy and neuropathy (2nd group) to the group with retinopathy, neuro- and nephropathy (3rd group). Analysis of isoenzymes profiles showed that the most abundant A isoenzyme of NAG in serum diabetes without and with complications was eluted by  $0.062 \pm 0.007$ ;  $0.063 \pm 0.011$ ;  $0.066 \pm 0.010$  mol/L NaCl, respectively. On the other hand, the A isoenzyme of urinary NAG in diabetes was eluted by significantly ( $p < 0.001$ ) higher concentrations of NaCl ( $0.057 \pm 0.006$ ;  $0.084 \pm 0.009$ ;  $0.095 \pm 0.016$  mol/L NaCl, resp.) compared to the control ( $0.068 \pm 0.009$ ) and serum form in diabetes. This analysis demonstrates that an increase in urine NAG activity in diabetes is a measure of altered function in the renal tubules and not simply result of glomerular damage.

## T-73

## QUICK LABORATORY SERVICES IN THE PHARMACY

Marini M.(1); Borgogni P.(2); Mora F.(2); Martini M.(2);

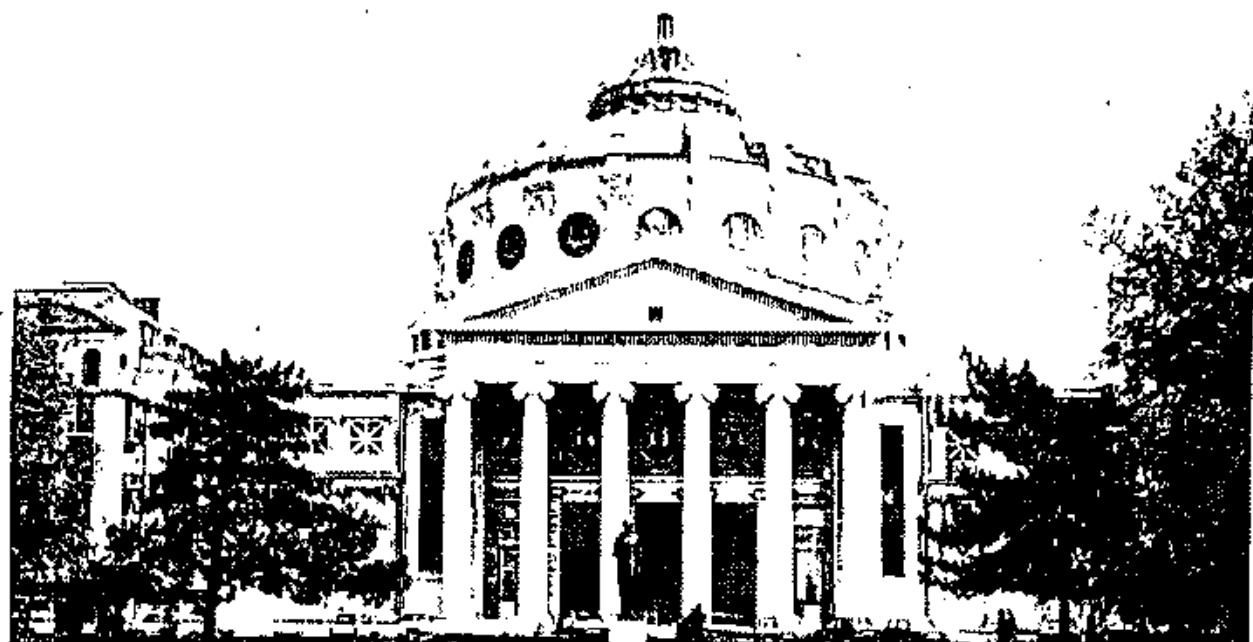
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Current very advanced technologies allow to exploit more and more precise, and simpler and simpler, methods to monitor and prevent diabetes complications, such that they can be used in settings different from a centralized laboratory, e.g. the pharmacy, ambulance, GP's office, etc. In particular, the pharmacist represents a reliable reference for people in the theme of health, as in the pharmacy they can save time and avoid bureaucratic steps. As an experiment in a public pharmacy, we used a DCA 2000 (Bayer) that measures HbA1c concentration in capillary blood and albuminuria. Comparisons with the reference methods (HPLC for HbA1c and nephelometry for albuminuria) were done previously at the laboratory of the Diabetology C.B. of Siena University. Upon informed consent, comparative tests were performed in 107 diabetic patients and they were comparable ( $p > 0.07$ ) and correlated ( $p < 0.0001$ ) for both HbA1c and albuminuria. The regression equations, according to Passing and Babcock, are  $y = -0.1736 + 1.015x$  and  $y = 1.8795 + 0.9593x$  ( $p < 0.0001$ ), respectively. The subsequent use of DCA 2000 in the pharmacy confirmed its features of ease of use, handiness and quickness of analytical times. Patients expressed very favorable opinions on this new opportunity offered through the pharmacy, which proved to be able to give a fast service, with high quality and low cost, in a setting the patients are accustomed to, easy to get to at any time, and qualified from the sanitary point of view.



# 12<sup>th</sup> BBBD BALKAN BIOCHEMICAL BIOPHYSICAL DAYS



## Molecular Biosciences in the Post-Genomic Era

BOOK OF ABSTRACTS

Organized by  
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Applied Biophysics  
Romanian Society of Immunology

May 10 -13, 2001  
Bucharest, Romania

►27

**CONTRIBUTIONS IN SPECIFICATION OF GENERAL VIEW OF THE GROSS LESIONS IN DOG'S ETHYLENE GLYCOL (ANTIFREEZE) POISONING**

ANCA LUNGU, CLAUDIA CONSTANTINESCU, GH. V. GORAN, I. R. DOBRE

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More frequently observed in the last years, usually in autumn and spring, ethylene glycol poisoning is an insufficient studied problem in carnivorous pathology.

In such cases, a proper treatment, suppose an exactly knowledge of injuries stages in different systems and organs.

The general lesions view which in the field literature was pointed out by hemorrhagic gastroenteritis, lung congestion and edema, severe dystrophy and cortical necrosis in kidney, extended toward the brain with medulla, it was completed with new observations.

In this paper, we mention severe vascular lesions, especially in the venous circulatory system, finally expressed by the presence of esophageal varicosity. These lesions are correlated with liver injuries represented by important damages of the parenchyma.

General view of the gross lesions is completed with information concerning the nervous system and adrenal gland.

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►28

**THE INFLUENCE OF GLUCOSE CONTENT ON THE ISOENZYME PROFILES OF N-ACETYL- $\beta$ -D-GLUCOSAMINIDASE IN DIABETES**

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Determination of serum and urinary N-acetyl- $\beta$ -D-glucosaminidase (EC 3.2.1.30, NAG) activity as an important parameter for diagnosis and prognosis of diabetes has been underlined by many authors. There are several reasons explaining the increase in the activity of NAG. The activity of NAG was correlated with the degree of metabolic control (1, 2) and with the presence or absence of diabetic complications (3, 4). In this paper the isoenzyme profiles of urinary NAG were investigated in order to understand better the correlation between the activity of NAG and glycemic control.

The activity of urinary NAG as well as of its isoenzyme forms in the control group (n=25) and IDDM diabetics (n=28) before and after adequate hospital treatment were determined. The synthetic substrate 2-methoxy-4-(2'-nitrovinyl)-phenyl-N-acetyl- $\beta$ -D-glucosaminide was used for the determination of NAG activity. The column with DEAE-cellulose was used for separation of NAG isoenzymes.

The average content of glucose in IDDM patients was  $13.24 \pm 3.58$  mmol/L. The contribution of the urinary A form activity to total NAG activity of diabetics ( $86.04 \pm 7.28\%$ ) was not significantly different from the corresponding values for the control group ( $84.64 \pm 2.75\%$ ). A significantly higher contribution of the B form activity was found in diabetics ( $14.1 \pm 3.9\%$ ) compared to the healthy individuals ( $8.62 \pm 2.19\%$ ). On the other hand the contribution of A<sub>2</sub> form in diabetics was significantly lower ( $2.38 \pm 1.02$ ) compared to the control ( $6.73 \pm 2.15$ ). These changes of isoenzyme forms in diabetes are interesting in view of the fact that there is no similarity in structure between B and A<sub>2</sub> forms. These results indicate that some of differences between the isoenzymes result from perturbations of the process of post-translational modifications as well as of variable glycosylation. The contribution of A, B and A<sub>2</sub> isoenzymes in selected group of IDDM patients



after hospital treatment did not change considerably ( $84.68 \pm 5.41\%$ ;  $13.95 \pm 5.20\%$ ;  $1.74 \pm 1.95\%$ , respectively). The content of glucose after treatment was  $9.18 \pm 3.25$ . These results indicated that the changes of glucose did not influence the isoenzymes profiles of NA(G).

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► 29

#### BCL-2 EXPRESSION IN MYELODYSPLASTIC SYNDROME

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The myelodysplastic syndrome (MDS) is a heterogeneous group of clonal stem cell disorders (refractory anemia-RA, refractory anemia with ringed sideroblasts-RARS, refractory anemia with excess of blasts-RAEB, refractory anemia with excess of blasts with transformation-RAEB-t, chronic myelomonocytic leukemia-CMML) characterized by trilineage dysplasia, peripheral pancytopenia and normo- or hypercellular bone marrow (BM) and an approximately 30% incidence of eventual transformation into acute myeloid leukemia (AML) (1,2). Recent data have provided evidence supporting the hypothesis that increased levels of apoptosis occur early in MDS bone marrow cells, but then diminish as the disease progresses (3,4).

We have studied Bcl-2 a protein with antiapoptotic and antioxidant potential, and its expression in hematopoietic cells is inversely related to maturation (5,6,7). The BM was directly aspirated into a syringe containing sodium citrate. Red blood cells were lysed and leukocytes were isolated by density gradient centrifugation on Sepcel. For the quantitative determinations of Bcl-2 in the bone marrow aspirate we worked with peroxidase labeled anti-Bcl-2 monoclonal antibodies, 2,2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) being peroxidase substrate. Peroxidase activity was quantitatively estimated by spectrophotometric activity at 415 nm. Minimal values of Bcl-2 expression were obtained in patients with CMML and maximum values in those with acute myeloid leukemia arising from MDS (AML-MDS).

The cases of RAEB and RAEB-t exhibited higher values than RA. The bcl-2 expression in myelodysplastic cells is inversely correlated with the rate of cells' apoptosis. We found that the rate of apoptosis is associated with Bcl-2 expression. The suppression of apoptosis in cases with leukemic transformation is associated with enhanced bcl-2 expression.

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► 30

#### BIOCHEMICAL RESEARCHES IN PIGS WITH OSTEOARTHROPATHIES

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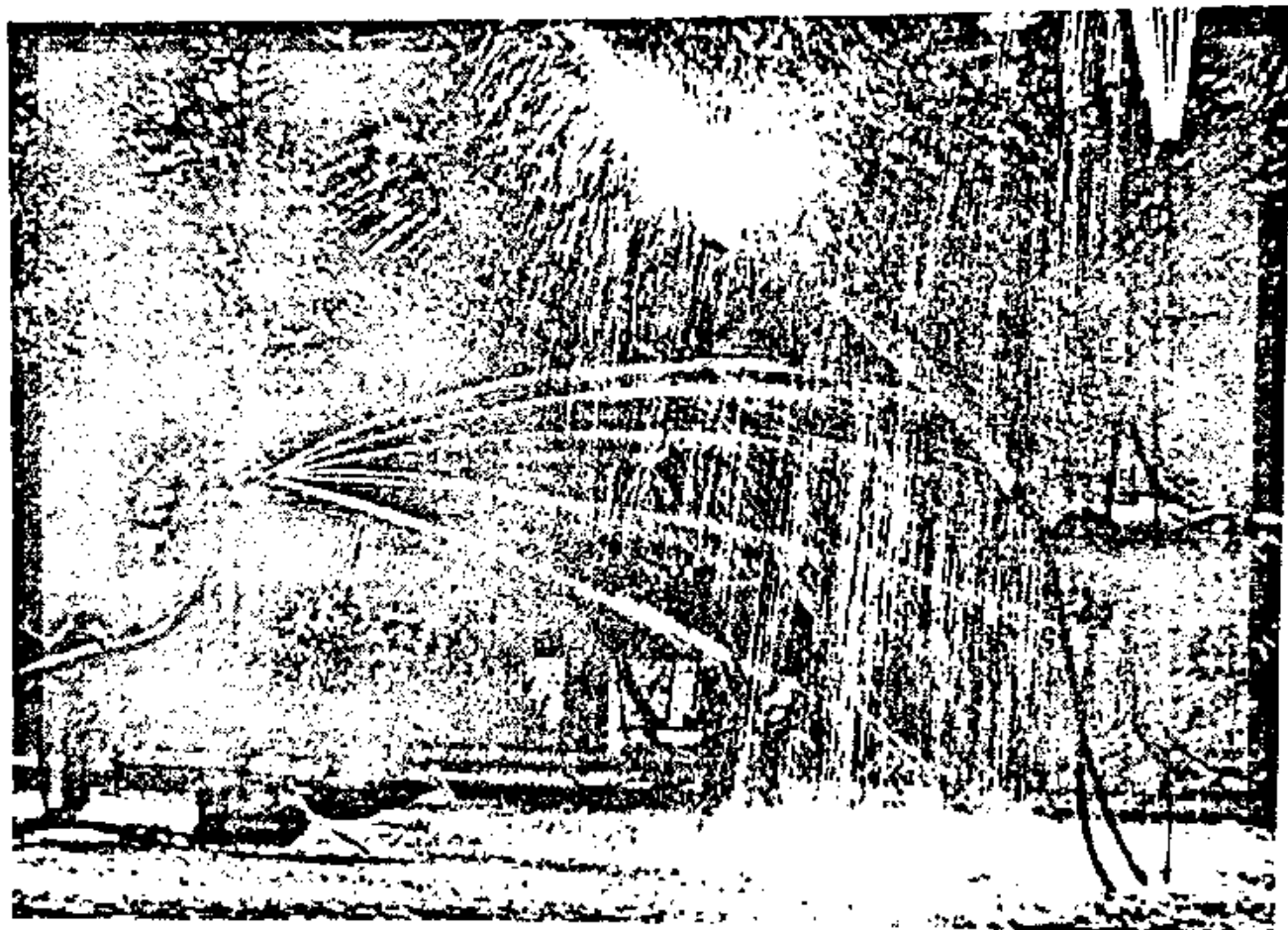
<sup>2</sup>Faculty of Veterinary Medicine, Bucharest, Romania

"Intensive breeding pathology" is a well known problem in large farms with a high density of animals. Osteoarthropathies is a part of these of nutritional and metabolic disorders. These injuries have a high incidence and produce economical problems represented by a lower daily weight growth, necessity slaughtered or even by mortality.

We studied some biochemical changes in a few osteoarthropathies at large swine farms, to explain some pathogenic aspects. Blood samples was taken on EDTA, between 9-10 a.m., in conditions of stress

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# **PBA 2000**

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Nowadays, the chromatographic, electrophoretic and immunological methods and their modifications are mostly used, because of the heterogeneity associated with the biological production. The side reactions may arise with activities that can be shown to be comparable to the active ingredient as related potency, clearance, safety and specific immunotoxicity eventually. A heterogeneity as a molecular variants have been shown and the side molecules present with active substance(s) often have a comparable effects.

Then, limits for purity can be established and justified based upon preclinical, clinical and manufacturing experience with the product. This limits must reflect the acceptability of product-related substances that may be consistently generated during the manufacture or increase upon storage.

A various molecular exchanges as a decomposition or polymerization have been shown and than, in addition to evaluation of the purity of the active ingredient and product-related substances, eventually, that have biological effect comparable with active substance(s), it is necessary to evaluate and calculate the impurities that may be present in the product and in the medicine(s).

Impurities may be either known defined chemical structure, partially characterized, or unidentified. Impurities that are selected for inclusion in the specifications are typically specific impurities for which a specific limits is regularly established. The same evaluation is necessary for product-related impurities as a molecular variants of the active substance that have not comparable effect(s) to the biological activity of the active substance. These impurities are considered specified impurities for which either individual or complete limits need to be established.

The impurity profile and its qualification is determined by a combination of molecular characterization of the product and throughout process validation studies in which removal and clearance of specific production-related impurities are evaluated. These studies provided the scientific rationale for the design of the control systems used to evaluate the levels of product-related substances, product-related impurities or product-degraded impurities.

## P 309

### ANALYSIS OF ISOENZYMIC PROFILES OF URINARY N-ACETYL- $\beta$ -D-GLUCOSAMINIDASE

(J. Mandić, V. Jovanović, J. Lilić, M. Jeremić)

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A great number of papers has been devoted to the importance of determining the activity of N-acetyl- $\beta$ -D-glucosaminidase (NAG, EC 3.2.1.35) as well as its isoenzymic profiles in body fluids of diabetic patients (1). Isoenzyme profile of urinary NAG was found to serve as sensitive indicator in monitoring diabetes and in the development and prediction of nephropathy (2).

In this paper a simple chromatographic method on small DEAE cellulose column (dimensions 0.8 cm x 5 cm) for the separation and determination of isoenzymic forms of urinary

NAG was developed. Prior to separation of isoenzymic forms urine was concentrated by ultrafiltration and then dialysed. The volume of dialysate used for separation was 150 - 500  $\mu$ l. The optimum separation of the NAG isoenzymic forms was achieved when B, A and M forms were eluted with 16 ml 0.01 mol/l phosphate buffer, pH 7.0; 20 ml 0.170 mol/l NaCl in phosphate buffer and 10 ml 0.4 mol/l NaCl in phosphate buffer, respectively. The activity of NAG in fractions was assayed by 2-methoxy-4-(2'-nitrovinyl)-phenyl-N-acetyl- $\beta$ -D-glucosaminide as substrate.

The above separation conditions were applied in urine samples in which isoenzymic profiles were already done on a big column. A good agreement in the contributions of NAG isoenzymic forms was obtained. The contributions of B, A and M forms on the big (8.0; 83.8 and 8.2%, res.) and on the small column (7.5; 84.2 and 8.2 %, res.) were approximately equal. As this method for separation of the urinary NAG isoenzymes is faster than commonly used and reproducible it is proposed for clinical practice.

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## P 310

### N-HETEROCYCLIC DERIVATIVES OF 2,4-DIHYDROXYTHIOBENZAMIDE AS ANTIMYCOTIC AGENTS

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One of the essential problem in chemotherapy which has not been solved yet, is the chemotherapy of systemic fungal infections. Because of it is need of modern, effective and nontoxic agents. In search for new chemical compounds possessing antifungal activity among thioamides, some

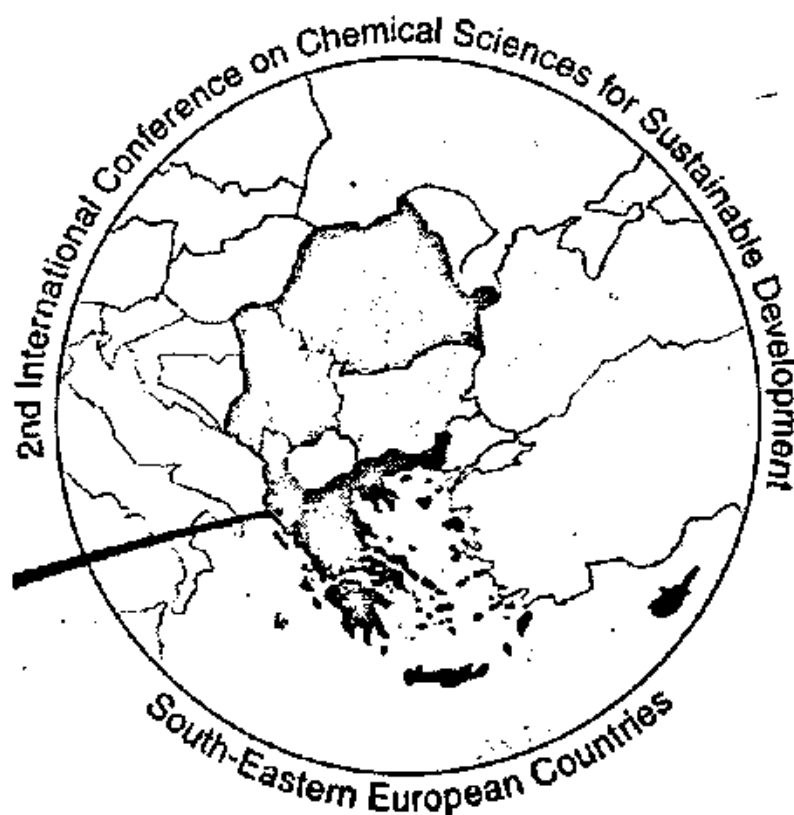
N-heterocyclic derivatives of 2,4-dihydroxy-benzencarbothioamide were obtained. The MIC assessment were used for the estimation of potential activity in vitro against dermatophytes: *Epidermophyton*, *Microporum*, *Trichophyton* and yeasts: *Candida*, *Cryptococcus*, *Geotrichum*, *Trichosporon*. Compounds studied, depending on kind of N-substitution, show activity 20.49 mg/ml against dermatophytes and 37.8 mg/ml against yeasts. In order to compare antifungal activity of chemicals under tests batraion and griseofulvin were used as standards.

# **2<sup>nd</sup> International Conference**

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## **BOOK OF ABSTRACTS VOLUME I**

**June 6-9, 2000, Halkidiki**

**GREECE**

**Under the Auspices of the "Eugenidis" Foundation**

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Although very toxic, inorganic mercury compounds have various applications and dealing with them is inevitable. Mercury is easily absorbed by skin, and gastrointestinal and respiratory tracts and is selectively concentrated in kidneys, which are particularly sensitive to its toxic effect[1]. It was shown that urinary N-acetyl- $\beta$ -D-glucosaminidase (NAG) can serve as a sensitive indicator of early renal injury [2]. Some results suggest that after exposure to mercury at levels below the biological exposure index, a transient increase in NAG can be observed, but is not an early indicator of developing renal dysfunction. Since in the individuals exposed to mercury the maximum concentration is found in lysosomal tubular cells of kidneys and as the isoenzymic NAG forms in lysozymes are differently bound, in this work the isoenzymes of urinary NAG were separated and determined. For the determination of NAG activity a synthetic substrate 2-methoxy-4-(2'-nitrovinyl)-phenyl-N-acetyl- $\beta$ -D-glucosaminide was used. To separate isoenzyme NAG forms a column filled with DEAE-cellulose was used. The study has been performed on two groups of workers exposed to various concentrations of inorganic Hg-compounds: the first group of workers periodically manufacturing with mercury (n=87), the second group of workers permanently manufacturing with mercury (n=25) and control group (n=15). Hg concentrations were determined in blood and urine by AAS. Urinary NAG activities were elevated in both groups occasionally and continually exposed to Hg > MAC, but statistically significant increase of NAG activity was found only in the second group. The results of the analysis of NAG isoenzymic profiles show that the contribution of the B form activity to the total NAG activity is statistically considerably decreased compared to the control group. Therefore, it is proposed to use the activity of NAG B form as an early parameter in determining tubular injuries.

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# CLINICAL CHEMISTRY AND LABORATORY MEDICINE



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## ABSTRACTS VOLUME



Wiley at Guyton Berlin New York

## - H179 -

## SERUM CHOLINESTERASE DOES NOT FORM MACROENZYME COMPLEXES.

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Many serum enzymes, with the main exception of cholinesterase (CHE), have been reported to link immunoglobulins or other macromolecules, to yield "macroforms". We recently reported the suitability of the polyethyleneglycol (PEG) precipitation technique to show the occurrence of several enzymes macroforms in human serum, but no macro-CHE occurred in a limited number of samples. In this work 11, 185 and 47 human serum samples (total 243), exhibiting respectively low ( $< 5.3$  kU/L), normal and high ( $> 12.3$  kU/L) CHE activity (substrate: butyrylthiocholine), were supplemented with PEG at 90 and 120 g/L final concentrations. The activities measured in the supernatants were expressed as a percentage of the original activity. After discarding two outliers, the unprecipitated activities (mean  $\pm$  SD) were  $100.2 \pm 2.3$  (PEG 90 g/L) and  $93.4 \pm 5.4$  (PEG 120 g/L). A very low-activity serum ( $1.25$  kU/L) behaved as outlier; however, in spite of values outside the  $m \pm 3SD$  interval, its behavior was not consistent with a macroform. Present data show the formation of CHE macroforms, if any occurs, is by far rarer as compared to other serum enzymes.

## - H180 -

## EVALUATION OF THE MDX™ G6PD-MED KIT FOR QUALITATIVE DETECTION OF THE MOST COMMON G6PD DEFICIENCY IN ITALIAN POPULATION

Madaia Francesca M.F., Ivaldi Giovanni I.G., Parodi Maria Isola P.M.I., Leone Daniela L.D., Pascotto Dino P.D., Perroni Lucia P.L., Argenti A.

Laboratorio Genetica Umana, Ospedali Galliera, Genova (Italy)

Glucose 6-phosphate dehydrogenase (G6PD) deficiency is an X-linked genetic abnormality. The diagnosis of G6PD is made normally by demonstrating markedly decreased or nearly absent activity of this enzyme in red cells. However, in some patients the test may give false-normal results when performed during a hemolytic attack, in double heterozygous females for G6PD deficiency and  $\beta$ -thalassaemia trait or in newborn females with reticulocytosis. G6PD deficiency is genetically heterogeneous. Other 403 different variants have been reported on the basis of diverse biochemical characteristics. A common G6PD variant characterized by severe enzyme deficiency and B-like electrophoretic mobility is called G6PD-Mediterranean (G6PD-Med). The use of molecular techniques has been widely documented to confirm the diagnosis of enzymatic deficiency in several G6PD variants. Here we report a study conducted to evaluate the performance characteristics of the Bio-Rad mDx™ G6PD-Med kit (this kit utilizes the principle of allele-specific oligonucleotide (ASO) hybridization) compared with restriction enzyme analysis results (Libo®). All reagents and equipment for this study were supplied by Bio-Rad Laboratories (Hercules, CA). The method comparison study was performed on whole blood samples from 65 individuals (35 normal, 6 hemizygoes, 22 heterozygoes and 2 homozygoes for G6PD). Genomic DNA was prepared using InstaGene whole blood kit (Bio-Rad). The data from this study show that this method was found to be sensitive, specific reproducible and rapid for correct identification of the most common G6PD variant in Italian population.

## - H181 -

CHANGES OF ISOENZYMES OF URINARY N-ACETYL- $\beta$ -D-GLUCOSAMINIDASE IN DIABETES

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1) Biochemistry, Faculty of Chemistry, Belgrade (Yugoslavia)

2) Endocrinology, Faculty of Medicine, Belgrade (Yugoslavia)

Increased activity in serum and urinary N-acetyl- $\beta$ -D-glucosaminidase (NAG, EC 3.2.1.30) in diabetic patients was found. To understand better the cause of the increase in the activity of urinary NAG, the isoenzyme forms of NAG were separated by means of ionexchange chromatography on DEAE cellulose in control group and IDDM patients with various secondary complications. For the determination of NAG activity a synthetic substrate 2-methoxy-4-(2-nitrovinyl)-phenyl-N-acetyl- $\beta$ -D-glucosaminide was used.

From the urine mainly three most abundant isoenzyme forms of NAG, A, B and M, were separated. The contribution of the A form activity ( $80.2 \pm 3.9\%$ ) to total NAG activity of IDDM diabetics does not change considerably compared to the control ( $84.2 \pm 3.2\%$ ). The increase in total activity of urinary NAG in diabetics is proportional to the A form activity ( $r=0.997$ ,  $p<0.0001$ ). The contribution of the B form activity depends on the state of metabolic monitoring and diabetic complications. A significantly higher activity of the urinary B form was found in IDDM diabetics compared to the healthy individuals. An increase B form is correlated with the occurrence and abundance of the M form. Isoenzyme profiles of NAG can serve as sensitive indicators in monitoring diabetes and in the development and prediction of microangiopathies.

## - H182 -

## LIQUID STABLE REAGENTS FOR ENZYME MEASUREMENT

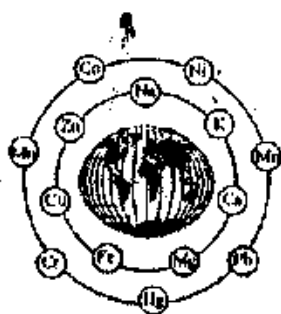
McCartney, B.A., Fitzgerald, S.P., Campbell J.J., Lamont, J.V.

R&amp;D, Randox Laboratories Ltd., Crumlin (UK)

Liquid stable reagents have been developed for measuring the clinically significant enzymes Alanine Aminotransferase (ALT); Aspartate Aminotransferase (AST); Alkaline Phosphatase (ALP), both AMP and DEA buffer based; Amylase; Pancreatic Amylase; Creatine Kinase-NAC activated (CK-NAC); Gamma-Glutamyltransferase (GGT) and Lactate Dehydrogenase (LD-L and LD-P). Our aim was to develop liquid stable reagents which offer advantages over equivalent freeze-dried reagents, such as less preparation time, better working reagent stability, less wastage and less risk of contamination. All reagents were evaluated on Hitachi 717 analyzer. Performance was comparable to lyophilized equivalents. Intra-assay precision (CV%) is  $< 3\%$  at normal levels;  $< 2\%$  at abnormal. Inter-assay precision is  $< 5\%$  (normal);  $< 4\%$  (abnormal). All reagents correlated well to commercially available equivalents, with all  $r$  values  $> 0.985$ . Linearity is  $> 800$  U/L for ALT and AST;  $> 1500$  U/L for ALP;  $> 1500$  U/L for GGT;  $> 3000$  U/L for LD-P;  $> 600$  U/L for LD-L; and  $> 2000$  U/L for CK-NAC, Amylase and Pancreatic Amylase. No significant interference was observed from Triglyceride up to  $1000$  mg/dl or Bilirubin up to  $13.5$  mg/dl with any of these reagents. Haemoglobin up to  $500$  mg/dl shows no interference in Amylase, Pancreatic Amylase, CK-NAC or GGT reagents. In summary, these reagents offer the advantages of being stable, ready-to-use liquids without compromising performance.



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## ISOENZYMIC FORMS OF N-ACETYL- $\beta$ -D-GLUCOSAMINIDASE IN THE URINE OF THE INDIVIDUALS EXPOSED TO MERCURY

Mandic Ljuba<sup>1</sup>, Sandor Livla<sup>2</sup>, Jagodic Vesna<sup>1</sup>, Liric Ivana<sup>1</sup>

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Mercury compounds are toxic environmental pollutants and cumulative poisons concentrating in the kidney. In order to find a parameter appropriate for early discovery of an injured kidney in individuals exposed to mercury effect, the activities of isoenzymic forms of urinary N-acetyl- $\beta$ -D-glucosaminidase (NAG, EC 3.2.1.30) were being determined in this work.

For the separation and determination of the activities of isoenzymic forms a simple, fast and reproducible method was developed, suitable for toxicological and clinical practice. The analysis of the obtained isoenzymic urinary NAG profiles indicated that the B form contribution to the total NAG activity is statistically considerably increased under mercury effect ( $p < 0.05$ ) compared to the control group. The increase in total NAG activity is correlated with the B form activity ( $r = 0.583$ ). Since the NAG B form is bound with lysosomal membrane its increase in urine is an early parameter indicating the injuries of lysosomes. The contributions of the A ( $64.13 \pm 16.51\%$ ) and M ( $21.32 \pm 6.32$ ) forms activity to the total NAG activity do not change considerably compared to the controls.

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**53. SAVETOVANJE**  
**SRPSKOG HEMIJSKOG**  
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**53<sup>rd</sup> Meeting of**  
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**Book of Abstracts**

**Prirodno-matematički fakultet, Kragujevac 10. i 11. jun 2016.**

**Faculty of Science, Kragujevac, Serbia, June 10 and 11, 2016**

### **Vezivanje slobodnih masnih kiselina i bakar(II)-jona za HSA dovodi do promena u karbonilaciji i reaktivnosti Cys34 tiolne grupe sa metilglioksalom**

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Ljudski serumski albumin (Human serum albumin, HSA) predstavlja glavni antioksidant plazme zahvaljujući redukovanom Cys34. Vezivanje masnih kiselina (FAs) za HSA može dovesti do promene u dostupnosti i reaktivnosti Cys34 tiolne grupe, njenog kapaciteta kao hvatača/antioksidanta. Cilj ovog rada je bio da se ispita efekat izabranih FAs (stearinska, miristinska, oleinska, ekstrakt ribljeg ulja-FO i smeša-MixFAs) na vezivanje Cu(II) jona za HSA, kao i sinergistički uticaj na reaktivnost HSA-SH i modifikaciju HSA metilglioksalom (MG). Vezivanje FAs povećava reaktivnost HSA-Cys34-SH,  $k'$  vrednost (konstanta brzine pseudo prvog reda reakcije tiola sa Elmanovim reagensom) je bila povećana u nizu: MixFAs, oleinska, stearinska, FO i miristinska. Vezivanje Cu(II) jona (0,1 mol/mol HSA) dovelo je do povećanja  $k'$  vrednosti ako je bio prisutan FO, ali je bila smanjena kada su ostale FAs bile prisutne. Sadržaj slobodne HSA Cys34-SH je bio smanjen za 10% posle vezivanja Cu(II) jona, a dalje tokom inkubacije 24 h na 37 °C, bio je dodatno smanjen za 10% (stearinska, MixFAs) i 20% (miristinska, FO, oleinska). Karbonilovanje FA-HSA-Cu(II) kompleksa sa MG (20 mol/mol HSA) u toku 24 h, dovelo je do smanjenja sadržaja Cys34-SH 30-40% zavisno od prisutne FA. Karbonilovanje FA-HSA-Cu kompleksa može doprineti povećanju karbonilnog i oksidativnog stresa u dijabetesu i drugim bolestima.

### **Binding of FAs and Cu(II)-ions to HSA changes its carbonylation pattern and Cys34 thiol group reactivity with methylglyoxal**

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Tamara Uzelac, Ljuba Mandić

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*\*Institute for the application of nuclear energy, INEP, Belgrade*

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Human serum albumin (HSA) represents a major plasma antioxidant due to its Cys34 reduced/sulfhydryl form. FAs binding to HSA could lead to changes of Cys34 thiol group accessibility and reactivity, i.e. its scavenger capacity/antioxidant properties. The aim of this work was to investigate the effects of selected FAs (stearic, myristic, oleic, fish oil extract-FO, FAs mixture-MixFAs), on Cu(II) ion binding to HSA, and the synergistic influence on the reactivity of HSA-SH and HSA modified with methylglyoxal (MG). Binding of FAs increased HSA-Cys34-SH reactivity,  $k'$  values (pseudo first order rate constant for thiol reaction with Ellman's reagent) increased in the order: MixFAs, oleic, stearic, FO and myristic. Binding of Cu(II) (0.1 mol/mol HSA) led to an increase of  $k'$  value if FO was present, but for other bound FAs  $k'$  value decreased. The content of free HSA-Cys34-SH decreased 10% after Cu(II) ion binding, and during 24 h incubation at 37 °C, it further decreased for another 10% (stearic acid, MixFAs) or 20% (myristic, FO, oleic). Carbonylation of FA-HSA-Cu(II) complexes with MG (20 mol/mol HSA) for 24 h, lead to a decrease in Cys34-SH content depending on FA present: 30-40%. Carbonylation of FA-HSA-Cu complexes could contribute to a further enhancement of the oxidative and carbonyl stress in diabetes, as well as other diseases.

**Srpsko hemijsko društvo**  
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# **52. SAVETOVANJE SRPSKOG HEMIJSKOG DRUŠTVA**

## **PROGRAM i KRATKI IZVODI RADOVA**

**52<sup>nd</sup> Meeting of  
the Serbian Chemical Society**

**Program  
&  
Book of Abstracts**

**Novi Sad, 29. i 30. maj 2015.  
Novi Sad, Serbia, May 29 and 30, 2015**

**Uticaj antipsihotika na reaktivnost tiolne grupe serum-albumina**

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Ljuba M. Mandić

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Oksidativni stres se navodi kao jedan od uzroka nastanka šizofrenije. Postoji neslaganje o tome da li primena antipsihotika (AP) u lečenju šizofrenije dodatno doprinosi oksidativnom stresu. Humani serum albumin (HSA) ima jednu slobodnu tiolnu grupu (Cys34) i pošto je najzastupljeniji protein u krvi, predstavlja najvažniji ekstracelularni antioksidans. HSA veže različite ligande (masne kiseline, lekove, metale) koji mogu da izazovu promenu konformacije HSA molekula i time promenu reaktivnosti HSA-SH grupe. Cilj ovog rada bio je da se ispita uticaj vezivanja AP na redoks homeostazu (određivanjem sadržaja ukupnih tiola i HSA-SH grupa u plazmi pacova) i na reaktivnost HSA-SH grupe (određivanjem konstante brzine pseudo prvog reda za reakciju HSA-SH sa DTNB) u *in vivo* i *in vitro* eksperimentima. Kod tri grupe pacova, nakon tretmana od 4 nedelje sa sertindolom (S), klozapinom (C) i ziprasidonom (Z), sadržaj ukupnih tiola u plazmi bio je niži u odnosu na kontrolnu grupu ( $0,188 \pm 0,041$  mmol/l). Smanjenje je bilo statistički značajno ( $p < 0,05$ ) samo u grupi tretiranoj sa C. Sadržaj HSA-SH grupa u kontrolnoj grupi ( $0,345 \pm 0,065$  mol-SH/mol HSA) bio je statistički značajno ( $p < 0,05$ ) niži u odnosu na grupu sa C, i statistički značajno ( $p < 0,05$ ) viši u odnosu na grupe sa S i Z. Ispitivanja *in vitro* su pokazala da AP utiču na reaktivnost HSA-SH grupe u stepenu koji zavisi od mesta vezivanja leka na molekulu HSA u odnosu na položaj Cys34 grupe.

**The influence of antipsychotics on serum albumin thiol group reactivity**

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Ljuba M. Mandić

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Oxidative stress is mentioned as one of the causes of schizophrenia. There is discrepancy whether the treatment of schizophrenia with antipsychotic (AP) additionally contributes to the oxidative stress. Human serum albumin (HSA) has one free thiol group (Cys34) and as the most abundant protein in the blood, it is major extracellular antioxidant. HSA binds various ligands (fatty acids, drugs, metals) which may cause change in the conformation of HSA molecules and therefore a change of HSA-SH group reactivity. The aim of this study was to investigate the influence of AP binding on redox homeostasis (determining of total thiol and HSA-SH group content in rat plasma) and reactivity of HSA-SH group (determining of the pseudo first order rate constant for the reaction of HSA-SH with DTNB) *in vivo* and *in vitro*. In three groups of rats, after 4 weeks treatment with sertindole (S), clozapine (C) and ziprasidone (Z), the total thiol content in plasma was lower than in the control group ( $0,188 \pm 0,041$  mmol/l). Decrease was statistically significant ( $p < 0,05$ ) only in the group treated with C. The content of HSA-SH group in the control group ( $0,345 \pm 0,065$  mol-SH/mol HSA) was statistically significant ( $p < 0,05$ ) lower than in the group with C, and statistically significant ( $p < 0,05$ ) higher compared to the groups with S and Z. Results of *in vitro* investigations shown that these AP affect HSA-SH group reactivity in the level depending on the distance between the positions of HSA-drug binding site and Cys34 thiol group.

**Kvantifikacija masnih kiselina vezanih za humani serum albumin**  
**Ivan D. Pavićević, Vesna B. Jovanović, Marija M. Takić\*, Jelena M. Acimović,**  
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Broj molekula masnih kiselina (MK) vezanih po molekulu humanog serum-albumina (HSA) varira, zavisno od fiziološkog stanja i ishrane organizma. Tokom određivanja kinetičkih parametara reakcije slobodne HSA-Cys34 tiolne grupe sa Elmanovim reagensom utvrđeno je da vrsta i broj vezanih MK po molekulu HSA značajno utiče na kinetiku ove reakcije. Rezultati određivanja sadržaja MK vezanih za HSA mogu se primeniti za procenu reaktivnosti tiolne grupe, odnosno redukcionog kapaciteta HSA u cirkulaciji, što može imati klinički značaj u nekim patološkim stanjima (poput metaboličkog sindroma i dijabetesa) u kojima je metabolizam MK poremećen. Stoga je razvijena metoda kvantitativne tankoslojne hromatografije (qTLC) za određivanje MK vezanih za HSA. Kao standard upotrebljena je stearinska kiselina. Densitometrijska analiza rađena je ImageJ i statističkim programom, koji smo razvili u R jeziku. HSA je izolovan iz humanog seruma frakcionim taloženjem, MK su ekstrahovane smešom heptan-hloroforma i kiselog rastvora bakar(II)-sulfata, i njihov sadržaj je određen qTLC-om. Dobijeni rezultati se statistički ne razlikuju u odnosu na dobijene GC kvantifikacijom (sa internim C13 standardom). Razvijena metoda je tačna, reproduktivna, brza i jeftina, što je čini pogodnom za kliničku praksu.

**Quantification of fatty acids bound to human serum albumin**  
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The number of fatty acids (FA) molecules bound per HSA molecule is variable, depending on the physiological state and diet. During the investigation of kinetics parameters of the reaction of free HSA-Cys34 thiol group and Ellman's reagent it was found that FA bound to HSA lead to the significant change of reaction kinetic. Thus, the results of determination of FA content bound to HSA could be used for the estimation of thiol group reactivity, i.e. of the redox capacity of HSA in circulation, making them useable in clinical studies of some pathologies (eg. diabetes and metabolic syndrome) with disturbed FA metabolism. Therefore the quantitative thin-layer chromatography (qTLC) method for determination of FA bound to the HSA was developed. Stearic acid was used for preparation of the standard solution. For the FA quantification, a densitometric analysis with the ImageJ was performed, and a specialised program developed in the R language was used. The HSA was isolated from human serum by fractional precipitation, FA were extracted with heptane-chloroform and acidified copper(II)-sulfate mixture, and their content was determined using the qTLC. The results obtained by qTLC and referent GC method (with internal C13 standard) were statistically comparable. Developed qTLC method is accurate, reproducible, low time consuming and inexpensive, which makes it suitable for clinical practice.

# Serbian Biochemical Society Fifth Conference

Faculty of Chemistry, University of Belgrade,  
13.11.2015. Belgrade, Serbia.

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## Fatty acids change the reactivity of the human serum albumin Cys34 thiol group

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Human serum albumin (HSA) is the most abundant plasma protein and with free Cys34 thiol represents a significant pool of the anti-oxidative thiol in human circulation. Crystallographic studies showed that accessibility of HSA Cys34 residue to oxidation was significantly changed when free fatty acids (FAs) were attached to HSA. Therefore, our aim was investigation of the impact of myristic acid (MYR, C14:0), palmitic acid (PLM, C16:0), stearic acid (STE, C18:0), oleic acid (OLA, C18:1), and fatty omega-3 polyunsaturated fatty acids from fish oil diet supplement EPA (C20:5) [(5Z,8Z,11Z,14Z,17Z)-5,8,11,14,17-icosapentaenoic acid] and DHA (C22:6) [(4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoic acid] on reactivity of the Cys34 free thiol group.

Investigating of fatty acids content (bound to HSA) using GC analysis was time consuming process, and therefore we developed novel methodology utilizing quantitative TLC and flatbed scanner for densitometric quantification of FAs. Fast determination of the HSA saturation with FAs could be useful when estimation of the reactivity and redox capacity of the HSA-SH in circulation is performed, especially in some pathologies with elevated FAs (metabolic syndrome and diabetes).

Changes of thiol group reactivity before and after FAs binding was studied with DTNB (5,5'-dithiobis(2-nitrobenzoic acid)) reagent, at the concentration that represented pseudo-first order excess compared to HSA-SH. HSA changes were monitored using native PAG electrophoresis and fluorescence spectroscopy.

Time courses (0–10 min) of the reactions of FA-free HSA and FAs-bound HSA sulfhydryl group were monitored spectrophotometrically. Graphics obtained after linearization of kinetics data show that reactions followed pseudo-first order reaction kinetic. The values of rate constants ( $k'$ ) obtained for all FAs-bound HSA-SH (from  $14.58 \times 10^{-3}$  to  $26.02 \times 10^{-3} \text{ s}^{-1}$ ) were 2–3.5 times higher than for FA-free HSA-SH ( $7.52 \times 10^{-3} \text{ s}^{-1}$ ). Among saturated long-chain FAs tested, the shortest FA, MYR, had the strongest effect. STE and OLA show similar effects on HSA-SH reactivity ( $k'$  values of  $17.34 \times 10^{-3} \text{ s}^{-1}$  and  $16.97 \times 10^{-3} \text{ s}^{-1}$ , resp.) although the effect of polyunsaturated

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*Program  
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Book of Abstracts*

*Beograd, 14. i 15. Juni 2012.  
Belgrade, Serbia, June 14-15, 2012*

## Tioli malih molekularskih masa kao hvatači metilglioksala

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Reaktivni  $\alpha$ -oksoaldehidi, posebno metilglioksal (MG), stvaraju se u većoj meri u dijabetesu, uremiji, u uslovima oksidativnog stresa, inflamaciji i u procesu starenja. Reaguju s amino-, guanidino-, i tiol-grupom proteina, što dovodi do nastajanja krajnjih proizvoda glikacije i do umrežavanja proteina. U cilju sprečavanja pomenutih reakcija neophodno je razviti pogodne „hvatače“ MG. Ispitivanje mehanizma reakcije tiol-grupe i MG pokazalo je da bi tioli malih molekularskih masa, koji sadrže  $\alpha$ -amino- $\beta$ -merkaptotetansku grupu kao farmakoforu, mogli biti uspešni hvatači MG. Stoga je u ovom radu ispitana efikasnost Cys, penicilamina, glutatona i N-acetilcisteina kao inhibitora glikacije humanog serum albumina (HSA) u reakciji sa MG. Pored tiola, u eksperimentima inhibicije upotrebljen je i metformin, lek koji se koristi u terapiji metaboličkog sindroma i dijabetesa, i koji kao biguanidino reagens može biti dobar hvatač MG. HSA je inkubiran sa MG, u i bez prisustva inhibitora. Modifikacije HSA molekula praćene su tokom vremena, u alikvotima reakcionih smeša, spektrofotometrijskim određivanjem sadržaja neizreagovanih amino- i guanidino-grupa, spektrofotometrijski i nativnom i SDS PAGE. Utvrđeno je da se u prisustvu Cys i penicilamina postiže veoma efikasna inhibicija reakcije glikacije proteina, odnosno da bi se supstance koje sadrže  $\alpha$ -amino- $\beta$ -merkaptotetansku grupu kao farmakoforu mogle koristiti kao efikasni hvatači metilglioksala.

## Low molecular mass thiols as scavengers of methylglyoxal

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Reactive  $\alpha$ -oxoaldehydes, especially methylglyoxal (MG), are produced in higher quantities in diabetes, uremia, oxidative stress, inflammation and aging. They react with amino, guanidino and thiol groups of proteins which causes formation of advanced glycated end-products and cross-linking. In order to prevent these reactions it is necessary to develop convenient MG „scavengers“. Investigation of the reaction mechanism of thiol-groups and MG showed that low molecular mass thiols containing  $\alpha$ -amino- $\beta$ -mercapto-ethanoic group as pharmacophore could be successful MG scavengers. Therefore, in this paper the efficiency of Cys, penicillamine, glutathione and N-acetylcysteine as inhibitors of human serum albumin (HSA) glycation reaction with MG was investigated. As biguanidino reagent metformin (drug used in the treatment of metabolic syndrome and diabetes) could be a good scavenger of dicarbonyl compounds, it was also used in the inhibition experiments. HSA and MG were incubated with and without inhibitors. Modifications of HSA molecule were monitored over time, in aliquots of the reaction mixtures, by spectrophotometric determination of amino and guanidino groups content, by spectrofluorimetry, and native and SDS PAGE. It was found that inhibition of protein glycation reaction is very efficient in the presence of Cys and penicillamine. Therefore, the substances containing  $\alpha$ -amino- $\beta$ -mercapto-ethan as a pharmacophore could be used as efficient scavengers of MG.

This paper was supported by the Ministry of Education and Science of RS (Grant 172049)

Srpsko hemijsko društvo



**XLI SAVETOVANJE  
SRPSKOG HEMIJSKOG  
DRUŠTVA  
IZVODI RADOVA**

ISBN 86-7132-014-6

Beograd, 23. i 24. januar 2003.  
[www.shd.org.yu/S11D41](http://www.shd.org.yu/S11D41)

T<sub>5</sub>

# STABILNOST IZOENZIMA N-ACETIL- $\beta$ -D-GLUKOZAMINIDAZE U BAZNIM URINIMA

Stability of N-acetyl- $\beta$ -D-glucosaminidase isoenzymes in alkaline urine

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Saznanje da se aktivnost urinarne N-acetil- $\beta$ -D-glukozaminidaze (EC 3.2.1.30, NAG), parametra za rano otkrivanje renalnih oštećenja, menja u baznim urinima dovelo je do potrebe da se sagleda uzrok ovih promena<sup>1</sup>. Ispitivanje je urađeno za pet baznih pH vrednosti: 8,7; 9,0; 9,5; 9,9 i 10,0. Aktivnost NAG-a određivana je sa 2-metoksi-4-(2-nitrovinil)-fenil-N-acetil- $\beta$ -D-glukozaminidom kao supstratom.

Pri podešavanju pH kiselih urina do baznih vrednosti aktivnost NAG-a se drastično smanjuje (od 20% za pH 8,7 do 74% za pH 10,0). Zakišeljavanje "baznih" urina na početne kisele pH vrednosti dovodi do daljeg smanjivanja aktivnosti NAG-a za nekoliko procenata. Ovo ukazuje da podešavanje urina na bazne vrednosti pH dovodi do ireversibilne inaktivacije NAG-a i da određivanje aktivnosti u baznim urinima nije pouzdano. U cilju sagledavanja uzroka smanjenja ukupne aktivnosti NAG-a u baznoj sredini analizirani su izoenzimski profili posle hromatografskog razdvajanja na DEAE celulozi.

Na hromatogramima urina zaalkaliziranih na pH 8,7; 9,0 i 9,5 uočeno je da se sa porastom pH ravnomerno smanjuju pikovi koji odgovaraju B, A i A<sub>2</sub> obliku. Analiza zastupljenosti pojedinačnih izoenzima u ukupnoj aktivnosti NAG-a pokazala je da su procentualni udeli B, A i A<sub>2</sub> oblika u kiselim urinima i urinima podešenim na pH 8,7; 9,0 i 9,5 gotovo jednaki. Zastupljenost B oblika u urinu čija je pH vrednost podešena na 9,9, odnosno 10,0, povećava se za 1,62 puta, odnosno 2,68 puta. Pri pH 9,9, odnosno 10,0 udeo A oblika se smanjuje za 1,25 puta, odnosno za 1,79 puta. Ove promene udela pri pH vrednostima većim od 9,5 su posledica izraženije inaktivacije A oblika. Promene u zastupljenosti A<sub>2</sub> oblika u kiselim i "baznim" urinima su slične kao kod B oblika. Zakišeljavanjem "baznih" urina, procentualni udeli pojedinih oblika u ukupnoj aktivnosti NAG-a ostaju jednaki gotovo za sve ispitivane pH vrednosti. Na osnovu dobijenih rezultata zaključeno je da se ukupna aktivnost NAG-a u baznim urinima čiji je pH manji od 9,5 smanjuje zbog inaktivacije sva tri izoenzima u ovim uslovima. S obzirom na to da se procentualni udeli aktivnosti pojedinih izoenzima i njihovi odnosi menjaju tek pri pH vrednostima većim od 9,5 zaključeno je da izoenzimski profili mogu biti pouzdaniji parametar renalnih oštećenja u širem opsegu pH vrednosti urina u odnosu na ukupnu aktivnost NAG-a.

I. A. Morita, Y. Numata, et al, *Chin Chim Acta*, 278(1) (1998) 35-43



# JUGOSLOVENSKA MEDICINSKA BIOHEMIJA

Časopis Društva medicinskih biohemičara Jugoslavije  
Official Journal of the Yugoslav Society of Medical Biochemists

Godište: 21

Beograd, april – jun 2002

Broj: 2

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Jugoslav. Med. Biochem. 21 (2): 69–244, 2002

relaciji sa katalitičkom aktivnošću lizozomalnog enzima N-acetil- $\beta$ -D-glukozaminidaze ( $\beta$ -NAG) i enzima celikaznog pokrova tubula  $\gamma$ -glutamyl transferaze ( $\gamma$ -GT) u urinu. Aktivnosti enzima izražene su na mmol urinarnog kreatinina. Korističen je 24h urin, čime su izbegnute varijacije ekskrecije u toku dana. Ispitivani su bolesnici oboleli od *diabetes mellitus* tipa II. Pacijenti su podeljeni u 3 grupe: I grupa, pacijenti sa mikroalbuminurijom manjom od 0,01 g/24h; II grupa, sa mikroalbuminurijom od 0,01 do 0,3 g/24h; III grupa, sa mikroalbuminurijom od 0,3 do 3 g/24h. Za statističku obradu podataka korišćen je Mann-Whitney-ov neparametarski test i neparametarska analiza varijanse po Kruskal-Wallis. Rezultati pokazuju da je porast aktivnosti  $\beta$ -NAG u urinu dijabetičara u III grupi bila statistički značajna u odnosu na bolesnike I ( $0,45 \pm 0,23$ ) i II ( $0,85 \pm 0,39$ ) grupe. Bolesnici II grupe imali su statistički značajno veću aktivnost  $\beta$ -NAG u odnosu na bolesnike I grupe. Porast aktivnosti  $\gamma$ -GT bio je, statistički, značajno veći kod bolesnika III grupe ( $3,43 \pm 1,16$ ) samo u odnosu na bolesnike I ( $1,52 \pm 1,19$ ) grupe. Na osnovu navedenih rezultata zaključeno je da je  $\beta$ -NAG osetljiviji parametar nego  $\gamma$ -GT za procenu uticaja mikroalbuminurije na anatomske integritet tubula i procenu stepena oštećenja tubula.

ence of microalbuminuria on anatomic integrity of tubules in correlation with catalytic activity of lysosomal enzyme N-acetyl-beta-D-glucosaminidase ( $\beta$ -NAG) as well as catalytic activity of enzyme  $\gamma$ -glutamyl transferase ( $\gamma$ -GT) in urine. The activities of enzymes are represented in mmol of urinary creatinine. We used 24h urine, whereby daily variation of excretion were avoided. In this study, patients with type 2 diabetes mellitus were examined. Patients were divided in 3 groups: I group, patients with microalbuminuria less than 0.01 g/24h; II group, with microalbuminuria from 0.01 to 0.3 g/24h and III group, with microalbuminuria from 0.3 to 3 g/24h. For statistical analysis, Mann-Whitney non-parametric test and non-parametric analysis of variance according to Kruskal-Wallis were used. Results showed that  $\beta$ -NAG activity in urine of diabetic patients from III group ( $1.91 \pm 1.44$ ) was significantly higher compared with  $\beta$ -NAG activity in urine of patients from I ( $0.45 \pm 0.23$ ) and II group ( $0.85 \pm 0.39$ ). In patients from II group  $\beta$ -NAG activity ( $0.85 \pm 0.39$ ) increased statistically significant compared with  $\beta$ -NAG activity in patients from I group ( $0.45 \pm 0.23$ ). Activity of  $\gamma$ -GT significantly increased in patients from III group ( $3.43 \pm 1.16$ ) only compared with patients from I group ( $1.52 \pm 1.19$ ). Based on reported results, we concluded that  $\beta$ -NAG represent more sensitive parameter than  $\gamma$ -GT for evaluation of the influence of microalbuminuria on anatomic integrity of tubules and for evaluation of tubule damage degree.

## B27

### UTICAJ pH NA ODREĐIVANJE AKTIVNOSTI N-ACETIL- $\beta$ -D-GLUKOZAMINIDAZE

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V. Jovanović, Lj. Mandić

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Određivanje aktivnosti N-acetil- $\beta$ -D-glukozaminidaze (EC 3.2.1.30; NAG) u urinu pokazalo je da NAG može biti veoma koristan pokazatelj renalnog oštećenja do kojih dolazi u različitim patološkim stanjima i pod dejstvom različitih toksičnih supstanci. Pri određivanju aktivnosti urinarnog NAG-a kod zečeva koji su bili izloženi dejstvu teških metala radene su vrednosti niže od očekivanih za renalna oštećenja. Urini su imali boju od žute do mrke. U urinima mrke boje pH vrednost je bila veća od 8, što je dovelo do toga da se u ovom radu ispita zavisnost aktivnosti NAG-a od pH. Aktivnost NAG-a određivana je sa 2-metoksi-4-(2-nitrovinil)-fenil-N-acetil- $\beta$ -D-glukozaminidom kao supstratom. U slabo-kiseljoj sredini (pH oko 6) ukupna aktivnost urinarnog NAG-a je stabilna.

## B27

### THE INFLUENCE OF pH ON THE N-ACETYL- $\beta$ -D-GLUCOSAMINIDASE ACTIVITY DETERMINATION

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V. Jovanović, Lj. Mandić

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It was shown that activity of urinary N-acetyl- $\beta$ -D-glucosaminidase (EC 3.2.1.30; NAG) may be useful parameter of renal damage in some pathological states as well as under toxicological substances action. In rabbits exposed to heavy metals urinary NAG activities were smaller than expected for renal injuries. The color of urines was yellow to dark. pH of dark urines was higher than 8. To clear these findings the influence of pH on NAG activity was investigated in this paper. For determination of NAG activity 2-methoxy-4-(2-nitrovinyl)-phenyl-N-acetyl- $\beta$ -D-glucosaminide as substrate was used. Under mild acidic condition (about pH 6), there was no significant loss of total urinary NAG activity. In contrast, under alkaline condition (pH  $> 8$ ) the total enzymatic activity of

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sok se u alkalnoj sredini ( $\text{pH} > 8$ ) ona izrazito smanjuje. Da bi se sagledao uzrok ovog smanjenja aktivnosti ispitani su izoenzimski profili NAG-a u slabotlačnoj baznoj sredini (pri različitim  $\text{pH}$  vrednostima). Za razdvajanje izoenzimskih oblika primenjena je jonsmenjivačka hromatografija na DEAE celulozi. Analiza profila je pokazala je da se aktivnost A oblika NAG-a u baznoj sredini značajno smanjuje (inaktivacija A oblika). Budući da je u normalnom urinu ( $\text{pH}$  5-7) udio aktivnosti A oblika u ukupnoj aktivnosti NAG-a malen ( $84.64 \pm 2.75\%$ ) od udela B oblika ( $15.36 \pm 2.19\%$ ), određivanje ukupne aktivnosti NAG-a u baznoj sredini daje pogrešne rezultate. Stoga u slučaju baznih urina remena oštećenja treba procenjivati na osnovu aktivnosti B oblika.

NAG was rapidly lost. To understand the cause of NAG activity decrease, the isoenzyme profiles in mild acidic and alkaline conditions (under different  $\text{pH}$  values) were investigated. For separation of isoenzyme forms the ion-exchange chromatography on DEAE cellulose was applied. The analysis of profiles showed that activity of NAG isoenzyme A under alkaline condition significantly decreases (it seems to be inactivated). As isoenzyme A is a major form in normal urine ( $\text{pH}$  5-7) which contribution in total activity ( $84.64 \pm 2.75\%$ ) is higher than contribution of isoenzyme B ( $15.36 \pm 2.19\%$ ), the results obtained for total NAG activity under alkaline condition may be wrong. Thus our results suggest that for alkaline urine, NAG isoenzyme B should be measured for detection renal damage.

## B28

#### AKTIVNOST SUPEROKSID DISMUTAZE U PLAZMI I ERITROCITIMA PACIJENATA OBOLELIH OD ŠIZOFRENIJE

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Poremećaj antioksidativne odbrane i povećanje lipidne peroksidacije nađeno je u pacijenata obolelih od šizofrenije. Mogućnost da oštećena antioksidativna odbrana i izraženo peroksidativno oštećenje mogu biti integralni deo patofiziološkog procesa šizofrenog obojenja intenzivno se razmatra. Cilj ove studije je bio da se odredi aktivnost superoksid dismutaze (SOD, EC 1.15.1.1) u plazmi i eritrocitima: 1-pacijenata sa prvom epizodom psihoze ( $n=20$ ) pre primene terapije i zatim posle 7, 14 i 21. dana tretmana; 2-hroničnih lečenih pacijenata ( $n=52$ ) u fazi egzacerbacije. Svi pacijenti su razvrstani u grupe prema Crow-u, kao Tip-I i Tip-II šizofrenije (SCH). Aktivnost superoksid dismutaze je merena spektrofotometrijski na osnovu autooksidacije epinefrina. Dobijeni rezultati pokazali su da je u grupi pacijenata sa prvom epizodom psihoze, pre lečenja, aktivnost SOD u eritrocitima i plazmi bila značajno snižena ( $p < 0.05$ ) u poređenju sa kontrolnom, zdravom grupom pacijenata sa Tip-I i Tip-II SCH. Posle prve tri nedelje tretmana, aktivnost SOD u plazmi i eritrocitima bila je u okviru kontrolne vrednosti u obe ispitivane grupe. Kod hroničnih pacijenata koji su dobijali neuroleptike u periodu dužem od jedne godine, zapaženo je statistički značajno pove-

## B28

#### SUPEROXIDE DISMUTASE ACTIVITY IN PLASMA AND ERYTHROCYTES OF PATIENTS SUFFERING FROM SCHIZOPHRENIA

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Impaired antioxidant defense and increased lipid peroxidation has been reported in schizophrenic patients. The possibility that an impaired antioxidant defense and increased peroxidative injury might be integral to the schizophrenic disease process is under intensive consideration. The aim of the present study was to assess activity of superoxide dismutase (SOD, EC 1.15.1.1) in plasma and erythrocytes of: 1-patients with first episode of psychosis ( $n=20$ ) before medication and then after 7, 14 and 21. days of treatment; 2-chronic medicated schizophrenic patients ( $n=52$ ) in the phase of exacerbation. All patients were selected in groups with Crow's Type-I or Type-II of schizophrenia (SCH). Superoxide dismutase activity was measured by spectrophotometric assay based on epinephrine autooxidation. Our results revealed that in the group of patients with first episode of psychosis, before medication, SOD activities in erythrocytes and plasma were significantly decreased ( $p < 0.05$ ) comparing to controls, only in patients with Type-I of SCH. After first 21 days of treatment the activities of SOD in plasma and erythrocytes were in the range of control values in both investigated groups. In chronic patients that have received neu-





# JUGOSLOVENSKA MEDICINSKA BIOHEMIJA

Časopis Društva medicinskih biohemičara Jugoslavije  
Official Journal of the Yugoslav Society of Medical Biochemists

Godište: 19

Beograd, jul – septembar 2000

Broj: 3

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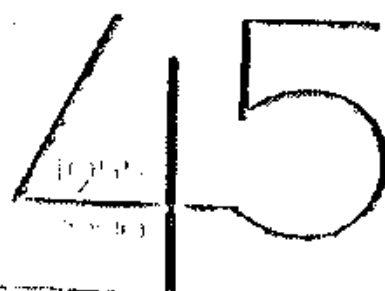
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YEARS OF YUGOSLAV  
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MEDICAL BIOCHEMISTRY

## YUGOSLAV MEDICAL BIOCHEMISTRY

Volume: 19

Belgrade, July – September 2000

No: 3

Jugosl. Med. Biochem. 19 (3): 145-362, 2000

C23

# IZOENZIMSKI PROFILI URINARNE I SERUMSKE N-ACETIL- $\beta$ -D-GLUKOZAMINIDAZE U DIJABETESU

Lj. Mandić<sup>1</sup>, V. Jagodić<sup>1</sup>,  
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Aktivnost urinarne N-acetil- $\beta$ -D-glukozaminidaze (NAG, EC 3. 2. 1. 30) je značajno povećana kod osoba obolelih od dijabetesa. Povećanje aktivnosti moglo bi da bude posledica oštećenja glomerula (prolaz serumskih oblika NAG-a) i proksimalnih tubula. Cilj da se pronikne koji je primarni uzrok povećane aktivnosti urinarne NAG-a u ovom radu razdvajani su izoenzimski oblici urinarne i serumskog NAG-a kod osoba obolelih od IDDM i NIDDM sa i bez komplikacija. Za razdvajanje izoenzimskih oblika NAG-a razvijena je brza, jednostavna i reproduktivna hromatografska metoda na DEAE celulozi. Ukupna aktivnost NAG-a i aktivnosti izoenzimskih oblika određene su sa 2-metoksi-4-(2'-nitrovini)-fenil-N-acetil- $\beta$ -D-glukozaminidom kao supstratom. Udeo aktivnosti urinarne i serumskog A oblika u ukupnoj aktivnosti NAG-a, kod dijabetičara sa i bez komplikacija, gotovo se ne menja u odnosu na kontrolu. Ovo ukazuje da se s povećanjem aktivnosti A oblika kod obolelih od dijabetesa proporcionalno povećava i ukupna aktivnost NAG-a ( $r=0.997$ ,  $p<0.001$ ). Aktivnosti B, M, I<sub>1</sub> i I<sub>2</sub> oblika u ukupnoj aktivnosti NAG-a menjaju se u zavisnosti od komplikacija. Udeo aktivnosti urinarne B oblika je statistički značajno povišen u odnosu na kontrolu, dok je udeo serumskog B oblika smanjen ( $p<0.001$ ). Promene u aktivnosti B oblika su povezane s promenama intermedijernih oblika u serumu i M oblika u urinu. Dobijeni izoenzimski profili serumskog i urinarne NAG-a odražavaju lizosomnu disfunkciju epitelijalnih ćelija i glomerula i proksimalnih tubula.

C23

# ISOENZYME PROFILES OF URINARY AND SERUM N-ACETYL- $\beta$ -D-GLUCOSAMINIDASE IN DIABETES

Lj. Mandić<sup>1</sup>, V. Jagodić<sup>1</sup>,  
V. Dimitrijević<sup>2</sup>, P. Đorđević<sup>2</sup>

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An increased activity of urinary N-acetyl- $\beta$ -D-glucosaminidase (NAG, EC 3. 2. 1. 30) was found in diabetic patients. The increase in activity could be the result of the glomerular damage (passage of serum isoenzymes of NAG) and proximal tubular damage. In order to better understand the cause of the increased activity in urinary NAG in diabetes, in this study the isoenzyme forms of NAG were separated in IDDM and NIDDM patients with and without complications. For the separation of isoenzymic forms of NAG a simple, fast and reproducible chromatographic method on DEAE cellulose was developed. For the determination of NAG activity a synthetic substrate 2-methoxy-4-(2'-nitrovinyl)-phenyl-N-acetyl- $\beta$ -D-glucosaminide was used. The contribution of the urinary and serum A form activity to total NAG activity of diabetics with and without complications were not significantly different from the corresponding values of control group. These results suggested that increase in the total activity of serum NAG in diabetics was due to the specific increase in isoenzyme A activity ( $r=0.997$ ,  $p<0.001$ ). The activity of B, M, I<sub>1</sub> and I<sub>2</sub> within the total activity of urinary NAG changed according to complications. The activity contribution of the urinary B form in diabetics was statistically significantly higher in comparison to healthy individuals, while the contribution of the serum B form was lower ( $p<0.001$ ). The changes in B form activity were correlated with the occurrence and abundance of the intermediary forms in serum and M form in urine. The obtained isoenzyme profiles of serum and urinary NAG reflected lysosomal dysfunction of both glomerular and proximal tubular epithelial cells.



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## IZOENZIMSKI PROFILI URINARNE N-ACETIL- $\beta$ -D-GLUKOZAMINIDAZE

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Veliki broj radova posvećen je značaju određivanja aktivnosti N-acetil- $\beta$ -D-glukozaminidaze (NAG, EC 3.2.1.30) u telesnim tečnostima i tkivima, u raznim obolenjima. Kod osoba obolelih od dijabetesa nađena je povišena aktivnost serumskog i urinarnog NAG-a. Ovo povećanje može biti posledica: aktivacije enzima uslovljene stanjem hiperglikemije, aktivacije enzima uslovljene povećanom akumulacijom glikomaterijala za degradaciju nastalog u promenjenom metabolizmu glukokonjugata, povećane biosinteze NAG-a, smanjene brzine degradacije enzima zbog promena na receptorima ili enzimu, smanjene stabilnosti lizozima. U cilju da se pronikne koji je primarni uzrok povećane aktivnosti urinarnog NAG-a u dijabetesu u ovom radu su razdvajani izoenzimski oblici NAG-a kod osoba obolelih od insulin-zavisnog i insulin-nezavisnog dijabetesa. Takođe je ispitivano u kojoj meri izoenzimski profili NAG-a zavise od vrste i stepena sekundarnih komplikacija kao i od stanja metaboličke kontrole obolelih.

Za radvajanje izoenzimskih oblika NAG-a razvijena je brza, jednostavna i reproduktivna hromatografska metoda na DEAE celulozi, pogodna za kliničku praksu. Aktivnost ukupnog urinarnog NAG-a i aktivnosti izoenzimskih oblika NAG-a određene su sa 2-metoksi-4-(2'-nitrovinil)-fenil-N-acetil- $\beta$ -D-glukozaminidom kao supstratom u kontrolnoj grupi (n=8) i kod dijabetičara (n=53) sa i bez sekundarnih komplikacija (retinopatija, nefropatija i neuropatija).

Pored A i B oblika u urinu dijabetičara izdvojen je i najkiseliji izoenzimski oblik NAG-a - M-oblik. Udeo aktivnosti A oblika, kod dijabetičara sa i bez komplikacija ( $85,1 \pm 6,5\%$ ), u ukupnoj aktivnosti NAG-a se gotovo ne menja u odnosu na zdrave osobe ( $84,2 \pm 3,2\%$ ). Ovo ukazuje da se sa povećanjem aktivnosti A oblika NAG-a kod obolelih od dijabetesa proporcionalno povećava i ukupna aktivnost, što je potvrđeno njihovim poređenjem. Za celu ispitivanu populaciju dobijena je veoma značajna pozitivna korelacija ( $r=0,997$ ,  $p<0,001$ ).

Procentualni udeli aktivnosti B i M oblika u ukupnoj aktivnosti NAG-a, za razliku od A oblika, su promenljivi. Udeo aktivnosti B oblika je statistički značajno povišen u odnosu na kontrolnu grupu. Promene u aktivnosti B oblika zavise od vrste i intenziteta sekundarnih komplikacija u dijabetesu. Najviše vrednosti za aktivnosti B oblika su dobijene kod osoba sa nefropatijom. Budući da je B oblik vezan za membranu, povećanje njegove aktivnosti ukazuje da je došlo do oštećenja epitelnih ćelija proksimalnih tubula. Aktivnost B oblika NAG-a nije zavisila od stanja metaboličke kontrole, odnosno nije nađena razlika u udelima B oblika kod osoba sa lošom i dobrom glukoregulacijom.

Kako promene u aktivnosti A izoenzimskog oblika NAG-a mogu biti povezane sa oštećenjima glomerula (prolaz serumskog oblika NAG-a), a B izoenzimskog oblika sa oštećenjem proksimalnih tubula može se zaključiti da izoenzimski profili NAG-a odražavaju lizozomalnu disfunkciju epitelnih ćelija i glomerula i proksimalnih tubula.

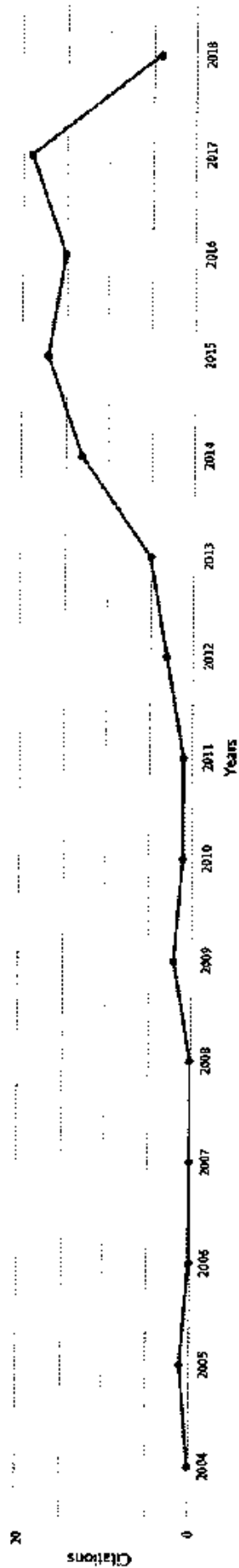
Saznanje da se aktivnost M izoenzimskog oblika urinarnog NAG-a u dijabetesu ( $2,4 \pm 0,5\%$ ) značajno smanjuje u odnosu na kontrolnu grupu ( $8,2 \pm 1,3\%$ ) je situaciju još više usložilo. Pretpostavljeno je da razlike, posebno u aktivnostima B (povećanje) i M oblika (smanjenje), mogu biti rezultat perturbacija u procesu post-translacionih modifikacija i da se možda radi o različitom glikozilovanju. Budući da u literaturi ima veoma malo podataka o M obliku NAG-a, deo rada posvećen je njegovom karakterisanju, posebno karakterisanju ugljenohidratnog dela.

Očito da je sinteza i ekskrecija intermedijarnih oblika NAG-a posebno indukovana stanjem glikemije, ali i mikroangiopatskim promenama. Zato izoenzimski profili NAG-a, a samim tim i odnosi aktivnosti izoenzimskih oblika mogu poslužiti kao osetljivi indikatori kontrole dijabetesa i razvoja i prognoze mikroangiopatija u dijabetesu.

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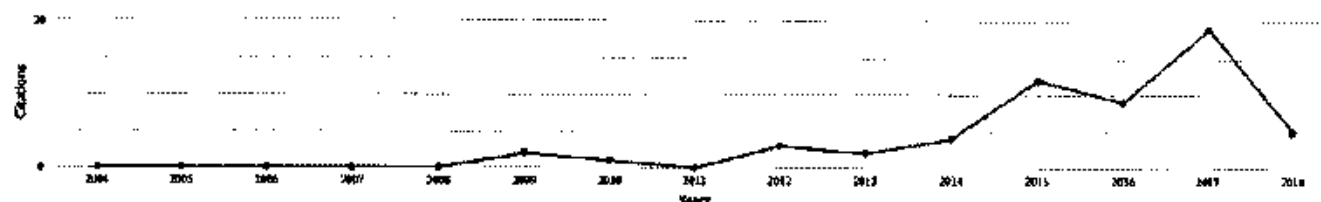
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## #7094 Review


### Submission To Be Reviewed

Title Gas chromatography – mass spectrometry system  
applied to determine botanical origin of various types of  
edible vegetable oils

Journal Section Analytical Chemistry

#### Abstract

This study presents a new strategy for discrimination of 59 samples of various cold-pressed, virgin and refined edible vegetable oils according to the corresponding botanical origin. Samples were produced from 17 plant species: olive, sunflower, safflower, flax, pumpkin, sesame, hemp, walnut, hazelnut, almond, grapeseed, black cumin, apricot seed, plum seed, soybean, wheat germ and rapeseed. A GC/MS device performing in a ion current (IC) mode, combined with multivariate clustering, was employed in the analysis. Derivatization reaction occurred in the injector of a gas chromatograph. The discriminations between species were based on marker-peaks of 9 molecular ions of dominant fatty acid methyl esters (FAMES), which were chosen as descriptors. These results demonstrate that IC-GC/MS approach with cluster analysis could be a useful tool in rapid semi-quantitative screening for botanical origin of commercial samples of various edible vegetable oils.

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#### New Article OLF

The application of alkane blot markers in the assessment of origin of oil pollutants in the recent river sediments (river V Bosna and Herzegovina)  
Ivan Samelak, Milica Balaban,



5. In addition, you can upload files for the editor and/or author to consult.

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## Reviewer Guidelines

### **Make sure the article you have been asked to review truly matches your expertise**

The Editor who has approached you may not know your work intimately, and may only be aware of your work in a broader context. Only accept an invitation if you are competent to review the article.

### **Avoid a potential conflict of interest**

A conflict of interest will not necessarily eliminate you from reviewing an article, but full disclosure to the editor will allow them to make an informed decision. For example; if you work in the same department or institute as one of the authors; if you have worked on a paper previously with an author; or you have a professional or financial connection to the article. These should all be listed when responding to the editor's invitation for review.

### **Check that you have enough time**

Reviewing an article can be quite time consuming. The time taken to review can vary greatly between disciplines and of course on article type, but on average, an article will take about 3 hours to review properly. Will you have sufficient time before the deadline stipulated in the invitation to conduct a thorough review?

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Deadlines for reviews vary per journal. The editors will provide information on deadline expectations with the review request. Let them know within a day or two that you got the request. They will appreciate being informed in a timely manner if you are able to complete the review or not. There are no consequences for refusing to review a paper.

If you feel the review will take you longer to complete than normal, please contact the editor to discuss the matter. The editor may ask you to recommend an alternate reviewer, or may be willing to wait a little longer (e.g., if the paper is highly specialized and reviewers are difficult to find). As a general guideline, if you know you will not be able to complete a review within the time frame requested, you should decline to review the paper.

**IMPACT FACTOR 0.797** (139 of 171 journals)  
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## ORGANIZACIJA

Organi Biohemijskog društva Srbije su:

- Skupština,
- Upravni odbor (koga čine predsednik, potpredsednik, sekretar, blagajnik i sedam članova),
- Nadzorni odbor

Funkciju zastupnika vrši Predsednik Društva, a u njegovom odsustvu potpredsednik i sekretar Društva.

Rad ostvarivanja ciljeva Biohemijskog društva Srbije, članovi Društva se mogu u dogovoru sa Upravnim odborom organizovati po dva osnova: (a) formirati regionalne ogranke (po regionalnom principu) i (b) formirati sekcije po naučnim oblastima.

Skupštinu Društva čine svi njegovi članovi. Skupština se redovno sastaje jednom godišnje. Vanredna sednica Skupštine može se zakazati na obrazloženi predlog Upravnog odbora, kao i na inicijativu najmanje jedne trećine članova Skupštine.

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## STRUKTURA I ORGANI BIOHEMIJSKOG DRUŠTVA SRBIJE USVOJENI NA IZBORNOJ SKUPŠTINI 31.3.2017. GODINE

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| <b>Generalni sekretar:</b>          | Milan Nikolić (Beograd)             |
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**GHENT UNIVERSITY**  
**GLOBAL CAMPUS**

**Ghent University Global Campus,**

**Incheon**

**South Korea**

**June, 28. 2018**

**Dr. Vesna Jovanović**  
**Department of Biochemistry**  
**Faculty of Chemistry**  
**University of Belgrade**  
**Serbia**

**Subject: Invitation letter for Guest Speaker**

**Dear dr. Jovanović,**

I would like to invite you on behalf of the Food Chemistry and Technology Research Center of Ghent University Global Campus to be a guest speaker and give a lecture and share experience with students and researchers of our Center in the field of food safety.

The lecture will be held on July 6, 2018 at GUGC, room 902, Incheon, South Korea.

Looking forward for your cooperation for the promotion of professional education.

If you have any question, please contact me.

Sincerely,

**Prof. dr. Ir. Sami GHNIMI**

**Ghent University Global Campus**

**Department of Environmental Technology, Food Technology and Molecular Biotechnology**

**119 Songdomunhwa-Ro, Yeonsu-Gu, Incheon, 21985, South Korea**

**E: [sami.ghnimi@ghent.ac.kr](mailto:sami.ghnimi@ghent.ac.kr)**

**T: +82 32 626 4212**

**F: +82 32 626 4109**



**Ghent University Global Campus, Incheon**  
**South Korea**

#### **Lectures Announcement**

We would like to kindly invite you to join at a Guest Lecture by **Dr. Vesna Jovanovic** from the Faculty of Chemistry University of Belgrade, Department of Biochemistry. Dr.Jovanovic share her experience with students and researchers of our Center in the field of food safety.

The lecture is titled **"Chemical contaminants and residues in the seashells"**.

The lecture will be held on July 6, 2018 at 15:00, in Gh902.

All are more than welcome to attend.

**Ghent University Global Campus**  
**Department of Environmental Technology, Food Technology and Molecular Biotechnology**

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Кандидат #03  
**Др Петер РАСПОР**

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**Prof. Dr. Peter Raspor, Dr.h.c.mult., Uni. dipl. eng**

[Peter.raspor@guest.arnes.si](mailto:Peter.raspor@guest.arnes.si)

Guest professor of Food Safety at The University of Natural Resources and Life Sciences, Vienna, 2006-

Guest professor of Modern Bio-Technology in Food Production at University Vienna, 2008-

Guest professor of Food Quality and Safety at Faculty of Biosystemic Sciences at University of Maribor, 2009-

Guest professor of Food Safety at Faculty of Health Sciences at University of Ljubljana, 2011-

Retired professor of Microbiology and Food safety from University of Primorska, 2014-2016

Retired professor of Food Biotechnology from Budapest Corvinus University, 1994-2006

Retired professor of Industrial Microbiology and Biotechnology from University of Ljubljana, 1986-2013

## **6 Lista predmeta nastave**

### **Pedagogical interests**

- New concepts of knowledge transfer in food, nutrition and health for graduate and postgraduate studies
- New approaches for practicum in field of food safety
- Concept and design of university courses on B.Sc and M.Sc level for food, nutrition and health according to Bologna principles
- Concept and design of university study program at doctoral level
- New concepts of transfer of food safety knowledge and skills in life long learning education

### **Current Pedagogical activities**

**University of Natural Resources and Life Sciences (BOKU) in Vienna, from 2006** MSc programme "Biomarkers in Food Characterisation", Euro league for Life science programme "Safety in the food chain", International study programme

**Teaching obligations at University of Vienna, Austria from 2008** MSc programme Modern Biotechnology in food processing

**Teaching obligations at University of Maribor from 2008**

Faculty of Agriculture and Life Sciences of the University of Maribor, MSc programme Food Safety and Quality

**Teaching obligations at University of Ljubljana, Faculty of Health Sciences**

MSc programme, Good practice in the food chain *from 2010*

**Teaching obligations at University of Primorska, Faculty of Health Sciences**

MSc programme dietetics, Microbiology *from 2014-2016*

**Teaching obligations at University of Ljubljana, Biotechnical faculty from 1987-2013**

He established around 30 courses and two study programs, graduate and postgraduate program in biotechnology and he was had or leading person on biotechnology and microbiology at university of Ljubljana till 2013

1993-1994 Conducting group for post diploma studies in Biotechnology at University Ljubljana

1998-2002 Conducting groups for post diploma study in Microbiology at University Ljubljana

1995-2003 Conducting group for diploma study in Biotechnology at Biotechnical Faculty, University Ljubljana

2004-2006 Conducting group for reformation diploma study in Biotechnology at Biotechnical Faculty, University Ljubljana to Bologna standards

1990 Concept and design of university courses on graduate study of microbiology and food technology: Biomass and secondary metabolites, Bioprocess, Biotechnology, Food Biotechnology, Industrial biotechnology, Microbial biotechnology, Microbial biotechnology of food, Introduction to biotechnology with bioethics, Water and waste management;

- 1994 Concept and design of university courses on post - graduate study of biological and biotechnical sciences and at Biomedicine, Biotechnology, Microbiology and in parasitology: Microbial biotechnology, Fermented food and feed Technology, Starter culture Technology, Food safety;
- 1994-2004 Concept and design of university courses for biotechnology and in 2004 transformation to Bologna principles - Bsc level for biotechnology Bioethics, Biotechnology of microorganisms,
- Biotechnology technique, Bioterrorism, Method for efficient study, Introduction to biotechnologies, Quality assurance, Food biotechnology;
- 1994-2004 Concept and design of university courses for biotechnology and in 2004 transformation to Bologna principles - Msc level for biotechnology Analytical biotechnology, Biomass and secondary metabolites, Bioproducts Design, Enzyme technologies, Industrial biotechnology, Up and downstream processes, System biotechnology, Innovations management, Intellectual law and invention protection

### **Teaching obligations at University**

#### **Graduate studies at University of Ljubljana**

##### **Bologna study – BSc 2006-2013, Biotechnical faculty Food Technology study:**

- Basic Biotechnology (75h)

##### **Microbiology study**

- Industrial Microbiology (75 h) Biotechnology study:
- Introduction to biotechnologies (55 h), - Biotechnology technique (110 h), - Biotechnology of microorganisms (150 h), - Quality assurance (40 h), - Food biotechnology (75 h)

##### **Bologna study – MSc 2006-2013, Biotechnical faculty Food Technology study:**

- Food safety (75 h) Microbiology study
- Processing (75 h), - Biomass and secondary metabolites (75 h), - Microbiology and biotechnology of food (75 h)

##### **Biotechnology study:**

- Industrial biotechnology (75 h), - System biotechnology (75 h), - Biomass and secondary metabolites (75 h), - Up and downstream processing in biotechnology (75 h)

#### **Postgraduate studies University of Ljubljana. Biotechnical faculty Biosciences**

- Microbial biotechnology (10 KP), White biotechnology (5 KP), Microbiology and Biotechnology of yeasts (5 KP)

### **Educations programs before Bologna reform from 1990- 2011**

#### **Graduate studies**

##### **Food Technology study:**

- Food Biotechnology (100 h) Microbiology study
- Biomass and Secondary Metabolites (70 h), - Bioprocessing (65 h), - Food Microbiology (70 h)

##### **Biotechnology study:**

- Industrial Biotechnology (90 h), - Quality assurance (45 h)

#### **Postgraduate studies**

##### **a) Post graduate study of Biological in Biotechnical Sciences Field Biotechnology**

- Biotechnology (90 h), - Microbial Biotechnology (60 h), - Technology of Fermented Foods (45 h)
- Technology of Production Starter Cultures (30 h), - Food Biotechnology (60 h) Food Safety (45 h) Field Food science

##### **b) Biomedicine – Postgraduate Education Programme in the Biomedical Sciences Microbiology**

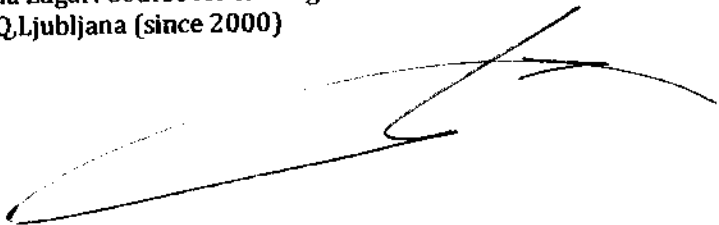


in Parasitology

### **The other university teaching activities**

- biology, MSc programme in Oenology, Universitat Rovira i Virgili Facultate d'Enologia de Tarragona, Spain
- Masters Degree in Wine Biotechnologies: University of the Basilicata (Italy), The University of Debrecen (Hungary) The University of Ljubljana (Slovenia) (since 2003)
- Job Creation Oriented Biotechnology International First Level Degree, BIOTECHNET - Biotechnology Thematic NET work-Biotech: Microbiology and virology course (since 2001)
- Doctoral studies in Microbiology: University of Pécs Department of General and Environmental Microbiology: Metals/ minerals and microbiology (since 1996)
- Doctoral studies in genetics: The international center for genetic engineering and biotechnology, ICGEB, Trieste: Design of Fermentation Processes, (since 1994)
- Doctoral studies in Food science: University of Budapest, Department of Microbiology and Biotechnology, Horticulture and Food Industry Sci. and Techn. Dept., Starter cultures in food processing (since 1993)

### **Lifelong teaching activities**

- excellence ensuring food product safety, consumer protection and competitiveness in Western Balkans, HACCP school education programme -Republic of Macedonia, Bosnia and Herzegovina and Serbia, March - June 2012
  - "Food Safety and Security -Rapid detection methods, policy making and emergency response, Serbia, 2009
  - Institute for food safety and consumer's health protection, ZAZA, Dobrovnik, Slovenia (initiator and member of executive committee (since 2006)
  - SO 22000 Training for increased competitiveness in South Serbia
  - 2nd ICFMH- Workshop on Food Safety in Africa, Stellenbosch University, South Africa, 2007
  - Raspor P; Traceability in food industry. SIQ, Ljubljana in last decadea (since 2001)
  - Raspor P. and Tatjana Žagar: Course for leading assessors in HACCP - Hazard Analysis Critical Control Points, SIQ, Ljubljana (since 2000)
- 

**Prof. Dr. Peter Raspor, Dr.h.c.mult., Uni. dipl. eng**

**Peter.raspor@guest.arnes.si**

Guest professor of Food Safety at The University of Natural Resources and Life Sciences, Vienna, 2006-

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Retired professor of Food Biotechnology from Budapest Corvinus University, 1994-2006

Retired professor of Industrial Microbiology and Biotechnology from University of Ljubljana, 1986-2013

## **7 Lista PhD, MSc i BSc studenata**

### **Supervisor for Doctoral Dissertations**

1486. TOMIČIĆ, Ružica. Uticaj faktora sredine na adheziju kvasaca na abiotičke površine : doktorska disertacija. Novi Sad: [R. Tomičić], 2018. X, 80 f., [8] f. pril., ilustr. [COBISS.SI-ID 4919928]

1487. TOMIČIĆ, Zorica. Uticaj probiotkog kvasaca *Saccharomyces boulardii* na adheziju *Candida glabrata* : doktorska disertacija. Novi Sad: [Z. Tomičić], 2018. X, 74 f., [6] f. pril., ilustr. [COBISS.SI-ID 4920184]

1488. OVCA, Andrej. Skladnost formalnega izobraževanja na področju varnosti živil s potrebami živilsko-prehransko-oskrbovalne verige : doktorska disertacija = Compliance of formal education with food safety needs within food supply chain : doctoral dissertation. Ljubljana: [A. Ovca], 2017. XI, 135, [27] f., ilustr. <https://repozitorij.uni-lj.si/IzpisGradiva.php?id=96659>. [COBISS.SI-ID 4855672]

1489. AMBROŽIČ, Mateja. Sistemsko vrednotenje in preprečevanje virusne okužbe s hrano v živilsko predelovalni oskrbovalno prehranski verigi : doktorska disertacija = Systemic evaluation and prevention of viral foodborne infection in food supply chain : doctoral dissertation. Ljubljana: [M. Ambrožič], 2016. X, 99 f., ilustr. [COBISS.SI-ID 4701816]

1490. KOVAČ, Katarina. Application of non-thermal procedures for inactivation of food- and water-borne viruses : doctoral dissertation = Uporaba netermičnih postopkov za inaktivacijo virusov v vodi in hrani : doktorska disertacija. Ljubljana: [K. Kovač], 2013. XIV, 162, [50] f., ilustr. [COBISS.SI-ID 4219512]

1491. PIVK KUPIROVIČ, Urška. Impact of in-mouth structure of lipids on texture perception in food : doctoral dissertation = Vpliv strukture lipidov v ustih na zaznavo teksture hrane : doktorska disertacija. Ljubljana: [U. Pivk Kupirovič], 2013. VIII, 96 str., ilustr. [COBISS.SI-ID 4199544]

1492. BIZAJ, Etjen. Interactions between contaminants and starter cultures during alcoholic fermentation : doctoral dissertation = Medsebojni vpliv kontaminantov in starterskih kultur med alkoholno fermentacijo : doktorska disertacija. Ljubljana: [E. Bizaj], 2013. 91 f., graf. prikazi, tabele. [COBISS.SI-ID 4237176]

1493. AVBELJ, Martina. Komunikacijske molekule pri izbranih vinskih kvasovkah : doktorska disertacija = Communication molecules among selected wine yeasts : doctoral dissertation. Ljubljana: [M. Avbelj], 2013. XVII, 194, [10] f., ilustr. [COBISS.SI-ID 4201080]

1494. MEDVED DJURAŠINOVIČ, Petra. Razvoj novih živilskih proizvodov in tradicionalna živila : doktorska disertacija = New food product development and traditional foods : doctoral dissertation. Ljubljana: [P. Medved Djurašinovič], 2013. XI, 119, [24] f., graf. prikazi, tabele. [COBISS.SI-ID 774775]

1495. SMREKAR, Franc. Razvoj tehnologije čiščenja plazmidov in bakteriofagov namenjenih humani uporabi : doktorska disertacija = Development of technology for plasmid DNA and bacteriophages applied in human treatments [!]: doctoral dissertation. Ljubljana: [F. Smrekar], 2011. XIII, 74 f., ilustr., tabele. [COBISS.SI-ID 3966072]

1496. ZUPAN, Jure. Opredelevitev invazivne rasti v populaciji kliničnih in nekliničnih sevov kvasovke *Saccharomyces cerevisiae* : doktorska disertacija (s področja biotehnologije) = The

determination of invasive growth in the population of clinical and non-clinical strains of *Saccharomyces cerevisiae* : doctoral dissertation. Ljubljana: [J. Zupan]: [Biotehniška fakulteta, Podiplomski študij bioloških in biotehniških znanosti], 2010. XV, 136 f., ilustr. [COBISS.SI-ID [3756152](#)]

1497. JEVŠNIK, Mojca. Integralno vrednotenje vključitve sistema HACCP pri zagotavljanju varnih živil : doktorska disertacija = Integral evaluation of HACCP system for food safety management : doctoral dissertation. Ljubljana: [M. Jevšnik], 2008. VII f., 176 str., ilustr., preglednice. [http://www.digitalna-knjiznica.bf.uni-lj.si/dd\\_jevsnik\\_mojca.pdf](http://www.digitalna-knjiznica.bf.uni-lj.si/dd_jevsnik_mojca.pdf). [COBISS.SI-ID [238862336](#)]

1498. KUŠČER, Enej. Regulacija biosinteze rapamicina pri bakteriji *Streptomyces hygroscopicus* : doktorska disertacija = Regulation of rapamycin biosynthesis in *Streptomyces hygroscopicus* : doctoral dissertation. Ljubljana: [E. Kuščer], 2008. VI, 61 f., ilustr. [COBISS.SI-ID [238334720](#)]

1499. PLAHTA, Primož. Tehnološko-ekonomska analiza uporabe genetsko spremenjenih organizmov pri predelavi vina : doktorska disertacija (s področja biotehnologije) = Technological and economic analysis of GMO application in winemaking : doctoral dissertation. Ljubljana: [P. Plahuta]: [Biotehniška fakulteta, Interdisciplinarni podiplomski študij biotehnologije], 2008. X, 114 f., ilustr. [COBISS.SI-ID [3463544](#)]

1500. MIKLİČ MILEK, Damjana. Vpliv biokontrolne aktivnosti kvasovk na rast nitaste glive *Botrytis cinerea* Pers. : doktorska disertacija = Affect of yeasts biocontrol activity on growth of filamentous fungi *Botrytis cinerea* Pers. : dissertation thesis. Ljubljana: [D. Miklič Milek], 2007. XIV, 133 f., [17] f. pril., ilustr., preglednice. [COBISS.SI-ID [3309176](#)]

1501. ČUŠ, Franc. Delovanje nekaterih fungicidov na kvasovke s površine grozdne jagode pri sorti Rebula (V. vinifera) : doktorska disertacija = Influence of some fungicides on yeasts isolated from grape berries of variety Rebula (v. vinifera) : doctoral dissertation. Ljubljana: [F. Čuš], 2005. XVIII, 168 f., ilustr., graf. prikazi, tabele. [COBISS.SI-ID [3102584](#)]

1502. ČADEŽ, Neža. Opredelevitev vrste kvasovk rodov *Hanseniaspora* in *Kloeckera* na osnovi polifazne taksonomije : doktorska disertacija = Species definition of yeast genera *Hanseniaspora* and *Kloeckera* on the basis of polyphasic taxonomy : doctoral dissertation, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Doktorske disertacije, 124). Ljubljana: [N. Čadež]: [BF, Interdisciplinarni podiplomski študij biotehnologije], 2005. XII, 120 f., ilustr., preglednice. [COBISS.SI-ID [3062648](#)]

1503. LENASSI ZUPAN, Ana. Izražanje fuzijskih proteinov označenih z zelenim fluorescirajočim proteinom v metilotrofni kvasovki *Pichia pastoris* : doktorska disertacija = Expression of GFP-fusion proteins in methylotrophic yeast *Pichia pastoris* : doctoral dissertation, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Doktorske disertacije, 110). Ljubljana: [A. Lennasi Zupan]: [BF, Interdisciplinarni podiplomski študij biotehnologije], 2004. XIX, 170 f., ilustr., preglednice. [COBISS.SI-ID [2983544](#)]

1504. BAVEC, Saša. Obseg patentne zaščite biotehnoloških izumov : doktorska disertacija = Scope of patent protection of biotechnological inventions : doctoral dissertation, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Doktorske disertacije, 111). Ljubljana: [S. Bavec]: [BF, Interdisciplinarni podiplomski študij biotehnologije], 2004. XIII, 124 str., ilustr., preglednice. [COBISS.SI-ID [2983800](#)]

1505. BURICA, Olga. Optimizacija bioprocesa čiščenja odpadne vode z imobilizirano biokulturo : doktorska disertacija = Bioprocess optimisation of wastewater treatment with immobilized culture : doctoral dissertation, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Doktorske disertacije, 105). Ljubljana: [O. Burica]: [BF, Interdisciplinarni podiplomski študij biotehnologije], 2004. XXIII, 140 f., [24] f. pril., ilustr., preglednice. [COBISS.SI-ID [129259520](#)]

1506. JENKO-BRINOVEC, Špelca. Vpliv Cr(III) na znotrajcelične procese v prokariotskih in eukariotskih celicah : doktorska disertacija = The influence of Cr(III) on intracellular processes in prokaryotic and eukaryotic cells : doctoral dissertation, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Doktorske disertacije, 109), (Krkina nagrada). Ljubljana: [Š. Jenko-Brinovec]:

[BF, Interdisciplinarni podiplomski študij biotehnologije], 2004. XVI, 165 f., ilustr., preglednice. [COBISS.SI-ID [2983288](#)]

1507. POVHE JEMEC, Katja. Mikrobna združba kvasovk v moštu malvazije in njen vpliv na potek alkoholne fermentacije : doktorska disertacija = Yeast population in Malvasia must and its influence on wine fermentation : doctoral dissertation, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Doktorske disertacije, 92). Ljubljana: [BF, Interdisciplinarni podiplomski študij biotehnologije]: [K. Povhe Jemec], 2003. XIII, 134 f., graf. prikazi, tabele. [COBISS.SI-ID [2828152](#)]

1508. ČARMAN, Irena. Učinkovitost združene starterske kulture v dvostopenjskem bioprocesu odstranitve dušikovih spojin iz odpadne vode farmacevtske industrije : doktorska disertacija = The mixed starter culture efficiency in two-stage bioprocess for elimination nitrogen compounds from pharmaceutical wastewater : doctoral dissertation, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Doktorske disertacije, 89). Ljubljana: [BF, Interdisciplinarni podiplomski študij biotehnologije]: [I. Čarman], 2003. XIX, 141 f., ilustr., graf. prikazi, pril. [COBISS.SI-ID [1259855](#)]

1509. SKRT, Mihaela. Izolacija in označitev nizkomolekulskih kromovih zvrsti iz kvasovke *Candida intermedia* : doktorska disertacija = Isolation and characterization of low molecular weight chromium binding species from yeast *Candida intermedia* : doctoral dissertation, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Doktorske disertacije, 80). Ljubljana: [BF, Interdisciplinarni podiplomski študij biotehnologije]: [M. Skrt], 2002. XV, 207 f., graf. prikazi, tabele. [COBISS.SI-ID [2655864](#)]

1510. JAMNIK, Polona. Odziv kvasovke *Candida intermedia* na Cr(VI) kot stresni dejavnik : doktorska disertacija = The response of yeast *Candida intermedia* to Cr(VI) as a stress factor : doctoral dissertation. Ljubljana: [P. Jamnik], 2002. XIV, 123 f., [11] f. pril., ilustr., preglednice. [COBISS.SI-ID [2670968](#)]

1511. ZAGORC, Tatjana. Konstrukcija rekombinantne kvasovke *Schizosaccharomyces pombe* za heterologno produkcijo proteinov : doktorska disertacija = Construction of recombinant yeast *Schizosaccharomyces pombe* for heterologous protein production : doctoral dissertation, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Doktorske disertacije, 76). Ljubljana: [BF, Interdisciplinarni podiplomski študij biotehnologije]: [T. Zagorc], 2001. XIII, 109 f., graf. prikazi, tabele. [COBISS.SI-ID [2537848](#)]

1512. RECEK, Marjeta. Vpliv faktorjev okolja na fiziologijo rasti in sprejemanje kromovih ionov pri kvasovki *Candida intermedia* : doktorska disertacija = The effect of environmental factors on *Candida intermedia* growth and chromium ions uptake : doctoral dissertation, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Doktorske disertacije, 78). Ljubljana: [BF, Interdisciplinarni podiplomski študij biotehnologije]: [M. Recek], 2001. XIV, 120 f., graf. prikazi, tabele. [COBISS.SI-ID [2598264](#)]

1513. PAŠ, Maja. Vključevanje Cr(III) in Cr(VI) zvrsti v celično zgradbo kvasovke : doktorska disertacija = Cr(III) and Cr(VI) uptake in the yeast cell structure : dissertation thesis, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Doktorske disertacije, 66). Ljubljana: [BF, Oddelek za živilstvo]: [M. Paš], 2000. XIII, 106 f., graf. prikazi, tabele. [COBISS.SI-ID [2442104](#)]

1514. TRČEK, Janja. Molecular identification of the acetic acid bacteria isolated from vinegar and plasmid vector construction for *Acetobacter* spp. : dissertation thesis = Molekularna identifikacija očetnokislinskih bakterij izoliranih iz kisa in razvoj plazmidnega vektorja za *Acetobacter* spp. : doktorska disertacija, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Doktorske disertacije, 61). Ljubljana: [Biotechnical Faculty]: [J. Trček], 1999. XII, 121 f., graf. prikazi, tabele. [COBISS.SI-ID [2291064](#)]

1515. TRKOV, Marija. Molekularsko biološka označitev in prepoznavanje bakterij rodu *Salmonella* v vzorcih živil : doktorska disertacija = Molecular biological characterization and detection of the genus *Salmonella* in food samples : dissertation thesis, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Doktorske disertacije, 64). Ljubljana: [BF, Oddelek za živilstvo]: [M. Trkov], 1999. XII, 139 f., graf. prikazi, tabele. [COBISS.SI-ID [2332792](#)]

1516. PETKOVIĆ, Hrvoje. An investigation of the molecular genetics of the gene cluster for oxytetracycline biosynthesis from *Streptomyces rimosus* : dissertation thesis = Študij molekularne genetike skupine genov za biosintezo oksitetraciklina iz *Streptomyces rimosus* : doktorska disertacija. Ljubljana: [H. Petković], 1998. XIX, 224 f., ilustr., tabele. [COBISS.SI-ID [2129272](#)]
1517. ŠTRANCAR, Aleš. Separation of biopolymers with different techniques of liquid chromatography : dissertation thesis = Ločevanje biopolimerov z različnimi tehnikami tekočinske kromatografije : doktorska disertacija. Ljubljana: [A. Štrancar], 1997. VIII, 99 f., ilustr., tabele. [COBISS.SI-ID [1912184](#)]
1518. KOVAČ, Boris. Združene biokulture gliv in mlečnokislinskih bakterij v procesu obogatitve škrobnoceluloznih substratov : doktorska disertacija = Cocultures of fungi and lactic acid bacteria in the process of the supplementation of lignocelulose substrates : dissertation thesis. Ljubljana: [B. Kovač], 1997. XV, 125 f., graf. prikazi, tabele. [COBISS.SI-ID [1910648](#)]
1519. SMOLE MOŽINA, Sonja. Molekularno-biološko določanje sorodnosti izbranih askomicetnih kvasovk : doktorska disertacija = Molecular biological determination of relationships among selected ascomycetous yeasts : dissertation thesis, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Doktorske disertacije, 36). Ljubljana: [S. Smole Možina], 1996. XVI, 139 f., graf. prikazi, tabele. [COBISS.SI-ID [61815](#)]
1520. KNEŽEVIĆ, Miomir. The establishment of the production process for recombinant proteins expressed in mammalian cells : dissertation thesis = Postavitev procesa za produkcijo rekombinantnih beljakovin eksprimiranih v celicah sesalcev : doktorska disertacija, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Doktorske disertacije, 28). Ljubljana: [M. Knežević], 1995. XIII, 96 f., graf. prikazi, tabele. [COBISS.SI-ID [51726336](#)]
1521. BATIČ, Martin. Yeast cultivation in media loaded with zinc and chromium : dissertation thesis = Kultivacija kvasovk pri povišanih koncentracijah cinka in kroma : doktorska disertacija, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Doktorske disertacije, 27). Ljubljana: [M. Batič], 1995. XIII, 140 f., graf. prikazi, tabele. [COBISS.SI-ID [51727104](#)]
1522. HOLOBAR, Andrej. Design and characterization of opto-chemical sensors and instrumentation for measurement of pH in bioreactors = dissertation thesis, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Doktorske disertacije, 24). Graz: [A. Holobar], 1994. 105 f., graf. prikazi, tabele. [COBISS.SI-ID [1794936](#)]

#### **Supervisor for Doctoral Dissertations (Bologna study programme)**

1523. TOME, Miha. Mechanism of azole resistance in *Candida glabrata* in the presence of immunosuppressant mycophenolic acid : doctoral dissertation = Mehanizem odpornosti proti azolom v kvasovki *Candida glabrata* v prisotnosti imunosupresiva mikofenolne kisline : doktorska disertacija. Ljubljana: [M. Tome], 2018. XXVIII, 163, [95] str., ilustr. [COBISS.SI-ID [926071](#)]
1524. KRANJČ, Luka. Heterologous expression of vinylphenol reductase of yeast *Brettanomyces bruxellensis* in yeast *Saccharomyces cerevisiae* : doctoral dissertation = Heterologna ekspresija encima vinilfenol reduktaze kvasovke *Brettanomyces bruxellensis* v kvasovki *Saccharmyces cerevisiae* : doktorska disertacija. Ljubljana: [L. Kranjc], 2016. XIV, 119, [16] f., ilustr. [COBISS.SI-ID [886135](#)]
1525. BEZEK, Katja. Preprečevanje filmotvornosti bakterij *Campylobacter jejuni* na abiotskih površinah ter adhezivnosti in invazivnosti na modelu celičnih linij : doktorska disertacija = Control of biofilm forming ability of *Campylobacter jejuni* on abiotic surfaces and adhesion and invasiveness in cell lines model : doctoral dissertation. Ljubljana: [K. Bezek], 2016. XVI, 96, [2] f., ilustr. [COBISS.SI-ID [884599](#)]
1526. KOSEL, Janez. Vpliv mešane fermentacije kvasovk *Saccharomyces cerevisiae* in *Dekkera bruxellensis* na biosintezo aromatskih snovi : doktorska disertacija = The influence of mixed fermentations of yeasts *Saccharomyces cerevisiae* and *Dekkera bruxellensis* on the biosynthesis

of aromatic compounds : doctoral dissertation. Ljubljana: [J. Kosel], 2014. XIII, 117, [15] f., ilustr. [COBISS.SI-ID 817783]

1527. BUTINAR, Bojan. Zasnova analitičnega postopka ugotavljanja pristnosti in stopnje predelave bučnega olja : doktorska disertacija = The establishment of an analytical procedure for assessment of the genuineness and the degree of processing of pumpkin seed oil : doctoral dissertation. Ljubljana: [B. Butinar], 2012. X, 77 f., ilustr. [http://www.digitalna-knjiznica.bf.uni-lj.si/dd\\_butinar\\_bojan.pdf](http://www.digitalna-knjiznica.bf.uni-lj.si/dd_butinar_bojan.pdf). [COBISS.SI-ID 4137080]

### **Supervisor for Master's Theses**

1528. TOMŠE, Romana. The presence of contaminants in food in Slovenia before and after joining the European Union : food safety in small and large systems : master's thesis. Nova Gorica: [R. Tomše], 2012. XII, 154, [25] str., ilustr. <http://www.ung.si/~library/magisterij/okolje/26Tomse.pdf>. [COBISS.SI-ID 2614523]

1529. KOČAR, Nataša. Vpliv zaporedne uporabe kvasovke *Saccharomyces pastorianus* na dinamiko izkoriščanja sladkorjev iz pивine : magistrsko delo = The impact of serial repitching of *Saccharomyces pastorianus* on sugar uptake dynamics from wort : M. Sc. thesis. Ljubljana: [BF, Podiplomski študij biotehnologije]: [N. Kočar], 2012. XVII, 96 f., [6] f. pril., ilustr. [COBISS.SI-ID 754807]

1530. ĐUKIĆ, Branka. Dejavniki, ki vplivajo na odnos potrošnikov do gensko spremenjenih živil v Sloveniji : magistrsko delo = Impacts on Slovenian consumers' attitude towards genetically modified food : master of science thesis. Ljubljana: [BF, Podiplomski študij bioloških in biotehniških znanosti, področje biotehnologije]: [B. Đukić], 2009. X, 117 str., ilustr., preglednice. [COBISS.SI-ID 3610488]

1531. RAMUŠ, Jana. Učinkovitost delovanja sistema HACCP v proizvodnji in distribuciji jajc : magistrsko delo = Effectiveness of HACCP system in eggs' production and distribution : master of science thesis. Ljubljana: [BF, Podiplomski študij bioloških in biotehniških znanosti, področje živilstva]: [J. Ramuš], 2009. XXIV, 207 str., ilustr., preglednice. [http://www.digitalna-knjiznica.bf.uni-lj.si/md\\_ramus\\_jana.pdf](http://www.digitalna-knjiznica.bf.uni-lj.si/md_ramus_jana.pdf). [COBISS.SI-ID 3590776]

1532. JUTERŠEK, Borut. Molekularna tipizacija in opis ESBL producirajočih sevov bakterije *Klebsiella pneumoniae* : magistrsko delo = Molecular typing and characterisation of ESBL producing strains of *Klebsiella pneumoniae* : master of science thesis, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Magistrska dela, 134). Ljubljana: [B. Juteršek]; [Biotehniška fakulteta, Podiplomski študij biomedicine], 2005. XII, 106 f., graf. prikazi, tabele. [COBISS.SI-ID 3072120]

1533. JERMAN, Sergej. Izolacija kakovostne DNA s CIM diski za odkrivanje gensko spremenjenih organizmov v živilih : magistrsko delo = Isolation of quality DNA with CIM disks for the detection of genetically modified organisms in food : master of science thesis, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Magistrska dela, 124). Ljubljana: [Biotehniška fakulteta, Podiplomski študij bioloških in biotehniških znanosti]: [S. Jerman], 2004. XIV, 92 str., graf. prikazi, tabele. [COBISS.SI-ID 2934904]

1534. RACMAN, Artur. Optimizacija bioprocasa klavulanove kisline : magistrsko delo = Clavulanic acid bioprocess optimization : master of science thesis. Ljubljana: [A. Racman], 2003. XIII, 102 f., ilustr., graf. prikazi, pril. [COBISS.SI-ID 1336911]

1535. RIJAVEC BREGAR, Andreja. Optimizacija medija za proizvodnjo fitaz pri izbranih nitastih glivah : magistrsko delo = Media optimization for phytase production from selected filamentous fungi : master of science thesis, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Magistrska dela, 86). Ljubljana: [BF, Oddelek za živilstvo]: [A. Rijavec Bregar], 1999. XIII, 96 f., graf. prikazi, tabele. [COBISS.SI-ID 2169208]

1536. REŽIĆ-DEREANI, Vesna. Vpliv kromovih spojin na dednino kvasovke *Saccharomyces cerevisiae* : magistrsko delo = Influence of chromium compounds on the genome of *Saccharomyces cerevisiae* yeast : master of science thesis, (Biotehniška fakulteta, Oddelek za

živilstvo, Ljubljana, Magistrska dela, 93). Ljubljana: [BF, Oddelek za živilstvo]: [V. Režić-Dereani], 1999. XII, 81 str., graf. prikazi, tabele. [COBISS.SI-ID 2340728]

1537. ZAGORC, Tatjana. Karakterizacija vinskih zimocidnih kvasovk in njihova uporaba v modelni fermentaciji : magistrsko delo = Characterisation of wine killer yeasts and their application in a model fermentation : master of science thesis, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Magistrska dela, 83). Ljubljana: [BF, Oddelek za živilstvo]: [T. Zagorc], 1998. XIV, 81 f., graf. prikazi, tabele. [COBISS.SI-ID 2133880]

1538. BERNIK, Borut. Optimizacija bioprocesa proizvodnje gentamicina v industrijskem merilu : magistrsko delo = Optimisation of the bioprocess for gentamicin production on industrial scale : master of science thesis, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Magistrska dela, 74). Ljubljana: [B. Bernik], 1997. IX, 83 f., graf. prikazi, tabele. [COBISS.SI-ID 1912440]

1539. KOŠMERL, Tatjana. Flokulacija med izolati vinskih kvasovk v Sloveniji : magistrsko delo = Flocculation among isolates of wine yeasts in Slovenia : master of science thesis, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Magistrska dela, 62). Ljubljana: [T. Košmerl], 1996. XVI, 144 f., graf. prikazi, tabele. [COBISS.SI-ID 39543]

1540. TRČEK, Janja. Izolacija in karakterizacija oetnokislinskih bakterij iz kisa : magistrsko delo = Isolation and characterization of acetic acid bacteria from vinegar : master of science thesis, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Magistrska dela, 69). Ljubljana: [J. Trček], 1996. XI, 80 f., graf. prikazi, tabele. [COBISS.SI-ID 255352]

1541. PLAHUTA, Primož. Uporaba prostih in imobiliziranih celuloz za čiščenje belih moštov : magistrsko delo = White grape musts clarification with free and immobilized cellulases : master of science thesis, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Magistrska dela, 63). Ljubljana: [P. Plahuta], 1996. XIII, 102 f., graf. prikazi, tabele. [COBISS.SI-ID 39799]

1542. KOVAČ, Boris. Obogatitev mlevskih odpadkov z biomaso plesni *Aspergillus oryzae* : magistrsko delo = Supplementation of milling industry wastes by *Aspergillus oryzae* biomass : master of science thesis, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Magistrska dela, 49). Ljubljana: [B. Kovač], 1993. XV, 105 f., graf. prikazi, tabele. [COBISS.SI-ID 235384]

1543. SMOLE MOŽINA, Sonja. Katalazna aktivnost bakterij *Staphylococcus simulans* in *Staphylococcus carnosus* : magistrsko delo = Catalase activity in bacteria *Staphylococcus simulans* and *Staphylococcus carnosus* : master of science thesis, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Magistrska dela, 32). Ljubljana: [S. Smole-Možina], 1990. II-XIX, 118 f., graf. prikazi, tabele. [COBISS.SI-ID 31795712]

#### **Supervisor for Master's Theses (Bologna study programme)**

1544. KONJAR, Matic. Odpornost na protiglivične učinkovine in invazivnost probiotičnih kvasovk *Saccharomyces boulardii* (nom. nud.) : magistrsko delo = Resistance to antifungal agents and invasiveness of probiotic yeast *Saccharomyces boulardii* (nom. nud.) : M. Sc. Thesis, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Magistrsko delo magistrskega študija - 2. stopnja Prehrana, 16). Ljubljana: [M. Konjar], 2015. XII, 89 f., ilustr. [http://www.digitalna-knjiznica.bf.uni-lj.si/zivilstvo/du2\\_konjar\\_matic.pdf](http://www.digitalna-knjiznica.bf.uni-lj.si/zivilstvo/du2_konjar_matic.pdf). [COBISS.SI-ID 4559224]

1545. BEZEK, Katja. Vloga signalnih molekul pri alkoholni fermentaciji s čisto in združeno starter kulturo : magistrsko delo = The role of signaling molecules in alcoholic fermentation with pure and mixed starter culture : M. Sc. Thesis, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Magistrsko delo magistrskega študija - 2. stopnja Mikrobiologija, 1). Ljubljana: [K. Bezek], 2012. XIV, 85 f., [19] f. pril., ilustr., tabele. [http://www.digitalna-knjiznica.bf.uni-lj.si/du2\\_bezek\\_katja.pdf](http://www.digitalna-knjiznica.bf.uni-lj.si/du2_bezek_katja.pdf). [COBISS.SI-ID 4146040]

1546. GAJŠEK, Sabina. Analiza baze podatkov Veterinarske fakultete o varnosti živil živalskega izvora v obdobju od 2004 do 2009 : magistrsko delo = Analysis of the Veterinary faculty database about the safety of food products of animal origin in the period between 2004 and 2009 : master's thesis. Maribor: [S. Gajšek], 2011. VI, 49 f., [8] f. pril., graf. prikazi. <http://dkum.uni-mb.si/Dokument.php?id=23812>. [COBISS.SI-ID 3190572]

### **Supervisor for Undergraduate Theses**

1547. LUKAN JEZERČIĆ, Tina. Klasične in sodobne metode določanja števila kvasovk vrst *Saccharomyces cerevisiae* in *Dekkera bruxellensis* v realnih vzorcih : diplomsko delo, univerzitetni študij = Traditional and recent techniques for enumeration of yeasts species *Saccharomyces cerevisiae* and *Dekkera bruxellensis* in real samples : graduation thesis, university studies, (Biotehniška fakulteta, Enota medoddelčnega študija mikrobiologije, Ljubljana, Diplomske naloge, 507). Ljubljana: [T. Lukan], 2012. XII, 54 f., [3] f. pril., graf. prikazi, tabele. [http://www.digitalna-knjiznica.bf.uni-lj.si/dn\\_lukan\\_tina.pdf](http://www.digitalna-knjiznica.bf.uni-lj.si/dn_lukan_tina.pdf). [COBISS.SI-ID 4060280]
1548. CIRINGER, Mateja. Ugotavljanje koncentracije in velikosti virusnih delcev z metodo sledenja nanodelcem (NTA) : diplomsko delo, univerzitetni študij = Determination of virus particle size distribution and concentration with nanoparticle tracking analysis (NTA) method : graduation thesis, university studies, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Diplomske naloge, 1495). Ljubljana: [M. Ciringer], 2012. IX, 52 f., [1] f. pril., graf. prikazi, tabele. [http://www.digitalna-knjiznica.bf.uni-lj.si/dn\\_ciringer\\_mateja.pdf](http://www.digitalna-knjiznica.bf.uni-lj.si/dn_ciringer_mateja.pdf). [COBISS.SI-ID 4192376]
1549. TOMC, Urška. Izolacija mikroorganizmov iz obrata proizvodnje vodnih barv in analiza učinkovitosti biocidov : diplomsko delo = Isolation of microorganisms from water-based paint manufacturing plant and analysis of efficiency of biocides : graduation thesis, (Biotehniška fakulteta, Enota medoddelčnega študija mikrobiologije, Ljubljana, Diplomske naloge, 479). Ljubljana: [U. Tomc], 2011. XII, 76 f., [8] f. pril., ilustr. [http://www.digitalna-knjiznica.bf.uni-lj.si/dn\\_tomc\\_urska.pdf](http://www.digitalna-knjiznica.bf.uni-lj.si/dn_tomc_urska.pdf). [COBISS.SI-ID 3926392]
1550. KRANJC, Luka. Vpliv žganih pijač na preživetje bakterij seva *Salmonella enteritidis* v modelu želodca : diplomsko delo, univerzitetni študij = Effect of spirits on survival of *Salmonella enteritidis* strain in model stomach : graduation thesis, university studies, (Biotehniška fakulteta, Oddelek za živilstvo, Ljubljana, Diplomske naloge, 1434). Ljubljana: [L. Kranjc], 2011. XI, 49 f., [5] f. pril., graf. prikazi, tabele. [http://www.digitalna-knjiznica.bf.uni-lj.si/dn\\_kranjc\\_luka.pdf](http://www.digitalna-knjiznica.bf.uni-lj.si/dn_kranjc_luka.pdf). [COBISS.SI-ID 3944312]
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#### **Supervisor for Undergraduate Theses (1st cycle Bologna study programme)**

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#### **Supervisor - Other**

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A handwritten signature in black ink, consisting of a long horizontal stroke with a small loop at the end and a few vertical strokes above it.

## Научно-истраживачки рад (1986 – 2018)

|   |              |
|---|--------------|
| <b>УКУПНО ОСТВАРЕНА ВРЕДНОСТ РЕЗУЛТАТА</b>                                    | <b>885.1</b> |
| <b>Вредност у претходних 5 година (01.01.2014 -данас)</b>                     | <b>192</b>   |
| <b>Коефицијент научне компетентности до научног сарадника (до 31.12.2013)</b> | <b>693.1</b> |

| Категорија публикације   | 1986-2014            | ≥ 2014             | УКУПНО     |
|--|----------------------|--------------------|------------|
| <b>Радови објављени у научним часописима међународног значаја (M20)</b>  |                      |                    |            |
| Рад у међународном часопису изузетних вредности (M21a)                   | 14 x 10 = 140        | 5 x 10 = 50        | 19         |
| Рад у врхунском међународном часопису (M21)                              | 17 x 8 = 136         | 5 x 8 = 40         | 22         |
| Рад у истакнутом међународном часопису (M22)                             | 35 x 5 = 175         | 9 x 5 = 45         | 44         |
| Рад у међународном часопису (M23)  | 37 x 3 = 111         | 14 x 3 = 42        | 51         |
| <b>Зборници међународних научних скупова (M30)</b>                       |                      |                    |            |
| Саопштење са међународног скупа штампано у целини (M33)                  | 7 x 1 = 7            |                    | 7          |
| Саопштење са међународног скупа штампано у изводу (M34)                  | 8 x 0.5 = 4          | 3 x 0.5 = 1.5      | 11         |
| <b>Радови и часописима националног значаја (M50)</b>                     |                      |                    |            |
| Рад у научном часопису (M53)   | 69 x 1 = 69          | 12 x 1 = 12        | 81         |
| <b>Предавања по позиву на скуповима националног значаја (M60)</b>        |                      |                    |            |
| Предавање по позиву са скупа националног значаја штампано у целини (M61) | 33 x 1.5 = 49.5      |                    | 33         |
| Саопштење са скупа националног значаја штампано у целини (M63)           | 2 x 0.5 = 1          | 3 x 0.5 = 1.5      | 5          |
| Саопштење са скупа националног значаја штампано у изводу (M64)           | 3 x 0.2 = 0.6        |                    | 3          |
| <b>УКУПНО</b>  | <b>Укупно: 693.1</b> | <b>Укупно: 192</b> | <b>276</b> |
| <b>Одбрањена докторска дисертација (M70)</b>                             | <b>1 x 6 = 6</b>     |                    |            |

## Научно-истраживачки рад (од почетка каријере закључно са 31.12.2013. годином)

### Рад у међународном часопису изузетних вредности (M21a)

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9. JEVSNIK, Mojca, HLEBEC, Valentina, RASPOR, Peter. Survey of safe and hygienic practices among Slovenian sauerkraut growers. *Food control*, Jul. 2009, vol. 20, no. 7, str. 677-685 (M21a-2009: IF-2.463)
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15. ZAKRAJSEK, Teja, RASPOR, Peter, JAMNIK, Polona. *Saccharomyces cerevisiae* in the stationary phase as a model organism - characterization at cellular and proteome level. *Journal of proteomics*, 2011, vol. 74, str. 2837-2845 (M21-2011: IF-4.878)
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## **Научно-истраживачки рад у претходном петогодишњем периоду (01.01.2014-2018)**

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237. KURINCIC, Marija, JERSEK, Barbara, KLANCNIK, Anja, SMOLE MOZINA, Sonja, FINK, Rok, DRAZIC, Goran, RASPOR, Peter, BOHINC, Klemen. Effects of natural antimicrobials on bacterial cell hydrophobicity, adhesion, and zeta potential = Vpliv naravnih protimikrobnih snovi na bakterijsko hidrofobnost, adhezijo in zeta potencial. *Arhiv za higijenu rada i toksikologiju*, 2016, vol. 67, str. 39-45 (M22-2016: IF-1.395)

238. BOHINC, Klemen, DRAZIC, Goran, ABRAM, Anze, JEVSNIK, Mojca, JERSEK, Barbara, NIPIC, Damijan, KURINCIC, Marija, RASPOR, Peter. Metal surface characteristics dictate bacterial adhesion capacity. *International journal of adhesion and adhesives*, 2016, vol. 68, str. 39-46 (M22-2016: IF-2.211)
239. TOMICIC, Zorica, ZUPAN, Jure, MATOS, Tadeja, RASPOR, Peter. Probiotic yeast *Saccharomyces boulardii* (nom. nud.) modulates adhesive properties of *Candida glabrata*. *Medical mycology*, 2016, vol. 54, no. 8, str. 835-845 (M22-2016: IF-2.377)
240. SIMS, Jason, BRUSCHI, Carlo V., BERTIN, Chloe, WEST, Nicole, BREITENBACH, Michael, SCHROEDER, Sabrina, EISENBERG, Tobias, RINNERHALER, Mark, RASPOR, Peter, TOSATO, Valentina. High reactive oxygen species levels are detected at the end of the chronological life span of translocant yeast cells. *Molecular genetics and genomics*: 2016, vol. 291, iss.1, str. 423-435 (M22-2019: IF-2.979)
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242. ZUPAN, Jure, TOMICH, Zorica, RASPOR, Peter. Determination of MICING: a new assay for assessing minimal inhibitory concentration for invasive growth. *European journal of clinical microbiology and infectious diseases*, 2015, vol. 34, issue 5, str. 1023-1030 (M22-2015: IF-2.857)
243. FINK, Rok, ODER, Martina, RANGUS, Dusan, RASPOR, Peter, BOHINC, Klemen. Microbial adhesion capacity: influence of shear and temperature stress. *International journal of environmental health research*, 2015, vol. 25, no. 6, str. 656-669 (M22-2015: IF-1.582)
244. CADEZ, Neza, PAGNOCCA, Fernando C., RASPOR, Peter, ROSA, Carlos. *Hanseniaspora nectarophila* sp. nov., a new yeast species isolated from ephemeral flowers. *International journal of systematic and evolutionary microbiology*, 2014, vol. 64, str. 2364-2369 (M22-2014: IF-2.511)

### **Рад у међународном часопису (M23)**

245. STERNISA, Meta, SMOLE MOZINA, Sonja, LEVSTEK, Sonja, KUKEC, Andreja, RASPOR, Peter, JEVSNIK, Mojca. Food safety knowledge, self-reported practices and attitude of poultry meat handling among Slovenian consumers. *British food journal*, 2018, str. [1-14, in press], (M23-2017: IF-1.289)
246. OVCA, Andrej, JEVSNIK, Mojca, RASPOR, Peter. Food safety practices of future food handlers and their teachers, observed during practical lessons. *British food journal*, 2018, vol. 120, no. 3, str. 531-548. (M23-2017: IF-1.289)
247. OVCA, Andrej, JEVSNIK, Mojca, RASPOR, Peter. Future professional food handlers' perspectives towards food safety. *British food journal*, 2018, vol.119, iss. 2, str. 411-424 (M23-2017: IF-1.289)
248. PAS, Maja, VOGRINC, Janez, RASPOR, Peter, UDOVC-KNEZEVIC, Nada, CEHOVIN ZAJC, Jozica. Biotechnology learning in Slovenian upper-secondary education: gaining knowledge and forming attitudes. *Research in science & technological education*, 2018, vol., no., str. 1-16, (M23-2017: IF-0.513)
249. TOMICIC, Ruzica, TOMI CIC, Zorica, RASPOR, Peter. Adhesion of *Candida* spp. and *Pichia* spp. to wooden surfaces. *Food technology and biotechnology*, 2017, vol. 55, no. 1, str. 138-142 (M23-2017: IF-1.168)
250. SURANSKA, Hana, RASPOR, Peter, UROIC, Ksenija, GOLIC, Natasa, KOS, Blazenka, MIHAJLOVIC, Sanja, BEGOVIC, Jelena, SUSKOVIC, Jagoda, TOPISIROVIC, Ljubisa, CADEZ, Neza. Characterisation of the yeast and mould biota in traditional white pickled cheeses by culture-dependent and independent molecular techniques. *Folia microbiologica*, 2016, vol. 61, str. 455-463 (M23-2016: IF-1.521)
251. KRANJC, Luka, CADEZ, Neza, SERGAN, Matej, GJURACIC, Kresimir, RASPOR, Peter. Physiological profiles relevant for novel alcoholic beverage design among *Dekkera bruxellensis* strains from different provenances. *Journal of the Institute of Brewing*, 2016, vol. 122, iss. 3, str. 536-542 (M23-2016: IF-0.859)
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Viral contamination in mussel production chain on the Slovenian coastline. Slovenian veterinary research, 2016, vol. 53, no. 4, str. 195-204 (M23-2016: IF-0.250)

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255. BIZAJ, Etjen, CURTIN, Chris D., CADEZ, Neza, RASPOR, Peter. Interactions between industrial yeasts and chemical contaminants in grape juice affect wine composition profile. Food technology and biotechnology, 2014, vol. 52, no. 2, str. 222-231. (M23-2014: IF-0.920)
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### **Саопштење са међународног скупа штампано у изводу (M34)**

259. RASPOR, Peter, TOME, Miha, TOMICH, Ruzica, TOMICH, Zorica, ZUPAN, Jure. Candida gfabrata: novel view on treating yeast infections. V: *Book of abstracts= Knjiga rezimea*, The 6th International Scientific Meeting Mycology, Mycotoxicology, and Mycoses, 27-29 September, 2017, Novi Sad, Serbia= Sesti međunarodni naucni skup mikologija, mikotoksikologija i mikoze, 27-29. septembar 2017., Novi Sad, Srbija. Novi Sad: Matica srpska. 2017, str. 31
260. RASPOR, Peter. Bread and bread like foods in the changing world. V: DURAGIC, Olivera (ur.). *Celebrating food: abstract book*, FoodTech Congress, 25-27. 10. 2016, Novi Sad, Serbia[:] XVII International Symposium Feed Technology [and] III International Congress Food Technology, Quality and Safety. Novi Sad: Institute of Food Technology. 2016, str. 11-12.
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### **Рад у научном часопису (M53)**

262. RASPOR, Peter, JEVSNIK, Mojca, RASPOR LAINSCEK, Petra. Food risk communication in the case of microbial contamination. Acta microbiologica Bulgarica, 2018, vol. 34, no. 1, str. 1-9.
263. OVCA, Andrej, JEVSNIK, Mojca, RASPOR, Peter. Curriculum analysis of food safety competences at elementary and upper-secondary level of formal education inside food-related programs in Slovenia. Journal of food science education, 2018, vol. 17, iss. 2, str. 42-51.
264. RASPOR, Peter. Priprava fermentirane hrane iz zit: fermentirana kruhom podobna zivija iz zit v azijsko-pacifiskem obmocju. Mlinarstvo in pekarstvo: slovenska strokovna revija za mlinarstvo in pekarstvo, 2017, letn. 18, st.104, str. 6-9, ilustr., 2017, letn. 18, st. 105, str.11-14.
265. VUCKOVIC, Darinka, SIKIC POGACAR, Maja, RASPOR, Peter, ABRAM, Maja, SMOLE MOZINA, Sonja, KLANCNIK, Anja. Virulence comparison of human and poultry Campylobacter jejuni isolates in a mouse model. Medical research archives, 2017, vol. 5, iss. 10, str. 1-11,



266. JEVSNIK, Mojca, OVCA, Andrej, RASPOR, Peter. A comparison of three different cleaning methods for reducing contaminants on contact surfaces - a preliminary study. *Sanitarno inženirstvo*, 2017, vol. 11, no. 1, str. 55-66
267. SORONJA SIMOVIC, Dragana, SMOLE MOZINA, Sonja, RASPOR, Peter, MARAVIC, Nikola R., ZAHOREC, Jana J., LUSKAR, Lucija, SERES, Zita. Carob flour and sugar beet fiber as functional additives in bread. *Acta periodica technologica*, 2016, vol. 47, str. 83-93,
268. LUSKAR, Lucija, SMOLE MOZINA, Sonja, AVBELJ, Martina, RASPOR, Peter. Dodatek ali surovina, tradicija ali inovacija?: rozic v pekarstvu. *Mlinarstvo in pekarstvo: slovenska strokovna revija za mlinarstvo in pekarstvo*, 2016, letn. 17, st. 102, str. 6-10
269. RASPOR, Peter. Priprava fermentirane hrane iz zit : fermentirana kruhom podobna zivila iz zit v azijsko-pacifiskem obmocju. *Mlinarstvo in pekarstvo : slovenska strokovna revija za mlinarstvo in pekarstvo*, 2016, letn. 17, st. 102, str. 22-24, ilustr., 2016, letn. 17, st. 103, str. 6-9.
270. TOMSE, Romana, CANCER, Vesna, RASPOR, Peter. Flows of raw materials and food safety of products of Slovenian manufacturers after EU entry. *Sanitarno inženirstvo*, 2012, vol 6, no. 1, str. 37-57.
271. AMBROZIC, Mateja, KUKEC, Andreja, JEVSNIK, Mojca, SMOLE MOZINA, Sonja, RASPOR, Peter. Food safety expertise among professional food handlers and consumers related to food borne viruses: case Slovenia. *Sanitarno inženirstvo*, 2016, vol. 10, no. 1, str. 4-19
272. ZIVKOVIC, Milica, CADEZ, Neza, UROIC, Ksenija, MILJKOVIC, Marija, TOLINACKI, Maja, DOUSOVA, Petra, KOS, Blazenka, SUSKOVIC, Jagoda, RASPOR, Peter, TOPISIROVIC, Ljubisa, GOLIC, Natasa. Evaluation of probiotic potential of yeasts isolated from traditional cheeses manufactured in Serbia and Croatia. *Journal of intercultural ethnopharmacology*, 2015, vol. 4, iss. 1, str. 12-18
273. BASNEC, Kristina, BEZEK, Katja, HAJZERI, Metka, RASPOR, Peter, KLANJSEK GUNDE, Marta. Chromogenic indicators for temperature control in the food cold chain. *Journal of hygienic engineering and design*, 2014, vol. 8, str. 53-60

**Саопштење са скупа националног значаја штампано у изводу (М64)**

274. RASPOR, Peter. Risk communication in cases of contamination: an industrial perspective. V: OBRADOVIC, Dragojlo (ur.), RANIN, Lazar (ur.). *Simpozijum Dani mikrobiologa Srbije 2016, Beograd, 12-13. maj 2016*. Beograd: Udruzenje mikrobiologa Srbije. 2016, str. 63
275. RASPOR, Peter. Klimatske spremembe, mobilnost in globalizacija: vpliv na varnost zivil. V: KUSAR, Darja (ur.), OCEPEK, Matjaz (ur.). *Knjiga povzetkov: kongres SMD 2014, 6. kongres Slovenskega mikrobioloskega drustva, 24.-26. september 2014, Bled, Slovenija*. Ljubljana: Veterinarska fakulteta. 2014, str. 28
276. BARLIC-MAGANJA, Darja, AMBROZIC, Mateja, RASPOR, Peter. Virusna in mikrobioloska kontaminacija skoljk: prenos izkusenj iz evropskega projekta FP7 vita v slovenski prostor. V: KUSAR, Darja (ur.), OCEPEK, Matjaz (ur.). *Knjiga povzetkov: kongres SMD 2014, 6. kongres Slovenskega mikrobioloskega drustva, 24.-26. september 2014, Bled, Slovenija*. Ljubljana: Veterinarska fakulteta. 2014, str. 35

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Guest professor of Food Quality and Safety at Faculty of Biosystemic Sciences at University of Maribor, 2009-

Guest professor of Food Safety at Faculty of Health Sciences at University of Ljubljana, 2011-

Retired professor of Microbiology and Food safety from University of Primorska, 2014-2016

Retired professor of Food Biotechnology from Budapest Corvinus University, 1994-2006

Retired professor of Industrial Microbiology and Biotechnology from University of Ljubljana, 1986-2013

## **4 spisak konferencija organizacija ili ucestvovanje**

### **Organizational activities**

**-Organizer (member, chair, president) of scientific meetings, congresses (for period 1992-2017) approx. 70**

**The most important in last decade**

- First CEFood Congress 2002, Ljubljana, Slovenia - approximately 600 participants
- First EFFoST Congress: First European Food Congress, 4. November 2008 - 9. November 2008, Ljubljana, Slovenia- approximately 1000 participants
- 1st FEMS Congress of European Microbiologists, Ljubljana, Slovenia, June 29 - July 3, 2003- approximately 1800 participants
- Regular annual meetings Microbiology and biotechnology for future 2000-2013

### **Selection for 2017**

- *Food microbiology education : new tools and technologies : at the 29<sup>o</sup> Congresso Brasileiro de Microbiologia, de 22 a 25 de Outubro de 2017, Foz de Iguaçu, Paraná, Brasil*
- 10th Balkan Congress of Microbiology [also] Microbiologia Balkanica '2017, Sofia, November 16th-18th, 2017.
- The 6th International Scientific Meeting Mycology, Mycotoxicology, and Mycoses, 27-29 September, 2017, Novi Sad, Serbia = Šesti međunarodni naučni skup mikologija, mikotoksikologija i mikoze, 27-29. septembar 2017., Novi Sad, Srbija. Novi Sad: Matica srpska. 2017.
- *The challenges for quality and safety along the food chain : abstract book, FOOD-3 International Conference, 23 - 25 March 2017, New Bulgarian University, Sofia, Bulgaria. Sofia: New Bulgarian University. 2017.* <http://ebox.nbu.bg/3foodconference/index.html>.
- *23. slovenski festival znanosti z mednarodno udeležbo, Ljubljana, 25. - 27. 9. 2017. [Ljubljana]: Slovenska znanstvena fundacija (SZF), 2017*

### **Selection for 2016**

- XVII International Symposium Feed Technology [and] III International Congress Food Technology, Quality and Safety. Novi Sad: Institute of Food Technology. 2016,
- *Simpozijum Dani mikrobiologa Srbije 2016, Beograd, 12-13. maj 2016. Beograd: Udruženje mikrobiologa Srbije. 2016,*
- *Inovacije u proizvodnji i preradi šilve : trening materijal za radionicu, Trening radionica, program Trafoon, Čačak, 26. februar 2016. [Čačak: Institut za voćarstvo. 2016],*
- *From tradition to innovation - in buckwheat, oats and gluten-free-strategies for SMEs in production, processing and marketing, Warszawa, 29. 06. 2016. Olsztyn: Instytut Rozrodu Zwierząt i Badań Żywności Polskiej Akademii Nauk: = Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences. 2016,*
- 25th International ICFMH Conference [also] Food Micro 2016, 19-22 July, 2016, Dublin, Ireland. Dublin: University College Dublin. 2016, [http://www.foodmicro2016.com/wp-content/uploads/2016/07/FoodMicro\\_2016\\_BookofAbstracts.pdf](http://www.foodmicro2016.com/wp-content/uploads/2016/07/FoodMicro_2016_BookofAbstracts.pdf).
- *ICY 2016, 14th International Congress on Yeasts, September 11-15, 2016, Awaji Yumebutai, Hyogo, Japan. Nara: Nara Institute of Science and Technology (NAIST). 2016,*

- **Symposium Power of Microbes in Industry and Environment** September 28 - October 1, 2016  
Krak, Croatia. Zagreb: Croatian Microbiological Society.

#### **Selection for 2015**

- *Mediterranska hrana in prehrana : svetovni dan hrane 2015 : zbornik povzetkov = Mediterranean food and nutrition : world food day 2015 : book of abstracts*, 2. letna konferenca Hrana in prehrana za zdravje, Portorož, 16 in 17. oktober 2015 = 2nd Annual Conference Food and Nutrition for Health, Portorož, 16 & 17 October 2015. Izola: Fakulteta za vede o zdravju, Inštitut za živila, prehrano in zdravje. 2015
- *Inovativne fermentacije za razvoj novih funkcionalnih živil = Innovative fermentation technologies for new functional food development*. V: RASPOR, Peter (ur.), et al. *Ajda med tradicijo in inovacijo : zbornik povzetkov*, Trafoon delavnica, Maribor, 3. in 4. junij 2015. Izola: Fakulteta za vede o zdravju, Inštitut za živila, prehrano in zdravje. 2015
- **Education session-6th Congress of European Microbiologists**, Maastricht, 7-11 June 2015

#### **Selection for 2014**

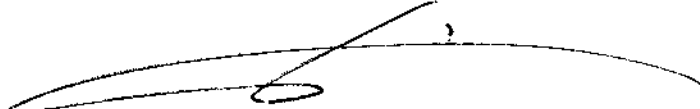
- *Strategic workshop: "Food waste in the European Food Supply Chain"*, Food and Agriculture (FA) Domain Committee, COST, Athens, Greece, 12 and 13 May 2014,  
Co-chair and Member of organizing committee
- **World Food Day 2014**, Food and Nutrition for Health Year Conference entitled, How much food do we waste?, Izola, Slovenia, 16 October 2014, Chair
- *WPAEFM symposia: Food microbiology education in practice, FOOD MICRO 2014: (ICFMH) Nantes, 5 september 2014*, Chair
- *Round table: International project sand collaboration*, II International Congress Food Technology, Quality and Safety, Novi Sad, 28-30 October Serbia, Chair

#### **Selection for 2013**

- **Slovenian Congress on Food and Nutrition**, 22. 9. 13-24. 9. 2013, Zreče, Slovenia, Member of organizing committee
- **FABE 2013**, International conference, Skiathos, Greece, Member of organizing committee
- **ISSY 2013**, 30<sup>th</sup> International Specialized Symposium on Yeast: Cell Surface and Organelles in Yeast- From Basics to Applications, 18. 6. 2013- 22. 6. 2013, Stara Lesna (High Tatras), Slovakia, Member of organizing committee
- **The 2nd International Symposium Vera Johanides- Biotechnology in Croatia by 2020**, 10. 5. 2013-11. 5. 2013, Zagreb, Croatia, Co-Chairperson
- **4th MoniQA International Conference**: 26. 2. 2013-1. 3. 2013, Budapest, Hungary, Co-Chairperson for session: 'Food safety, Climate Change, Mobility and Globalization'

#### **Selection for 2012**

- **Conference Biotechnology and Microbiology for Knowledge and Benefit**, 27. 9. 2012-28. 9. 2012 (organized by Chair of Biotechnology, Microbiology and Food Safety), Ljubljana, Slovenia
- **Congress FoodMicro 2012**, 3. 9. 2012- 8. 9. 2012, Istanbul, Turkey, member of executive board of ICFMH
- **Congress ICY 2012**, 24. 8. 2012 -31. 8. 2012, Madison, Wisconsin, USA, member of organizing committee
- **Congress CEFood 2012**, 21. 5. 2012- 26. 5. 2012, Novi Sad, Serbia, chair of session: COST actions related to food and feed issues
- **International food agricultural and gastronomy congress**, 13. 2. 2012-19. 2. 2012, Antalya, Turkey, congress ambassador for Slovenia, reporter and delegate for EFTN



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Guest professor of Food Safety at The University of Natural Resources and Life Sciences, Vienna, 2006-

Guest professor of Modern Bio-Technology in Food Production at University Vienna, 2008-

Guest professor of Food Quality and Safety at Faculty of Biosystemic Sciences at University of Maribor, 2009-

Guest professor of Food Safety at Faculty of Health Sciences at University of Ljubljana, 2011-

Retired professor of Microbiology and Food safety from University of Primorska, 2014-2016

Retired professor of Food Biotechnology from Budapest Corvinus University, 1994-2006

Retired professor of Industrial Microbiology and Biotechnology from University of Ljubljana, 1986-2013

### **5 lista projekata in last decade**

Research activities (Major research projects and activities- chair, coordinator or principal investigator)

1. TRAFooN- Traditional Food Network to improve the transfer of knowledge for innovation (FP7-KBBE 1.1.2014- 31.12.2017)
2. PROMISE - Protection of consumers by microbial risk mitigation through combating segregation of expertise (FP7-KBBE, 1. 1. 2012 -1.1. 2015)
3. Microbiology and biotechnology of food and environment (P4-0116, 1.1.2009 - 31.12. 2014)
4. Yeast Flavour Production - New Biocatalysts and Novel Molecular Mechanisms (COST FA0907, 7. 6.2010 – 6. 6. 2014)
5. Use of specific methods for detection and prevention of milk and milk products frauds (V4-1108, 1.10. 2011 - 30. 9. 2014)
6. FOOD-SEG - Strengthening cooperation in food safety research in the enlarged European Union (1.5. 2011- 30. 4. 2014)
7. Microbial adhesion management on material surfaces (L1–4067, 1. 7. 2011-30. 6. 2014)
8. Evaluation of antifungal resistance in emerging opportunistic yeast (Z7 4248- 1. 7. 2011- 30. 6.2013)
9. DREAM - Design and development of realistic food models to allow a multidisciplinary and integrated approach to food quality and nutrition ( FP7-222654, 1. 5. 2009 - 31.10.2013)
10. Food safety management: Problematic of poultry and poultry meat contamination with Campylobacter in Slovenia
11. Homologic recombinations in evolution of polyketide synthases
12. Contribution of efflux mechanisms to antimicrobial resistance and pathogenesis-related functions of Campylobacter spp (bilateral project SI-USA, 1.1. 2011-31.12. 2012)
13. Plant natural products as new agents to combat Campylobacter infections – evaluation of antimicrobial activity, resistance modulation and inhibition of biofilm formation (bilateral project SI-AU, 1.1. 2011-31.12. 2012)
14. Conservation and standardization of traditional technologies of fermented milk products based on autochthonous lactic acid bacteria (Joint Call of the SEE-ERA.NET PLUS, 1.10. 2010 -31.12. 2012)
15. Phytochemicals for food safety and shelf-life improvement (V4-1079, 1.10. 2010 – 30. 9. 2012)
16. Viral and microbiological contamination of bivalves and presence of marine biotoxins in bivalves (V4-1085, 1.10. 2010 - 30. 9. 2012 )
17. Antibiotic resistance of bacteria of animal origin (V4-1080, 1. 10. 2010-30. 9. 2012)
18. Tuning and Upgrading The Food Safety Education Curricula for BSc (EU-US ATLANTIS PROGRAMME, 16. 9. 2010 - 15. 9. 2012)
19. Microbiological (Campylobacter) risk assessment and management in poultry meat production chain (bilateral project SI-Serbia, 1.1. 2010-31.12.2011)
20. Exploitation of waste plant material after distillation of essential oil (bilateral project SI-Serbia, 1.1. 2010-31.12.2011)
21. TRACK\_FAST - Training Requirements and Careers for Knowledge-based Food Science and Technology in Europe FP7-227220, (1. 9. 2009 - 1. 9. 2012)
22. Virulence properties of Campylobacter in the model: environmental stress - cell lines (Z1- 2190, 1.5. 2009 - 1. 6. 2012)

23. Modulation of microbial intercellular communication by environmental factors (J4-2154, 1. 5.2009 - 30. 4. 2012)
24. Study of erythromycin biosynthesis by proteomic tools (J4-2195, 1. 5. 2009 - 30. 4. 2012)

## 2.12 Final research report- national projects

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