TECHNOLOGICA ACTA

JOURNAL OF SCIENCE PROFESSIONAL FROM CHEMISTRY AND TECHNOLOGY - FACULTY OF TECHNOLOGY TUZLA

ISSN 1840-0426
ISSN 2232-7568

Vol. 4
Broj 1
Juni 2011. godine

UNIVERSITAS STUDIORUM TUZLAENSIS MCMLXXVI

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Vol. 4 Number 1, page 1 – 68, Tuzla, june 2011. year

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Vol. 4 Number 1, page 1 – 68, Tuzla, june 2011. year

Publisher / Izdavač
Faculty of Technology University inTuzla

Lector / Lektor
Milja Jogunčić, teacher of english languige

Technical Editor / Tehnički urednik
Mirsad Fejzić

Printing / Štampa
IN SCAN d.o.o. Tuzla

Journal prints two times of year

Technologica Acta is indexed in the following database: CAB Abstracts, COBISS, Index Copernicus Journal Master List, EBSCO

Edition / Tiraž: 200

Editorial Office / Uredništvo
Secretary / Sekretar: Nermina Jahić
Fakulty of Technology, University in Tuzla
Univerzitetska 8, 75000 TUZLA
Tel/fax: +387 35 320 740

CONTENS

NEW GENERATION LEARNING – OPEN QUESTIONS

REVIEW

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ABSTRACT

Although there are obvious advantages in the use of technology in education there are also some serious problems, which have to be resolved in order to prepare young generation for living and working in the 21st century. It is the truth that new generation learns differently because of easy access to the ICT. However, they lack direct, face to face communication and are not prepared for it. This article discusses this as well as similar problems generated in education with paradigm change, influence of ICT on all segments of life, suggesting possible solutions.

Keywords: learning, skills, technology, addiction, creativity

The authors have been involved in different forms of education for a long time. Although they do not participate in formal educational system on regular basis, they often discuss with teachers, principals, parents and colleagues the changes which happened in educational process in the last two decades. Based on these discussions and on experience, acquired by participating in educational workshops organized for students of elementary and of high schools, they think that there are several serious open questions in the new generation learning as follows:

- too much technology from very early years
- much less human communication
- less creativity
- development of ctrlC & ctrlV culture
- less reading habits
- escape into virtual world
- students are less persistent in searching solution.

This short educational form was usually organised in the same school once a year for several years, which enabled them to note significant changes in students' behaviour and attitude, and which were caused by using ICT both at home and school in Slovenia.

Slovenia started to implement internet in early 1990s. The ARNES (Academic Research Network) played extremely important role in penetration of Internet in research and educational institutions. The access was free for those institutions as well as for their employees and students. Only few educational institutions had fast internet connections and computers in the first years of Internet in Slovenia. However the situation changed very fast, resulting in having well equipped computer laboratories, classrooms and having a good connection to Internet in almost all schools at the end of millennium.

The same process occurred in households too, with a short delay. Today in Slovenia, 59% of households have Internet access and 50% of them broadband connection. According to CIA fact book data there are 1.300.000 users of Internet and 1.928.000 users of cellular phones while the population of Slovenia is 2.005.000. 13,5% of the whole population in Slovenia are young between age 0-14.

According to data obtained by RIS (Research of Internet in Slovenia) there are more than 70.000 users of Internet, under age of 10, which means that almost all children at that age use internet.

What follows are data collected after we had conducted workshops in the secondary schools including students at the age between 12-14.

All of the students have a cellular phone and use them intensively both for calling and for texting; almost all have computers and Internet connection at home and use it couple of hours per day. Furthermore, messengers, playing games, browsing websites with music, sport, porno or some extravagant contents are the most used web applications. They find already prepared solution like essays covering different topics for their school assignments while only few of them are using email.

What is found out by observing students' behaviour and comparing it to generations of a decade ago is that those young people are less focused, worse in communication. Moreover their writings are more aggressive and a great number of them are the internet addict especially using internet for playing games. It is also found out that a violent way of competition pattern definitely has strong influence on their behaviour. Young people are influenced by messages and images, which are not characterized with a positive personal empowerment. These inhibit them from accessing many creative tools and from rising human potentials. So the excessive usage of the modern technology causes nervousness, anxiety and aggressiveness.

Almost the same pattern has been noticed working with high school students and primary school students. They are better in using technology, searching the web, preparing PowerPoint presentation, yet most of the time spent on web was used for playing game or watching some very special events on the internet.

The authors made an interesting experiment a month ago. A workshop from Idea to Patent was organized at the vocational high school for car mechanics. On the first day the basic knowledge about intellectual rights, patent and patent office was presented. On the second day students generated the ideas and were searching web trying to find whether the generated ideas were already known or were developed by someone else in the world. The pattern was very similar to the acquired experience when working with the elementary school students. After some time of searching, most of them started to watch car races on YouTube, or to play web games. Whereas only few were still trying to search patent databases and to get information they needed to continue with their work.

On the third day the two hours long workshop was organized, covering the basic issues of communication. All technology was excluded, only paper and pencils were used. At the beginning it was not easy to have their attention, to listen and to participate. However, students started to participate after playing the simple social games without any technology. At the end of the workshop they were divided in two groups. One was defending the VW cars and the other was attacking it. There was a moderator and after

some time the groups changed their roles. The students were extremely creative in arguing which we all enjoyed very much.
Later, analyzing this event the students said: »We should have such staff at secondary school; why don't we have more of such events. If there were more of these things, I would love to go to school...«

When people think about fast and formal penetration of ICT in the educational institutions, they do not take into account High Tech – High Touch trend, observed and explained by John Naisbitt more than three decades ago[1]. High technology requires high touch – a strong human relationship. And it was not done a lot to help pupils and students to gain soft skills required for more efficient communication, learning, presentation, working in team, relaxing and reducing stress. If there were such topics in curriculum, pupils and student would be better prepared for both work and life in fast changing society and would also use ICT more efficiently.

Another growing problem in the educational institutions is »copy and paste« culture. There are several websites like www.cheathouse.com offer solutions from an essay to PhD thesis for acceptable price. Using this students lose their creativity, their writing skills and are not persistent enough in searching for solution.

The solution for this problem lies in using of Edward de Bono »design principle« which is best explained in a popular article[2]:

"In a stable world, knowledge of standard situations and the routine ways of dealing with them is sufficient. Not so much in a changing world. The routines and category judgments from the past may be inadequate, misleading and dangerous. Instead of analysis and judgment we need design. We need to be able to "design" ways forward.

Traditionally we have solved problems by analysing them and seeking to identify and then to remove the cause of a problem. It often works however on the other hand there are too many causes to remove or we cannot simply remove it because it is a part of human nature.

Most of the world's major problems such as poverty, crime, conflict and pollution will not be solved by more analysis and more information. We need to design ways forward - leaving the cause in place. Unfortunately, the traditions of education and the thinking culture of society make no provision for "design" - we see it as applying only to buildings, furniture and Christmas cards. This is a fundamental weakness, derived from the last Renaissance, which never encouraged innovation or design but believed that truth was enough."

If we use the "design principle" in combination with operacy (defined in the same article by de Bono "In education we are concerned with literacy and numeracy. That leaves out the most important aspect of all, which I call "operacy". The skills of action are every bit as important as the skills of knowing. We neglect them completely and turn out students who have little to contribute to society. ") then we can change "the copy paste culture" to the creative one in which pupils and students would design new own solutions. The creativity process would substitute copying of existing solution and would make them happy, because research in creativity found out that finding solutions to problem makes people happy[3]. We think that we have to find right balance how much ICT technology we shall use in formal education. We believe that what we need in elementary and even in high school is much more art, physical education and development of personality rather than technology. We are not against technology but we have to find right solutions and to use them in most efficient way preventing young people from becoming technology addicted, so they can use it only as a tool. It is a difficult task which has to be solved soon.

REFERENCES:

1 J. Naisbitt,"High-Tech High-Touch", Broadway, New York, 1999 pp. 31-57.

2 E. De Bono, Away with the Gang of Three. Guardian, January 25, 1997 URL**[**http://www.edwdebono.com/debono/guard.htm], 5/2/2011]

3 M. Chikszentmihalyi, "Finding Flow" Basic Books, New York, 1997 pp. 17-34.

MICROBIOLOGICAL CHARACTERISTICS OF THE WATER IN MINE PIT LAKES IN THE TUZLA COAL BASIN

PROFESSIONAL PAPER

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ABSTRACT
In the last three decades, as a result of coal surface exploitation in the area of Tuzla Canton, formation of new water bodies - so called pit lakes, has occurred. Lake water may be contaminated with a large number of various microorganisms, which represent a potential threat to human health. This paper presents results of microbiological analysis of water from seven pit lakes in Tuzla Canton. These lakes were: Suhodanj, Požar, Sjerkovača, Brestovica, Mušići, Ramići and Bešin. Water samples were collected at 27 sites during 2008, and analyzed by standard microbiological methods. The analysis results show that in all lakes, except the lake Ramići, fecal contaminants were present: *Escherichia coli* or *Streptococcus faecalis*. On the basis of the most probable number of coliform bacteria in a 1000 ml water sample, lakes of Suhodanj, Ramići and Bešin have water quality class I-II; whereas the other investigated lakes have water quality class II.

Keywords: Mine pit lakes, Microbiological analysis, fecal indicators, water quality

INTRODUCTION

Due to surface coal exploitation formation of new water bodies-mine pit lakes has occurred on the degraded surfaces of the Tuzla area. These lakes are created by filling the final crater with water upon completion of drainage and exploitation process, or by partitioning of surface water courses by waste material [1]. The lakes appeared in 1980s and have their own water regime [2]. As they represent significant water resources, some of them are used for recreation, sport and fishing. The published data on water regime, hydrobiological researches and possibilities of usage the mine pit lakes of the Tuzla area are quite insufficient. Pašić-Škripić and associates[3] present the data on hydro-geological characteristics of existing accumulations on the open mining Turija within the Banovići basin. The researches Velagić-Habul and associates[4] on influence of the waste banks on quality of running waters and mine pit lake in the Đurđevik coal-bearing basin are also known, in which the authors prove that the lake water has a neutral reactions, and that processes of acidification and neutralisation run parallel. Kamberović [5] performs hydro-biological researches of phytobentos and macrophyte vegetation composition and researches of the conservation potential of the mine pit lakes in a broader area of Tuzla. However, micro-biological analyses of water from mine pit lakes of the Tuzla basin have not been performed so far, except for Šićki Brod and Bistarac [6] lakes, which are located in the Kreka coal-bearing basin.

Having in mind that water may be a carrier of toxic substances, pathogenic microorganisms and a cause of mass epidemics, monitoring of the lakes that are utilised by humans and are located in the direct vicinity of households is indispensable.

According to valid Water Classification Regulation of RB&H No.19/80, categorysation of water quality is performed on the grounds of physical-chemical, biologic and microbiologic indicators. As a part of biotic component in lake ecosystems, microorganisms are responsible for processes of disintegration of organic matter, due to which determination of state of microorganism populations is a realistic indicator of various and specific courses of biochemical processes, and also the first indicator of eutrophication, which reflects their significance within hydrobiologic researches. Lake water may be contaminated by a large number of different microorganisms, which represent a potential treath for human health. The most significant contaminant of surface waters are microorganisms that arrive from human and animal excretes. Microbiological examination determines whether the water represents a threat to human health, respectively whether it is contaminated by any of pathogenic microorganism. A simple and reliable method of proving the eventual presence of pathogenic microorganisms is proving the bacterial indicators of fecal contamination. The bacteria, indicators of fecal contamination, are those types that are, as normal microflora, predominantly located in digestive tract of humans and animals and arrive to water currents through fecal waters. Among these are coliform bacteria, streptococci of fecal origin, sulphite-reducing clostridia and the species from *Salmonella* and *Proteus* [7]genera.

The objective of this research work is to determine microbiological and physical-chemical characteristics of waters from seven mine pit lakes of the Tuzla coal-bearing basin and to estimate the condition of water quality from the obtained results.

Description of the researched area

Research of microbiological characteristics of water was conducted in seven mine pit lakes of a broader area of Tuzla: lakes of Suhodanj, Požar, Sjerkovača and Brestovica, located in the Đurđevik coal-bearing basin and Mušići, Ramići and Bešin lakes, situated in the Banovići coal-bearing basin.

Lake Suhodanj was formed in 1985 by partitioning the stream Donja Brnjica by waste material, on the location of inactive open mining Suhodanj [5]. It has area of 2.5 ha and maximum depth up to 18 meters [8].
Lake Požar is situated on south border of the active open mining Potočari, near Beširovići. It was formed in 1985 by partitioning the smaller stream with waste material, so that it has a maximum depth up to 14 metara [5.8]. The lake area is 1.38 ha [2].

The lake Sjerkovača is located on south border of open mining Višća II, near the settlement of Gornja Višća. It was formed in 1982 by filling up the waste material and partitioning of the stream having the same name. The lake has a maximum depth up to 20.5 meters [5.8].

The lake Brestovica was formed in 1982 on west border of exploitation field of the Đurđevik basin, by partitioning of the stream having the same name with waste material of argillaceous marls. The lake has maximum depth up to 8 meters [5.8]. It is characterised by humus base and a large variation in water level during the year, due to poor inflow of water in summer months. Mušićko Lake was formed in 1982 in final crater upon completion of coal exploitation in the shut down Ravne open mining [5.8]. It is in the direct vicinity of Banovići. The area of the water mirror is 5.53 ha [2].

Ramićko Lake was formed in 1983 by partitioning the river Razlaštica by cover of marlaceous composition. It is situated on south border of the open mining Turija and has a depth up to 18 meters [5.8].

The lake Bešin was formed in 1982 and is situated in the active part of the open mining Turija. It has depth up to 6 meters and is supplied by water from surface flows and precipitation [5.8].

Figure1.

MATERIAL AND METHODS

Water samples for microbiological analyses were taken in May and June of 2008 in sterile glass bottles of volume 1000 ml, at 27 measuring sites within seven researched lakes. The water samples were transported to Microbiologic laboratory of the Faculty of Science in Tuzla, where the analysis of the following microbiological indicators was performed: total number of aerobic mesophilic bacteria in 1 ml of water, the most probable number of coliform bacteria in 1000 ml of water, coliform bacteria of fecal origin, streptococci of fecal origin and sulphite-reducing clostridia. During the analysis, the bases of ‘Torlak’ Belgrade and ‘Oxoid’ England were used. The methods determined by the Regulation on method of sampling [9] and methods for bacteriologic examination of drinking water [10, 11, 12] were used in the work.

Just before taking the samples, physical-chemical parameters were measured: water temperature and pH of water. Collecting the samples for laboratory determining the physical-chemical characteristics of water was performed in the bottles of inert plastics and Winkler bottles, at depth of 20 cm on each lake. According to standard methodology for research of water of the American Health Organisation [13], eight physical-chemical indicators of water quality were analysed: pH value, oxygen (mg/l), turbidity (NTU), consumption $KMnO_4$ (mg/l), total nitrogen (mg/l), ammonium nitrogen (mg/l), nitrites (mg/l) and nitrates (mg/l). As a criterion for evaluation of water quality, the guidelines of Water Quality Regulation No. 19/80[14] were used.

Method for determining the total number of aerobic mesophilic bacteria

This method implies determining the total number of all living bacteria in 1 ml of water and is performed for orientation toward the basic parameter according to which hygienic properness of water is determined. Total number of bacteria is useful information on water quality and is a support of colimetry result. For determining the total number of aerobic mesophilic bacteria dilutions of the sample in sterile physiological solution in the ratio 1:10 and 1:100 were performed. From well homogenized dilution, as well as from undiluted sample, 1 ml of the sample was taken in a sterile manner and transferred to empty Petri dish. Thereupon, the Petri dishes were covered by the sterilized agar cooled at around $45^{o}C$ for total number of bacteria. After the agar had been poured out, the contents of Petri dishes was carefully stirred by circular movements on the flat table in order to obtain the best mixture of agar with inoculum. Thus planted bases were incubated in the thermostat at $37^{o}C/48^{h}$, for the purpose of growing predominantly mesophilic bacteria, which are the most important from hygienic aspect. Counting the grown colonies was performed by binocular magnifying glass. Number of living cells in the sample is determined on the basis of grown colonies (CFU - colony forming units) according to formula:

CFU = number of grown colonies / volume of sample x reciprocal value of dilution.

Method for proving the coliform bacteria in water

MPN method (The most probable number)
For determining the coliform bacteria, the base Andrade lactose 1% peptonic water (ALPA) has been used. Planting the certain dilutions of sample as well as a calculation of MPN coliform bacteria was performed according to Swaroop table for less polluted river and questionable well waters [11].

Test tubes with Andrade lactose 1% peptonic water in which upon incubation at 37°C/48 hours a change of color (red) and gas production in Durham tubes occurred, were planted on Endo agar. The grown, characteristically red colonies, with or without methalic glow, were identified after incubation at 37°C/24 hours by testing the biochemical properties (lactose, maltose, saccharose, urea, citrate, indole, manit, H_2S, esculin, VP/MC (Vogues-Proskauer/methyl order) and FD (phenylalanin deaminase) and the catalase test.

Method for proving coliform bacteria of fecal origin – Eikman test

For proving the coliform bacteria of fecal origin, test tubes with positive reaction in the base with Andrade lactose 1% pepton water were used. The contents of these test tubes (1 ml) were planted in clean, sterile test tubes also with Andrade lactose 1% pepton water, which were then incubated at 44°C/48 hours. A positive experiment is a change of indicator colour in the base with the gas production.

Method for proving the streptococci of fecal origin

For proving streptococci of fecal origin Azide Dextrose broth was used. In 9 ml of Azide Dextrose broth 1 ml of the tested sample was planted, which was incubated at 37°C/24 respectively 48 hours until appearance of turbidity. From the turbid test tubes one drop of material was taken and inoculated on Slanetz-Bertley agar and incubated at 37°C/24 hours. The increase of tiny purple colonies indicate to presence of fecal streptococci. For identification of *Streptococcus faecalis* a bile esculin test was performed. Appearance of black colour from the formed iron esculatine is a sign of positive reaction.

Method for proving sulphite-reducing clostridia

Before planting, the sample of tested water needs to be heated for 15 minutes in water bath at 80°C in order to destroy asporogenic microorganisms, which have an ability of sulphite reduction.

In a 1 ml water sample, which is previously thermically treated, 15 ml of the warmed up sulphite polymiksin agar is added. The contents of the test tube is mixed well with the base and cooled under jet of water. The planted bases are incubated at 37°C/ to 5 days. The increase in black colonies indicates to presence of sulphite-reducing clostridia. Microscopic preparations (sporogenic gram-positive rods) are made of black colonies from sulphite agar and planted aerobically on blood agar. Absence of growth after 24 - 48 hours confirms that these are sulphite-reducing clostridia.

Methods of identifications of other present bacteria

The grown lactose negative colonies (colourless colonies) on Endo agar, asporogenic Gram negative rods, are furher identified by testing their biochemical properties. Biochemical properties were tested on the following biochemical multicoloured series: (lactose, maltose, saccharose, urea, citrate, indole, manite, H_2S, esculin, VP/MC (Vogues-Proskauer/methyl order) and FD (phenylalanin deaminase), as well as catalase and oxidase tests.

Antracoids were identified on the basis of morphology of colonies formed on blood agar aerobically (large, rugose, β hemophitic colonies), microscopic preparation (sporogenic Gram positive rods) and the catalase test.

RESULTS

Results of microbiological analyses of water of the researched mine pit lakes were shown in the tables 1-7.

On Suhodanj Lake five samples of water taken on north (L1), west (L2), north-east (L3) and south (L4) side of the lake were analysed, and the stream Brnjica which supplies the lake with surface water (L5).

Table1. Results of microbiological analysis of Suhodanj Lake water

Microbiological parameters	**Sampling sites on Suhodanj Lake**				
	L1	**L2**	**L3**	**L4**	**L5**
Total number of aerobic mesophyllic bacteria in 1 ml	62	45	65	70	20
The most probable number of coliform bacteria in a 1000 ml MPN	500	220	880	1500	220
Coliform bacteria of fecal origin (Eikman test)	-	-	-	*Escherichia coli*	-
Fecal Streptococci	-	-	-	-	-
Sulphite-reducing clostridia	-	-	-	-	-

With microbiological analysis of the water from Suhodanj Lake it was determined that a number of aerobic mesophyllic bacteria in 1 ml ranges from 20-70, while the most probable number of coliform bacteria in 1000 ml of water on all localities, except on locality 4, refers to water quality class I. In water sample on south side of the lake, MPN amounts to 1500, which corresponds to water quality class II. Detection of the bacterium *Escherichia coli* with no other bacterial indicators of fecal contamination indicates to temporally indefinite, but certain pollution of water by feces [7].

On Požar Lake 4 samples of water were analysed, taken on west (L1), north (L2), east (L3) and south side of the lake (L4).

With microbiological analysis of Požar lake water it was determined that a number of aerobic mesophyllic bacteria in 1 ml of the tested water range from 25-161. The most probable number of coliform bacteria in a 1000 ml water sample on north and east side of the lake is 880, while the water on west and south side of lake is more burdened by coliform bacteria, whose MPN is 3800.

Table 2. Results of microbiological analysis of Požar Lake water

Microbiological parameters	**Požar Lake sampling sites**			
	L1	**L2**	**L3**	**L4**
Total number of aerobic mesophyllic bacteria in 1 ml	45	25	161	50
The most probable number of coliform bacteria in a 1000 ml MPN	3800	880	880	3800
Coliform bacteria of fecal origin (Eikman test)	*Escherichia coli*	*Escherichia coli*	*Escherichia coli*	-
Fecal Streptococci	-	-	*Streptococcus faecalis*	*Streptococcus faecalis*
Sulphite-reducing clostridia	-	-	-	-
Other identified bacteria	*Klebsiella* sp.	*Klebsiella* sp.	*Klebsiella* sp.	

The most probable number of coliform bacteria in 1000 ml of the tested water indicates to water quality class II. Detecting the bacterium *Escherichia coli* with other bacteria indicators of fecal pollution (*Streptococcus faecalis*) refers to more recent polluting the water with feces [7].

On Sjerkovača Lake, four samples of water were analysed: on west (L1), east (L2), north (L3) and south (L4) side of the lake.

Table 3. Results of microbiological analysis of Sjerkovača Lake water

Microbiological parameters	**Sjerkovača Lake sampling sites**			
	L1	**L2**	**L3**	**L4**
Total number of aerobic mesophyllic bacteria in 1 ml	106	161	101	96
The most probable number of coliform bacteria in a 1000 ml MPN	3800	3800	3800	1500
Coliform bacteria of fecal origin (Eikman test)	*Escherichia coli*	*Escherichia coli*	*Escherichia coli*	*Escherichia coli*
Fecal Streptococci	-	-	-	*Streptococcus faecalis*
Sulphite-reducing clostridia	-	-	-	-
Other identified bacteria	*Citrobacter* sp.	*Klebsiella* sp.		*Enterobacter* sp.

Microbiological analysis of Sjerkovača Lake water shows that a number of the aerobic mesophyllic bacteria in 1ml range from 96 to 161, while the most probable number of coliform bacteria varies in the range of 1500-3800. On the basis of colimetry results, Sjerkovača Lake water meets the criteria of water quality class II. In all samples of water, coliform bacteria of fecal origin (*E. coli, Citrobacter sp., Klebsiella sp., Enterobacter sp.*) were isolated, and in the sample on south side of the lake *Streptococcus faecalis* was isolated. These findings indicate to more recent fecal contamination of water, which is epidemiologically dangerous.

On Brestovica Lake five samples of water were analysed: on west (L1), east (L2), north (L3) and south (L4) side of the lake and the Brestovica stream (L5).

Table 4. Results of microbiological analysis of Brestovica Lake water

Microbiological parameters	**Brestovica Lake sampling sites**				
	L1	**L2**	**L3**	**L4**	**L5**
Total number of aerobic mesophyll bacteria in a 1 ml	160	443	353	58	304
The most probable number of coliform bacteria in a 1000 ml MPN	-	24000	500	2700	880
Coliform bacteria of fecal origin (Eikman test)	-	*Escherichia coli*	*Escherichia coli*	*Escherichia coli*	*Escherichia coli*
Fecal Streptococci	-	*Streptococcus faecalis*	-	-	-
Sulphite-reducing clostridia	-	-	-	-	-
Other identified bacteria	-	*Enterobacter* sp.	-	*Citrobacter* sp.	*Providentia* sp.

Microbiological analysis of Brestovica lake water shows that a number of aerobic mesophyllic bacteria in 1ml are in range of 58 - 443. The most probable number of coliform bacteria in 1000 ml of the examined water significantly varies in dependence of the locality, and the highest number has been determined for the samples on east bank (24000), while on west bank of the lake coliform bacteria have not not isolated.

Table 5. Results of microbiological analysis of Mušići Lake water

Microbiological parameters	**Mušići Lake sampling sites**			
	L1	**L2**	**L3**	**L4**
Total number of aerobic mesophyll bacteria in a 1 ml	170	1616	360	975
The most probable number of coliform bacteria in a 1000 ml MPN	500	8800	8800	3800
Coliform bacteria of fecal origin (Eikman test)	-	*Escherichia coli*	-	*Escherichia coli*
Fecal Streptococci	-	-	*Streptococcus faecalis*	-
Sulphite-reducing clostridia	-	-	-	-
Other identified bacteria	*Pseudomonas* sp.	-	*Seratia* sp.	-

Coliform bacteria of fecal origin (*E. coli, Enterobacter sp., Citrobacter sp., Providentia sp.*) have been isolated on the localities on east, north and west side of the lake.

According to mentioned parameters, Brestovica lake water can be included into water quality class II.

On Mušići Lake, four water samples have been analysed: on south (L1), north (L2), west (L3) side of the lake and the stream flowing into the lake L4).

Microbiological analysis of Mušići Lake water shows that the numbers of aerobic mesophyllic bacteria in 1 ml range from 170 to 1616. The largest number of coliform bacteria has been isolated on west and north side of the lake (8800), while the smallest number has been identified in the stream that supplies the lake with surface water.

Coliform bacteria and fecal streptococci have been isolated in the samples of water, as well as *Pseudomonas sp. and Seratia species.* Results of colimetry indicate to water quality class II.

On Ramići Lake, three samples of water have been analysed: on north (L1), east (L2) and west (L3) side of the lake.

Table 6. Results of microbiological analysis of Ramići Lake water

Microbiological parameters	**Ramići Lake sampling sites**		
	L1	**L2**	**L3**
Total number of aerobic mesophyllic bacteria in a 1 ml	22	49	120
The most probable number of coliform bacteria in a 1000 ml MPN	1500	500	1500
Coliform bacteria of fecal origin (Eikman test)	-	-	-
Fecal Streptococci	-	-	-
Sulphite-reducing clostridia	-	-	-
Other identified bacteria	*Antrakoidi*	*Providentia* sp.	*Klebsiella* sp.

The microbiological analysis of Ramići Lake water shows that numbers of the aerobic mesophyllic bacteria in 1 ml range from 22 to 120. Good quality of Ramići lake water confirms the absence of streptococci and coliform bacteria of fecal origin. The most probable number of coliform bacteria in 1000 ml ranges from 500 to 1500, which indicates to water quality class I-II. With regard to microbiological parameters, the lake has a good potential for utilisation in sports-recreational purposes.

On Bešin Lake, three samples of water have been analysed: on east (L1), west (L2) and north (L3) side of the lake.

The microbiological analysis of Bešin lake water shows that the largest number of aerobic mesophyllic bacteria in 1ml has been isolated on east side of the lake (1940), and the smallest on north side (475). Of coliform bacteria of fecal origin *Escherichia coli* has been isolated, while the most probable number of coliform bacteria in 1000 ml of the examined water indicates to a water quality class I-II.

Table 7. Results of microbiological analysis of Bešin Lake water

Microbiological parameters	**Bešin Lake sampling sites**		
	L1	**L2**	**L3**
Total number of aerobic mesophyll bacteria in a 1 ml	1940	586	475
The most probable number of coliform bacteria in a 1000 ml MPN	220	1500	220
Coliform bacteria of fecal origin (Eikman test)	-	*Escherichia coli*	*Escherichia coli*
Fecal Streptococci	-	-	-
Sulphite-reducing clostridia	-	-	-

Results of research of physical-chemical analysis of water

The water of all researched lakes has neutral to weak alkaline reaction, whose pH value ranges from 7.8 to 8.3. The largest turbidity of water has been measured on Brestovica Lake (12 NTU) and Bešin lake (8 NTU), while the least values of turbidity have been recorded on Suhodanj Lake (1.5 NTU). The water of all researched lakes has a favourable oxygen regime; whose concentration ranges from 8.5 to 10.2 mg/l. Total nitrogen (N) is made of the sum of concentrations of nitrogen of inorganic and organic origin. The largest concentration of total nitrogen has been determined on Mušići Lake (1.46 mg/l) and Bešin Lake (0.42), while the smallest concentrations have been recorded on Suhodanj Lake (0.18 mg/l). Observed from aspect of total nitrogen, all lakes are of oligotrophic-mesotrophic character.

Table 8. Physical-chemical indicators of researched lake water in the period of June 2008

Item number	Indicators	Unit	Lake Suhodanj	Lake Požar	Lake Sjerkovača	Lake Brestovica	Lake Mušići	Lake Ramići	Lake Bešin
1.	Water temperature	°C	20	22	21	24	19	21	22
2.	Turbidity	NTU	1,5	2	3,4	12	2,5	3,5	8
3.	pH value		8,1	8,3	8,3	8	7,8	8,3	7,9
4.	Oxygen	mg/l	10	10,2	9,6	8,7	10,2	8,5	9
5.	Consumption of $KMnO_4$	mg/l	10,5	16,3	16,9	24	12,6	17,2	15,1
6.	Total nitrogen	mg/l	0,18	0,32	0,15	0,27	1,46	0,184	0,42
7.	Ammonium nitrogen	mg/l	0,16	0,31	0,1	0,22	0,59	0,16	0,31
8.	Nitrites	mg/l	0,018	0,013	0,016	0,007	0,424	0,004	0,06
9.	Nitrates	mg/l	0	0	0,03	0,04	0,45	0,02	0,05

DISCUSSION

Due to increasing anthropogenic influence, contamination processes of surface waters have been significantly accelerated lately, particularly under influence of waste and communal waters, which empty directly into the water recipients. Mine pit lakes, which most frequently represent the water gates of mine waters and waters from the surrounding settlements, are increasingly used by people in the function of bathing beaches or locations for sports and fishing.

All reasearched mine pit lakes are mostly supplied with water from precipitation and surface flows, which partially flow through inhabited places with fewer numbers of households. On some lakes the outlets have been formed by which permanent water level is maintained during the year, as in the case of Ramići Lake. Water level on Bešin Lake is constant as the water is drawn by pumps for needs of the open mining. However, on other lakes, water level is rather variable during the year, and the water flow depends on permeability and composition of the waste material by which the lake has been partitioned. So, Suhodanj Lake was partitioned by marlaceous limestone cover, while the other lakes have been partitioned by argillaceous marls, which are less permeable [5]. Weaker fluidity of water in lakes can be a cause of retention of a larger number of bacteria, which confirms poorer quality of water from Požar, Sjerkovača, Brestovica and Mušići lakes. On these lakes, along with coliform bacteria of fecal origin fecal streptococci have been also identified, which certainly indicates to recent water contamination by feces. The water of these lakes is epidemically dangerous and can be a sourse of various deseases.

On the other hand, much better condition has been found on the lakes of Ramići, Suhodanj and Bešin, where low values of total number of aerobic mesophyllic bacteria, as well as of coliform bacteria, have been determined. In Ramići lake water, no bacteria of fecal origin and sulphite-reducing clostridia have been identified, and the samples have low values of total number of coliform bacteria that are within the limits of water classes I-II. According to valid regulations on water categorisation in the Federation of Bosnia and Herzegovina [14], the first class is made of waters that are in natural condition, and can be used for drinking and in food industry by eventual desifection, and the surface waters can be used for growing purebred sorts of fish (salmonid), while the second class is made of waters that can be used in natural condition for bathing and recreation, for water sports, for growing other sorts of fish or which can be used, with usual measures of purification, for drinking and in food industry. As the works on arrangement of coast have already started on Ramići Lake, for the purpose of its utilisation as a bathing beach, results of microbiological analysis of water of this lake are encouraging and are in favour of realisation of this goal.

On the lakes of Suhodanj and Bešin, along with fewer numbers of aerobic mesophyillic bacteria and coliform bacteria, *Escherichia coli* have been isolated. Finding of this bacterium, as well as of other coliform bacteria that are in intestines of humans and animals, certainly indicates to presense of fecal contamination[7]. Contamination of these lakes with bacteria of fecal origin can be a consequence of rinsing the surrounding agricultural surfaces, which are manured with organic fertilizer, as the lakes are not in vicinity of inhabited places.

With analysing the physical-chemical parameters of water, it is noticeable that a larger number of aerobic mesophyllic bacteria comes in water samples with higher values of total nitrogen, so that the largest values of these two parameters have been recorded on Mušići Lake, and the lowest on Suhodanj Lake.

It is impossible to make a comparison of the results of microbiological analysis of water of the researched mine pit lakes in function of time, because no researches of microbiological nature have been conducted on these localities until now. Researches of bacteriological characteristics of the biggest mine pit lake are known in the Tuzla area (Šićki Brod Lake) [6] during 2005, in which coliform bacteria of fecal origin (*Escherichia coli*) have been isolated and where it has been determined that the most probable number of coliform bacteria ranged up to 2000/l. The finding of lonely bacterium *Escherichia coli,* without other bacteria indicators of fecal contamination, is regarded as a sign of temporally indefinite, but certain contamination of water with feces. In our researches, similar results were determined for the lakes of Suhodanj and Bešin.

Results of microbiological researches mainly correspond to results of saprobiological researches of phytobentos samples conducted in 2008 [5]. According to these resultats, Suhodanj, Požar and Bešin lakes on the ground of values of saprobity index have characteristics of oligo-betamesosaprobic level, while the othere examined lakes (Sjerkovača, Brestovica, Mušići and Ramići) have water of betamesosaprobic level of saprobity. Deviations and inversion in water categorization are noticeable for two lakes, Požar and Ramići, which may be explained by different approach in research methodology, as the phytobentos samples in the mentioned research were taken in summer period (August) on the spots differently covered with water vegetations.

CONCLUSION

Microbiological analysis of mine pit lake waters of the Tuzla coal-bearing basin shows that lakes of Suhodanj, Ramići and Bešin have water quality class I-II, while the other researched lakes have water quality class II.

The worst quality in Banovići coal-bearing basin has Mušići Lake and the best water quality, respectively the smallest number of colifom bacteria and aerobic mesophyllic bacteria have Ramići and Suhodanj lakes. In all lakes, except in Ramići Lake coliform bacteria of fecal origin have been isolated, which indicates to an evident negative anthropological influence on water quality of mine pit lakes. Sulphite-reducing clostridia have not been isolated in any of these localities.

Worse status of the lake water regarding microbiological contamination may be a consequence of increased anthropological influence and nonexistence of the arranged sewer network. Weak fluidity of water in the researched lakes, particularly in summer period, can also cause longer retention and proliferation of bacteria in water. In this regard, responsible institutions and the managing companies should pay more attention to establishing the overflow systems on existing mine pit lakes at minimum water level, in order to ensure higher fluidity of water in the summer period.

REFERENCES

1. S. Barudanović, J. Kamberović, Potentials of tourism and environ-ment of Bosnia and Herzegovina – restauration of the deserted open minings. International conference proceedings ‘The protected areas in function of sustainable development’. Fram Ziral, Bihać, 2008, p. 497 – 507.
2. S. Smajić, Geographic aspect of surface coal exploitation in the area of Tuzla basin. Master thesis. Faculty of Science, University in Tuzla, 2007.
3. D. Pašić-Škripić, I. Žigić, A. Adilović, Study on prognosis of surface waters balance in the upper river basin of the Turija River. Faculty of Mining-Geology-Civil Engineering, University in Tuzla, 2008.
4. E. Velagić-Habul, M. Bašagić, E. Omanović, Influence of waste banks of open minings on quality of running waters. The works of Faculty of Agriculture of the University in Sarajevo, L. 56. 2005.
5. J. Kamberović, Anthropogenic marsh habitats as conservation potential of the Tuzla area, Master thesis, Faculty of Science, University in Sarajevo, 2010.
6. M. Amela, Bacteriological character-ristics of lake waters in the area of Tuzla canton. Veterinaria, 58, 3-4, Sarajevo, 2009, p.219-228.
7. M. Hukić, Bacteriology, Jež, Sarajevo, 2005, p. 305-309.
8. Maps of aerophotogrammetric survey 1973. The Đurđevik coal-bearing basin, Institute of Photogrammetry, Belgrade.
9. Regulations on sampling and methods for laboratory analysis of drinking water. Official Gazette SFRJ, 33/87, p. 856-873.
10. B. Poček, Methods for bacteriological, virological and parasitological examination of drinking water, Belgrade, 1990, p.628-640.
11. B. Karakašević, Handbook manual of methods for microbiological routine work. Medicinska knjiga Belgrade-Zagreb, 1967. p. 1453-1489.
12. S. Duraković, Food microbiology. Medicinska Naklada, Zagreb, 2003, p.15-21.
13. D. A. Eaton, L. S. Clesceri, E.W. Rice, A. E. Greenberg, Standard methods for the examination of water & wastewater, American Public Health Association, American Water Works Association, Water Enviroment Federation, 2005, 9,34-9,36.
14. Regulation on classification of waters and waters of coastal sea of Yugooslavia within boundaries of SR B&H "Official Gazette SR BiH" 19/80, p. 853-855.

SUSTAINABLE TREATMENT OF UNDERGROUND COAL DEPOSITS AS A CO_2 SEQUESTRATION POTENTIAL

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

The presented best practise case of reducing GHG footprint of Šalek Valley is a transparent example applicable also to the landscape units with the similar geological and infrastructure (very close location of the CO_2 resource and underground coalmine) conditions. The sustainable aspect of the exploitation of the biggest Slovene non-renewable lignite stock is set out in the paper. We also discuss the potentially produced electricity and the potentially emitted CO_2 as one of the main environmental issues regarding the Kyoto and other European GHG legislative documents when the researched lignite deposit would be used in the nearest Thermal power plant (TPP) Šoštanj. The experiment was carried out in 2004 to estimate the suitability of the coal bed studied for CO_2 storage whereas in 2008 it was upgraded with the study of permeability of Velenje coal seam for CO_2.

Key words: sustainable approach, reducing CO_2 emissions, underground resources, Velenje Coalmine

INTRODUCTION

The material goods of the natural environment (raw materials and energies) are defined as natural sources (air, water, soil, ground, minerals, ores…), when a man recognizes them as useful and starts to use them according to the human race, the technical and the economic level of development – all three kinds of development determine the value of the source. When there is no knowledge and no technical method to extract the natural source and no demand for the material and services, the environmental components remain ‘’the neutral goods’’ (Rees, 1990).

Contemporary definitions distinguish between non-renewable and renewable natural sources, the landscape (Haggett, 2001) and the ecosystems’ services (the nutrient cycles, the balance of gases, climate and water). So the concept of natural sources includes all the environmental sources (Plut, 2004). We can also talk about environmental capital which forms, together with the man made and human capital, the complexity of landscape structure.

$$\boxed{LS = EC+MC+HC}$$

LS=landscape structure; all landscape sources/capitals
EC=environmental capital (biodiversity, water, ground, material goods)
MC=manmade capital (buildings, machines, infrastructure)
HC=human capital (knowledge, skills)

Figure 1: The complexity of landscape structure (after Pearce et al., 1994; Freedman, 1995)

The non-renewable sources are environmental components, which take shape slowly in geological history. When we consider them anthropocentrically (from the aspect of their usefulness to man) we can talk of stock, namely, quantitatively limited (finite) sources. The area researched had the mineral raw material lignite naturally present in it. It became a natural source at the end of the19th century when it was discovered and started to be used in energy production. But the awareness of the non-renewable characteristics of the underground natural source has appeared only recently, when global nature and its sources have run short so intensively that the self-purifying capabilities of the natural ecosystem's structure have been exceeded. After Plut (2004) a man became a 'geological power' and we can already see the catastrophic consequences of nature degradation because the ecosystem's benefits (goods and services) have not been considered and evaluated properly. They used to be regarded as self-evidently available and free of charge.

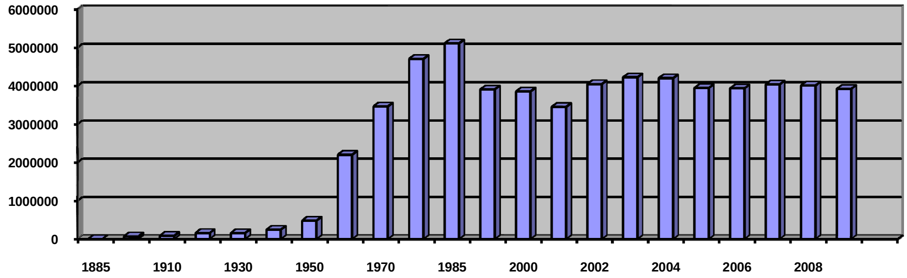

Figure 2: Lignite excavation in the Šalek Valley (in tons) (Source: Website 2)

The location between two faults determined the landforms of the Velenje basin. The area was subsiding in the Pliocene. The sedimentary area was mainland firstly, but later on changed to swamp and lake area (lush vegetation altered to lignite), which finally dried to land again (Brezigar, 1987). The geological background section shows some 200 m of uppermost layer, more than 100 m of lignite seam and around 400 m of lowermost layer. The Pliocene sediments are mostly of clays, silts and sands. The thickness of the lignite layer is outsize considering even the global scale. Its maximum exceeds 160 m whereas the average is approximately 60 m. Much less lignite settled right below the surface and was encountered by river Paka during its erosion.

The discovery of lignite in the river basin resulted in many (drilling) researches that have been taking place since 1873. The shape, dimensions, quantity and quality of the lignite seam have been well stated.

The research on lignite stock established that after 119 years 200 Mt tones of lignite have been excavated (Figure 2), and another 580 Mt tones of lignite stay settled under the surface of Šalek Valley. After up-to-date plans some 80 Mt tones of lignite will be removed, so around 500 Mt tones of lignite will be kept for our progeny (Speh, Veber, 2006).

The Velenje coalmine implemented a project of Clean Coal Technology (CCT). The possibility of using coal bed methane (CBM) is one of these CCT projects. A research has been carried out starting from the year 2001. In 2001, the content of methane was measured on existent vertical wells at the Velenje coalmine. In 2002, an estimation of quality and quantity of emitted gas from horizontal and cross-measure wells was performed. Gas samples were taken from three different locations: coalseam at the G plate, during mining at area –20a and at collapsed area -90c (Zapušek, Kozinc, 2003). In the year 2003 the measurements of gas quality from horizontal wells at the G plate and at the collapsed area were continued. The gas was of medium quality and methane content was between 33 and 70 % (Zapušek, Kozinc, 2004).

In 2004 an experiment was performed on sequestration of carbon dioxide (CO_2) in the coal seam. The aim of the experiment was to estimate the suitability of the coal bed for storage of CO_2 released during combustion of coal and the usage of subsequently released CH_4 (Orešnik, 2005). The wells were 3000 m far from the mine entrance. The first three wells were 6 m apart from each other, the other three 3 m. In the wells the amounts of CH_4, CO_4, dimethylsulfide, oxygen, carbon monoxide and hydrogen, as wel las flow, humidity and temperature of desorbed gases were measured. CO_2 was sequestered in the second and fifth well for four hours. In the remaining wells desorbed gases were sampled in 2 l tedlar bags. During sequestration of CO_2 gases were sampled from the wells every half hour. Eight weeks after the experiment had been performed, the composition of gases was remeasured.

The sequestration of CO_2 in coal seams was seen as a possible way to mitigate the rising atmospheric concentrations of CO_2 (Reichle et al. 1999). One of the earliest studies of the adsorption of CO_2 on coal used BET equation to calculate the CO_2 surface areas of anthracites (Walker and Geller, 1956). That the diffusion of CO_2 through coals of various ranks is an activated process was established not long afterward (Nandi and Walker 1956). The adsorption of CO_2 and other gases, especially methane, has also been studied in an effort to increase the safety of coal mining (Kim and Kissell 1986). Recently, CO_2 adsorption on coal has been studied as means of enhancing the gas production in coal bed methane projects. Burlington Resources, the largest producer of coal-bed methane, has been injecting CO_2 in the USA' San Juan basin, New Mexico to enhance methane production since 1996 (Stevens et al. 1998).

MATERIALS AND METHODS

Sampling

In 2004 an experiment was performed on sequestration of carbon dioxide (CO_2) in coal seam. The wells were 3000 m far from the cave's exit. The first three wells were 6 m apart from each other, the other three 3 m.

In the wells the amounts of CH_4, CO_2, dimethylsulfide, oxygen, carbon monoxide and hydrogen, as well as flow, humidity and temperature of desorbed gases were measured (Figure 3). CO_2 was sequestered in the second and fifth well for four hours. In the remaining wells desorbed gases were sampled in 2 l tedlar bags. During sequestration of CO_2, gases were sampled from the wells every half hour. Eight weeks after the experiment had performed, the composition of gases was remeasured. In Figure 3, the situation in the mine where the experiment of CO2 sequestration was performed is presented.

Figure 3: Situation in the mine where CO_2 sequestration was performed

The collected gases during the experiment and the calibration gases were analysed under the same conditions in the ERICo laboratory.
The calibration curves were linear; the limits of detection were 0.01% for CH_4 and CO_2, 1.0 ppm for dimethylsulfide (DMS) and hydrogen (H_2), 0,5 ppm for carbon monoxide (CO), and 0,5 % for oxygen (O_2). The measured components were determined by methods validated under ISO 17025 requirements (Kozinc, Zupancic 2002).

GC analysis

The gas samples were analysed using a Perkin Elmer AutoSystem XL chromatograph. A 100-μL loop connected to a 6-port gas sampling valve for injection of gas samples was used. Behind the injector, the sample was delivered into two capillary columns of the same dimensions. CO_2 and CH_4 were separated on a Carboxen 1006 PLOT capillary column, 30 m in length (530 μm id.), connected to a flame ionisation detector (FID) equipped with a Zr/Ni catalytic reactor. The catalytic reactor reduced CO and CO_2 to CH_4, which could be detected at considerable lower concentrations by FID. A Supel Q PLOT capillary column was used for separation of DMS. This column was connected to a Flame Photometric Detector (FPD), which is a highly sensitive and selective detector for sulphur compounds and is appropriate for detecting low concentrations of DMS. The other hydrocarbons and sulphur species were not determined at the operating conditions, which were as follows:

Carrier gas: helium flow: 8 ml min-1
Gas sampling valve on: 0.01 min to 0.05 min
Oven temperature (start): 50°C, 1 min
Heating rate: 18°C min-1 to 170°C
Detectors: FID with catalytic reactor ZR/Ni, FPD
FID: Air flow: 400 ml min-1, Hydrogen flow: 40 ml min-1
FPD: Air flow: 100 ml min-1, Hydrogen flow: 75 ml min-1
Messer’s secondary gas standards of various concentrations (as expected in our samples) for each gas component were used for qualification and quantification.

Electrochemical analysis
Concentrations of CO, H_2 and O_2 were measured by electrochemical sensors, built into a special device, made by the ECHO Company, Slovenia. With this device it is possible to measure concentrations of CO and H_2 from 1 to 500 ppm and O_2 from 0.5 to 30 %.

RESULTS AND DISCUSSION

In gas desorbed from the wells after sequestering CO_2, the amount of methane reached the value of 78,2 %, which is essentially higher than in previous research, when the amount of methane was on average between 55 and 65%. It was shown in the experiment that the methane concentration increased during the experiment while the CO_2 concentration remained at the same level (18%).

During the CO_2 sequestering the gas flow from the production wells was between 24 and 33 l/h. In the weeks after sequestering, the flow from the production wells was in general lower (22 l/h) than it was on the day of CO_2 sequestering.

In Figure 4 the numbers of samples with a certain concentration of methane are presented. The most frequent methane concentration was from 70 to 75 %. In theprevious investigations (in the period 2001 – 2003) only three samples reached this concentration level on the G plate.
The average concentration of methane during CO_2 sequestering was 71% and 69%

eight weeks after sequestering. The average content of methane was higher in this experiment than it was in previous investigations, where the average content of methane was 61%. In the wells which were at a distance 3 m from the central well where CO_2 was sequestered, higher individual (78 %) and average concentrations (74 %) of methane were determined in comparison to those wells, which were at a distance of 6 m from the central well (75 % in the individual samples and 70 % on average). The measurements confirmed the data from literature, where the optimal distance between wells from the central well is 3 m.

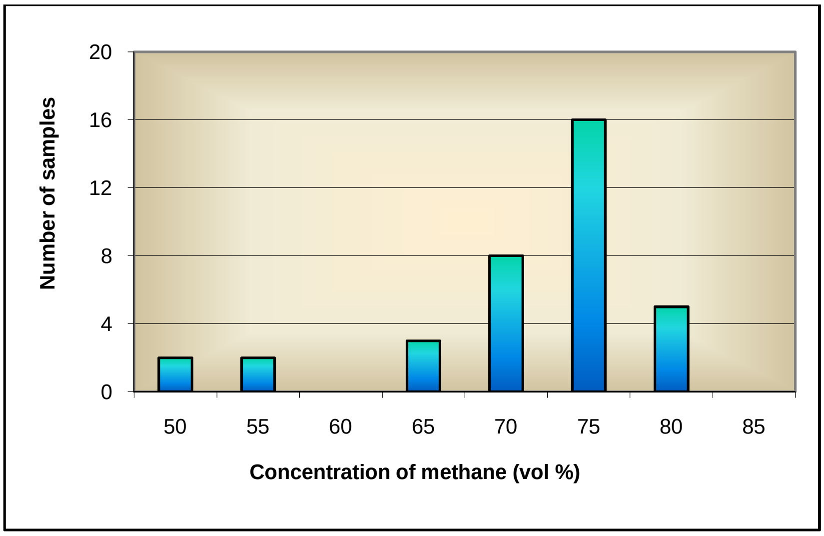

Figure 4: The number of samples, containing methane in the interval from 50 to 80 % during CO_2 sequestering in the wells jv 3242-II/04 and jv 3243-II/04

The higher concentrations of methane in released gases were the consequence of CO_2 sequestering in the coal seam. Regarding the fact that a released molecule of methane needs two to three molecules of carbon dioxide (Hamelinck, 2001) and 150 kg of the sequestered amount of carbon dioxide, between 18 to 27 kg (25 – 40 m3) of methane were released. From the results determined in the experiment, 10 – 15 kg of methane were daily released from wells jv 3242-I/04, jv 3242-III/04, jv 3243-I/04 and jv 3243-III/04. If the released amount of methane remained at the same level for the whole year it would mean 3,5 – 5,5 t of released methane of medium quality.

The average concentration of carbon dioxide was 17 % during sequestration, which increased to 18 % in the two-month interval after sequestration. The determined concentrations of CO_2 and O_2 from the four wells in the experiment are comparable to the CO_2 and O_2 concentrations from the investigations from the G plate in previous years (Speh et al, 2007).

EXPERIMENT IN 2008

The aim of the experiment in the year 2008 was to check the characteristics of unbroken layer of very qualitative lignite of Velenje Coal Basin as the potential geological reservoir, that would suit the CO_2 storage demands. The indicators' list was established at the basis of similar researches worldwide and is assumed to present a realistic estimation on suitability of the reservoir conditions (Justin et al., 2008).

The experiment was carried out as an Workpackage 1 activity of 6th Frame Programme European Project MOVECBM and was lead by Shell International Exploration & Production Netherlands. It means an upgrade of the 2004 findings and realizations. Final results should give the data on the lignite layer permeability for CO_2 in Velenje Coal Mine.

The experiment was formed of one injection and three production wells of 30 m length and of 40° incline. As at the 2004 experiment the monitoring was focussed on the CH_4 and CO_2 concentrations in the production wells as the consequence of the CO_2 sequestration; 60 hours after the experiment had started the CH_4 maximum concentration values wells exceeded 60 % whereas the maximum concentration values of CO2 were around 40 % in all three production wells (Orešnik et al., 2008).
Guidelines: the use of methods to increase of the coalseam permeability for CO_2

CONCLUSIONS

The sequestration of CO_2 in the coalmine is one of the possibilities for CO_2 (re)use. In the year 2004 an experiment was performed on sequestration of carbon dioxide in coal seam.
While CO_2 has a higher adsorption affinity than CH_4, it helps to desorb CH_4 from the coal. For the experiment, wells 3242-II/04 and 3243-II/04 on the G plate were used.
The composition of desorbed gases was measured from wells 3242-I/04, 3242-III/04, 3243-I/04 and 3243-III/04. The amount of methane was between 60 and 78%, which was essentially higher than in previous research. Gas desorbed from these four wells was still of medium quality and can be used for electricity production or for space and water heating on-site.
When Thermal power plant Šoštanj installs a gas turbine, the degasification system in Velenje coalmine will be an interesting option due to coal bedmethane use in Thermal power plant Šoštanj.

Table 1: Main points and outcomes of the discussed project activities

PERIOD/PROJECT TOPIC	GOALS	RESULTS
Until y. 2003 CBM projects	To get the information about the methane quality and quantity in the natural state regarding the GHG impacts in landscape and security of the underground minning (the possibilities of the gas outburst forecasting)	Data on methane quality and quantity in the natural state
Y. 2004-2005 ECBM projects; ERICo and Velenje Coalmine cooperation	The experiments of sequestering of CO_2	The ECBM process takes place; methane is produced at 40° inclination of the wells
Y. 2006-2008 Cooperation with partners in the 6th European Framework programme project MOVECBM	• To research the best conditions where CH_4 would be released with the most qualitative characteristics • Micropilot test, that would conect the laboratory research and field test of CO_2 sequestration	• Low permeability of the coal seam • ECBM takes place with the delay

The other possibilities for CO_2 usage are also in the food and chemical industries, in agriculture, for extinguishing fire, for production of dry ice or it can be used as a solvent at supercritical conditions.

CO_2 is the most important anthropogenic greenhouse gas. Over the past 2000 years its concentration in the atmosphere has increased from 0,0275% to 0,0370%, mostly as a result of the combustion of fossil fuels (Website 3). Šoštanj power plant (PP) as the largest Slovene thermal power plant in the vicinity of Velenje coalmine is known to be the biggest source of CO_2 emissions in Slovenia (Figure 5). Since approximately 4 Mt of the lignite coal per year is used in Šoštanj PP it also emits CO_2 every year. Although they have already installed the desulphurisation devices which decreased the sulphur pollution by 90% the problem of NOx and especially the CO_2 emissions remain (Speh et al, 2007). The data of the 2008 experiment will be used for the computer modeling of the CO_2 permeability of the Velenje coal seam.

The emitted value of carbon dioxide in the Šalek Valley in 1990 was 3.782.023 tons or 94.551 kg per capita. In the same year there was a Slovene average value of 6800 kg, and European one of 9216 kg per capita (Rode, Šušteršic, 1997).

In 2002 the Šalek Valley still had a very high value of 96795 kg per capita. In fact the emissions trend points to an increase in CO2 pollution (Table1), also in Slovenia in a general way.

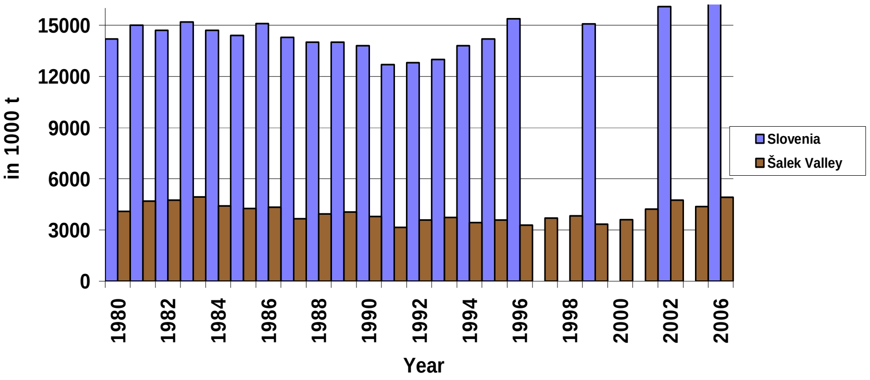

Figure 5: CO_2 emissions in Slovenia and in the Šalek Valley for the period 1980-2006 (data for the years 1997, 1998, 2000, 2001, 2003 and 2006 is also available just for Šalek Valley)
(Sources: TPP Annual Report, 2003; Ministry of environment and energy Republic of Slovenia: http://www.gov.si/mop/zakonodaja/zakoni/okolje/op_zmanjsevanje_toplog_plinov.pdf)

Slovenia has ratified the Kyoto protocol regarding the United Nations Framework Convention on Climate Changes in the year 2002. With the ratification we agreed on decreasing GHG emissions in the period 2008 – 2012 by 8% compared to the 1986 emissions.

In 1999 CO_2 presented 80% of GHG emissions,mostly because of fossil fuels combustion.

Although the Slovenian government passed a law on Order about taxes for air pollution with CO_2 (Ur. l. RS 91/2002, 8/2003, 67/2003), the data for 2002 show an increase in CO_2 emissions (Figure 5 and Table 2). It could be a sign that CO_2 emissions license trading does not operate in the appropriate manner, namely the polluters in Slovenia payed less than 10 EUR/t CO_2 in 2008, when the estimated price for the 2nd Kyoto Protocol period 2008-2012 will be between 20 and 21 EUR/t CO_2. The uncertainties and obstacles at the emissions' trading from the 1st Kyoto

Protocol period have already been discussed, also a higher penalty of 40 – 100 EUR is expected for each additional ton of CO_2 not covered by the emission coupon (Website 1). Recently, the groups of Climate Change 'deniers' have been organized. They present all the CO_2 emissions issues as a natural process and deny the scientific proves of anthropogenic reason for global climate changes.

But still, the idea of the external costs' concept in energy management should be accepted, since we want to follow the sustainable development paradigm. The ecosystem's goods and services should not be free of charge. After all we can see more and more biota responses originate from devalued environment resources, degraded health and climatic conditions. It has been already published about the limits of the ability of our current technology to provide solutions to energy supply and global warming as we attempt the transition to a carbon neutral civilisation (Moriarty, Honnery, 2010).

And more challenges will happen to be cope with in the post Kyoto period 2012-2020, because the emission trade will get new dimensions and climate changes, too.

Table 2: The Šoštanj TPP emissions at turning points (in 1000 tons)

YEAR	**1983** (the record year)	**1991**	**1995** (after the first desulp. device)	**1999** (the second desulph. device was built)	**2000**	**2003**	**2006**
CO_2	4931	3143	3581	3335	3596	4366	4906

(Source: Website 4)

ACKNOWLEDGEMENT

We wish to thank the staff of the Coal Mine for giving us the permission and help to prepare the experiment's area, sample the holes and to monitor the gases after the sequestration. Special thank also to the technical support staff of ERICo who carried out the measurements of gas flows. The financial support of the Šoštanj Thermal Power Plant and Velenje Coal Mine are gratefully acknowledged.

REFERENCES

1. Brezigar, A. 1987. The lignite layer of Velenje Coalmine Company. Geologija 28/29. Ljubljana, pp. 319-336.
2. Freedman, B., 1995: Environmental Ecology, The Ecological Effects of Pollution, Disturbance and Other Stresses, Second edition, Academic Press Limited, London, pp 606.
3. Haggett, P. 2001: Geography – a Global Synthesis. Prentice Hall, Harlow, pp 633.
4. Hamelinck c.n. Potencial for COs sequestration and Enchanced Coalbed Methane Production in the Netherlands. NOVEM

Programme Nr. 234.1., Uterecht, March 2001.

5. Kim A.G. and Kissell F.N. 1986: Methane formation and migration in coalbeds. Methane Control research: summary of results, 1964-80.

6. Kozinc, Janez, Zupancic-Kralj, Lucija. Validation of the gas chromatographic method for determination of methane, carbon dioxide and dimethylsulphide in coalmine atmosphere. V: VEBER, Marjan (ur.). 4th Mediterranean Basin Conference on Analytical Chemistry (MBCAC IV), Portorož, September 15-20, 2002. Book of abstracts. Ljubljana: Slovensko kemijsko društvo, 2002, 2002, pp 230.

7. Justin, B., Orešnik, K., Mazej, Z., 2008: The possibilities of CO2 storage on Šalek Valley, ERICo d.o.o., DP 341/03/08.

8. Ministry of environment and energy Republic of Slovenia, 2003;
http://www.gov.si/mop/zakonodaja/zakoni/okolje/op_zmanjsevanje_toplog_plinov.pdf

9. Moriarty, P., Honnery D., 2011: Rise and Fall of the Carbon Civilisation, Resolving Global Environmental and Resource Problems. Series: Green Energy and Technology. Springer.

10. Nandi S.P. and Walker, Jr.P.L. 1965: The diffusion of nitrogen and carbon dioxide from coals of various rank, Fuel 44, pp 757-764.

11. Orešnik K. 2005 : Use of coal bed methane. Report for year 2004. ERICo Velenje DP 41/03/05.

12. Oresnik K., Justin, B., Mavec, M., 2008: Research studies on incidence and exploitation of gases in Velenje Coalmine. International Workshop, Velenje Coal Mine.

13. Pearce, D., Turner, R., K., O'Riordan, T., Adger, N., Atkinson, G., Brisson, I., Brown, K., Dubourg, R., Fankhauser, S., Jordan, A., Maddison, D., Moran, D., Powell, J., 1994: Blueprint 3, Measuring Sustainable Development, Earthscan Publications, Ltd London, pp 224.

14. Plut, D. 2004: Zeleni planet?. Population, energy and environment in 21st century. Didakta. Radovljica, pp 239.

15. Rees, J. 1990: Natural Resources, Routledge, London – New York, pp 499.

16. Reichle D. Houghton J., kane B., Ekmann J., Benson S., Clarke J., Dahlman R., Hendry G., Herzog H., Hunter-Cevera J., Jacobs G., Judkins R., Ogden J., Palmisano A., Socolow R., Stringer J., Surles T., Wolsky A., Woodward N., York M., 1999: Carbon Sequestration Research and Development, U.S. Department of Energy Report DOE/SC/FE-1.

17. Rode, B., Šušteršic A., 1997: The role of thermal power plants at the air pollution in Slovenia and the comparison to Europe. Rogaška Slatina, Slovenija, 28. - 30. May 1997, pp 319-327.

18. Stevens S.H., Kuuskraa V.A., Spector D., Riemer P. 1998: CO2 Sequestration in Deep Coal Seams: Pilot Results and Worldwide Potential. GHGT-4 Conference Interlaken, Switzerland.

19. Špeh, Natalija, Orešnik, Klara, Kozinc, Janez. The emmissions of CO2 as the chance for the sustainable management with natural sources. Acta chim. slov., 2007, vol. 54, no. 2, pp 392-396

20. Špeh, N., Veber, I., 2006. The evaluation of nonrenewable sources of Šalek Valley from the aspect of sustainable regional development. Šalek and Savinja Valley. [Proceedings]. Velenje: Erico, Inštitut za ekološke raziskave, 2006, pp194-211. [COBISS.SI-ID 728022]

21. Thermal power plant (TPP) Annual Report, 2003. Editors: Rotnik, U., Ribaric-Lasnik C.. Šoštanj.

22. Zapušek, A., Kozinc, J., 2003: Use of coal bed methane. Report for year 2002. ERICo Velenje DP66/03/03.

23. Zapušek, A., Kozinc, J., 2004: Use of coal bed methane. Report for year 2003. ERICo Velenje

24. Walker, Jun. P.L. and Geller I. (1956): Change in surface area of anthracite on heat treatment. Nature 178, 1001.
25. Website 1:
http://www.focus.si/index.php?node=156
26. Website 2:
http://www.rlv.si/default.asp?id=95
27. Website 3:
http://www.iea.org/Textbase/stats/index.asp
28. Website 4: www.te sostanj.si /filelib/ cd_finalpopravki.pdf

STABILITY OF α-THUJONE AND ß-THUJONE IN BITTER LIQUER "PELINKOVAC"

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

Since 19th century, a controversial discussion is going on about thujone, a characteristic component of the essential oil of the wormwood plant *Artemisia absinthium L.*, *Salvia officinalis* and other, which are commonly used in production process of alcoholic beverages such as wormwood flavored spirit or bitter liqueur. After consuming alcoholic beverages, thujone is considered to be responsible for possible mental disorder, including the symptoms of hallucinations, sleeplessness and convulsions.

In order to define the stability of α-thujone and β-thujone this research has been conducted by using Croatian traditional bitter liqueur "Pelinkovac". The results showed that the portion of α-thujone and β-thujone in Croatian traditional bitter liqueur "Pelinkovac". decrease as product becomes more mature. Portion of α-thujone decrease from 24,52 -24,53 mg/l a.a., to 15,81 -22,09 mg/l a.a. after first year and to 14,03 -19,97 mg/l a.a. after three years. Content of β-thujone decrease from 24,38 – 24,39 mg/l a.a., to 15,83- 21,98 mg/l a.a. after first year and to 14,13 - 20,03 mg/l a.a. after three years. More significant decrease has been recorded if samples were kept in oak barrels in comparison to decrease which happened when samples were kept in stainless steel barrels or commercial glass bottles.

Key Words: α-thujone, β -thujone, bitter liqueur

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INTRODUCTION

Thujone is a chemical substance naturally found in a number of aromatic plants like *Artemisia absinthium*, *Salvia officinalis* and others, which are commonly used in production process of alcoholic beverages, such as wormwood flavored spirit or bitter liqueur.

Thujone is a bicyclic monoterpene ketone that occurs in two stereoisomeric forms: α-thujone and β –thujone[1].
This substance fell under scrutiny at the beginning of the 20th century due to its association with the adverse effects following the consumption of the wormwood flavored spirit *absinthe*[2,3]. Symptoms of so-called "absinthism" included convulsions, blindness, hallucinations and mental deterioration[4]. Absinthe and the use of wormwood extracts for food purposes were prohibited around the years 1910 - 1920 in many countries[5].
In 1979 the Codex Alimentarius Commission proposed the following maximum thujone limits in food and beverages: 0.5 mg/kg for ready-to-eat foods and beverages in general; 5 mg/kg in alcoholic beverages containing less than 25% vol; 10 mg/kg in alcoholic beverages above 25% vol; 25 mg/kg in food containing sage; 35 mg/kg in bitters and 250 mg/kg in sage stuffing’s[6]. The Codex Alimentarius proposal was introduced into the European Union law in 1998, which re-legalized the production of *absinthe* from wormwood as well as the food use of other thujone-containing plants. European regulation recently has been amended to maximally 35 mg/kg for all *Artemisia*-derived alcoholic beverages[7]. However, the specific limits for sage preparations and the general limit for foods were removed from the regulation.
Majority of companies that produces branded alcoholic beverages and bitter liqueur use old recipes in the production process as well as tested ingredients which have been used as medicinal substance for centuries before. So it is possible to find unacceptable level of α-thujone and β - thujone in modern branded products[8,9]. Because of that it is very important for modern production process to define maximum α-thujone and β-thujone in the final product and to find suitable technological procedure to reduce their level to acceptable one, without changing the original recipes.
The aim of this research was to define the level and stability of α -thujone and β - thujone in Croatian traditional bitter liqueur “Pelinkovac”.

MATERIALS AND METHODS

Each set of samples of Croatian traditional bitter liqueur “Pelinkovac” are taken from the production process at “Zvečevo d.d. Food Industry”, in period from 2006 to 2010. Sample preparation for GC analyses was done using liquid–liquid extraction with 1,1,2-trichloro-1,2,2-trifluoroethane after a method of Rapp et al[10].

GC analyses

Gas chromatography (GC) analyses were performed on a Chrompack 437A gas chromatograph with a CTC Combi PAL auto sampler, split/splitless injector and an FID detector. Data acquisition and analysis were performed using standard software supplied by the manufacturer. Substances were separated on a fused silica capillary column (HP-Innowax, 60 m _ 0.25 mm i.d., film thickness 0.25 mm). Temperature program:

45°C hold for 1 min, 5°C/min up to 180°C, 25°C /min up to 240°C, hold for 5 min.

The temperatures for the injection port, ion source, quadruple and interface was set at 220, 230, 150 and 250°C, respectively. Split/splitless injection mode (1 ml, split ratio 5:1) and helium with a flow rate of 1.0 ml/ min as carrier gas was used.

Qualitative analysis of α-thujone and ß-thujone was done by comparing the standard retention times (analytical grade from "Merck", Darmstadt, Germany) with the corresponding peaks of samples. Calibration curves were constructed and quantification was carried out by comparing the peak areas to those of the Merck standards.

All analyses were performed in duplicates, and average values were used in further data elaboration. Concentrations were expressed in mg/l of absolute alcohol (mg/l a.a.).

RESULTS AND DISCUSSION

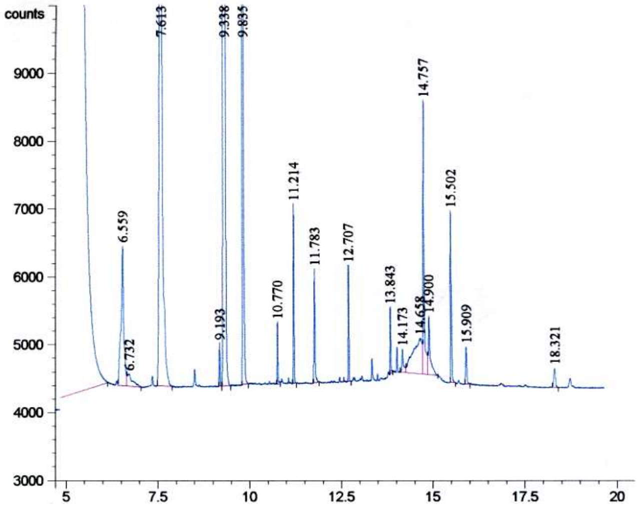

Figure 1. Chromatogram of *α-thujone* and *β -thujone* for **S 1,** (starting day, *α-thujone* 11.783 and *β –thujone* 12.707).

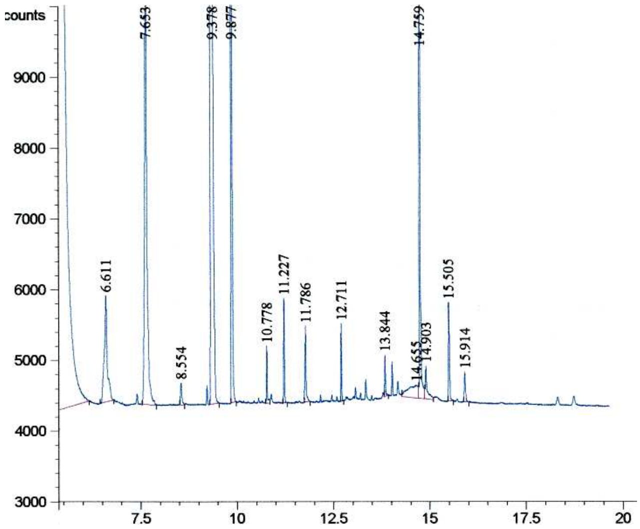

Figure 2. Chromatogram of *α-thujone* and *β –thujone* for **S 2** after 30 days in Oak barrels (*α-thujone* 11.786 and *β –thujone* 12.711)

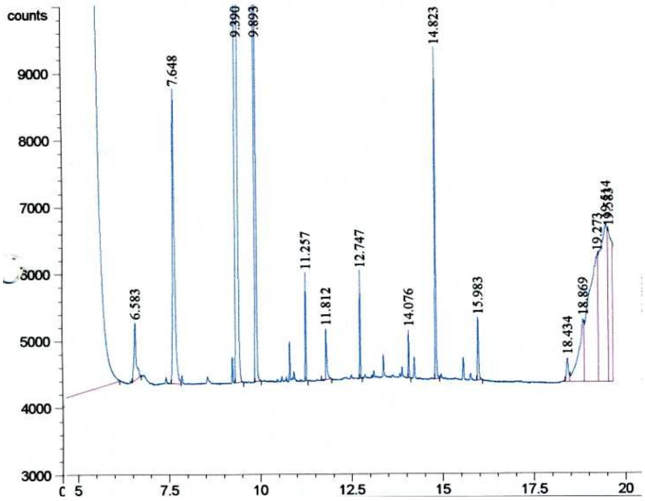

Figure 3. Chromatogram of *α-thujone* and *β –thujone* for **S 3** after 1 year in Oak barrels (*α-thujone* 11.812 and *β –thujone* 12.747)

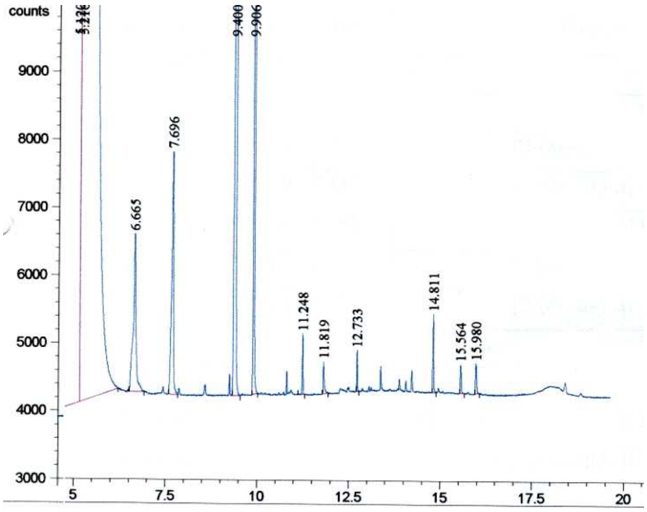

Figure 4. Chromatogram of *α-thujone* and *β –thujone* for **S 4** after 3 years in Oak barrels (*α-thujone* 11.819 and *β –thujone* 12.733).

Figure 1, 2, 3, 4 and Table 1 shows the level of α-thujone and β-thujone in Croatian traditional bitter liqueur "Pelinkovac" and the way it changes during the process of maturation. Beside that, in table 1 is visible how the level of α-thujone and β-thujone in Croatian traditional bitter liqueur "Pelinkovac" changes, if product is kept in oak barrels, stainless steel barrels or in commercial glass bottles.

It was possible to predict that the rate of α-thujone and β-thujone will faster decrease because of ultraviolet light irradiation in commercial glass bottles[4,11]. The results of this research show that there wasn't any significant difference between samples. It is probably because the research team conducts analyses during extreme values of ultraviolet radiation[4].

So it's possible to compare the results of this research with results of similar experiments which prove stability of α-thujone and β-thujone in very old samples of commercial products[12].

Table 1. Concentration means ± standard deviations of α-thujone and β-thujone in different sample fractions of Croatian traditional bitter liqueur "Pelinkovac".

Samlpe	**Oak barrels**		**Stainless steel barrels**		**Commercial glass bottles**	
	α-thujone	***β-thujone***	***α-thujone***	***β-thujone***	***α-thujone***	***β-thujone***
S 1 (starting day)	24.52±0.03	24.38±0.03	24.53±0.09	24.39± 0.31	24.52±0.13	24.38±0.07
S 2 (after 30 days)	21.14±0.04	20.96±0.01	24.44±0.05	24.31±0.09	24.46±0.01	24.36±0.09
S 3 (after 1 year)	15.81±0.14	15.83±0.03	22.32±0.22	21.98±0.10	22.09±0.31	21.73±0.18
S 4 (after 3 years)	14.03±0.23	14.13±0.03	19.97±0.06	19.97±0.13	19.83±0.08	20.03±0.41

*All analyses were performed in duplicates. Concentrations were expressed in mg/l of absolute alcohol (mg/l a.a.).

The results (Table 1) also show that no significant difference could be established between the stability of α- and β-thujone, which is in line with previously published results[13].

In this research, the only significant difference was noticed when samples were kept in oak barrels, when the rate of both isomers, α-thujone and β-thujone has fallen straight after first year from starting rates 24.52 – 24.53; 24.38 – 24.39 ml/l a.a to 15.81 - 22.32; 15.83- 21.98 ml/la.a (Figure 1, 2, 3 and 4). These results are in line with the results described by Fröhlich and Shibamoto[14], Skurihin[15], Singleton[16]. In alcohol beverages some substances content changes because of natural oxidation process in oak barrels[15,16].

CONCLUSIONS

According to the results of this research it can be concluded that there isn't significant difference in stability of α-thujone and ß-thujone during maturity period of Croatian Traditional biter bitter liqueur "Pelinkovac".

If the product is kept in oak barrels, the levels of α and β thujone would be significantly reduced after one year of storage.

REFERENCES

1. Stapleton, M. Absinthe and thujone. Chem. Eng. News 86 (2008) 9.
2. D.W. Lachenmeier, Thujone's properties. Chem. Eng. News 86 (2008) 7–8.
3. L. Kroner, S.A. Padosch, M.S Bruckner, B. Madea, XIII GTFCh-Symposium: Ausgewa¨ alte Aspekte der Forensischen Toxikologie, Verlag Dr. Dieter Helm, Heppenheim, Germany, (2004) pp 354–360.
4. D.W. Lachenmeier, J. Emmert, T. Kuballa, G. Sartor, Thujone's cause of absinthism, Forensic Sci. Int. 158 (2006) 1–8.
5. S.A Padosch, D.W Lachenmeier, L.U. Kröner, Absinthism: a fictitious 19th century syndrome with present impact, Subst. Abuse Treat. Prev. Policy 1 (2006) 14-15.
6. Codex Alimentarius, (1979) Report of the 13th session of the codex committee on food additives. Alinorm 79/12-A. Codex Alimentarius

Commission of the FAO/WHO, Rome, Italy.

7. Council of Europe, Committee of Experts on Flavouring Substances, Alpha- and beta-Thujone, in: ctive principles (constituents of toxicological concern) contained in natural sources of flavourings, Strasbourg, France, (2008) pp 143-148.
8. C. Bauer-Christoph, C. Fauhl, Laborvergleichsuntersuchung der Arbeitsgruppe Spirituosen: Bestimmung von Stoffen der Anlage Aromen in einer Krauterspirituose, Lebensmittelchemie 59 (2005) 46-47.
9. N. Schafer, D.W. Lachenmeier, Determination of α - and β -thujone in spirit drinks using headspace solid-phase microextraction (HS-SPME): comparison between the new HS-SPME and conventional extraction, Dtsch. Lebensm.-Rundsch. 101 (2005) 534 –539.
10. A. Rapp, H. Hastrich, I. Yavas, H. Ullemeyer, Zureinfachen, schnellen Anreicherung ("Kaltronmethode") und quantitativen Bestimmung von flu¨chtigen Inhaltsstoffen aus Spirituosen: Bestimmung von Thujon, Safrol, Isosafrol, α - Asaron, Pulegon und Cumarin, Branntweinwirtschaft 134 (1994) 286–289.
11. R.H. Eastman, J.E. Starr, R.S. Martin, M.K. Sakata, The photolysis of thujone, J. Org. Chem. 28 (1963) 2162–2163.
12. M. Lang, C. Fauhl, R. Wittkowski, Belastungssituation von Absinth mit Thujon (BgVV-Hefte), Bundesinstitut fur gesundheitlichen Verbraucherschutz und Veterinarmedizin, Berlin, Germany (2002).
13. J. Emmert, G. Sartor, F. Sporer, J. Gummersbach, Determination of α -/ β -thujone and related terpenes in absinthe using solid phase extraction and gas chromatography, Dtsch. Lebensm. Rundsch. 100 (2004) 352–356.
14. O. Frohlich, T. Shibamoto, Stability of pulegone and thujone inethanolic solution, J. Agric. Food Chem. 38 (1990) 2057–2060.
15. I.M. Skurihin, Himija konjačnog proizvodstva, Piščevaja promišlenost, Moskva (1967).
16. Singleton, V.L. (1995): Maturation of wines and spirits comparisons, facts, and hypoteses, Am. J. Enol. Vitic. 1 (1995) 98-112.

INFLUENCE OF BUCKWHEAT FLOUR ON THE DOUGH RHEOLOGY AND SENSORY PROPERTIES OF WHEAT BREAD

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT
Farinograph and extensograph properties were determined in the prepared mixtures in which wheat flour was partially replaced with 15%, 30% and 40% of buckwheat flour. Wheat flour was used for comparison of results. The results showed that the increase of buckwheat flour up to 40% increases water absorption, development time and dough stability. Add 40% buckwheat flour significantly reduces the amount of wet gluten, which affects the decrease in resistance (from 380 to 280 EU) and extensibility of dough (from 125 to 71 mm). The ratio of 15% of buckwheat flour did not significantly affect the rheological properties. The dough for bread making was prepared from a mixture of wheat and buckwheat flour with the addition of salt, yeast and water. The control sample was prepared from wheat flour without the addition of buckwheat flour. Sensory evaluation of bread was conducted by a scoring method, by five assessors who evaluated the following sensory properties: external appearance, appearance of crumb, taste and flavour of crust and crumb. Given the proportion of buckwheat flour, the highest sensory evaluation was given to the bread with 15% buckwheat flour. Compared with wheat bread, sensory evaluation indicated change in color of bread with 15% of buckwheat flour, while the taste of bread was not changed in a negative sense to the replacement of 40%. Based on the results, we can conclude that it is possible to produce quality and acceptable bread for consumers by the replacement of wheat flour with buckwheat flour up to 30%.

Keywords: buckwheat flour, dough, rheology properties, bread, sensory evaluation

INTRODUCTION

Although the most bakery products are made from white flour, need and demand for products with enhanced nutritional and functional properties, present in the bakery industry sector[1] , is growing caused by increasing awareness of the importance of proper nutrition and healthy lifestyle. Buckwheat is a rich source of proteins, carbohydrates, minerals, fiber, flavonoids, flavones and other compounds which are considered to lower blood pressure, reduce cholesterol levels, control blood glucose and prevent cancer [2,3,4,5]. Unlike to wheat and other cereals, buckwheat proteins have a well balanced amino acids composition and contain all essential amino acids[6]. Buckwheat also contains a number of bioactive components which show significant antioxidant activity [7,8]. Phenolic compounds, rutin and quercetin are the main antioxidants in buckwheat that prevent lipid peroxidation and activity of free radicals [9,10,11]. Given the unfavorable mineral and amino acid composition of the white flour products, buckwheat as a natural source of these compounds represents a potentially valuable raw material that may be used in the baking industry. The use of buckwheat flour can make a useful contribution to assortments of potentially useful components in the production of enriched bakery products[12]. The aim of the study was to examine how much of wheat flour in the basic recipe could be replaced with buckwheat flour in order to obtain a loaf with enhanced nutritional value and of acceptable sensory properties for the human diet.

MATERIAL AND METHODS

Water content, ash, the degree of acidity and amount of wet gluten[13] were determined in wheat flour, and in mixtures of wheat and buckwheat flour prepared in the ratios 85:15, 70:30 and 60:40 (w / w). Extensograph and farinograph test for rheological properties of dough were made according to a standard procedure for wheat (ICC Standard No. 114/1 and 115/1)[14]. The basic formulation of baking is shown in Table 1.

Table 1: The formulations of breads (% on the flour basis)

Wheat flour (%)	100	85	70	60
Buckwheat flour (%)	-	15	30	40
Salt (%)	2	2	2	2
Yeast (%)	3,5	3,5	3,5	3,5
*Water (%)	WA	WA	WA	WA

*WA – water absorption (according to farinograph test)

Bread making was conducted under the following conditions: dough temperature 27°C ± 1; initial fermentation + intermediate fermentation: 15+15 min; temperature of chambers for the final fermentation: 35°C ± 2; relative humidity: 75% - 80%; baking: 250°C. After the baking, the bread samples were cooled, packaged in plastic bags and stored at a room temperature. A sensory evaluation of bread quality was determined 24 hours after baking by a scoring method (weighted score) according to the references[13, 15]. The sensory evaluation was conducted by a panel of five experienced assessors. The following sensory properties were evaluated: external appearance, appearance of crumb, taste and flavour of crust and crumb. Each sensory property was rated by score 1-5 applying the factors of significance (FS). Total grade was obtained as the sum of individual properties. Weighted mean (WM) was calculated by dividing the total grade and sum of factors of significance.

A statistical analysis of sensory evaluation of bread samples for determining the existence of several differences between arithmetic means was performed by an analysis of variance (ANOVA). Duncan test of significance level 0,05 was used to determine which individual samples statistically significantly were different from each other. Pearson's correlation coefficient (r) was used for the analysis of linear correlation between certain parameters. The data were processed using software package SPSS V.15.

RESULTS AND DISCUSSION

For the used wheat flour (WF) and mixture of wheat and buckwheat flour (BF), the physicochemical, farinograph and extensor-graph analyses were performed, but also content of wet gluten was determined. The results presented in Table 2 showed that with the addition 15% of buckwheat flour wet gluten content already decreased signifycantly compared to wheat flour. It was not possible to detect percentage of wet gluten in the samples with 30% and 40% of buckwheat flour because the samples disintegrated. Due to higher free fatty acids content buckwheat flour increases the acidity of a buckwheat and wheat flour mixture from 2,2 to 2,8 compared to wheat flour.

Table 2: The results of physical and chemical analysis of flour

Flour	Water (%)	Ash (%)	Acidity	Wet gluten (%)
WF (100%)	13,40	0,50	1,8	27,5
WF + BF (85 % + 15 %)	12,86	0,83	2,2	21
WF + BF (70 % + 30 %)	12,09	1,13	2,6	ND*
WF + BF (60 % + 40 %)	11,68	1,28	2,8	ND*

WF – wheat flour; BF – buckwheat flour; ND* - not detected

The changes in rheological properties of wheat dough by adding buckwheat flour are shown in Tables 3 and 4 addition of buckwheat flour into wheat flour significantly influences the changes of all farinographs parameter. According to farinograph tests data which show the behavior of the dough during mixing, with the increase of buckwheat flour from 15% to 40%, water absorption (WA) was increased for 1% to 2,5% , compared to wheat flour. Addition of 15% of buckwheat flour increased dough stability for 1,4 minutes. While the increase of the buckwheat flour up to 40% decreased dough stability indicating that the wheat dough with a lower content of buckwheat flour in the final fermentation is more stable and more suitable for processing. Buckwheat flour prolongs time to achieve maximum consistency of dough. Development time for dough with 15% buckwheat flour was extended for 5,4 minutes, while the extension for the samples with 30% and 40% of buckwheat flour was 4,4 minutes. Extension of dough develop-ment required the extension of mixing time for 3 to 4 minutes, compared to wheat flour dough.

The results of extensograph test indicating the characteristics of the dough during rest and fermentation are shown in Table 4. The energy, extensibility and dough resistance were reduced by increasing the proportion of buckwheat flour from 15% to 40%, in comparison with pure wheat flour dough. Reduction of energy required during dough mixing is the result of reducing the resistance of dough.

The correlation coefficients showed a statistically significant correlation between the energy and resistance of dough ($r = 0{,}998$; $p \leq 0{,}01$), energy and extensibility ($r = 0{,}983$; $p \leq 0{,}05$), extensibility and resistance ($r = 0{,}973$; $p \leq 0{,}05$).

Table 3: Effect of buckwheat flour on the farinograph parameters of rheological properties of wheat Dough

Flour	Water absorption (%)	Dough development (min.)	Stability (min.)	Degree of softening (FU)	Quality number	Quality group
WF (100%)	58	2	0,9	15	80	A2
WF + BF (85 % + 15 %)	59	7,4	2,3	64	76,4	A2
WF + BF (70 % + 30 %)	60	6,4	1,9	68	75	A2
WF + BF (60 % + 40 %)	60,5	6,4	1,5	60	76,4	A2

WF – wheat flour; BF – buckwheat flour; FU – farinograph unit

Significant extensibility decrease is the result of reducing the amount of gluten in the dough with the addition of buckwheat flour.

The calculated correlation coefficients showed a statistically significant and nega-tive correlation between the proportion of buckwheat flour and energy ($r = -0,990$; $p \leq 0,01$), extensibility ($r = -0,987$; $p \leq 0,05$) and resistance of dough ($r = -0,980$; $p \leq 0,05$). Increasing the ratio R/E (up to 3,94 by adding buckwheat flour to 40%) compared to the value of R/E for wheat flour (3,04) indicates a strengthening of the dough.

In comparison with white wheat flour, all samples of dough with addition of 15%, 30% and 40% of buckwheat flour required the longer final fermentation.

Table 4: Effect of buckwheat flour on the extensograph parameters of rheological properties of wheat dough

Flour	Energy (cm^2)	Extensibility (mm)	Resistance (EU)	Max. resistance (EU)	Ratio resistance/ extensibility (R/E)
WF (100%)	74	125	380	410	3,04
WF + BF (85 % + 15 %)	64	110	360	370	3,27
WF + BF (70 % + 30 %)	43	92	300	310	3,26
WF + BF (60 % + 40 %)	34	71	280	290	3,94

WF – wheat flour; BF – buckwheat flour; EU – extensograph unit

Increasing the proportion of buckwheat flour up to 30%, the final fermentation was prolonged for 30 minutes. Depending on proportion of buckwheat flour, baking time was longer for all bread samples from 3 to 7 minutes, compared to wheat bread (Table 5).

Table 5: Time of the final fermentation and baking of bread samples

Buckwheat flour	Fermentation (min.)	Baking time (min.)	Baking temperature (°C)
0 %	55	22	250
15 %	70	25	250
30 %	85	28	250
40 %	80	29	250

Increasing the proportion of buckwheat flour from 15% to 40%, yield of dough was increased for 3% to 10,84%, while the yield of bread increased for 2,79% to 9,7% as compared to wheat bread (Table 6). Increasing of amount of water needed for mixing leads to increase of dough and bread yield. The correlation coefficient showed a statistically significant and positive correlation between WA and the yield of bread ($r = 0{,}989$ $p \leq 0{,}05$).

Table 6: Effect of buckwheat flour on dough yield, bread yield, losses during baking and cooling, degree of acidity and the ratio of height and width (h/w)

Bread	Yield of dough (%)	Yield of bread (%)	Losses (%)	Acidity	Ratio of height and width (h/w)
White wheat bread	157,16	135,42	13,83	2,1	0,68
Bread with 15% BF	160,16	138,21	13,7	2,8	0,667
Bread with 30% BF	163,33	142,04	13,03	3,6	0,659
Bread with 40% BF	168	145,12	13,6	4,3	0,35

BF – buckwheat flour

The aroma and taste improvement could result of be achieved by acidity increase in the bread samples with 15%, 30% and 40% of buckwheat flour. Height (h) and width (w) of cross-section were decreased proportionally by addition of buckwheat flour up to 30% in comparison to wheat bread, while the addition of 40% buckwheat flour significantly reduced height but increased bread width. By comparing the ratio h/w values (Table 6), it can be concluded that the only addition of the maximal tested buckwheat flour dose (40%) significantly affected the reduction in the h/w value indicating a negative influence on the shape of bread.

Table 7: Sensory evaluation of wheat bread made with buckwheat flour addition

Bread	External appearance	Appearance of crumb	Flavour of crust and crumb	Taste of crust and crumb	WM
White wheat bread	$4{,}3^{a}$	$4{,}9^{a}$	$4{,}6^{a}$	$4{,}2^{a}$	$4{,}51^{a}$
Bread with 15% BF	$4{,}6^{a}$	5^{a}	$4{,}9^{a}$	$4{,}7^{a}$	$4{,}81^{a}$
Bread with 30% BF	$4{,}1^{a}$	$4{,}7^{a}$	$4{,}9^{a}$	$4{,}4^{a}$	$4{,}53^{a}$
Bread with 40% BF	$2{,}9^{b}$	$4{,}1^{b}$	$4{,}8^{a}$	$3{,}3^{b}$	$3{,}75^{b}$

BF - buckwheat flour; WM – Weighted mean

Values in the same column marked with different letters are statistically significantly different (Duncan test $P < 0{,}05$)

Crumb of bread becomes less porous and less elastic while these changes are much less pronounced with the addition of 15% and 30% buckwheat flour. Sensory evaluation (Table 7) showed that the bread made of wheat flour supplemented with 40% of buckwheat flour is significantly different in the negative terms from the bread with 15% and 30% of buckwheat flour and from wheat bread. Using 40% of buckwheat flour, significantly affects the sensory properties of bread, especially the appearance and taste of crust and crumb.

CONCLUSION

Addition 15%, 30% and 40% of buckwheat flour to wheat flour tends to decrease the amount of gluten, which leads to changes in rheological properties. Buckwheat flour increases water absorption, stability and development time of dough. Extensibility reduction, resistance, and energy of dough are especially pronounced with the addition of 40% of buckwheat flour. Buckwheat flour extends the mixing time and final fermentation of dough. By using buckwheat flour in the production of bread, the following effects were achieved: the bread yield increase (2,79% to 9,7%), acidity of bread crumb increase, flavor and taste improvement (when using 15% and 30% buckwheat flour), crust color and shine improvement, reduction of losses during baking. Given the results of sensory evaluation, the addition of buckwheat flour up to 30% does not significantly affect the bread quality. Overall, buckwheat flour can be used as a supplement in wheat bread production.

REFERENCES

1. R. Schönlechner, Proceedings of 4th International Congress Flour-Bread '07 (2007) 58-61.

2. C.Quettier-Deleu,B. Gressier, J.Vasseur, Journal of Ethnopharmacology 72 (2000) 35–42.

3. K. J. Steadman, M. S. Burgoon, B. A. Lewis, S. E. Edwardson, R. L. Obendorf, J. Cereal Sci. 33 (2001) 271-278.

4. M. Holasova, V. Fiedlerova, H. Smrcinova, M. Orsak, J. Lachman, S. Vavreinova, Food Research International 35 (2002) 207–211.

5. S. Gorinstein, E. Pawelzik, E. Delgado-Licon, International Journal of Food Science and Technology 39 (2004) 183–189.

6. H. H. Wijngaard, E. K. Arendt, Cereal Chem. 83 (2006) 391–401.

7. D. Dietrych-Szostak, W. Oleszek, J. Agric. Food Chem. 47 (1999) 4384-4387.

8. K. Christa, M. Soral-Śmietana, Czech J. Food Sci. 26 (2008) 153–162.

9. S. Kreft, M. Knapp, I. Kreft, J. Agric. Food Chem. 47 (1999) 4649–4652.

10. Z. Li, L. Zhixi, G. Fengqiu, Proceedings of the l0th international symposium on buckwheat (2007) 427-432.

11. H. Zieliński, H. Kozłowska, J. Agric. Food Chem. 48 (2000) 2008–2016.

12. L. Y. Lin, H. M. Liu, Y. W. Yu, S. D. Lin, J. L. Mau, Food Chemistry 112 (2009) 987-991.

13. G. Kaluđerski, N. Filipović, Metode ispitivanja kvaliteta žita, brašna i gotovih proizvoda, Tehnološki fakultet Univerziteta u Novom Sadu, Novi Sad (1998).

14. Standard Methods of the International Association for Cereal Science and Technology (ICC). Methods 114/1, 115/1. (1998).

15. R. Radovanović, J. Popović-Raljić, Senzorska analiza prehrambenih proizvoda, Poljoprivredni fakultet Beograd – Tehnološki fakultet Novi Sad (2000/2001).

DIRECT SPECTROPHOTOMETRIC DETERMINATION OF L-ASCORBIC ACID IN THE PRESENCE OF ALANINE AS A STABILIZER

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT
A simple, accurate and sensitive direct spectrophotometric method was developed for the determination of L-ascorbic acid (vitamin C) in pharmaceuticals. Alanine (0.18 mol/dm^3) in phosphate buffer was used to stabilize L-ascorbic acid in aqueous medium. The molar absorptivity of the L-ascorbic acid solution, which does not require an extraction procedure, was 1.397 x 10^4 dm^3 mol^{-1} cm^{-1} at 266 nm. Beer's law was obeyed in the concentration range of 1.02 – 12.0 µg ascorbic acid/cm^3. The detection limit was 0.305 µg/cm^3 and the relative standard deviation 0.94 % for the determination of 8.00 µg ascorbic acid/cm^3 (n = 7). Many of the substances commonly found in vitamin C products do not interfere with the determination of L-ascorbic acid. The proposed procedure was successfully applied to the determination of L-ascorbic acid in vitamin C preparations. The results obtained by the proposed method showed good agreement with those provided by the titrimetric method using iodine.

Keywords: L-Ascorbic acid, Alanine, Ultraviolet spectrophotometry, pharmaceutical preparations

INTRODUCTION

L-Ascorbic acid (2-oxo-L-threo-hexono-1,4-lactone-2,3-enediol) is an essential vitamin which participates in many different biological processes. It occurs naturally in most fruit juices and vegetables. The reversible oxidation of L-ascorbic acid to dehydro-L-ascorbic acid is the basis for its physiological activities and technical applications. L-Ascorbic acid is widely utilized as an additive in juices, soft drinks and foods, and is also used in pharmaceutical preparations. Sometimes, its addition is carried out during product manufacturing in order to increase the nutritional value or as an antioxidant to lengthen the lifetime of the industrialized products.

Many analytical methods have been reported in the literature for the determination of the ascorbic acid contents in different pharmaceutical products, fruits, vegetables and biological fluids. These include fluorimetric[1], electrochemical[2], highperformance liquid chromatographic[3,4], spectrophotometric[5-8] and chemiluminescent[9] methods. Of all these methods, spectrophotometric methods are, perhaps, the most commonly used.

Direct ultraviolet spectrophotometry can provide a fast, simple and reliable method for the determination of L-ascorbic acid. The use of direct UV spectrophotometry for the assay of ascorbic acid has not been easy due to its instability in aqueous solutions. The instability of L-ascorbic acid is due to its oxidation to dehydroascorbic acid which is a reversible reaction and subsequently to 2,3-diketo-

L-gulonic acid. These reactions can be inhibited by stabilizers.

The purpose of this work was to develop a direct and simple ultraviolet spectrophotometric method for the determination of L-ascorbic acid in pharmaceuticals with alanine as a stabilizer. The effects of a number of substances commonly encountered in pharmaceutical preparations on the proposed method were studied.

MATERIAL AND METHODS

Reagents

All reagents used were of analytical-reagent grade.

Buffer solution (pH = 5.4). A mixture of potassium dihydrogenphosphate (0.03 mol/dm^3) and disodium hydrogenphosphate (8.99x10^{-4} mol/dm^3) was prepared by dissolving 4.08 g of KH_2PO_4 (Fluka) and 0.16 g of $Na_2HPO_4 \cdot 2H_2O$ (Merck) in 1000 cm^3 of distilled water.

Alanine solution (0.18 mol/dm^3). Prepared by dissolving 16.00 g of alanine (Sigma) in 1000 cm^3 of the buffer solution.

L-Ascorbic acid solution (1.13x10^{-3} mol/dm^3). A 0.05 g amount of L-ascorbic acid (Riedel-de Haën) was dissolved in 250 cm^3 of the alanine solution.

Solutions of metal ions, anions, organic acids, amino acids and sugars were prepared by dissolving calculated amounts of these substances in the (0.18 mol/dm^3) alanine solution.

Apparatus

All absorbances were determined on Cecil 2021 (UK) single beam spectrophotometer using 1 cm path length.

General procedure

Transfer a portion of the sample solution containing 60 – 300 µg of L-ascorbic acid to a 25 cm^3 standard flask. Dilute to the mark with the (0.18 mol/dm^3) alanine solution and measure the absorbance at 266 nm against the alanine solution as a blank.

Determination of L-ascorbic acid in tablets

Transfer an accurately weighed amount of powder obtained from 10 tablets into a 100 cm^3 volumetric flask, dissolve and make up to the mark with the (0.18 mol/dm^3) alanine solution. Filter and dilute a suitable aliquot of the filtrate to 50 cm^3 with the stabilizer solution. Take an aliquot of the final solution and determine the ascorbic acid content as described under general procedure.

RESULTS AND DISCUSSION

Optimization of conditions

Absorption properties of L-ascorbic acid are dependent upon the pH of the aqueous media. Above pH 5.0, L-ascorbic acid exists predominantly as the monoanion and has maximal absorption at 265 nm. Undissociated, at more acid pH levels, maximal absorption occurs around 245 nm[10]. Since the position of maximum absorbance is pH-dependent, the potassium dihydrogenphosphate – disodium hydrogenphosphate buffer solution (pH = 5.4) was used throughout this work.

L-Ascorbic acid is readily and reversibly oxidized to dehydroascorbic acid, which is present in aqueous media as a hydrated hemiketal. The biological activity is lost when the dehydroascorbic acid lactone ring is irreversibly opened, giving rise to 2,3-diketogulonic acid. The oxidation of

L-ascorbic acid to dehydroascorbic acid and its further degradation products depends on several factors. Oxygen partial pressure, pH, temperature, light, and the presence of heavy metal ions are of great importance. Metal-catalyzed destruction proceeds at a higher rate than noncatalyzed spontaneous autoxidation. Traces of heavy metal ions, particularly Cu^{2+}, result in high losses. Therefore, a major problem with the analysis of L-ascorbic acid in real samples concerns the prevention of the degradation of the vitamin.

In the present work, alanine in the buffer solution was used to stabilize L-ascorbic acid in the aqueous media. The effect of alanine concentration on the stability of L-ascorbic acid was studied in the range of 0.045 to 0.19 mol/dm^3 in the presence of KH_2PO_4 (0.03 mol/dm^3) and Na_2HPO_4 (8.99×10^{-4} mol/dm^3). When the concentration is within the range of 0.17 – 0.19 mol/dm^3, solutions of L-ascorbic acid remain stable for at least 30 minutes at room temperature. Therefore, 0.18 mol/dm^3 alanine concentration was selected for further investigation.

Analytical characteristics of the proposed method

The calibration curve is linear up to an ascorbic acid concentration of 12.00 µg/cm^3. A least-square analysis of Beer's plot gave the following linear regression equation (n = 7):

$$A_{266} = 0.0319 + 0.0793\,C$$

where A_{266} is the absorbance at 266 nm and C is the concentration of L-ascorbic acid in µg/cm^3. The detection limit (three times the standard error of the intercept/slope), quantification limit (ten times the standard error of the intercept/slope), molar absorptivity (ε), as well as other analytical characteristics are summarized in Table 1[11].

The precision of the proposed method, expressed as relative standard deviation, for the determination of 8.0 µg/cm^3 ascorbic acid, was 0.94 % (n = 7). The molar absorptivity calculated from the slope of the calibration graph shows that the proposed method is highly sensitive. This procedure is more sensitive than other spectrophotometric methods, using 4-chloro-7-nitrobenzofurazane ($\varepsilon = 6.49 \times 10^3$ dm^3 mol^{-1} cm^{-1})[12], zinc chloride salt of diazotized 1-amino-anthraquinone ($\varepsilon = 4.07 \times 10^3$ dm^3 mol^{-1} cm^{-1})[13], gold(III) ion ($\varepsilon = 2.30 \times 10^3$ dm^3 mol^{-1} cm^{-1})[14], *peri*-naphthindan-2,3,4-trione ($\varepsilon = 3.18 \times 10^3$ dm^3 mol^{-1} cm^{-1})[15] and iodate-fluorescein ($\varepsilon = 8.81 \times 10^3$ dm^3 mol^{-1} cm^{-1})[16].

Table 1. Analytical characteristics of the proposed method

Slope of the calibration line	0.0793
Intercept of the calibration line	0.0319
Standard error of the slope of the calibration line	0.00099
Standard error of the intercept point of the line	0.00807
Correlation coefficient (r)	0.9997
Limit of detection	0.305 µg/cm^3
Limit of quantification	1.02 µg/cm^3
Linear dynamic range	1.02 – 12.0 µg/cm^3
Molar absorptivity (ε)	1.397×10^4 dm^3 mol^{-1} cm^{-1}
Relative standard deviation	0.94 %

Interference studies

To assess the selectivity of the proposed method, interferences caused by those foreign species that are commonly found with L-ascorbic acid in the samples analyzed were studied by adding different amounts of other species to a solution containing 8.00 µg/cm^3 of ascorbic acid. The criterion for the interference was an absorbance varying by 5 % from the expected value. The results are listed in Table 2.

Table 2. Effect of foreign substances on the determination of L-ascorbic acid

Foreign substance added	**Mass ratio (foreign substance:ascorbic acid)**	**Error, %**
Cl^-	10	0.00
NO_2^-	2	-2.02
PO_4^{3-}	5	0.00
HCO_3^-	10	1.57
Benzoate	2	> 5.00
Acetate	20	0.00
Citrate	20	0.00
Iron(II)	0.02	3.00
Copper(II)	0.02	1.06
Calcium(II)	2	0.00
Magnesium(II)	2	-1.46
Zinc(II)	2	0.00
Manganese(II)	1.3	0.00
Nickel(II)	0.4	0.00
Tartaric acid	2	-1.79
Citric acid	5	-1.63
Sucrose	200	0.00
Glucose	200	0.00
Fructose	200	0.00
L-Proline	10	0.00
L-Arginine	10	0.00
L(+)-Asparagine	10	0.00

None of the metal ions investigated interfered with the determination at the levels studied. The positive errors (all within 5 %) caused by iron(II) and copper(II) may be ascribed to the absorption of UV light by these substances. The results in Table 2 demonstrate that alanine at a concentration of 0.18 mol/dm^3 is a suitable stabilizer for L-ascorbic acid in the developed method. This stabilizer together with metal ions forms complexes, that are no longer effective catalysts. Alanine prevents the ascorbic acid-metal ion complex formation and therefore inhibits effectively the oxidation of L-ascorbic acid.

The anions tested except benzoate did not interfere with the determination of ascorbic acid using the proposed method. Benzoate interfered seriously because of the absorption of UV light. The negative error caused by nitrite may be ascribed to the oxidation of L-ascorbic acid with this oxidant in an acidic medium. Since absorption properties (λ_{max} and ε) of L-ascorbic acid depend on the pH of the aqueous media[10], the positive error caused by hydrogencarbonate may be ascribed to an increase in the pH of the L-ascorbic acid solution. The experimental results revealed that a 200-fold excess of sucrose, glucose and fructose and 10-fold proline, asparagine and arginine had no effect on the determination of L-ascorbic acid using the proposed method. Negative errors caused by citric and tartaric acids may be ascribed to a decrease in the pH of the ascorbic acid solution after the addition of organic acids.

Analysis of pharmaceutical samples

The proposed method was applied to the determination of the ascorbic acid contents in commercial pharmaceutical preparations (tablets). The results obtained are shown in Table 3. In every case, the sample was analyzed by both the proposed and the titrimetric method using iodine as titrant[17]. The last one, used as a reference method, is a procedure based on the oxidation of L-ascorbic acid to dehydro-L-ascorbic acid by iodine.

The statistical study of precision and accuracy of the proposed method was made from *F* criterion and the *t*-test, respectively. The *t*-test was applied to the results obtained by the proposed and the iodine method, and it showed that calculated *t* values were lower than the tabulated *t* value (t = 2.31, P = 0.05). This suggested that at 95 % confidence level differences between the results obtained by the two methods were statistically not significant.

The *F*-test revealed that there is no difference between the precision of the two methods. In every case, the calculated value of *F* was lower than the critical value (F = 6.39, P = 0.05). Thus, the proposed method can be successfully applied to real samples.

Table 3. Determination of L-ascorbic acid in vitamin C preparations

Commercial name (Supplier)	L-Ascorbic acid (mg/tablet)				
	Claimed value	Proposed method*	Iodine method*	$t_{exp.}$	$F_{exp.}$
Vitamin C (Krüger)	180	184.28 ± 2.76	183.28 ± 1.93	2.07	0.82
Plivit C (Pliva)	500	499.25 ± 4.74	498.33 ± 5.12	1.17	0.36
Vitamin C (Galenika)	500	502.12 ± 6.56	498.93 ± 5.38	1.48	1.04

Theoretical value for *F* is 6.39 (P = 0.05) and for *t* is 2.31 (P = 0.05).
*The 95 % confidence limits of the mean (n = 5).

CONCLUSIONS

Alanine at a concentration of 0.18 mol/dm^3 is a suitable stabilizer for L-ascorbic acid in a UV method of assay. The proposed method using the stabilizer is simple, sensitive, precise and accurate. Many common ingredients present in pharmaceutical preparations do not interfere. The results of applying the proposed method showed good agreement with those provided by the reference method. The results obtained by the proposed method also agreed well with the claimed values on the labels in all instances. Thus, the proposed method can be applied to the determination of vitamin C in commercial pharmaceutical preparations.

REFERENCES

1. X. Wu, Y. Diao, C. Sun, J. Yang, Y. Wang, S. Sun, Talanta 59 (2003) 95.
2. C. X. Li, Y. L. Zeng, Y. J. Liu, C. R. Tang, Anal. Sci. 22 (2006) 393.
3. J. Lykkesfeldt, Anal. Biochem. 282 (2000) 89.
4. H. Iwase, Talanta 60 (2003) 1011.
5. E. K. Janghel, V. K. Gupta, M. K. Rai, J. K. Rai, Talanta 72 (2007) 1013.
6. S. P., Arya, M. Mahajan, P. Jain, Anal. Chim. Acta 427 (2001) 245.
7. M. Salkic, R. Kubicek, Eur. J. Sci. Res. 23 (2008) 351.
8. M. Salkic, A. Selimovic, Technologica Acta 2 (2009) 13.
9. T. Kato, O. Ohno, T. Nagoshi, Y. Ichinose, S. Igarashi, Anal. Sci. 21 (2005) 579.
10. R. R. Eitenmiller, W. O. Landen, Vitamin Analysis for the Health and Food Sciences, CRC Press, USA, 1999, pp. 224-228.
11. D. A. Skoog, D. M. West, F. J. Holler, Foundamentals of Analytical Chemistry, Sanders College Publishing, 1999, pp. 31-56.
12. O. H. Abdelmageed, P. Y. Khashaba, H. F. Askal, G. A. Saleh, I. H. Refaat, Talanta 42 (1995) 573.
13. E. Y. Backheet, K. M. Emara, H. F. Askal, G. A. Saleh, Analyst 116 (1991) 861.
14. T. Pal, P. K. Das, Anal. Lett. 21 (1988) 2333.
15. S. S. M. Hassan, M. M. Abd El Fattah, M. T. M. Zaki, Fresenius Z. Anal. Chem. 277 (1975) 369.
16. N. Balasubramanian, S. Usha, K. Srividya, Indian Drugs 32 (1995) 78.
17. J. S. Fritz, G. H. Schenk, Quantitative Analytical Chemistry, Prentice Hall, New Jersey, 1987, pp. 594-597.

ETHANOL PRODUCTION FROM CORN SEMOLINA BY BAKER'S AND WINE YEASTS

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

Higher fuel prices and global warming have brought upon the need for alternative fuels. The goal of this work was to optimize a two-step production of ethanol from mixtures of corn cultivars specific for Croatia (Pioneer PR36B08, PR36R10, and Osječka OS 596, OS 602), for possible application in the bio-ethanol production. Saccharification was conducted using commercial amylases Endozyme Alphamyl SB1 and Attenuzyme, while fermentations were performed using baker's and wine's strains of yeast *Saccharomyces cerevisiae*. Results showed that the chosen enzymes and the method applied are suitable for the saccharification of starch from these corn cultivars and could be applicable in the industrial production. The chosen yeast strains are technologically suitable, because of their fast fermentation and good tolerance on high sugar concentration at the beginning of the process and the ethanol concentration at the end of fermentation. The ethanol yield of 9.2 % vol/vol, achieved in 20 hours of fermentation in media with 17% of sugar, is economically sufficient for distillation process. The process required 2.7-2.8 kg of corn mixture for 1 litre of bio-ethanol.

INTRODUCTION

Oil crisis in the last period of time, which are partly the consequence of lower amounts of fossil fuels, have resulted in the higher prices of crude oil on world market. Beside that, the global warming and greenhouse gases have forced the need for the use of alternative fuels, what include the production of bio-fuels. Namely, EU and many other developed countries all around the world have introduced many regulations and directives in law, e.g. "2003/30/EC of the European parliament and of the Council" on the promotion of the use of bio-fuels or other renewable fuels for transport and Kyoto Protocol (2005) for the protection of greenhouse gases. Promoting the use of bio-fuels in the transport made a step toward the wider application of bio-ethanol and bio-diesel. According to 2003/30/EC directive, the countries that signed this directive have to use 5.75 % of bio-fuels in total amount of fuel for transport starting with 2010. As a result of technological advances, most vehicles currently in use are capable of using bio-fuels. Some countries are already using bio-fuel blends up to 10% without any problem. Recent technological developments make it possible to use even higher percentages of bio-fuel in the blend. However, higher percentages of bio-fuel are possible to use only with new constructed FF-vehicles (Flex-fuel-vehicles).

Brazil, USA, Canada, Japan, India, Kina and EU are main countries in the production and development of bio-fuel market[1].

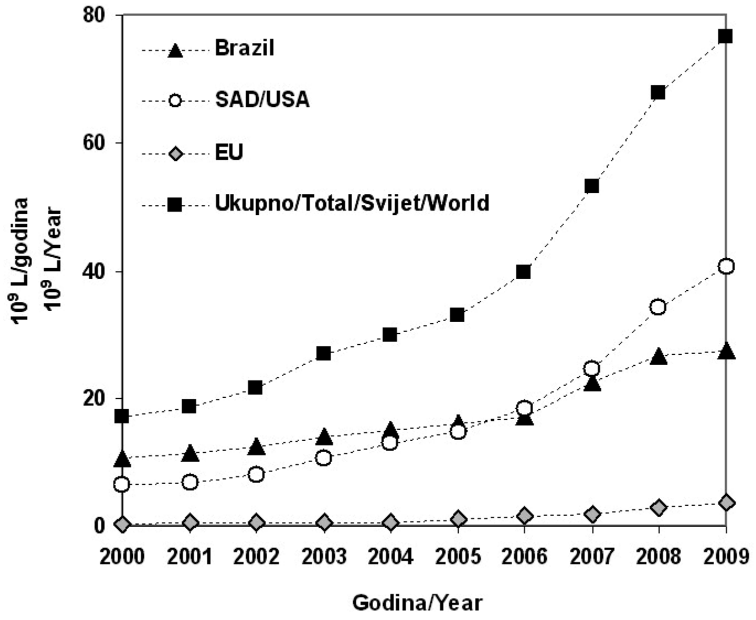


Figure 1. Dynamics of bio-ethanol production in the world

The main advantage of bio-fuel is lower produ-ction of carbon dioxide during burning, without generating of carbon monoxide[2]. Bio-ethanol is favourable alternative fuels for the transport today. The world production of bio-ethanol is shown in figure 1 and table 1.

There are many substrates for the mass-production of ethanol: corn and other grains, sugar cane and sugar beet, molasses[4]. The highest yields of ethanol per hectare can be obtained from sugar beet and sugar cane. However, plants for bio-ethanol production in the world mostly use corn (USA, China, and EU) and sugar cane (Brazil). Sugar beet is still not used enough (except France), because it is mostly used for the more economic production of sugar sucrose.

The use of bio-ethanol for fuel was widespread in United States until the early 1900s.

Ford Company has been used ethanol as fuel in the United States since 1908 with the **Ford Model T** which could be modified to run on either gasoline or pure alcohol.

Table1.World production of bio-ethanol in 2009s ([3])

Country	Production (10^9 L)	Production (%)
SAD	40,13	54
Brazil	24,90	34
China	2,05	3
Canada	1,35	2
France	1,25	2
Germany	0,75	1
Spain	0,47	1
Thailand	0,40	1
India	0,35	<1
Columbia	0,31	<1
Australia	0,22	<1
Austria	0,18	<1
Sweden	0,18	<1
Poland	0,17	<1
Hungary	0,15	<1
Others	1,11	2
Total (world)	73,95	100

Production of ethanol for fuel retained only in Brazil and the beginning was registered in

1927. Since ethanol was more expensive to produce than petroleum-based fuel, especially after World War II, bio-ethanol was ignored until the first big oil crisis of the 1970s. From this time countries (including Brazil and the United States) have promoted bio-ethanol production. Since 1976 Brazil government made the mandatory to blend dehydrated ethanol with gasoline, fluctuating between 10% and 22%, and just a minor adjustment on regular gasoline engines was requested. Since 2007, mixture of 25% of anhydrous ethanol and 75% gasoline (E25) is used in Brazil; the new constructed car engines are requested. For that reason, Brazilian car industry developed FFV that can use any proportion of gasoline and ethanol. Introduced in the market in 2003, flex vehicles became a commercial success.

Since the 1980s, there has been an increased interest in USA for the use of bio-ethanol as an alternative transportation fuel. Very soon, just in a few years, USA increased fuel ethanol production to the large volumes, and today is the biggest producer in the world. Unlike Brazil, in USA E-10 is used in old cars, but E-85 only in FFV.

Brazil and USA are still the biggest producers of bio-ethanol for fuel in the world, which share is almost 85-90%. Total world production of fuel ethanol is constantly increased from 2000s (figure 1). It increased to 40x 10^9/year in 2005s, what was 2 % of gasoline used for the transport. Production in 2008 increased up to 65x10^9/year, and in 2009 to about 75x10^9/year ([3]). Today, many countries all around the world produce and use a significant amount of bio-ethanol (table 1). Distribution of the world production is changing very little during last few years, and it is assumed that moreover Brazil and SAD will be the main producer of bio-ethanol.

The production of bio-ethanol has three main phases: preparation of media, fermentation, distillation and dehydration. Bio-ethanol can be produced from many different substrates. Depending of the used substrates, preparation of media can be completely different[3,6] . The simplest production is on the sugar substrates: juices and/or syrups of sugar cane and sugar beet, molasses and whey. The preparation of media requires hydrolysation of starch and other polysaccharides to fermentable sugars.

In the case of bio-ethanol production from corn, the process begins by wet or dry milling the grains to semolina[7]. It is necessary to obtain defined particle size. If the particles are too small quick increase in viscosity because of hitting and gelatinizing during saccharyfication can happen, what could stop the hydrolysis of starch. On the other hand, if corn particles are to big the extraction of starchy molecules is too slow, what can result in lower yield. To improve the saccharification some researchers used hitting treatment before drying[8] and application of ultrasound[9] .With the aim to increase yield of ethanol production from corn per hectare, new idea is to produce ethanol from both, corn stalk and corn together, which could make double yield[10-12].

Bio-ethanol production from lignocelluloses is the second generation of bio-alcohol production for fuel. It is very interesting all around the word because of large amounts of renewable biomass (corn stalk, straw, bagasse, wood and sawdust). Since 30 years ago many researchers have investigated production of bio-ethanol from such material and noted difficulties of these processes due to the media preparation. For this reason up today bio-ethanol from these materials is more expensive then ethanol produced from corn or sugar’s[1,13] . Anyhow, during last period new pilot-plants for the production of bio-ethanol from lignocelluloses are made in USA, Canada, Japan, Spain, Sweden and Denmark with the capacity of 3-5 millions of litres per year[5, 6] .

Republic of Croatia is following the trends of bio fuel use and signed the EU protocol of obligation that will use 5.75% of bio fuels from 2010s in transportation. Unfortunately, there is no plant for the production of bio-ethanol in Croatia; in spite of enough raw materials (Croatia has two rather small factories for the production of bio-diesel, a product with much lower profitability). Therefore, the aim of this work was to research the possibility of the production of bio-ethanol from corn semolina, which consist from mixture of corn (Pioneer PR36B08 i PR36R10 i OSS 596 i 602) produced in region of Slavonija in Croatia. As control substrate the corn semolina for beer production was used. Since commercial yeast can not ferment any kind of starch, specific method of saccharification has been done. For the fermentation of hydrolyzed starch two strains of yeast *Saccharomyces cerevisiae* were tested, to improve the ethanol yield and the concentration of ethanol at the end of fermentation process.

MATERIALS AND METHODS

Microorganism

The following species of *Saccharomyces cerevisiae* were used throughout the study: baker’s yeast *S. cerevisiae* obtained from the factory KVASAC-Lessafre in Savski Marof (Croatia), and vine yeast (Zymaflore, Laffort) taken from the culture collection of the Faculty of Food Technology and Biotechnology, University of Zagreb. The vine yeast was maintained on agar slants at 4°C.

Substrates

Two types of corn semolina, brewer's semolina (K1), and semolina (K2) obtained by milling of mixture of Croatian corn cultivars Pioneer PR36B08, Pioneer PR36R10, Osječka OS 596 and Osječka OS 602, were tested for their impact on the ethanol production. The basic features of K1 and K2 semolina’s are presented in Table 1.

Table 2. Content of dry matter and starch in corn semolina’s

Vrsta kukuruzne krupice/ Corn semolina type	**Količina suhe tvari/ Dry matter (%)**	**Količina škroba/ Starch content (/%)**
K1	**87.2**	**78.12**
K2	**86.9**	**67.72**

Enzymes used for the semolina starch saccharification:

1. Thermo-stabile α-amylase Endozyme Alphamyl SB1 (France)
2. A mixture of α-amylase and gluco-amylase Attenuzyme (Novoenzymes, Denmark)

Optimal pH value of α-amylase (Endozyme Alphamyl SB1) is 4.5-5.8, and the best activity between 80-90 °C. Contrary to that, optimal pH value of mixed enzymes α-amylase and gluco-amylase (Attenuzyme) is 4-5, and temperature of 60°C as well.

Corn semolina saccharification and basic substrate preparation

Medium for saccharification was prepared by suspending the corn semolina in water in concentrations of 150, 200 and 250 g/L. Starch hydrolysis was performed in Erlenmeyer's flask (1000 mL, working volume 500 mL). Amount of α-amylase was 0.35% (on the semolina), while content of the mixture of α-amylase and glucoamylase was 0.2 %. There was no need for correction of pH for K1, but with K2 pH was adjusted to 5.3 with phosphoric acid. Saccharification was performed on a laboratory shaker. The course and basic parameters of the saccharification are presented with Figure 2. Samples for measurement of glucose content were collected every 15 minutes. The Iodine test was used for the starch hydrolysis control.

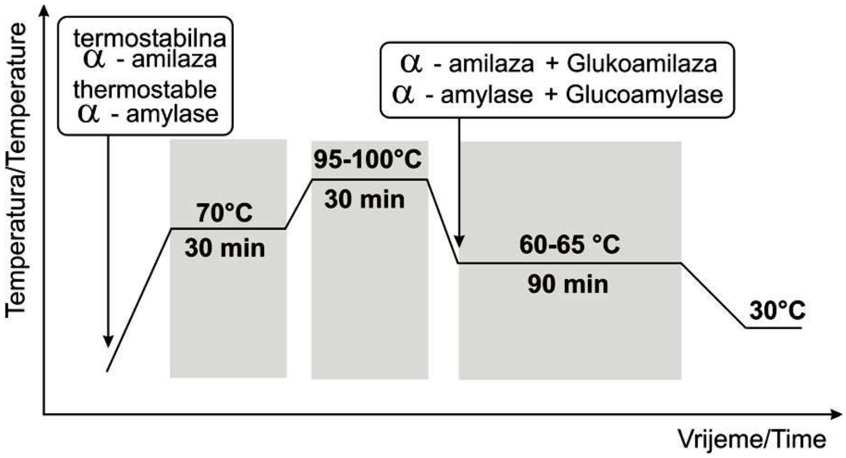


Figure 2. Parameters of corn semolina saccharification

Inoculum preparation

Fresh baker's yeast *S. cerevisiae* with 30% dm was maintained in refrigerator at 4°C. It was added in the media as a suspension with 15% dm , to make a concentration of 3 or 5 g dm/L at the beginning of fermentation.

Preparation of fresh vine yeast was started by transfer of yeast from agar slants into test tubes each containing 10 mL of sterile liquid yeast medium (YM), containing in g/L: glucose, 50; bacto-peptone, 10; yeast extract, 5), and incubated in a thermostat at 30^0 C for 24 h. The sterile 300 mL of liquid YM in 500 mL Erlenmeyer flasks were inoculated with 5% of the obtained inoculums. Flasks were shaken on a rotary shaker at 150 rpm and 30 ^{0} C for 24 h. After fermentation, yeast biomass was separated by centrifugation at

4000 rpm/10 min and used for preparation of 15% suspension to inoculate the starch hydrolysate.

Alcoholic fermentation

Fermentations were performed in corn hydrolysate with various sugar concentrations, in 500 mL Erlenmeyer's flasks with 200 mL of media. The pH was adjusted with phosphoric acid to 4.5, while 1g/L $(NH_4)_2SO_4$ was added as a nitrogen and phosphorus source, and pH stabilizer. The media were inoculated with 3 or 5 g dm/L of yeast. Fermentations were performed in thermostat at 30^0 C for 24 hours. Final fermentations were conducted in bioreactor Biostat A (Braun, Germany) with 2 L of substrates at 30 ^{o}C during 24 hours (Figure 3). Samples for the control of glucose consumption and ethanol production were collected every 3 hours.

Figure 3. Fermentation of the corn semolina hydrolysate in fermenter

Analysis

The corn semolina dry matter was determined gravimetrically (ISO 712, 1998), while starch content in corn semolina was measured by Ewers method (ISO 10520, 1997). Efficiency of the starch saccharification was monitored by Iodine test. Glucose content in filtrated samples was measured spectophotometrically by Glucose-PAP method. Ethanol concentra-tion in the medium was determined by Martin-Dietrich method[14] or by HPLC method[15].

RESULTS AND DISCUSSION

Saccharification

The main processes of ethanol production on starchy materials are: saccharification and fermentation. In some cases a very sophisticated one-process of simultaneous saccharification and fermentation could be used. For that kind of process thermo-tolerant yeasts are applied. The fermentation temperature is about 40^{o}C, what is more closed to optimal temperatures of amylolytic enzymes activity, α-amylase and gluco-amylase[16].

In this work classical two steps process was conducted and results contain the data obtained from starch hydrolysis and fermentation, as was described before. Since the kinetics of starch hydrolysis depends of many factors, like enzyme concentration, temperature, pH and time, specific process was optimised to get as much as possible of glucose in hydrolysates (figures 4 and 5). Two kinds of semolina (K1 and K2) were subjected to hydrolysis in tap water in concentrations of 150, 200 and 250 g/L. In these mixtures at the beginning of the process 0.35 % thermo stabile α-amylase was added (calculated on semolina amounts). Preparation of substrates and saccharification process were described in Material and methods chapter.

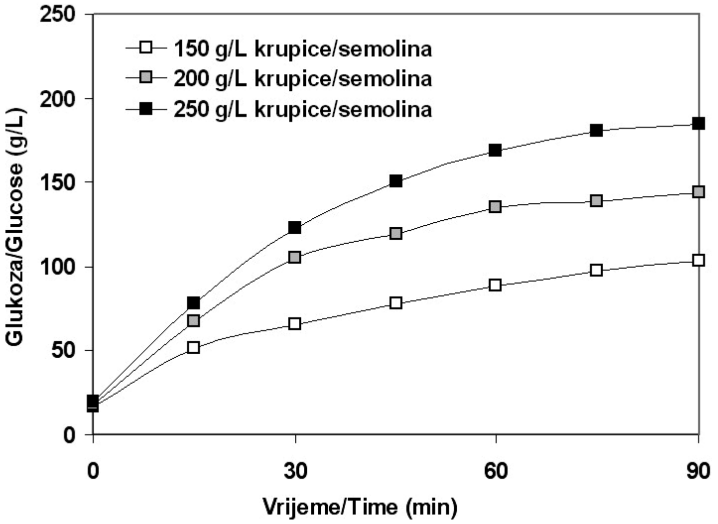


Figure 4. Saccharification of the corn semolina K_1 within 90 minutes

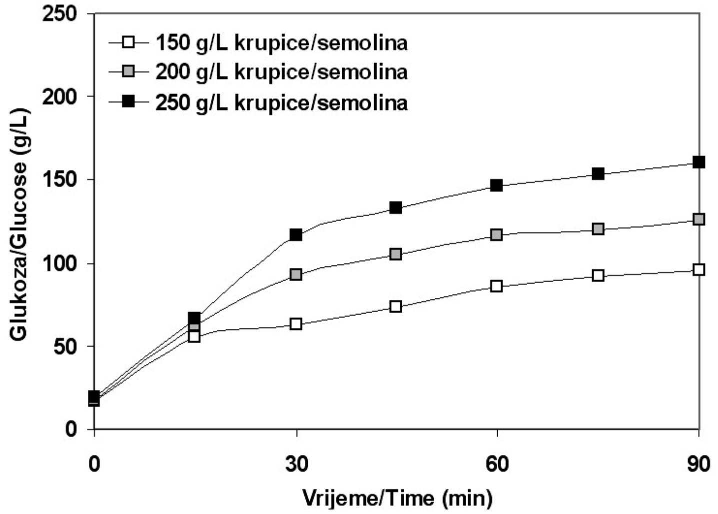


Figure 5. Saccharification of the corn semolina K_2, within 90 minutes

The achieved results have showed that both semolina types were suitable for saccharification with applied enzymes by proposed method. With higher amounts of semolina (250g/L) at the beginning of saccharification rather high yield of glucose was also obtained even dough some authors[17] mentioned lower yield of glucose with high concentration of starch in substrate at the start of hydrolyses. In the hydrolysate obtained from semolina K_1 concentration of glucose was higher (19-20%) than in hydrolysate obtained from semolina K_2 (16-7%), what was expected because of the concentration of starch in the semolina types (table 2).

Fermentation

After the hydrolysis of semolina types K_1 i K_2 and cooling, those media were suitable for the alcoholic fermentation. The aim of these experiments was to get at least 9 % vol/vol of ethanol in the fermented media, as the distillation could be profitable. For that reason baker’s yeast *S. cerevisiae* was used, with 3 or 5 g/L of dm. at the fermentation start (figure 6). Aimed concentration of ethanol was achieved in 24 h of fermentation, with higher amount of yeast produced (figure 6).

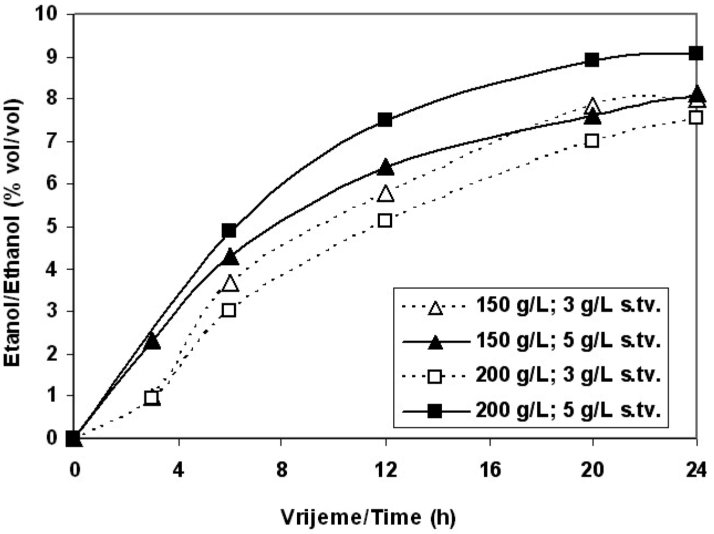


Figure 6. Fermentation of the corn semolina hydrolysates (with 150 and 200 g/L of corn semolina K_1), by baker yeast (3 and 5 g/L)

To get even higher concentration of ethanol in fermented media two kind of yeasts were tested, baker's and wine yeast as well. The results of the experiments are shown in figures 7-9.

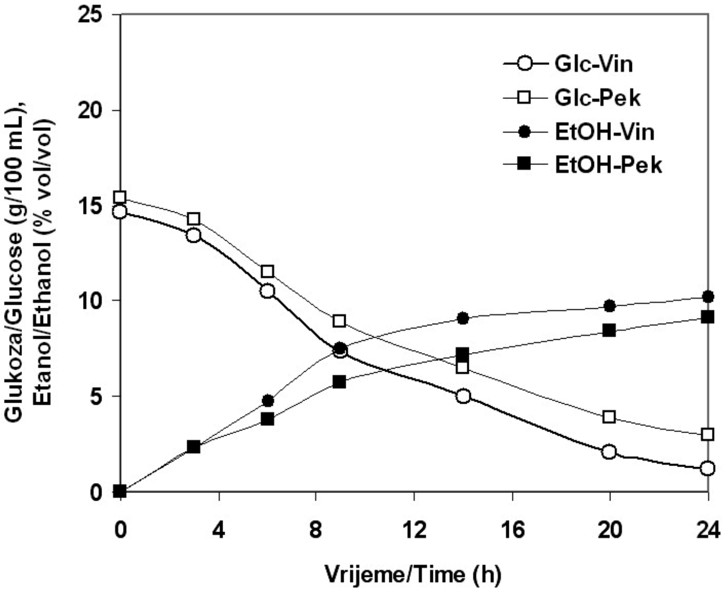


Figure 7. Fermentation of the corn semolina hydrolysate (with 200 g/L of corn semolina K_1), by 5 g/L of baker yeast (Pek) and wine yeast (Vin)

Both yeasts are suitable for the fermentation, but baker's yeast seems to be a little bit favourable, as it ferments corn hydrolysates with quite high concentration of sugar at the beginning of the process very well, and it has good tolerance to ethanol as a product. Using hydrolysates of semolina K1 with the higher glucose concentration at the start of fermentation (19-20%) the process was prolonged to 30 and 50 hours (figure 8).

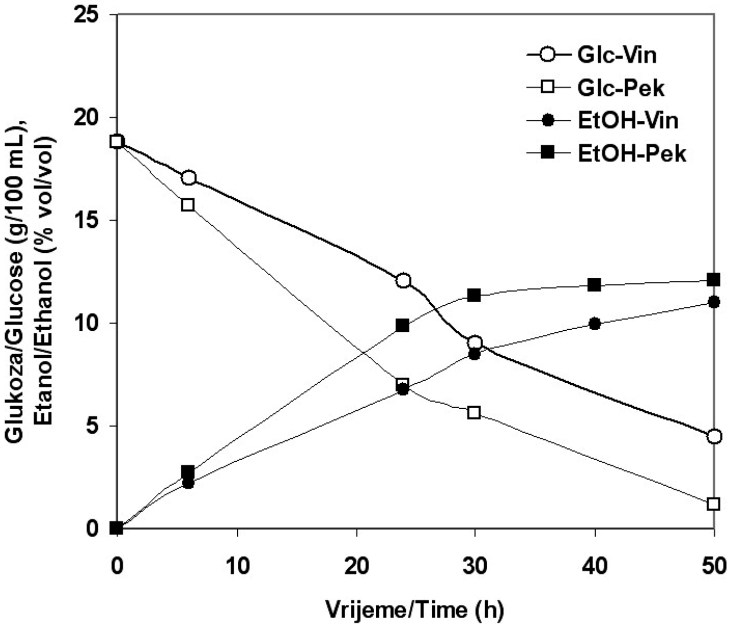


Figure 8. Prolonged fermentation of the corn semolina hydrolysate (with 250 g/L of corn semolina K$_1$), by 5 g/L of baker yeast (Pek) and wine yeast (Vin)

In this case baker's yeast fermented sugars much faster and yield reached 11.6 % vol/vol of ethanol after 30 hours. Wine yeast showed slower activity of fermentation, and get that concentration of alcohol after 50 hours. After 50 h baker's yeast produced 12.1 % vol/vol of ethanol. It is suggested that profitability of such process is very promising[18].

Very similar results were obtained with fermentation of semolina K2 hydrolysates (figure 9), but with a lower yield.

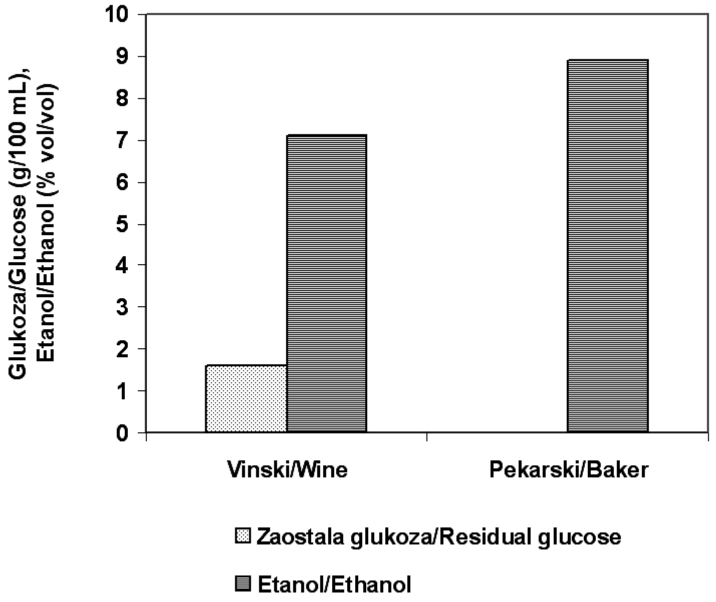


Figure 9. Ethanol yield and residual glucose after 24 hours of corn hydrolisate fermentation (250 g/L semolina) by 5 g/L of wine yeast and baker yeast

Better fermentation rate had baker's yeast and therefore it was used for the examination of the kinetics of process in bio-reactor (figure 10).

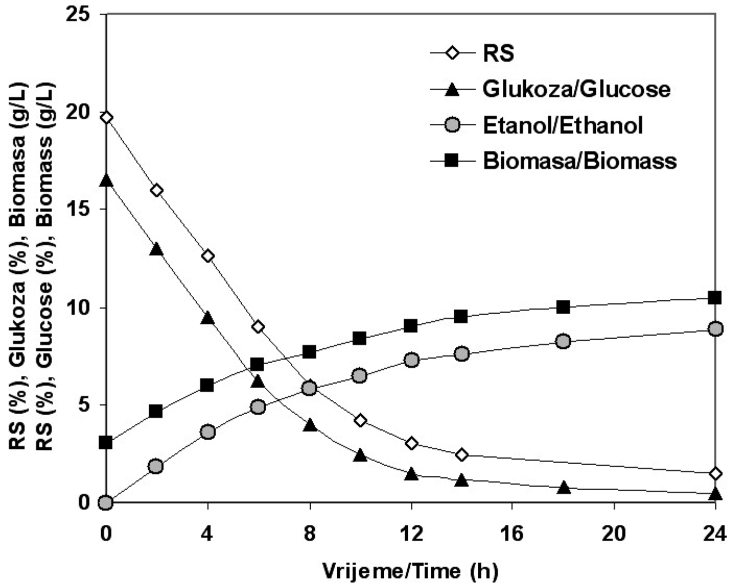


Figure 10. Fermentation of the corn semolina hydrolisate (with 250 g/L of corn semolina K_2), by 3 g/L of baker yeast, performed in a laboratory bioreactor Biostat A, with temperature and pH regulation.

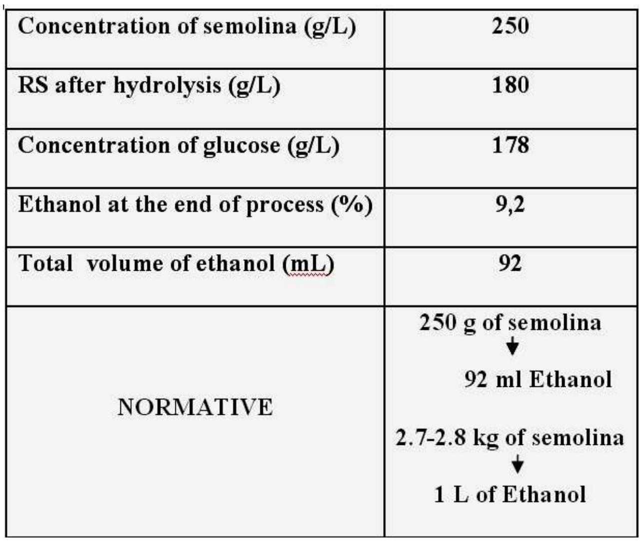
Using the hydrolysed substrate with 250 g/L of semolina K2 (mixture of corn), 9 % vol/vol of ethanol after 20 h of fermentation was achieved.

Growing interest for the production of bio-ethanol resulted in many research attempts to define activity of different yeast strains for the fermentation of specific substrates. Special attention was paid to the amylolytic yeast which could directly ferment starch [19] and yeasts for the fermentation of sugars from lingo-celluloses[3]. Mojovic et all.[20] and Rankin[21] used immobilised *S. cerevisiae,* what improved the ethanol tolerance. Mojovic et all.[16] have also demonstrated that higher concentration of yeast reached higher activity, what is in agreement with the results achieved in this work.

Calculation of normative

On the basis of the results in this work, the normative provision of corn semolina K2 for the production of ethanol was calculated and presented in table 3. Results are in good correlation with the results of some researchers[8], who examined exploitation of Croatian corn for ethanol production with different preparations of substrates.

Table 3. Normative for the production of ethanol from corn semolina K_2



Concentration of semolina (g/L)	**250**
RS after hydrolysis (g/L)	**180**
Concentration of glucose (g/L)	**178**
Ethanol at the end of process (%)	**9,2**
Total volume of ethanol (mL)	**92**
NORMATIVE	**250 g of semolina** ↓ **92 ml Ethanol** **2.7-2.8 kg of semolina** ↓ **1 L of Ethanol**

CONCLUSION

From the obtained results, it can be concluded that the examined cultivars of corn, produced in Croatia, are suitable for the potential production of bio-ethanol in the commercial-scale. The method and the used enzymes for the hydrolysis of corn semolina are adequate and should be adapted easily in plant process.

For the fermentation process *S. cerevisiae* preferable yeast, but strain of baker's yeast has better performances then wine yeast, since it has good substrate (glucose) tolerance, fast fermentation rate and also high tolerance to ethanol, up to 12% vol/vol. Since the concentration of starch in mixture of corn is 67-68 %, for the production of 1 L of bio-ethanol 2.7-2.8 kg of corn is required, what satisfies technological demands of Croatia. On the other hand, beside the advantages related to global warming and greenhouse gases, alternative fuels makes the country more energy independent.

REFERENCES

1. S.I. Mussatto, G. Dragone, P.M.R. Guimarães, J.P.A. Silva, L.M. Carneiro, I.C. Roberto, A.Vicente, L. Domingues, J.A. Teixeira, Biotechnol. Adv. 28 (2010) 817.
2. M.E.D. de Oliveira, B.E. Vaughan, E.J. Rykiel, BioScience 55 (7)(2005) 593.
3. Biofuels Platform, ENERS Energy Concept, Production of biofuels in the world (available online < http:// www.biofuels-platform.ch, 16 Feb 2011).
4. O.J. Sanchez, C.A. Cardona, Interciencia 30 (11) (2005) 671.
5. W.W. Institute, Biofuels for transportation: Global potential and implications for sustainable agriculture and energy in the 21st century, Washington, D.C., June7, 2006.
6. S. Grba, Proizvodnja alkohola, u knjizi Kvasci u biotehnološkoj proizvodnji, Izd. PLEJADA, Zagreb, 2010, pp.189- 225.

7. S. Grba, P. Horvat, J. Juračak, V. Marić, Studija izvodljivosti proizvodnje bio-etanola u Sladorani Županja, 2007.

8. N. Voca, B. Varga, T. Kricka, D. Curic, V. Jurisic, A. Matin, Bioresour. Technol. 100 (10) (2009) 2712.

9. Lj. Mojović, D. Pejin, O. Grujić, S. Markov, J. Pejin, M. Rakin, M. Vukašinović, S. Nikolić, D. Savić, CI&CEQ 15 (2009) 49.

10. B.S. Dien, X.Li, L. Iten, L.B. Jordan, D.B. Nichols, N.N O'Bryan, M.A. Cotta, Enzyme Microb. Tech. 39 (2006) 1137.

11. K.A Gray, L. Zhao, M. Emptage, Curr. Opin. Chem. Biol. 10 (2006) 141.

12. F. Torney, L. Moeller, A. Scarpa, K. Wang, Curr. Opin. Biotech, 18 (2007) 193.

13. J.Y. Zhu, X.J. Pan, Bioresour. Technol. 101 (2010) 4992.

14. H. Kretschmar, Hefe und Alcohol, Springer-Verlag, Berlin Heidelberg, 1955, pp. 597 – 600.

15. D. Lefebvre, V. Gabriel, Y. Vayssier, C. Fontagne-Faucher, Lebeusm. Wiss. Technol. 35 (2002) 407.

16. Lj. Mojović, S. Nikolić, M. Rakin, M. Vukasinović, Fuel 85 (2006) 1750.

17. V. Arasaratnam, K. Balasubramaniam, Starch 45 (1993) 231.

18. C. Berg, World Fuel Ethanol Analysis and Outlook (available online http: //www.distill.com/World-Fuel-Ethanol A&O-2004.html)

19. P.M. Kilonzo, A. Margaritis, J.T.Yu, Q. Ye, Int. J. Green Energy 4 (2007) 1.

20. Lj. Mojović, M. Rakin, M. Vukašinović, S. Nikolić, J. Pejin, D. Pejin, Chem. Eng.Transactions 21 (2010) 1333.

21. M. Rakin, Lj. Mojović, M. Vukašinović, S. Nikolić, M. Vukasinović, V. Nedović African J. Biotech. 8 (2009) 464.

TECHOLOGICAL NOTES

Low calorie diet may reverse diabetes: study

A new study has transformed thinking on type 2 diabetes by reporting that an extreme low calorie diet could reverse the condition in just eight weeks.

The research, published in the journal Diabetologia, reports that a low calorie diet of 600 calories a day for two months can remove excess fat clogging up the pancreas, thus allowing normal insulin secretion to be restored – overturning the long held belief that type 2 diabetes is a lifelong condition.

The early stage clinical trail in eleven people reported a 100 per cent reversal of diabetes symptoms during the two month diet period, with 64 per cent of the participants remaining diabetes free three months after the diet had finished.

“To have people free of diabetes after years with the condition is remarkable - and all because of an eight week diet,” said Professor Roy Taylor of Newcastle University, UK, who led the study. “This is a radical change in understanding Type 2 diabetes. It will change how we can explain it to people newly diagnosed with the condition.

“It has long been believed that someone with Type 2 diabetes will always have the disease, and that it will steadily get worse … we have shown that we can reverse the condition,” said Taylor

Study details

Under close supervision of a medical team, 11 people who had developed diabetes later in life were put on an extreme diet of just 600 calories a day, consisting of liquid diet drinks and non-starchy vegetables. They were matched to a control group of people without diabetes and then monitored over eight weeks to measure the production of insulin from their pancreas and fat content in the liver and pancreas.

After just one week, the research team found that pre-breakfast blood sugar levels had returned to normal, whilst scans revealed that fat levels in the pancreas had lowered from an elevated level of eight per cent to the healthy, normal level of six per cent.

In combination with the reduction of fat levels, the pancreas was found to regain its normal ability to produce insulin. As a result, blood sugar after meals was reported to steadily improve.

“We believe this shows that Type 2 diabetes is all about energy balance in the body,” explained Taylor, “if you are eating more than you burn, then the excess is stored in the liver and pancreas as fat which can lead to Type 2 diabetes in some people.”

The researchers said that the new insight “allows an understanding of the causality of type 2 diabetes in individuals as well as in populations.”

“It carries major implications for information to be given to newly diagnosed patients, who should know that they have a potentially reversible condition and not one that is inevitably progressive,” they concluded.

Source:
http://www.foodnavigator.com/Science-Nutrition/Gluten-powder-may-boost-pasta-quality-Study

Coca-Cola snaps up first Bonsucro certified sugarcane

The first products using sustainable sugarcane under a new certification scheme are about to hit the market after the initial batch was purchased by The Coca-Cola Company’s bottling system.

The Bonsucro environmental and social sustainability standard was used to certify more than 130,000 tons of sugar at a Raízen mill in Brazil in an initiative expected to “change the sugarcane industry forever”.

The certification is said to be “relatively inexpensive” and allows companies to demonstrate through measurable impacts that it is “against child labour, supports human rights and is concerned with implementing environmentally sound practices”.

Bonsucro is a multi-stakeholder organisation and a spokeswoman told FoodNavigator.com that it is the “only global metric standard on the market today”.

She added “It is unique because it can specifically measure the impact of the production of sugarcane. Bonsucro is the only standard in the market that covers all three elements of sustainability-environment, social and economic stability and growth.”

The spokeswoman said that members can choose whether or not to use the registered trademark Bonsucro either on product or off-product.

Kevin Ogorzalek, program officer, World Wildlife Fund and chairman of the Bonsucro Board, said: “This will change the sugarcane industry forever. Certification will drive the industry to greater sustainability, thereby preserving natural resources while upholding human and labour rights.”

The 130,000 tons of sugar and 63,000 cubic metres of ethanol were certified against the Bonsucro production standard by independent certification body SGS.

This may be a drop in the ocean considering that globally about 30 million hectares are farmed for sugarcane. However, Bonsucro said it plans to increase its membership by 25 per cent every year.

Its members are producers, growers, companies who purchase the product (including British Sugar, Cargill, Syngenta and Tate & Lyle Sugars) and NGO’s such as WWF.

Sugarcane is used as a table sugar, but also in the production of foods, sweetened beverages, livestock feed, molasses, rum and the production of biofuels.

The production standard assesses the biodiversity, ecosystem and human rights impacts of sugarcane production and demands legal compliance and continuous improvement throughout the production process.

This is assessed against key indicators, such as energy consumption, greenhouse gas emissions and water consumption. Sugarcane mills are required to be members of Bonsucro and certificates are valid for three years, with annual audits.

Bonsucro was originally the Better Sugarcane Initiative – an industry body set up to develop a productions standard with the aim to reduce the environmental and social impacts of sugarcane.

The first certification is said to be the conclusion of five years collaboration between the world's biggest sugarcane producers, corporations and influential NGOs, “providing a standard against which the sustainability of sugarcane derived products can be assessed by consumers, companies, governments and NGOs”.

Source:

Post a commentBy Sarah Hills, 22-Jun-2011

Related topics: Sustainable sourcing, Financial & Industry, Carbohydrates and fibres (sugar, starches)

Gluten powder may boost pasta quality: Study

The addition of gluten powder to pasta made from poor quality flour may lead to greater quality and improved consumer acceptance, says new research.

The study, published in Journal of Texture Studies, reports that the addition of three grams of powder to pasta made from poor quality, low protein flour, can increase the quality and colour of the cooked pasta.

“This study shows an easy and applicable method of improving the quality of pasta made from low protein wheat flour using gluten powder,” said the researchers, led by Mahsa Majzoobi from the department of food science and technology, at Shiraz University, Iran.

The researchers said that the findings to their study “may be useful for pasta producers in some parts of the world where ordinary wheat flour is the most available type of flour for pasta production.”

Pasta production

The researchers noted that one of the most important factors influencing the quality of pasta is the protein content and gluten quality of the flour used. They noted that the protein content of semolina is normally higher than 12.5 per cent, while almost all non-semolina flours have lower protein content, making them unsuitable for pasta production.

Majzoobi and colleagues added that glutenin, a low molecular weight fraction of gluten, is crucial for the production of high-quality pasta.

Due to the high price and falling supply of semolina and other gluten rich flours, in some parts of the world pasta’s have been partially or totally substituted with other flours, including as ordinary wheat flour, corn, rice, oats, legumes or pea flours. However, the replacement or partial substitution of semolina with other flours has adverse effects on the quality of the final product, said the authors.

“Common problems with these types of pasta are their unacceptable colour, high cooking

Beta-glucan could be used to partially replace salt while retaining taste and texture in high pressure processing of chicken breast meat, according to a new study in Food Chemistry.

Beta-glucan is a dietary fiber most often extracted from oats or barley. The study’s authors, from the University of Alberta’s Department of Agricultural, Food and Nutritional Science, said that increasing interest in minimally processed and additive free foods is spurring development of novel processing technologies that can improve textural properties and taste. They tested oat beta-glucan in temperature assisted high pressure processing of reduced sodium ground chicken breast meat.

“Temperature assisted high pressure processing of foods is a method to prepare gel type products with the desired gel texture,” the researchers wrote. “Further, such processing helps to inactivate microorganisms and hence extends the shelf-life of the product.”

Sodium chloride – NaCl, or common table salt – is a common additive in the meat industry for making protein soluble, thereby improving protein’s functional properties, the authors said. However, excessive sodium consumption has been linked to increased risk of hypertension.

To reduce the amount of sodium in meats, phosphates are often added to the mix, but in this latest study, researchers aimed to get a similar textural effect through processing with oat beta-glucan, to allow manufacturers to cut salt and eliminate phosphates.

They found the optimum conditions for taste and texture of the finished product to be 400/600 MPa pressure, and 40°C, in a formulation including 1 per cent salt and 0.3 per cent beta-glucan. Addition of salt favours gelation, an important factor for the texture of processed meats.

The authors wrote: “The solubility of all the samples decreased drastically at a processing temperature of 60°C compared to that at 20°C and 40°C, indicating that temperature assists formation of a gel network resulting in decreased total protein solubility…At higher pressure level (600 MPa) and temperatures (20°C and 60°), total protein solubility of NaCl and BG [beta-glucan] samples were statistically similar, indicating that BG can be used as a partial replacement for NaCl in temperature assisted high pressure processed products.”

In addition to its potential for reducing salt and phosphates in meat, the authors noted that the US Food and Drug Administration has approved a claim for oat beta-glucan on product labels, meaning that food manufacturers are authorized to say that the ingredient can have a beneficial effect on cholesterol levels at consumption levels of 3g or more per day.

Source:
Food Chemistry (2011)
doi: 10.1016/j.foodchem.2011.05.018
“The use of β-glucan as a partial salt replacer in high pressure processed chicken
breast meat”
Authors: Dileep A. Omana, Graham Plastow, Mirko Betti

New microbial enzymes aim to plug animal-free Italian cheese gap

Biocatalysts has launched a new range of microbial enzymes to produce Italian style cheese for processing applications without resorting to animal-derived products.

The new microbial pregastric esterases (PGEs) are used to make ‘enzyme modified cheeses’ which are very strong products that are then diluted and used in processed cheese, cheese sauces and snacks.

Market gap

Aelig Robin, a dairy scientist at Biocatalysts, told this publication that the new products fill a market gap for enzymes suitable for Italian style cheeses that are not derived from animals.

Robin said there were previously no microbial enzymes on the market for hard Italian style cheeses such as Provolone, Parmesan and Romano despite growing market interest.

“Demand for non-animal enzymes is increasing – there is a lot of need in the market for halal and kosher products and they can be used for vegetarian market applications as well,” said Robin.

Explaining why there have been no microbial launches for hard Italian style cheeses until now, she said: “It is not that easy to do all the development work – it has been a long term project at Biocatalysts.”

By studying the fatty acids that are released by the microbial enzymes and using sensory analysis, Robin said the company was able to ensure that the new enzymes match the specific flavours created by their animal-derived counterparts.

The PGEs are added to a cheese slurry and work to create a very potent product within 48 hours. Typically food manufacturers then use the enzyme modified cheese in formulation at a dosage level of 2 or 3 per cent with fresh cheese.

The new range of enzymes consists of three products: Flavorpro C, Flavorpro K & Flavorpro L.

http://www.foodnavigator.com/Science-Nutrition/Whey-protein-may-aid-low-fat-yoghurt-formulation-Study

Whey protein may aid low-fat yoghurt formulation: Study

By Nathan Gray, 14-Jun-2011

Related topics: Science & Nutrition

The addition of whey protein may boost the textural properties of low-fat yoghurt to levels comparable with its full-fat counterpart, according to new research.

They study, published in LWT - Food Science and Technology, investigates the effect of whey protein addition on textural properties of yoghurt at different protein and fat contents, finding that “the use of whey proteins impart the possibility to replace parts of fat.”

The team of researchers, led by Alina Krzeminski from the institute of food science and biotechnology at the University of Hohenheim, Germany, reported that the firmness and viscosity of yoghurt samples increased with the addition of whey protein.

“It is evident that the addition of whey proteins reinforces firmness properties oflow-fat yoghurts comparable to characteristics of full-fat yoghurt,” wrote Krzeminski and colleagues.

Low-fat demand

As consumer demand for healthy products grows, many food producers are being forced to develop new low-fat products without altering sensory and functional properties of products.

Hydrocolloids and stabilizers have been used to imitate the fat perception and enhance the stability of yoghurt, however the reduced fat levels still led to a loss in viscosity and structure, “resulting in an altered appearance, texture, and mouthfeel,” said the authors.

One alternative to fat replacement with hydrocolloids is the use of milk ingredients such as whey proteins. Krzeminski and colleagues noted that previous research has highlighted the potential application for the use whey proteins in cheese production.

Although several studies have been carried out on the textural effects of whey protein-enriched yoghurts, there is little knowledge about the characteristics of those yoghurt systems depending on protein and fat content, particularly for low-fat yoghurt products, said Krzeminski and co-workers.

Study details

The authors reported that the addition of whey protein increased the firmness (elastic modulus) and apparent viscosity of yoghurt samples. They noted that the increases were a result of increased inter particle interactions, “mainly caused by self-aggregation of whey proteins or aggregated whey protein-coated fat globules, respectively.”

Krzeminski and colleagues noted that as whey protein content raised, the particle size, viscosity, and network firmness also increased.

The research team concluded that that the use of whey proteins imparts the possibility to replace parts of fat in yoghurt.

Source:

LWT - Food Science and Technology

“Structural properties of stirred yoghurt as influenced by whey proteins”

Authors: A. Krzeminski, K. Großhable, J. Hinrichs

First Zero-Waste Biochemical Refinery In Production

The country’s first zero-waste biochemical refinery is now up and running in Missoula, Montana. The facility is owned and operated by Blue Marble Biomaterials and uses a combination of clean technologies to produce specialty biochemicals that can be used in the food, cosmetics and personal care markets. Each year the biorefinery will produce 72 tons of biochemicals, and its only “waste” is purified water and pellet fuel for wood boilers.

“This biorefinery is an excellent example of how the U.S. can ensure global leadership in advanced manufacturing: we have developed cutting edge technology which combine the fields of biology, chemistry, and industrial manufacturing to produce petroleum replacing chemicals,” said Blue Marble CEO Kelly Ogilvie. “These products will reinvigorate the meaning of Made in America: more lean, more clean and sourced from our abundant renewable natural resources.”

Last week, President Obama announced intentions to invest $500 million in U.S.-based advanced manufacturing technologies via an Advanced Manufacturing Program. The Blue Marble co-founders, Ogilvie and James Stephens, served on the council that worked with the president in forming the program.

The system uses a range of plant feedstocks to produce the biochemicals. The facility uses a photo-bioreactor containing algae to purify wastewater and waste gas from the fermentation system. In addition, the solid waste generated during the production process is pelletized for use in wood-burning furnaces and stoves. In the future, the company intends to power its facility with the waste gas and pellets created during the process in onsite gasifiers.

“Natural systems are the inspiration for our processes. Just as in nature, we see waste as nutritional; in this case, wastewater, waste gas and waste solids are industrial nutrients for both our system and the surrounding economy,” added Stephens. “We believe this principal is key to reducing not only environmental impact but also operational cost.”

New Laser-based Nanotechnology Could Grow Human Cells for Damaged Body Parts

New EUV laser technique could be a better and faster way of regrowing human cells European researchers working on the EUREKA project ModPolEUV have found a new method of rebuilding parts of the body that have been damaged using polymeric material.

Johannes Heitz, main coordinator of the ModPolEUV project and Senior Research Associate at the University of Linz, Austria, along with Dr. Henryk Fiederowicz, professor at the Military University of Technology in Warsaw, and a team of European scientists, have developed a new and better way of growing human cells in the event that the body has been damaged.

Using polymeric material to regrow human cells is not a new discovery. According to Heitz, researchers have known for a few years that synthetic polymeric materials have the ability to not only grow, but also multiply human cells significantly in order to develop new body parts faster. What's new about this study is the method that this particular group of scientists chose in which to grow the human cells.

Heitz and his team have created a new laser-based technology called EUV, which stands for Extreme Ultra-Violet. The beam of EUV light was made with a special mirror, and when this EUV light hits the material, new types of polymeric materials can be made. Specifically, nano-structured polymer surfaces were created by the EUV technique.

The EUV technique has a high level of precision ranging from 10 to 20 nanometers, while older techniques only had a precision level of 100 nanometers. This method also conserves the material's structure.

The greatest benefit of the EUV method is that the nano-structures made by this technique are capable of influencing organic cells' behavior, which can cause the cells to grow "better and faster depending on the type of polymer surface used."

"Using one type of polymer material or another will help you grow different types of muscle, nerves, cells adapted to a human heart, bone or any other part of the human body," said Heitz.

This method can be used in a number of medical-related ways. For instance, the World Health Organization estimates that 322,000 deaths worldwide per year are caused in part by severe fire-related injuries. Several of these deaths could have been prevented with surgery, but the problem is that patients with major burns do not have enough skin to graft and need to have skin regrown by skin cells. Prior to this new EUV technique, this process would take much longer, increasing risk of infection in the burn patients exposed wounds.

The EUV method can also be used to develop medical implants. In the future, it could be used in many fields such as microelectronics, micro-mechanics, and integrated optics.

The EUV technique is still in a testing phase, but has already been installed into laboratories in the United States, Germany, Japan, China, South Korea, the Czech Republic and France. The next step will be to prepare for the commercial phase.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