CECE 2014

11th International Interdisciplinary Meeting on Bioanalysis

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INVESTMENTS IN EDUCATION DEVELOPMENT

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Find the meeting history and more at www.ce-ce.org

Foreword

Welcome to CECE 2014. With this 11th CECE in a row we are entering the second decade of the conference. As in the previous year we start with lectures by young scientists (CECE Junior), followed by two days of invited lectures and poster sessions. This book of proceedings includes the program of all three days. This year the meeting is free of charge for all perticipants thanks to the financial support by the European Social Fund and the state budget of the Czech Republic (CZ.1.07/2.3.00/20.0182). Of course our original goal of "bringing together scientists who may not meet at specialized meetings, promote informal communication of researchers from different disciplines and map the current status of the fields shaping the bioanalytical science" remains intact. The organizers want to thank the invited speakers and all the participants and hope that you will enjoy the scientific presentations as well as personal contacts and informal discussions.

partil tout

Brno, October 18, 2014

independent branch of natural science and analysis as one of the philosophical domains. During his carreer he was a visiting scientist at the Technical University in Gdańsk (Poland), New York Academy of Sciences (New York, USA), Academia Sinica in Dalian (China), Laboratorio di Cromatographia CNR, Rome (Italy) and the Technical University in Delft (The Netherlands).

Jaroslav Janák was deeply involved in establishing reputable international journals such as, *Journal of Chromatography* and *Journal of Gas Chromatography*, now *Journal of Chromatographic Science*. He had a considerable influence on the scientific profiling of the first one, and served as an editorial board member or editor of several special issues and bibliographic service for many decades. He was advisor and co-author of the compendium *Encyclopedia of Separation Science* (London 1996, UK).

Professor Janák's contributions to the development of (analytical) chemistry, especially chromatography, have been recognized and honored with several awards and medals, namely, the M.S. Tswett Award for Distinguished Research in Chromatography (Munich, Germany 1975), the J. Heyrovsky Gold Medal (Prague, Czechoslovakia 1984), gold medal of University in Ferrara (Italy 1991), gold medal of Masaryk University in Brno (Czech Republic, 1991), medal of Faculty of Chemistry, Technical University in Brno (1992), gold medal, and "Leading Intellectual of the World" award of American Biographical Society (Raleigh, NC USA, 2004) and "De Scientia et Humanitate Optime Meritis" by the Academy of Sciences of the Czech Republic in 2005.

Since 1945, Professor Janák has been a member of the Czech Chemical Society and a long-term chairman of its office in Brno. He organized postgraduate and summer courses and due to his efforts the Chemical Faculty of Technical University in Brno was renewed. In recognition of his work he was awarded *doctor honoris causa*. This curriculum vitae would not be complete without mentioning Jaroslav's strong cultural background and his interests in everything taking place in the culture, politics, and society. For all his work and contributions to the city he was awarded the Prize of the City of Brno in 2009.

Program - CECE 2014

Monday, October 20

- 8:00 16:00 Registration
- 9:00 9:10 CECE Junior opening

9:10 – 9:25 LA-ICP-MS AS A TOOL FOR ELEMENTAL MAPPING <u>Tereza Warchilová^{1,2}</u>, Tomáš Vaculovič^{1,2}, Zuzana Čadková³, Vítězslav Otruba¹, Jiřina Száková⁴, Viktor Kanický^{1,2}

¹Department of Analytical Chemistry, Masaryk University, Brno, Czech Republic; ²Central European Institute of Technology, Masaryk University, Brno, Czech Republic; ³Department of Zoology and Fisheries, Czech University of Life Sciences, Praha, Czech Republic; ⁴Department of Agroenvironmental Chemistry and Plant Nutrition, Czech University of Life Sciences, Praha, Czech Republic

9:25 - 9:40THIOL-ENE-BASED MONOLITHIC MICROREACTORS
Jakub Novotný^{1,2}, Josiane P. Lafleur³, Jörg P. Kutter³

¹Institute of Analytical Chemistry of the ASCR, v. v. i., Brno, Czech Republic; ²Department of Biological and Biochemical Sciences, Faculty of Chemical Technology, University of Pardubice, Pardubice, Czech Republic; ³Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

9:40 – 9:55 POLYELECTROLYTE MULTILAYER COATINGS FOR THE SEPARATION OF PROTEINS BY CAPILLARY ELECTROPHORESIS: INFLUENCE OF POLYELECTROLYTE NATURE Samya Bekri, Laurent Leclercq, Hervé Cottet Institut des Biomolécules Max Mousseron (UMR CNRS 5247), Montpellier, France

9:55 – 10:10 IN VITRO RNA RELEASE OF A HUMAN RHINOVIRUS FOLLOWED VIA A MOLECULAR BEACON AND CHIP ELECTROPHORESIS <u>Victor U. Weiss</u>¹, Dieter Blaas², Guenter Allmaier¹ ¹Vienna University of Technology, Institute of Chemical Technologies and Analytics, Vienna, Austria; ²Max F. Perutz Laboratories (MFPL), Vienna Medical University, Vienna, Austria

10:10 - 10:25DEVELOPMENT OF A NOVEL RP-HPLC METHOD FOR
THE DETERMINATION OF AMINO SUGARS IN SAMPLES
OF ENVIRONMENTAL ORIGIN
Erik Beňo, Róbert Góra, Milan Hutta
Department of Analytical Chemistry, Faculty of Natural Sciences,

Comenius University in Bratislava, Bratislava, Slovak Republic

10:25 – 10:40 HPLC DETERMINATION OF METHIONINE, HOMOCYSTEINE AND CYSTEINE ENANTIOMERS IN SERUM OF PATIENTS AFTER STROKE <u>Zuzana Deáková^{1,2}</u>, Zdeňka Ďuračková², Ingrid Žitňanová², Jozef Lehotav¹

> ¹Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology, Bratislava, Slovak Republic; ²Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic

10:40 – 11:00 Coffee break

11:00 – 11:15 CAPILLARY ELECTROPHORETIC ANALYSIS OF SINGLE BREATH - POSSIBLE OR NOT?

Michal Greguš^{1,2}, František Foret¹, Petr Kubáň^{1,2}

¹Bioanalytical Instrumentation, CEITEC MU, Brno, Czech Republic; ²Department of Chemistry, Masaryk University, Brno, Czech Republic

11:15 – 11:30 ANALYSIS AND CHARACTERIZATION OF ANTIMICROBIAL PEPTIDES BY CAPILLARY ELECTROPHORESIS <u>Tereza Tůmová</u>^{1,2}, Lenka Monincová¹, Václav Čeřovský¹, Václav Kašička¹ ¹Institute of Organic Chemistry and Biochemistry AS CR, v.v.i., Prague, Czech Republic; ²Institute of Chemical Technology, Prague, Czech Republic

11:30 – 11:45 TAIL-LABELED OLIGONUCLEOTIDE PROBES FOR A DUAL ELECTROCHEMICAL MAGNETIC IMMUNOPRECIPITATION ASSAY OF DNA-PROTEIN BINDING <u>Monika Hermanová</u>, Jan Špaček, Petr Orság, Miroslav Fojta Institute of Biophysics, v.v.i., Academy of Sciences of the Czech Republic, Brno, Czech Republic

11:45 – 12:00 MAGNETIC BEAD-BASED IMMUNOCAPTURE OF CLINICAL BIOMARKERS IN MICROFLUIDIC DEVICES: FROM PEPTIDES TO WHOLE CELLS Zuzana Svobodova, Barbora Jankovičová, Jana Kučerová, Zuzana Bilkova Department of Biological and Biochemical Sciences, Faculty of

Department of Biological and Biochemical Sciences, Faculty of Chemical Technology, University of Pardubice, Pardubice, Czech Republic

12:00 – 12:15 DIODE LASER THERMAL VAPORIZATION – NOVEL SAMPLE INTRODUCTION TECHNIQUE FOR ICP MS <u>Antonín Bednařík¹</u>, Pavla Foltynová¹, Iva Tomalová¹, Viktor Kanický^{1,2}, Jan Preisler^{1,2}

¹Department of Chemistry, Faculty of Science, Masaryk University, Brno, Czech Republic; ²Central European Institute of Technology (CEITEC), Masaryk University, Brno, Czech Republic

12:15 – 13:45 Lunch break - poster session

13:45 – 14:00 A RAPID IDENTIFICATION OF TRIACYLGLICEROLS AND PHOSPHOLIPIDS USING MALDI-TOF MS Justyna Walczak, Bogusław Buszewski

Department of Environmental Chemistry and Bioanalysis, Faculty of Chemistry, Interdisciplinary Centre of Modern Technologies, Nicolaus Copernicus University, Torun, Poland

14:00 – 14:15 COMPARISON OF CHIRAL STATIONARY PHASES BASED ON CYCLOFRUCTAN IN NORMAL PHASE LIQUID CHROMATOGRAPHY

Marianna Moskaľová, Taťána Gondová

Department of Analytical Chemistry, Faculty of Science, P. J. Šafárik University, Košice, Slovak Republic

14:15 – 14:30 SIMULATION OF MICROFLUIDIC SYSTEMS WITH COMSOL MULTIPHYSICS <u>Andrea Nagy¹</u>, Eszter Tóth², Kristóf Iván², Attila Gáspár¹

> ¹Department of Inorganic and Analytical Chemistry, University of Debrecen, Debrecen, Hungary; ²Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest, Hungary

14:30 – 14:45 INFLUENCE OF MASS SPECTROMETRY RESOLUTION ON METABOLITE COVERAGE IN PLASMA Lukas Najdekr^{1,2}, David Friedecky¹, Ralf Tautenhahn³, Junhua Wang³, Tomas Pluskal⁴, Yingying Huang³, Tomas Adam^{1,2} ¹Laboratory of Metabolomics, Institute of Molecular and Translational Medicine, University Hospital and Palacky University in Olomouc, Olomouc, Czech Republic; ²Department of Clinical Biochemistry, University Hospital in Olomouc, Olomouc, Czech Republic; ³Thermo Fisher Scientific, San Jose, CA, USA; ⁴Okinawa Institute of Science and Technology, Okinawa, Japan

14:45 – 15:00 STUDY ON SILVER IMMOBILIZATION TO LACTOFERRIN

Paweł Pomastowski, Bogusław Buszewski

Department of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, Interdisciplinary Centre for Modern Technologies, Nicolaus Copernicus University, Toruń, Poland

15:00 – 15:20 Coffee break

15:20 – 15:35 DEVELOPMENT AND APPLICATIONS OF IONIZATION TECHNIQUES IN AMBIENT MASS SPECTROMETRY Jan Rejšek^{1,2}, Vladimír Vrkoslav¹, Josef Cvačka¹

¹Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i., Prague, Czech Republic; ²Department of Analytical Chemistry, Faculty of Science, Charles University in Prague, Prague, Czech Republic

15:35 – 15:50 COMPARISON OF ANTIOXIDANT PROPERTIES OF DIFFERENT MENTHA PIPERITA SPECIES AND COMMERCIAL TEAS BY CAPILLARY ZONE ELECTROPHORESIS AND SPECTROSCOPY Vendula Roblová¹, Miroslava Bittová¹, Petr Kubáň^{1,3}, Vlastimil Kubáň^{1,2}

¹Department of Chemistry, Faculty of Science, Masaryk University, Brno, Czech Republic; ²Department of Food Technology, Faculty of Technology, Tomas Bata University, Zlín, Czech Republic; ³Bioanalytical Instrumentation, CEITEC MU, Brno, Czech Republic 15:50 – 16:05 TOWARDS CYTOCHROME P450 IMER FOR KINETICS AND INHIBITION STUDIES USING CAPILLARY ELECTROPHORESIS IN ONLINE CONFIGURATION Jan Schejbal, Roman Řemínek, Zdeněk Glatz Department of Biochemistry, Faculty of Science and CEITEC, Masaryk University, Brno, Czech Republic

16:05 – 16:20 WATER UPTAKE ON SILICA-BASED STATIONARY PHASES IN HYDROPHILIC INTERACTION CHROMATOGRAPHY Jan Soukup, Pavel Jandera

Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Pardubice, Czech Republic

16:20 – 16:35 GRAPE POMACE APPLICATION IN ENVIRONMENTAL STUDIES: FROM WASTE TO NATURAL FOOD PRESERVATIVE AND SOURCE OF BIOFUEL Zorana Andonovic¹, Violeta Ivanova Petropulos²

> ¹Secondary School Yahya Kemal College, Skopje, Republic of Macedonia; ²Faculty of Agriculture, University "Goce Delčev", Štip, Republic of Macedonia

16:35 – 17:05 MULTILEVEL CHARACTERIZATION OF ANTIBODY THERAPEUTICS BY CESI-MS <u>András Guttman</u>

University of Pannonia, Veszprem, Hungary; University of Debrecen, Hungary; Sciex Separations, Brea, CA, USA

17:05 – 09:00 Poster session

Tuesday, October 21

- 8:00 15:00 Registration
- 9:00 9:15 CECE 2014 Opening remarks
- 9:15 9:45 NEXT-GENERATION PROFILING OF HUMAN IMMUNE REPERTOIRES Jan Berka

Roche Molecular Systems, Pleasanton, USA

9:45 - 10:15 THE EVOLUTION OF FORM AND FUNCTION SANS GENES <u>Keith Baverstock</u> Department of Environmental Science, University of Eastern

Finland, Kuopio, Finland

- 10:15 10:45 Coffee break
- 10:45 11:15 ANALYSIS OF BIONANOPARTICLES BY MEANS OF NANO ES/CHARGE REDUCTION COUPLED TO DIFFERENTIAL MOBILITY ANALYZER <u>Guenter Allmaier</u>¹, Victor Weiss¹, Marlene Havlik¹, Martina Marchetti-Deschmann¹, Peter Kallinger², Wladyslaw Szymanski² ¹Institute of Chemical Technologies and Analytics, Vienna

University of Technology (TU Wien), Vienna, Austria; ²Faculty of Physics, University of Vienna, Vienna, Austria

11:15 - 11:45 THE STUDY OF ULTRASMALL SAMPLES BY FAST CAPILLARY ELECTROPHORESIS - MASS SPECTROMETRY Frank-Michael Matysik, Jonas Mark, Marco Grundmann, Sven Kochmann, Andrea Beutner University of Regensburg, Institute for Analytical Chemistry, Chemo- and Biosensors, Regensburg, Germany

11:45 - 12:15 TIME-RESOLVED CRYO-ELECTRON MICROSCOPY OF MACROMOLECULES <u>Tanvir Shaikh</u> *CEITEC, Brno, Czech Republic*

12:15 – 14:15 Lunch break – poster session

14:15 – 14:45 IMPROVING ENANTIOSELECTIVITY AND RESOLUTION IN CEC AND NANO-LC: RECENT RESULTS Salvatore Fanali Institute of Chemical Methodologies, Italian National Research

Institute of Chemical Methodologies, Italian National Research Council (C.N.R.), Monterotondo, Italy

14:45 – 15:15 THE VALUE OF BIOBANK PATIENT SAMPLES IN PROTEIN EXPRESSION STUDIES <u>György Marko-Varga</u>

¹Clinical Protein Science & Imaging Group, BioMedical Center, University of Lund, Lund, Sweden; ²Dept. of Surgery, Tokyo Medical University, Tokyo, Japan

15:15 – 15:45 SEX DURING COMMUNISM. INTIMATE LIFE AND THE POWER OF EXPERTISE

<u>Kateřina Lišková</u>

Masaryk University, Brno, Czech Republic

- 16:10 City walk with invited speakers
- 19:00 Conference dinner with the traditional Moravian music

Wednesday, October 22

9:15 – 10:00 THE MONKEY KING AND PIGSY FERRYING THE PROTEOMIC SUTRAS INTO THE THIRD MILLENNIUM <u>Pier Giorgio Righetti</u> Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Milano, Italy

10:00 – 10:30 HYDROPHILIC INTERACTION CHROMATOGRAPHY-THE STATE OF THE ART <u>David McCalley</u> Faculty of Health and Life Sciences, University of the West of

Faculty of Health and Life Sciences, University of the West of England, Bristol, UK

10:30 – 11:00 Coffee break

11:00 – 11:30 MICROFLUIDICS COUPLED WITH MASS SPECTROMETRY FOR ONLINE MONITORING OF DYNAMIC BIOLOGICAL PROCESSES <u>Ryan T. Kelly</u> Pacific Northwest National Laboratory, Richland, USA

11:30 - 12:00 HARSH ENVIRONMENT CAPILLARY ELECTROPHORESIS <u>Mihkel Kaljurand</u> Tallin University of Technology, Tallinn, Estonia

12:00 – 14:00 Lunch break – poster session

14:00 – 14:30 EVOLUTION OF A MICROFLUIDIC LC/MS SYSTEM -FUNDAMENTAL TECHNOLOGY TO A COMPLETED SYSTEM <u>Geoff Gerhardt</u> *Waters Corporation, Milford, MA, USA*

14:30 – 15:00 A LIGHT AT THE END OF THE TUNNEL FOR THE COMPREHENSIVE COMPOUND IDENTIFICATION IN UNTARGETED METABOLOMICS <u>Robert Mistrik</u> HighChem Ltd., Bratislava, Slovak Republic cHILIC, shows the N_w equivalent to more than nine monomolecular water layers due to its enhanced affinity to water; followed by second zwitterionic sulfobetaine ZIC HILIC phase with the equivalent of six adsorbed water layers. Equivalent from three to five monomolecular water layers were observed on the stationary phases with bonded hydroxyl groups, Luna HILIC (4.72), YMC Triart diol (3.06) and Ascentis Express OH5 (3.98). Between one and two monomolecular water layers equivalents are adsorbed on XBridge HILIC, Atlantis HILIC, Ascentis Express HILIC and LiChrospher diol columns. The less-polar Ascentis Express ES-CN column shows very low water saturation capacity, like the hydrosilated Cogent columns.

4 Conclusions

The adsorption isotherms of water can be described by Langmuir model, like in nonaqueous normal-phase LC. At full column saturation, the excess adsorbed water fills 2.3 - 45.3% pore volume, which approximately corresponds to the equivalent of 0.25 - 9 water layer coverage of the adsorbent surface. It is assumed that the equivalent number of adsorbed water layers decreases with decreasing number of active silanols and hydroxyl groups. In the ANP mode, partition retention mechanism is probably the predominating retention mechanism on the stationary phases with higher number of water-layers, while the columns with less than one equivalent of water layer support the idea of predominating role of adsorption retention mechanism.

Acknowledgement

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References

[1] Alpert A.J., J. Chromatogr. 1990, 499, 177-196.

[2] Langmuir I., J. Am. Chem. Soc. 1916, 38, 2221-2295.

GRAPE POMACE APPLICATION IN ENVIRONMENTAL STUDIES: FROM WASTE TO NATURAL FOOD PRESERVATIVE AND SOURCE OF BIOFUEL

Zorana Andonovic¹, Violeta Ivanova Petropulos²

¹Secondary School Yahya Kemal College, Skopje, Republic of Macedonia ²Faculty of Agriculture, University "Goce Delčev", Štip, Republic of Macedonia zorana.andonovic@aol.com

1 Introduction

The geographic location of Republic of Macedonia is exceptional for breeding vine and specific grape varieties. But, the wine industry waste in general is a problem in Macedonia, since it does not have any usage. In the European Union, there is approximately 14.5 million tons of wine industry waste produced from wineries (http://www.academicwino.com/2012/11/grape-seed-extract-leather-production.html). In fact, the wine industry waste (grape pomace) contains primarily crushed grape skins and seeds rich in beneficial polyphenol compounds that act as antioxidants, antibacterial agents etc. The largest fraction of winery waste is pomace, or the solid remains of grapes (skins, stalks and seeds), which is thrown away ending up in landfills. From another point of view, transport is the third largest emitter of greenhouse gases and biofuels can significantly reduce transport's carbon footprint since it is dependent on finite fossil fuels such as oil and petroleum for its energy needs (R.E.H. Sims, et al.). Biodiesel, provides significantly reduced emissions of carbon monoxide; compared to petroleum diesel fuel.

2 Experimental

Totally, 4 types of Macedonian grape pomace, (from Zupjanka, Prokupec, Kadinal and Vranec varieties) as well as blueberry and aronia, were used. For the extraction of polyphenolics liquid-liquid extraction with ethanol/water/acetic acid, followed by decantation and filtration was used. The phenolic content of the obtained extracts was determined by the Folin-Ciocalteu method (Ivanova et al. 2010); and the total anthocyanins were realized by the Di Stefano et al. (1989) method. Afterwards, three different volumes of the obtained extracts (10, 50 and 100 mL) from each sample, were concentrated by rotoevaporation to dryness. The three concentrates were applied on milk together with the lactic bacteria to study the influence of polyphenolics during fermentation; as well as, applied on milk after the fermentation (into the obtained yoghurt) (Figure 1). The pH value of the newly generated yoghurts was observed by using a pH meter. In the second part, the seeds were separated from the grape pomace and served as a new source of oil, together with blueberry and aronia peels, that was to be transformed into biodiesel. Six organic solutions (ethanol, hexane, benzene, diethyl ether, acetone, acetic acid) were added to the dried seeds and then filtration and distillation followed for obtaining the oil. By transesterification reaction the biodiesel was divided and it was proved due to the combustion of the final product.

3 Results and Discussion



Fig. 1. Yoghurt enriched with polyphenols, from Aronia, added after fermentation.

Total phenolic assay (TP)					
Name of sample analyzed	TP (mg/L)	TP average value			
1 – Zupjanka	62.8	67.9			
	73.17				
2 - Kardinal	76.17	76.7			
	77.21				
3 - <u>Prokupec</u>	247.01	247			
	246.03				
4 - <u>Vranec</u>	152.18	152			
	151.56				
5 - Blueberry	190.42	192			
	194.21				
6 - Aronia	430.2	431			
	432.49				

Table 1. Comparison of the total phenols in the analysed samples (grape pomaces and berries).

Table 2. The pH variation between some of the samples.

pH values of yoghurt with polyphenols added during fermentation								
Polyphenols	31.01	02.02	04.02	06.02	09.02	15.02	22.02	02.03
added								
Zupjanka								
1-10	4.0	4.0	4.0	4.0	3.9	3.9	3.9	3.9
1-50	4.3	4.0	4.0	4.0	3.9	3.9	3.9	3.9
1-100	4.2	4.0	4.0	4.0	3.9	3.9	3.9	3.9
Kardinal								
2-10	4.1	3.8	4.0	3.8	3.8	3.9	3.9	3.9
2-50	4.2	3.9	4.1	3.9	3.9	3.9	3.9	3.9
2-100	4.0	3.9	4.0	3.9	3.9	3.9	3.9	3.9
Prokupec								
3-10	3.9	3.7	3.9	3.9	3.9	3.9	3.9	3.9
3-50	4.1	3.8	3.9	3.9	3.9	3.9	3.9	3.9
3-100	4.2	4.0	4.1	4.0	4.0	4.0	4.0	3.9
Vranec								
4-10	4.0	3.8	3.8	3.8	3.8	3.8	3.9	3.9
4-50	4.1	3.9	3.9	3.9	3.9	3.9	3.9	3.9
4-100	4.2	4.0	3.9	4.0	4.0	4.0	4.0	4.0
Blueberry								
5-10	4.0	3.7	3.9	3.9	3.9	3.8	3.9	3.9
5-50	4.1	3.9	4.1	4.0	4.0	3.9	3.9	3.9
5-100	4.2	4.0	4.01	4.0	4.0	4.0	4.0	3.9
Aronia								
6-10	4.0	3.7	3.9	3.9	3.9	3.9	3.9	3.9
6-50	4.1	3.8	4.0	3.9	4.0	3.9	3.9	3.9
6-100	4.1	3.8	4.0	4.0	4.0	4.0	4.0	3.9

Name of sample (source of	The amount of oil	Impure biodiesel
oil)	obtained (g)	obtained (g)
1 + ethanol	0.26	0.39
2 + ethanol	0.28	0.4
3 + ethanol	0.19	1.19
4 + ethanol	0.15	0.26
5 + ethanol	0.29	0.28
6 + ethanol	0.29	1.96
1 + acetone	0.2	0.32
2 + acetone	0.26	0.24
3 + acetone	0.36	0.43
4 + acetone	0.25	0.58
5 + acetone	0.13	2.43
6 + acetone	0.19	4.23
1 + acetic acid	0.28	1.54
2 + acetic acid	0.6	1.7
3 + acetic acid	0.31	3.72
4 + acetic acid	0.11	1.14
5 + acetic acid	0.29	4.54
6 + acetic acid	0.27	1.83

Table 3. Comparion in the oil and biodiesel (biodiesel + byproducts) obtained from the samples.

The dry phenolic extracts were applied on a home - made, traditional yoghurt during and after fermentation, to observe the change in the pH value (if there is any) of both types and of the three different concentrations. As it is already known, the varieties in the pH value of the yoghurt are directly proportional to the time of spoilage. Actually, a faster decrease in the pH values, indicates faster spoilage and lower shelf-life. With the help of polyphenols from the winery waste there should be a lower pH drop and a deceleration in the mold development in the new yoghurt. There is already a dairy in Macedonia, "Buchen Kozjak" that is interested in taking the method from this project as a base for manufacturing of a new yoghurt, in our country. Regarding the second part of the project, it is known that a larger amount of oil is present in the grape seeds, than in the stem and the peels. Five different organic solvents for extracting the oil contained in the seeds were applied. Later, it was observed that diethyl ether, hexane, and bensene had evaporated without extracting the oil. By use of transesterification method the oil obtained in 18 different amounts was converted into biodiesel and also some byproducts, soap, glycerol, excess alcohol, and trace amounts of water. Then the content was heated again until it stopped boiling, meaning that the byproducts were removed and biodiesel + glycerol was the only liquid left. The presence of biodiesel was proved by the 90% combustion of the remaining substance in all beakers.

4 Conclusions

All yoghurt samples containing polyphenolics applied before the fermentation, presented higher pH value compared to the control and samples with polyphenolics applied after fermentation. Zupjanka has shown the best results (pH-4.4 for 1-100) for all concentrations, which is even higher than aronia, which is known to contain the highest phenolic content. After obtaining the yoghurt, sensory analysis was performed, stating that the new product has creamy texture; it tastes good, without unpleasant smell or bitterness. The colour of some samples turned into red-violet, excluding the voghurt with dry extracts from: Zupjanka and Kardinal (white grapes). By microbiological analysis the presence of pathogen and other harmful bacteria was proved to be negative, showing that this yoghurt could be a main basis for manufacturing a more beneficial dairy product that could be soon available on the market. Concerning the biodiesel production, the oil content, depending on the solvent used, differed from 0.11 g to 0.36 g. During the extraction acetone and acetic acid showed better results than ethanol, which proved to be a better extractor of antioxidands. Thus, acetic acid was the most proper solvent for obtaining impure biodiesel with a yield of 90.8%. Overall, the sample which showed the highest percentage when mixed with acetic acid was blueberry. On the other hand, from the grape pomace, the type comprising the largest amount of impure biodiesel was aronia with the average yield of 53.46% and then followed: blueberry, Prokupec, Kardinal, Zupjanka and Vranec, respectively.

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[1] http://www.academicwino.com/2012/11/grape-seed-extract-leather-production.html.

[2] R.E.H. Sims, et al. *Energy supply*, Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, 2007.

[3] Ivanova V., Stefova M., Chinnici F. (2010) *Journal of the Serbian Chemical Society*, *75*, *45-59*.

[4] Di Stefano R., Cravero M. C., Gentilini N. (1989). Metodi per lo studio dei polifenoli dei vini, L'Enotecnico I, Maggio, *83-89*.