TECHNOLOGICA ACTA

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

ISSN 1840-0426
ISSN 2232-7568

Vol. 4
No. 2
December 2011.

TECHNOLOGICA ACTA

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

Vol. 4 Number 2, page 1 – 58, Tuzla, december 2011. year

TECHNOLOGICA ACTA

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

Vol. 4 Number 2, page 1 – 58, Tuzla, december 2011. year

Publisher / Izdavač
Faculty of Technology University in Tuzla

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

This number of Technologica acta is supported by the Federal Ministry of Education, Science and Culture of Bosnia and Herzegovina

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

C O N T E N S

MICROBIOLOGICAL ANALYSIS OF FAST FOOD MEAT PRODUCTS IN TUZLA AREA

PROFESSIONAL PAPER

Milan Perić [1], Elvira Lonić [2], Snježana Hodžić [2],
Marizela Šabanović [3], Edina Hajdarević [2]

[1]*UKC, Trnovac bb, Tuzla*
[2]*Faculty of Science, Univerzitetska 4, Tuzla*
[3]*Faculty of Pharmacy, Univerzitetska 4, Tuzla*

ABSTRACT

Food used to be and is still one of the key factors of human adaptation to surroundings. Human need for food is tightly connected with soil fertility, climate and finally with sources of nourishment. However, besides man, other living creatures, like microorganisms, insects, as well as other animals, know about available sources of nourishment on Earth. Human beings are the only species from the animal world that tend to adapt the surroundings to their particular needs. Apart from milk, the most appreciated foodstuff in nutrition, meat is very significant as well, because it is a source of easily digestible and biologically and energetically valuable ingredients. Therefore, meat is one of the most frequent foodstuffs in human nutrition and it is necessary to control it especially in places where people often eat.The results of the microbiological analysis of meat samples taken in 10 different fast food restaurants are shown in this study.
The selected samples were analyzed using the standard microbiological method, and the results of the analysis show that all samples were microbiologically sound since the total number of bacteria in 1g of the sample was within acceptable limits. In favor to this is the fact that the pathogenic microorganisms harmful to human health were not found.

Key words: food, meat, microbiological methods

INTRODUCTION

Microbiology of meat and meat products is a special sector of microbiology which identifies and determines the origin of microorganisms in these foodstuffs. Unlike the sanitary and veterinary microbiology, not only harmful to health spices but the entire population is researched, because most of present microorganisms may influence the quality of meat products. An important section of the microbiology includes analysis of interactions between microorganisms and meat or meat products, as a substrate in which all these living creatures develop.[1] Meat is rich in all nutrients necessary for microbial growth, and by superficial consideration one can conclude that meat is a common source of health and sanitary problems for people. Yet meat is not an ideal environment for the development of microorganisms, because a developed system of connective tissue membranes prevents their penetration into deeper layers. In addition, meat contains small quantity of low molecular weight compounds that microbes directly use for energy purposes and construction of cellular elements. During ripening the pH value decreases below a necessary optimum for the most of microorganisms, the meat surface dries and meat is stored at low

temperatures preventing or significantly slowing reproduction of microorganisms. During handling and processing of meat, conditions for subsequent contamination and reproduction of microorganisms are created. Meat and meat products may be contaminated when they come into contact with hands, working surfaces, additives etc. Contamination is increased by cutting and chopping meat. Elevated temperatures during certain phases of the technological process and extracted meat juice contribute to faster reproduction of the present microflora.[2]

A constant control of production is carried out in order to observe hygienic quality of meat, thus reducing the risk of deterioration and food poisoning, and extending meat usage.[3]

Meat is normally sterile in its deeper layers or has a very small number of bacteria (up to 10^2/g). Anaerobic, facultative anaerobic and microaerophilic bacteria from the genera *Clostridium, Bacillus, Streptococcus, Lactobacillus*, and possibly from the family *Enterobacteriaceae* can be found in deeper layers of meat. However, microorganisms are always present on the meat surfaces, and their number depends on work hygiene and slaughter technology, processing and cooling. There are normally 10^2 – 10^5 microorganisms / cm^2 on the surface. [4]

Microflora of warm meat is comprised mainly of mesophilic microorganisms from the genera *Micrococcus, Brevibacter, Moraxella, Lactobacillus, Bacillus, Streptococcus*, but less from genera *Acinobacter, Leuconostoc, Mycobacterium* and *Pseudomonas*. Representatives of the genera *Pseudomonas, Moraxella, Brochothrix* and some genera of the family *Enterobacteriaceae* are present on the surface of chilled meat. Species of genera *Flavobacterium, Alcaligenes, Vibrio, Aeromonas* and *Arthrobacter* were seldom isolated. As a consequence of bacteria reproduction meat can be spoiled or people who consume it can be poisoned.[3]

Meat safety is usually determined by bacteriological examination of samples or swabs from meat surface or from work surfaces. However, the results of bacteriological analyses do not have to indicate the source of infection. Therefore, a hygiene monitoring also includes the hygiene of hands, surfaces of technological equipment as well as other appliances. A constant bacteriological control quite contributes to meat safety, yet it is expensive and sometimes does not give satisfactory results[3].

Many pathogenic microorganisms that can be found in meat, such as *Salmonella, Shigella, Brucella*, etc, are usually destroyed by high temperatures during a usual heat treatment.

The toxin, produced by *Staphilococccus aureus*, is not normally destroyed by the heat treatment used in boiling sausages or other meat products. On the other hand, temperatures above 100^0 C destroy the toxin of *Clostridium botulinum*, but not its spores. Roasting is a less efficient way of destroying microorganisms than boiling. Thermophilic and thermoresistent strains are not destroyed by roasting [5].

RESEARCH GOALS

The main research goals are:

- Determining the microbiological characteristic of meat products using the microbiological methods
- Defining microbiological quality of meat in fast food products.

MATERIALS AND METHODS

Food samples, ready grilled meat products - kebab, were collected in 10 fast food restaurants in the area of Tuzla. The samples were labeled with numbers from 1 to 10 in this study. Microbiological tests were performed in a laboratory of the Department of Microbiology at University - Clinical Center in Tuzla and Faculty of Science of the University in Tuzla. All samples were taken following the relevant legislation[3,6]. Primers that were used for microbiological food testing were manufactured by „Torlak" Belgrade and "Oxoid" England. According to the Regulation on conditions and terms of microbiological safety which must be applied in foodstuffs (Official Gazette SR BiH 45/83), which was taken over by the Regulation on taking the federal regulations that are applied in the Republic of Bosnia and Herzegovina as a Republican legislation (Official Gazette SR BiH No. 2 / 92), and pursuant to Article 4, the microbiological methods include determination of the total number of live microorganisms in 1 g sample, coagulasa of the positive staphylococci, *Proteus* species, *Escherichia coli*, *Salmonella* species and sulfite-reducing clostridia [7].

The analyses were performed according to the methods prescribed by the Regulation on the methods of conducting microbiological analysis and super-analysis of food products (Official Gazette SR BiH No. 25/80) [6].

The samples were taken in aseptic conditions into sterile bags for food sampling. They were transported to the laboratory in a portable fridge and during the next 24 hours sown on bacteriological mediums. Primary dilution was made by homogenizing 20 g of the sample and 180 ml of normal saline producing a dilution of 0.1, which was used for preparing other dilutions depending on the type of food and bacteria that were tested.

Determining the total number of aerobic microorganisms in 1 ml sample

Certain volumes of the diluted sample were transferred into a sterile Petri plate, into which 15-20 ml of dissolved and at 45º C cooled agar was poured for total bacterial count. After the homogenization of sample and substrate, the Petri plates were incubated at 37º C / 48 hours. After incubation, colonies were counted with a magnifying glass and CFU (colony forming units) were determined according to the formula [8].

$$\text{CFU} = \frac{\text{number of grown colonies}}{\text{volumen of the sample}} \times \text{reciprocal value of the diluted sample}$$

Proving coagulasa positive staphylococci

Certain volume of diluted sample was sown in 9ml of salty bouillon and incubated at 37º C / 18-24 hours. Then 0,1ml of salty bouillon was transplanted on blood agar and incubated at 37º C / 18-24 hours. Grown colonies were further identified by examining biochemical characteristics. On the characteristic colonies (golden yellow, smooth, shiny with beta hemolysis), the test on the enzyme coagulase (+) and catalase (+) production and test on mannitol which was incubated aerobically (+) and anaerobic (+), were performed.

Proving *Proteus* species

Certain volume of diluted sample was sown in 9 ml glucose bouillon and incubated at 37º C / 18 - 24 hours. Then 0,1ml of blurred

glucose bouillon was transplanted on endo agar and incubated at 37º C / 18-24 hours. The increases of suspicious colonies on endo agar were lactose negative, pale pink, shiny and smooth colonies. The suspicious colonies on *Proteus* species were further identified by examining biochemical characteristics (H_2S test, lactose, mannitol, indole, urea, citrate). The test results that confirm *Proteus* species are: H_2S positive in the lower part of the test tube and intractable slope, lactose negative, mannitol negative, indole variable, urea positive, citrate negative.

Proving *Escherichia coli*

Certain volume of diluted sample was sown in 9 ml of brilliant green lactose bile bouillon (BGLBB) with Durham's tube and incubated at 44º C / 48 hours. If a blurring, color change and gas appear in Durham's tube, it is sown on endo agar, and the sown Petri dishes were incubated at 37º C / 18 - 24 hours. Lactose positive colonies red, smooth or shiny were further identified by examining biochemical characteristics of the media: triple Kligler's sugar (acidity and gas), lactose (+), mannitol (+), urea (-), citrate (-).

Proving *Salmonella* species

Salmonella species was detected by sowing 25 g of the sample directly in 225 ml of Selenite F bouillon. Sown bouillon was incubated at 37 º C / 18 - 24 hours. After incubation, selenite bouillon shined on *Salmonella - Shigella* agar (SS agar) and repeatedly incubated at 37º C / 18 - 24 hours. Suspicious colonies (pale pink with a black spot in the middle) were sown on the triple sugar and incubated at 37ºC / 18 - 24 hours. Colonies from the characteristiccally altered triple sugar (H_2S positive in the lower part of the test tube and intractable slope) were identified by further examining of biochemical characteristics of the substrates: lactose, mannitol, indole, urea and citrate. The results of a positive test for the bacteria are lactose negative, mannitol positive, indole negative, urea negative, citrate positive.

Determining sulfite reducing clostridia

Certain volume of diluted sample was transferred into an empty tube and treated for 10 minutes at 80º C in a water bath. Afterwards, melted and at 45-50º C chilled sulphite agar was poured in the tube, and incubated at 37º C / 3 - 5 days in aerobic conditions. Bacteriological smear, according to Gram, was made of the suspected colonies that can be black, cobweb and cleave the substrate. As the result, Gram positive rod bacteria with spores were sown on blood agar, which were incubated in aerobic conditions at 37º C / 24 hours and in anaerobic conditions at 37º C / 48 hours. The results of anaerobic growth of colonies are sulphate reducing clostridias. Rigid, large, hemolytic colonies on blood agar after aerobic incubation (antracoid) were tested on mannitol to exclude possible presence of *Bacillus cereus.*

RESULTS AND DISCUSSION

According to the performed laboratory analysis of the samples of the ready grilled meat product – kebab taken in ten fast food restaurants, all samples were microbiologically sound. The results of the analysis are shown in Table 1.

Based on the microbiological analysis of the ready meat product - kebab in fast food restaurants, the total number of aerobic mezophilic bacteria in 1g of the sample

ranged from 0 to 200, which corresponds to microbiologically safe product for this type of food. In 50% of samples (5 / 10) sporogenic bacilli (antracoids) were isolated, while the only one sample showed the presence of *Streptococcus faecalis*.

A relatively small number of microorganisms and absence of pathogenic bacteria in the tested samples showed the microbiological quality of the ready product in all the fast food restaurants. Raw meat contains a variety of microorganisms, both at the surface and in its deeper layers requiring certain conditions for their growth and reproduction. Temperature is one of the important factors for their survival. The most important prerequisite for the use of meat in diet is to reduce the number and types of microorganisms. The number of microorganisms and possibility of their further reproduction are significantly reduced by low temperature during storage of meat before its use.

However, high temperatures achieved during thermal processing of meat also have important effects on the micro flora of meat, besides the effects of low temperature. At tempera-tures below 100° C the vegetative forms of microorganisms are destroyed, while at temperatures above 100ºC spores and bacteria are destroyed. During the thermal processing of the samples (grilling) a temperature of 120-160^0 C was used reducing the number of bacteria, and a small number of aerobic bacteria was isolated in the tested samples. High temperature is achieved on the surface of food which is grilled, while the inner temperature of food is much lower and does not cause destruction of all bacterial species [9]. In our samples, a small number of sporogenic aerobic bacilli (antracoidi) and *Streptococcus faecalis* were identified.

Sporogenic bacilli (ex. *Bacillus subtilis*) are extremely resistant to high temperatures and can handle temperatures of 100° C during 18 minutes[10]. Insufficient heat treatment of the grilled products (short period, inadequate temperature) clarifies the high percentage (50%) of the bacteria positive samples. The results of the research on the bacterium *Listeria monocytogenes* also showed a lack of the heat treatment effectiveness and presence of bacteria in grilled meat products after heat treatment but depending on the initial number of bacteria [9].

Streptococcus faecalis detected in the tested sample most likely comes from the subsequent contamination of meat after the thermal treatment, caused primarily by unsanitary conditions created by a man and equipment.

The hygienic quality of ready meat products - kebab in this study are the result of continuous application of good hygiene procedures and detailed sanitary and hygienic control of facilities and staff conducted by the sanitary inspection.

Table 1. Microbiological analysis meat, ready product - kebab (meat rolls)

	Microbiological parameters	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10
1.	The total number of aerobic mesophilic bacteria to 1 g	5	Ø	10	5	200	65	5	55	Ø	Ø
2.	Bacteria *Salmonella* species	-	-	-	-	-	-	-	-	-	-
3.	Coagulase-positive *Staphilococci*	-	-	-	-	-	-	-	-	-	-
4.	Sulfite-reducing *Clostridia*	-	-	-	-	-	-	-	-	-	-
5.	*Proteus* species	-	-	-	-	-	-	-	-	-	-
6.	Escherichia coli	-	-	-	-	-	-	-	-	-	-
7.	Other isolated bacteria	Antrakoidi	-	-	-	Antrakoidi	Antrakoidi	Antrakoidi	*Streptococcus faecalis*	Antrakoidi	-

CONCLUSIONS

Based on the microbiological analysis of the samples of ready meat product – kebab taken in 10 fast food restaurants in the area of Tuzla, the following can be concluded:

- According to the Regulations on conditions of microbiological safety which must be applied in foods-tuffs, all the tested samples were microbiologically sound.
- Sporogenic aerobic bacills (antracoids) were identified in 50% of the tested samples, and their presence is most likely a consequence of the inadequate heat treatment.
- The presence of *Streptococcus faecalis* in one tested sample is the result of contamination of meat after the thermal treatment, and a particular attention should be given to the hygienic conditions of equipment and staff that come into contact with it.
- Since fast food restaurants are visited by a great number of people, it is necessary to conduct continu-ous microbiological anal-ysis of products and frequent sanitary hygienic measures of controlling facilities, staff and equipment in order to ensure prevention of possible human diseases which can be caused by microorga-nisms present in food.

REFERENCES

1.Z. Bem, J. Adamič, Mikrobiologija mesa i proizvoda od mesa, Tehnološki fakultet, Univerzitet u Novom Sadu. (1991) 92

2.M. Šolaja, Lj. Topalić-Trivunović, Mikrobiološke metode analize namirnica, Tehnološki fakultet, Univerzitet u Banja Luci. (2007) 6-9

3.R. Rede, Lj. Petrović, Tehnologija mesa i nauka o mesu. Prvo izdanje Novi Sad: Tehnološki fakultet. (1997) 3-7, 268–277

4.R.B. Tompkin, A.M. McNamara, G.R. Acuff, Meat and Poultry Products. In Compendium od Methods for the Microbiological Examination od Foods. Ed by Frances Pouch Downes and Keith Ito. APHA, Waschington, DC, (2001) 463-471

5.I. Savić, Ž. Milosavljević, Higijena i tehnologija mesa. Prvo izdanje Beograd: Privredni pregled. (1983) 181- 190

6.Pravilnik o metodama vršenja mikrobioloških analiza i super-analiza životnih namirnica (Sl. list SR BiH broj 25/80)

7.Pravilnik o uslovima u pogledu mikrobiološke ispravnosti kojima moraju odgovarati životne namirnice u prometu (Sl. list SR BiH 45/83), a koji je preuzet Uredbom o preuzimanju saveznih propisa koji se u R BiH primjenjuju kao Republički propisi (Sl. list SR BiH broj 2/92).

8.S. Duraković, Opća mikrobiologija, Zagreb: Medicinska naklada (1996).

9.P. G. Karaioannoglou, G. C. Xenos, Survival of Listeria monocytogenes in meatballs. Hell. Vet. Med. (1980) 23:111-117

10.S. Duraković, Prehrambena mikrobio-logija, Zagreb Medicinska naklada (1991) VI:199-218.


MODRIČA
OIL REFINERY
BIOPROIZVODI
-Dvotaktol bio
-Testerol bio
-Biohidraulik- HEES
-Biorazgradive mazive masti
Motorna ulja
Ulja za mjenjače i diferencijale
Ulja za automatsku transmisiju
Tečnosti za hlađenje i zaštitu motora
Maziva i tečnosti za industrijska postrojenja
Ulja za obradu metala
Mazive masti
Bazna ulja Grupa II i Grupa III
Bijeljeni parafini
57 godina iskustva u proizvodnji baznih ulja i maziva
31 godina iskustva u proizvodnji hidrokrekovanih baznih ulja
RAFINERIJA ULJA MODRIČA; Vojvode Stepe Stepanovića 49; 74480 Modriča, Bosna i Hercegovina
Tel: +387 53 810 111; Fax: +387 53 812 541 info@modricaoil.com www.modricaoil.com

EFFECT OF ACID AMLODIPINE SALT ANION ON CRYSTAL SIZE DISTRIBUTION DURING CRYSTALLIZATION FROM METHANOL AND ETHANOL

ORIGINAL SCIENTIFIC PAPER

Jozo Budimir[1], Mirza Nuhanović[2], Ranka Kubiček[1], Božo Banjanin[3], Jasmin Suljagić[1], Mersiha Suljkanović[3]

[1]*Faculty of Technology, University of Tuzla,* [2] *Bosnalijek, Sarajevo,*
[3]*Faculty of Science and Mathematic, University of Tuzla*

ABSTRACT

Acid salts of amlodipine are used as pharmaceutical bioactive components. They are obtained by the reaction of amlodipine with pharmaceutically suitable organic acids (benzenesulphonic, succinic, adipic, orotic, acetic, etc). Conditions of recrystallization of these salts affect their morphological characteristics and crystal size distribution, which is significant both from the aspect of performing technological operations in preparation of pharmaceutical products (pills), and from the aspect of drug dissolution speed during the drug application.

In this paper, the effect of anions and solvents on crystal size distributions, during the spontaneous recrystallization process at 25^{o}C, were investigated. The results (HPLC, DCS with TGA and HTSM) indicate that recrystallization process from concentrated ethanol and methanol resulted in the formation of predominantly anhydrous salts with morphological properties and purity that satisfy requirements of the pharmaceutical industry. Also, the obtained results from PSA analysis show that the crystal size and its distribution, depends on type of anion and type of alcohol.

Key words: amlodipine benzenesulphonate, amlodipine orotate, amlodipine adipate, amlodipine succinate, crystal size distribution, ethanol, methanol.

INTRODUCTION

Acid salts of amlodipine [(±)-3-ethyl-5-methyl-2-(aminoethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate] are used as pharmaceutical bioactive components in treatment of cardiovascular diseases (hypertension, angina pectoris, etc). Although amlodipine benzenesulphonate (amlodipine besylate) is a very efficient bioactive substance with good physical and chemical properties (thermostability, non-hygroscopicity, chemical stability, suitable for making tablets and soluble in aqueous solutions), disadvantages in its synthesis are related to toxicity and corrosiveness of benzenesulphonic acid. Therefore, other acid salts of amlodipine (adipate, succinate, sulphate, orotate, malonate, oxalate, tartarate, etc) were synthesized and pharmacologically investigated [1-10].

In the synthesis, various solvents as well as their mixtures (water, ethyl acetate, ethanol, methanol, isopropanol, acetonitrile, hexane, isopropyl ether, toluene, dimethyl sulphoxide) were used[10]. From aqueous solutions, the largest proportion of synthe-sized acid salts of amlodipine are crystallized hydrates

with a water content between 0.8 and 2.5 water molecules for one molecule of acid salt of amlodipine. Increasing the temperature and concen-tration of bioactive substance and solvent, the proportion of anhydrous crystalline form increases[11,12].

Previous studies were based on determining the influence of anions and solvents on the morphological characteristics, hygrosco-picity, photostability, thermostability and synthetic path of amlodipine acid salts, but not on their effect on crystall size and its distribution.

Crystall size and its distribution for active pharmaceutical substance are important factors both from the aspect of performing technological operations (mixing, sieving, drying, etc) in preparation of pharmace-utical products (pills), and from the aspect of drug dissolution speed during the drug application[14].

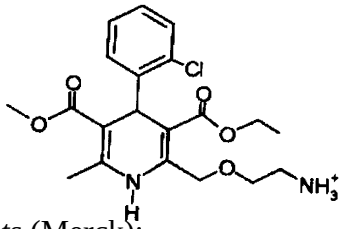
In this paper, the effect of amlodipine acid salt anions on crystall size and its distribu-tion during the recrystallizations from methanol and ethanol, were investigated. For this purpose, amlodipine benzenesul-phonate (AM-B), amlodipine orotate (AM-O), amlodipine adipate (AM-A) and amlodipine succinate (AM-S) were used. Structures of organic acids used in the experiment are shown in Table 1.

EXPERIMENTAL PART

Materials:

- *Acid salts of amlodipine* ***(AM-X):***

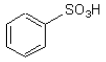
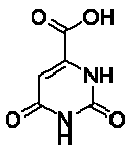
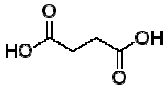
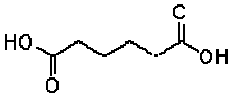
AM-B, AM-S, AM-O, AM-A, Bosnalijek d.d., Sarajevo, with general structure of amlodipinium cation (**AM**):



- *Solvents* (Merck):

Methanol, 98 % ; Ethanol, 96 %

Table 1. Structures of acids in amlodipine acid salts synthesis

Amlodipine acid salt	**Acid (HA)**	**Structure of acid (HA)**	**Anion (X^-)**
Amlodipine benzenesulphonate (**AM-B**)	Benzenesulphonic		Benzenesulphonate **(B)**
Amlodipine orotate (**AM-O**)	Orotic		Orotate (**O**)
Amlodipine succinate (**AM-S**)	Succinic		Succinate (**S**)
Amlodipine adipate (**AM-A**)	Adipic		Adipate (**A**)

Recrystallization process

The amount of exactly 5 g of appropriate amlodipine acid salt was added in 100 mL of solvent, then with reflux coolant at 50-60^{o}C, heating was performed during 3 hours. After heating, the solution was cooled to room temperature in stationary conditions for 2 days. The resulting crystalline product was filtered, then rinsed and dried under the vacuum at 40^{o}C

Methods:

1. High performance liquid chromatography (HPLC), Agilent 1100 with UV/VIS fluoroscent detector (with 237 nm range of detection) and Lichrospher 100 RP18 column (with diameter 125 x 0,4 mm): 5 µm with 1 mL/min flow and temperature of 25^{o}C [85].
2. Hot-stage thermal microscopy (HSTM), OLYMPUS BX 51 with polarized light was used for visual characterization of thermal transitions. The sample was placed on the holder and heated at a rate of 10^{o}C/min. Preliminary studies with different heating rates (10, 20, 30 and 40^{o}C/min) showed that the heating rate of 10^{o}C/min were best suited. Heating interval was 25 to 220 ^{o}C.
3. Differential scanning calorimetry (DSC), PERKIN ELMER DSC PYRIS DIAMOND, heating rate of 10^{o}C with sample amount of 3-5 mg. Scanning was carried out by heating the sample for one minute at 50^{o}C, and then in temperature range from 50 to 220^{o}C. During the work, inert atmosphere of dry nitrogen has been used. Obtained results were compared with USP grade standards.
4. Particle size analysis (PSA), MALVERN MSTERSIZER 2000, with possibility of the size and distribution of particle size measurements, both wet and dry procedures. For AM-B and AM-O dry method was used. The sample was placed on a dry dispersion unit and then under the compressed air of 1 bar passed through the optical unit (laser diffraction) and the size and distribution of particle size was detected at room temperature.

RESULTS AND DISCUSSION

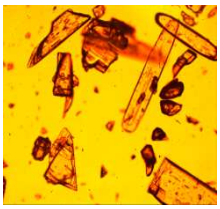
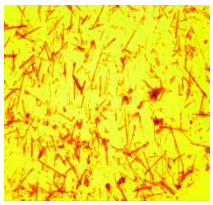
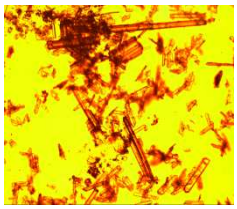
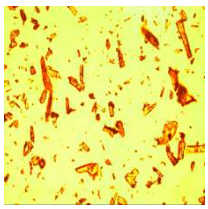
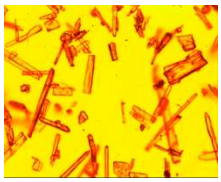
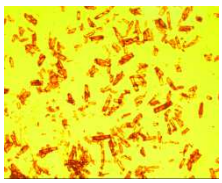
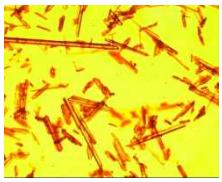
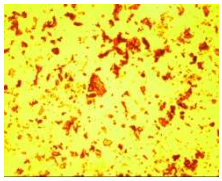
Purity of amlodipine acid salts after recrystallization from 98% methanol and 96% ethanol is determined using of HPLC method, and the results are presented at Table 2. During the recrystallizations from ethanol and methanol solutions, amlodipine acid salts of high purity were obtained. Content of impurities (D) is higher for all of the acid salts obtained from ethanol solutions. Impurity profiles, D (intermediates, initial components, products of side reactions, products of hydrolysis) are crucial from the point of health safety of drug and their content must be reduced to a minimum (< 1 %).

Also, each individual impurity which content exceeds 0,1 % must be identified[15-18].
Morphological characteristics of each of the acid salts after recrystallization were studied using the thermal analysis (DSC) and thermal microscopy (HTSM). The results are shown at Figures (1 and 2) and Tables (3 - 5).

Table 2. Purity of amlodipine acid salts after recrystallization

Acid salt	Methanol (98%)			Ethanol (96%)		
	Purity (%)	Impurities D (%)	Other impurities (%)	Purity (%)	Impurities D (%)	Other impurities (%)
AM-B	99,53	0,00	0,47	98,61	0,08	0,08
AM-O	97,60	0,19	0,11	99,98	0,29	0,06
AM-S	96,67	0,00	0,30	99,98	0,15	0,05
AM-A	99,86	0,07	0,04	96,69	0,09	0,05

Table 3. Shape of crystals of amlodipine acid salts

Solvent	Shape of crystals of amlodipine acid salts after recrystallization			
	AM-B	**AM-O**	**AM-S**	**AM-A**
Methanol (98%)				
Ethanol (96%)				

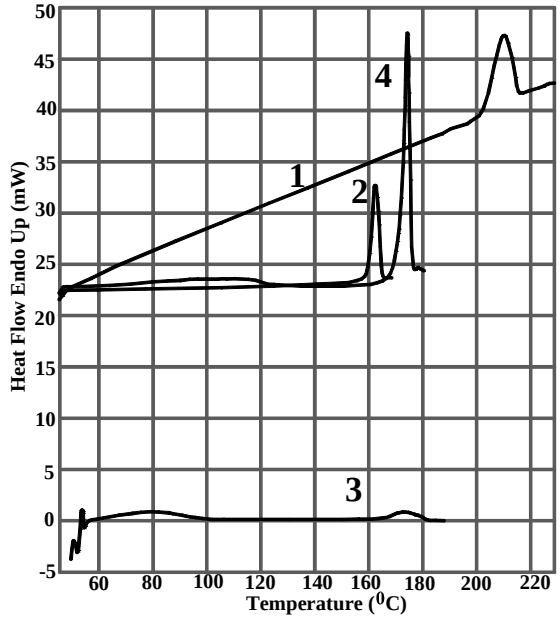


Figure 1. DSC-curve of amlodipine acid salts after recrystallization in 98% methanol:

1- AM-B, **2**- AM-S, **3**- AM-O, **4**- AM-A

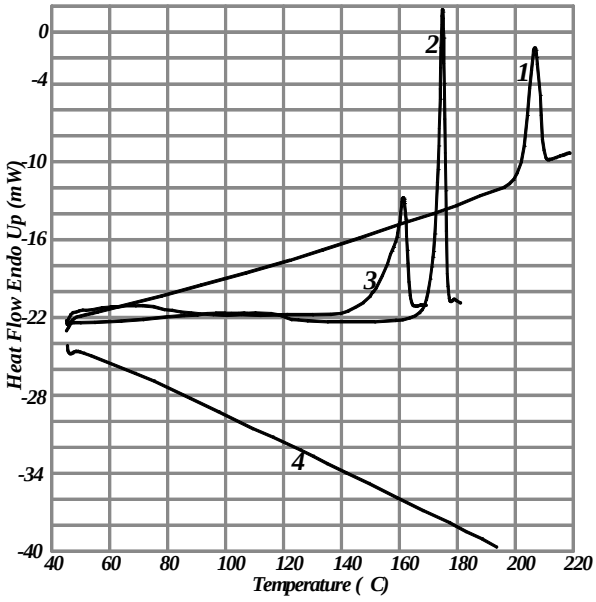


Figure 2. DSC-curve of amlodipine acid salts after recrystallization in 96% ethanol:

1- AM-B, **2**- AM-S, **3**- AM-A, **4**- AM-O

The results are revealing that from the concentrated solutions of methanol and ethanol, mixture of crystals in the form of needles and prisms are obtained. Shape and size of prismatic and needle forms are depending on the structure of anions of amlodipine acid salts, that is in compliance with the researches of other autors[19-21].

DSC-curves (curve 1 on Fig. 1 and curve 1 on Fig. 2) show that AM-B from the concentrated solutions of ethanol and methanol crystallize as an anhydrous form with melting point between 203 and 215^{o}C (from methanol) and between 201 and 208 ^{o}C (from ethanol).

Table 4. Thermal characteristics of amlodipine acid salts after recrystallization from 98% methanol

Salt	Thermal characteristics, 10 °C / min				
	m (mg)	T_{onset} (°C)	T_{peak} (°C)	T_{end} (°C)	ΔH_f (J/g)
AM-B	3,50	203,30	210,79	215,92	84,76
AM-O	1,98	169,30	175,15	180,3	85,67
AM-S	1,58	160,27	162,85	165,25	123,86
AM-A	3,45	174,44	176,61	177,82	124,63

Table 5. Thermal characteristics of amlodipine acid salts after recrystallization from 96% ethanol

Salt	Thermal characteristics, 10 °C / min				
	m (mg)	T_{onset} (°C)	T_{peak} (°C)	T_{end} (°C)	ΔH_f (J/g)
AM-B	3,41	201,20	206,89	208,53	78,74
AM-O	2,11	-	-	-	-
AM-S	3,01	156,20	161,02	163,14	124,19
AM-A	3,49	172,07	174,66	176,11	119,52

Previous studies of other authors[1,22,23] indicate that anhydrous form of AM-B derived from the water solutions or mixtures of water/alcohol (where the water was the dominant solvent), express a melting point between 201 and 205° C. Studies also showed that, depending on solvent, hydrated forms could also crystallize with a water content between 0.8 and 2.2 water molecules for one molecule of AM-B[23]. By heating, hydrated forms are turned into anhydrous, retaining the previous crystal form. In contrast, addition of water leads to the conversion of anhydrous form to hydrated. Using the X-ray analysis it was determined that the crystal structures contain „channels“ which binds water. This way, the water molecules can go out, or be incorporated into the crystal so that the resulting interactions between molecules of AM-B in crystal will not be disrupted. In the process of heating, monohydrated forms are more stable than dihydrated forms. More heat, the temperature generally higher than 65° C, and a longer heating time lead to the conversion of hydrated forms into anhydrous.

Studies (Rollinger et all)[18,19] showed that the microcrystalline aggregates of AM-B monohydrate melts at temperature range about 70 - 100°C, while the weight loss is from 2.9 to 3.1 %. It was also found that dihydrate lose the water at about 88 - 130°C,

and its enthalpy is very low (about 9,7 kJ/mol).

Endothermic dehydration and melting are combined with the exothermic crystallization of anhydrous forms, while in temperature range between 90 and 140^{o}C amorphous unstable phase also exists.

DSC-curves for AM-A (curve 4 on Fig. 1 and curve 3 on Fig. 2) indicate that proportion of hydrated forms is insignificant. At 100^{o}C incorporated water disappears, probably with combination of melting and crystallization processes of anhydrous form.

Researchers[12] have synthesised AM-A, with melting point at 164 to 186^{o}C after recrystallization from methanol. DSC-curve for AM-O displayed at Fig. 1 (curve 3) show stretched low intensity peaks, which probably match with anhydrous form (which melts at about 170 ^{o}C) and hydrates (which lose their water at 105^{o}C). DSC-curve for AM-O displayed at Fig. 2 (curve 4) doesn't show any specific peaks for melting of anhydrous forms or water loss of hidrated forms. By recrystallization of AM-O from water solutions, researchers[7] produced the hydrated and anhydrous forms of crystals with melting point at 223-225^{o}C. Hydrated form included 2,5 water molecules for a single molecule of AM-O.

DSC-curves for AM-S displayed at Fig. 1 (curve 2) and at Fig. 2 (curve 2), showed that the melting points of obtained crystals from both solvents are in agreement with the results for AM-S synthesized by authors[10] from ethanol/water mixture and recrystallized from methanol.

TGA-curves for AM-O are shown at Figures 3 and 4. The analysis of the curves shows an intense weight loss while heating to 120^{o}C (about 4,5 %), which corresponds to the loss of water and probably adsorbed solvent. With further heating, weight loss is not so intense, and at a temperature of about 180^{o}C, formation of a stable anhydrous form occurred.

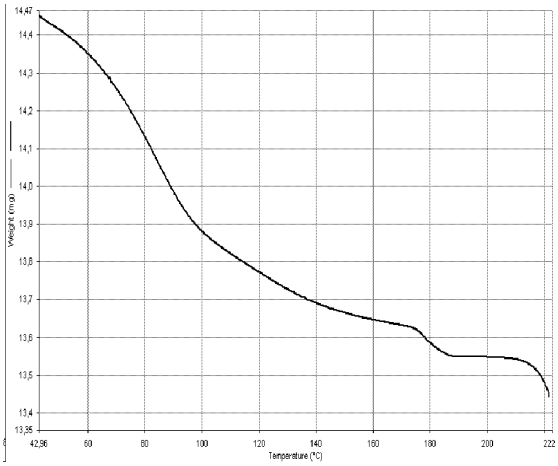


Figure 3. TGA-curve of amlodipine orotate after recrystallization in 98% methanol

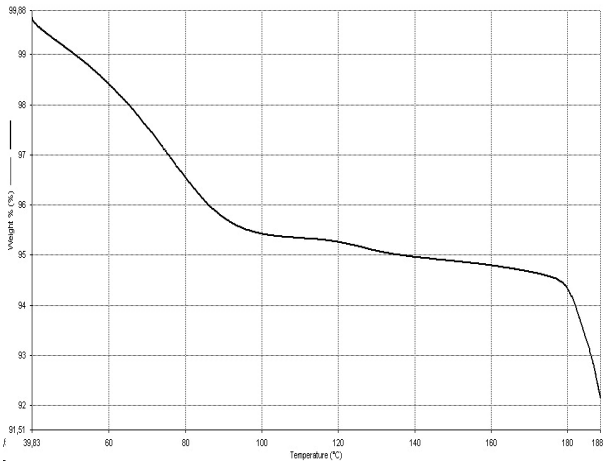


Figure 4. TGA-curve of amlodipine orotate after recrystallization in 96% ethanol

The results of PSA analysis are displayed at Figures 5 and 6 and the average values of crystal diameter are presented at Table 6. The results point to the relative uniformity of the crystal size for the appropriate amlodipine salts. Wide range of crystal sizes is evident only for the crystallization of AM-A from 96% ethanol (Fig. 6, curve 1). The results indicate that the size of crystals and their morphology depend on structure of anion and alcohol, i.e. the number and type of heteroatoms involved in the formation of hydrogen bonds and electrostatic interactions inside the molecules of amlodipine salts, as well as the interactions between molecules of solvents and water molecules.

Configuration of the molecule of salt as well as the interactions and dynamic equilibrium within the solvent and at the crystal/solvent interface, determines the speed of diffusion, desolvatation and crystal growth at the specific crystal surfaces. The researchers[25-28] also found a dominant impact of mutarotation to the establishment of the appropriate confor-mation. The mechanism of desolvatation and molecules /solvent interactions affect on the orienta-tion and incorporation of molecules into the crystal lattice, i.e. the formation of crystals with the appropriate morphological characteristics.

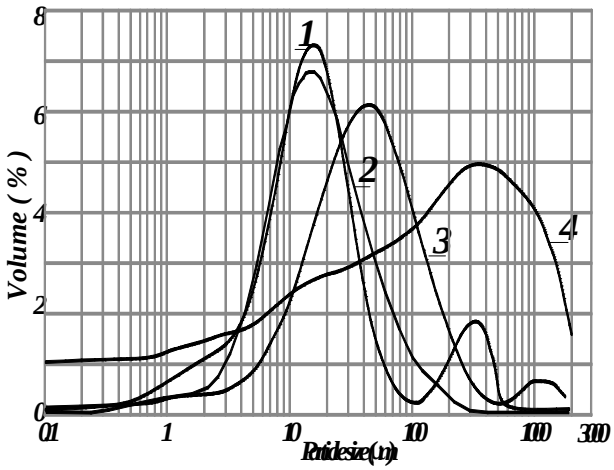


Figure 5. Crystal size distribution of AM-X after recrystallization in 96% methanol

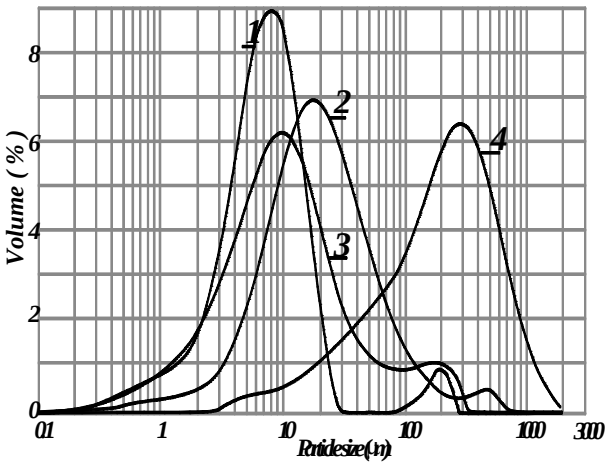


Figure 6. Crystal size distribution of AM-X after recrystallization in 96% ethanol

(1- AM-A, **2**- AM-S, **3**- AM-O, **4**- AM-B)

Table 6. Average amount of crystal size of acid salts of amlodipine, d(μm), after recrystallization in 98% methanol and 96% ethanol

Salt	Average amount of crystal after recrystallization					
	Methanol (98%)			Ethanol (96%)		
	d (0,9) (μm)	*d* (0,5) (μm)	*d* (0,1) (μm)	*d* (0,9) (μm)	*d* (0,5) (μm)	*d* (0,1) (μm)
AM-B	667,62	215,12	30,93	21,05	9,40	4,10
AM-O	60,07	9,92	2,17	22,09	12,14	7,68
AM-S	69,11	19,17	5,77	58,99	16,45	5,04
AM-A	17,09	7,30	2,15	952,38	179,11	10,62

CONCLUSIONS

Based on the results there are following conclusions:

- Researched amlodipine acid salts: AM-B, AM-S, AM-A and AM-O can be successfully recrystallized from concentrated methanol and ethanol solutions, providing products with purity which is in accordance with the pharmaceutical legislation
- Morphological characteristics of the resulting crystals depend on the anion structures in the establishment of interactions within the solvent and at the crystal/solutions interface, which results in a corresponding distribution of crystal size
- Thermal properties studied by DSC and TGA analysis show that besides the dominant anhydrous form which crystallize from the concentrated alcohols, small proportion of crystals belongs to hydrated forms
- Melting point depends on the structure of anions, while the temperature range and melting lengthiness depend on the size of crystal and its distribution, which is achieved in the appropriate alcohol
- An appropriate proportion of anhydrous forms of formed crystals probably contributes to slight difference between the melting temperature and melting point range for the same salt crystallized from the different solvents
- Determination of morphological and thermal characteristics of the analyzed amlodipine acid salts, besides its fundamental contribution to understanding the influence on the crystallization process, has its applicative contribution to the design of appropriate pharmaceutical products.

List of signs and abbreviations:

AM-A Amlodipine adipate
AM-B Amlodipine benzenesulphonate (besylate)
AM-O Amlodipine orotate
AM-S Amlodipine succinate
DSC Differential Scanning Calorimetry
HPLC High Performance Liquid Chromatography
HTSM Hot-stage Thermal Microscopy
PSA Particle Size Analysis
TGA Thermal Gravity Analysis

REFERENCES

1. U.S. Pat. No 4,879,303
2. Korean Patent Laid-OpenPublication No 1989-3375
3. U.S. Pat. No 6,291,490
4. U.S. Pat. No 6,080,761
5. U.S. Pat. No 6,057,344
6. EP Publication No 89,167
7. Korean Patent Laid-Open Publication No 10-2005-37498
8. Korean Patent Laid-Open Publication No 10-2005-37498
9. U.S Pat. 4,572,909
10. U.S. Pat. 6,608,206
11. U.S. Pat. 20100069642
12. U.S. Pat.7015238,
13. A. Burger, R. Ramberger, Mikrochim. Acta, 1979 II (1979) 259
14. S. Byrn, R. Pfeiffer, M. Graney, G. Poochikian, Pharm. Res., 12 (1995) 945
15. Guidance for Industry-ANDAs: Impurities in Drug Substance, Draft Guidance, U.S. Department of health and human services, Food and Drug Administration, Center for Drug Evaluation and Research, june 1998
16. ICH Guidance on Impurities-A, International Pharmaceutical regula-tory Monitor, 1997
17. Martindale, The Extra Pharmacopoeia, Royal Pharmaceutical Society, 1999
18. J.M. Rollinger, A. Burger, Journal of Thermal Analysis and Calorimetry, 68 (2002) 361-372
19. J.M. Rollinger, A. Burger, J. Pharm. Sci., 90 (2001) 949
20. A. Grunenberg, B. Keil, J.O.Henck, Int. J. Pharm., 118 (1995) 11
21. K. R. Morris, S.L. Nail, G.E. Peck, S.R. Byrn, U. J. Grisser, J.G. Stowell, S. J. Hwang, K. Park, PSTT, 18 (1998) 235
22. U.S.Pat. No 4,876,303
23. U.S. Pat. 6,828,339 B2
24. J.W. Mullin, Crystalization, 2nd ed., London: Butterworth; 1993
25. M.K. Cerreta, K.A.Berglund, The structure of aqueous solutions of some dihydrogen orthophosphates by laser Raman spectroscopy, J. Crystal Growth, (1987) 84-577
26. A. van Hook, Crystallization, New York, Reinhold, 1961
27. K. Sato, Crystallization of fats and fatty acids. In: n. Gatr, K.Sato, eds., Crystallization and Polymorphism of fats and fatty Acids, New York, Marcel Dekker, 1988
28. N.S. Tavare, Industrial Crystalliza-tion, New York, Plenum Press, 1995

SPECTROPHOTOMETRIC STUDY OF URANYL - ORCIPRENALINE COMPLEX AND ITS APPLICATION FOR ORCIPRENALINE DETERMINATION IN PHARMACEUTICAL PREPARATIONS

ORIGINAL SCIENTIFIC PAPER

Leposava Pavun, Snežana Uskoković Marković, Dušan Malešev

Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, Beograd, Serbia

ABSTRACT

The composition and stability constant of UO_2(II)-orciprenaline complex were determined using spectrophotometric methods and pH measurements. It was found that uranyl ion and orciprenaline form a 1:1 complex in the pH region 2.50 - 5.00, with apsorption maximum at 360.4 nm. The thermodynamic stability constant at room temperature of the UO_2(II)-orciprenaline complex is $\log\beta_1^0$ = 7.52 and ΔG_1^0 = -42.9 kJ mol^{-1}. A simple, accurate, sensitive and validated method was developed for the spectrophotometric determination of orciprenaline. A linear dependence at 360.4 nm of the absorbance on the concentration of orciprenaline was obtained in the range from 0.0020 mol L^{-1} to 0.0245 mol L^{-1} using a solution of 0.025 mol dm^{-3} $UO_2(NO_3)_2$ at pH 3.90. The LOD was 0.00052 mol L^{-1} and LOQ was 0.00174 mol L^{-1}. Recovery was 99.30% while the measurement error was 2.02 %.

Key words: *Orciprenaline, uranyl ion, complex formation*

INTRODUCTION

Orciprenaline ((±)-1-(3,5-hydroxyphenyl)-2-isopropylaminoethanol, $C_{11}H_{17}O_3N$) is a white crystaline powder and belongs to β-sympathomimetics, Figure 1. Orciprenaline is a bronchodilator and works by relaxing muscles in the airways to improve breathing. Orciprenaline is used to treat conditions such as asthma, bronchitis and emphysema[1,2]. Colorimetric[3], spectrophotometric[3], fluorimetric[3] and chemiluminiscence[4] methods for determination of orciprenaline have been reported. Orciprenaline has been investi-gated using interaction with phenantro- [9,10-*d*] imidazole-2-N-chloramide[5].

Due to presence of amino and phenyl groups in the molecule (pK_a (-OH) = 9.0 and pK_a ($-NH_2^+$-) = 10.1)[1], orciprenaline forms complexes with Fe^{3+} (1:1 complex)[6,7].

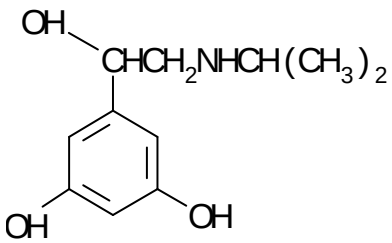


Figure 1: Structural Formula of Orciprenaline

However, there are no data in the literature about UO_2^{2+}- orciprenaline interaction. The purpose of present work is investigation of the UO_2^{2+}- orciprenaline complex and investigate the possibility to use the complex for spectrophotometric determination of orciprenaline in aqueous media and pharmaceutical dosage forms using UO_2(II)-ion.

EXPERIMENTAL PART

Apparatus

Spectrophotometric measurements were performed on a Beckman DU-650 spectrophotometer, using 1cm quartz cells.

IR spectra (KBr pellets, 1%) were recorded on a 983 G Perkin Elmer Spectrophotometer.
The pH values were measured using pH-meter (pHM-28 Radiometer) and combined electrode (accuracy of ±0.01 pH unit).

Materials and reagents

All the chemicals were of analytical grade, and also all solvents were spectroscopic grade.

Uranyl-nitrate (Fluka AG), HNO_3, NaOH and $NaNO_3$ (Merck) and orciprenaline sulphate ($C_{11}H_{17}O_3N\ H_2SO_4$, Sigma), all p.a. were used. Alupent tablets, Boehringer Ingelheim were used from commercial sources.

The uranyl-nitrate solution was standar-dized gravimetrically, by precipita-tion with oxine (8-hydroxidequinoline)[8].

The solution of orciprenaline sulphate was prepared by dissolving a precisely measured mass of orciprenaline sulphate in deionised water. The orciprenaline sulphate was dried in a desiccator over silica-gel. This solution was stored in a refrigerator.

All solutions were prepared by dilution of 0.01 mol L^{-1} $UO_2(NO_3)_2$ and 0.05 mol L^{-1} orciprenaline sulphate, respectivelly.
The pH of all solutions was adjusted using HNO_3 or NaOH solutions, and ionic strength of the final solutions was kept constant by addition of 1 mol L^{-1} $NaNO_3$.

Electronic spectra

The complexation of orciprenaline and uranyl ion was studied by spectrophoto-metric measurements. Each spectrophoto-metric measurement was repeated three times.

pH Measurements

Buffers solutions (Radiometer) pH 4.01 and pH 7.00 at 25 ^{0}C, were used for the calibration of the pH-meter.

Analysis of orciprenaline in pharmaceutical preparations

Orciprenaline in pharmaceutical was analysed by the proposed method. For the analyses, orciprenaline in tablets Alupent, Boehringer Ingelheim, each with a nominal content of 20 mg of orciprenaline per tablet, ten tablets were weighed and powdered using pestle and mortar. A portion of the powder, equivalent to weight of one tablet, was dissolved in 50 ml water, and the solution was filtered through an ordinary filter paper. An appropriate volume of the filtrate was diluted further with water so that the concentration of orciprenaline was approximately 5×10^{-3} mol L^{-1}.

RESULTS AND DISCUSSION

Absorption spectra

Orciprenaline and uranyl (II)-ion formed the complex in the range of pH 2.50- 5.00. The absorption spectra (Figure 2) were recorded using the solutions of 0.002 mol L^{-1} $UO_2(NO_3)_2$, and 0.020 mol L^{-1} orciprenaline, and the mixture where the concentrations of components are the same as in the single solutions at constant pH

values (4.00) and ionic strength (0.03 mol L^{-1}). Water was used as a blank.

Also, the calculated spectra of the complex, obtained by subtracting the corresponding absorbance of $UO_2(NO_3)_2$ (curve 2) and orciprenaline (curve 3) from absorbance (curve 1) of their mixture, at different wavelengths, has been obtained (Figure 2, curve 4). The absorption spectra of complex has maximum at 360.4 nm.

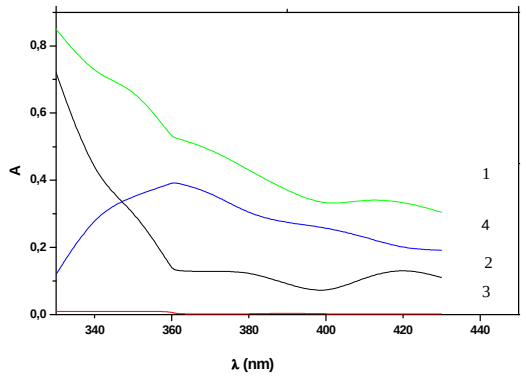


Figure 2: Absorption spectra: 1- mixture 0.002 mol L^{-1} $UO_2(NO_3)_2$ and 0.040 mol L^{-1} orciprenaline; 2- 0.002 mol L^{-1} $UO_2(NO_3)_2$; 3- 0.020 mol L^{-1} orciprenaline 4- Calculated absorption spectrum of complex (ΔA).

Composition of the complex

Stoichiometric ratio of uranyl-ion and orciprenaline in complex was determined by the method of molar ratios[9]. The absorbances of solutions containing a constant concentration of $UO_2(NO_3)_2$ (0.005 mol L^{-1}) and different concentrations of orciprenaline (0.0025 mol L^{-1} – 0.0200 mol L^{-1}) were measured at constant pH values (4.00) and ionic strength (0.03 mol L^{-1}) at 360.4 nm. A straight line, $A=f(c_{orc}/ c_{UO_2^{2+}})$, with intercept at $c_{orc}/ c_{UO_2^{2+}}=1$ was obtained and also proved that stoichiometric ratio of uranyl-ion: orciprenaline in complex is 1:1 (Figure 3).

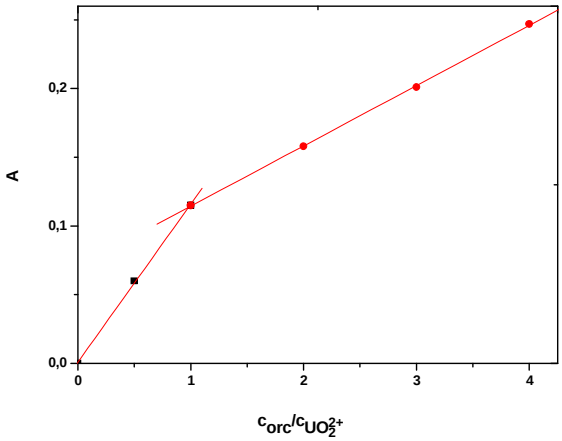


Figure 3: Method of molar ratios: dependence of absorbance on molar ratio.

By the method of variation of equimolar solutions[10] the composition of the complex was determined, too. The absorbances of a series of solutions formed by mixing equimolar solutions of $UO_2(NO_3)_2$ and orciprenaline (0.025 mol L^{-1}) at different ratios at constant pH values (4.00) and ionic strength (0.05 mol L^{-1}) were measured at 360.4 nm.

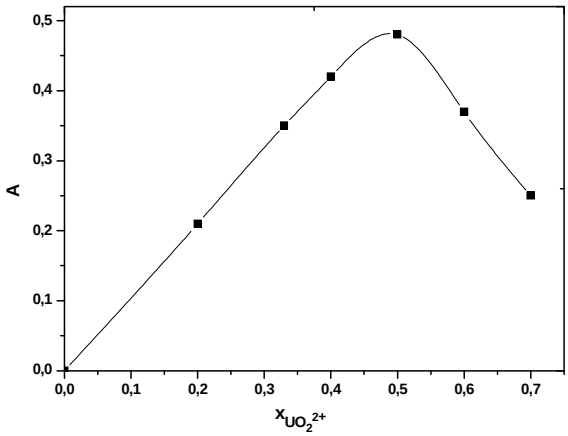


Figure 4: Method of variations of equimolar solutions. Change of absorbance on mol fraction of $x_{UO_2^{2+}}$.

On the plot of absorbance dependence on molar fractions of UO_2^{2+} ion, a curve with a maximum at $x_{UO_2^{2+}} = 0.5$ was formed. This confirms the previous method, that the complex composition was UO_2^{+}: orciprenaline = 1:1 (Figure 4).

Infrared spectra of orciprenaline and of the complex

More detailed information about complex formation could be obtained by IR spectroscopy, and for that purpose IR spectra of orciprenaline and of the isolated complex were recorded in the wave number region from 4000 to 800 cm^{-1}. The differences are most pronounced in the region below 2000 cm^{-1} (Figure 5), with bands at 1606s, 1470s, 1398m, 1341w, 1306w, 1154s, 1008m, 848s and 1610m, 1506s,b, 1389s, 1301m, 1279s, 1134m, 1027s, 963s, 931sh, 825w for orciprenaline and its UO_2^{2+} complex, respectively.

Comparing the IR spectra of the newly synthesized UO_2^{+} complex with the IR spectrum of the parent orciprenaline, differences that could be attributed to the formation of bonds between UO_2^{+} and orciprenaline were observed.

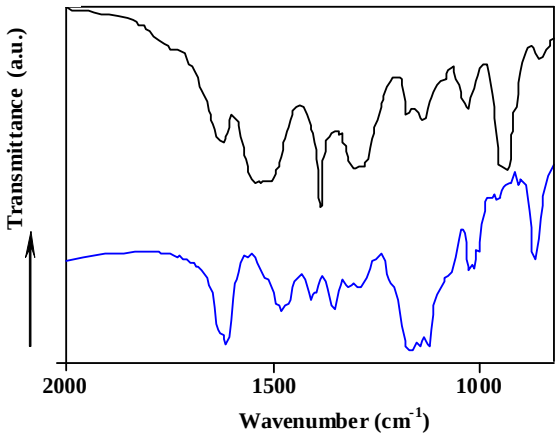


Figure 5: IR spectra of orciprenaline (lower) and isolated UO_2^{2+} - orciprenaline complex (upper).

The most notable distinctions observed in the IR spectrum of complex are bands prescribed to bending vibrations of OH^{-} group in wave number region 1280 – 930 cm^{-1} which disappeared, indicating that complex formation of uranyl-ion with orciprenaline could be between OH groups, as well as the stretching and bending vibrations that correspond to hydrogen bonds.

Effects of pH

While measuring the pH of solutions of 0.005 mol L^{-1} $UO_2(NO_3)_2$, orciprenaline solution of 0.005 mol L^{-1}, and the mixture containing the same concentrations as in single solutions, it was found that $[H^+]_{mixtures} > [H^+]_{uranyl} + [H^+]_{orc}$. It means that orciprenaline in anionic form occurred in composing the complex to give a singly charged cation according to the reaction:

$$UO_2^{2+} + C_{11}H_{17}O_3N \Leftrightarrow UO_2C_{11}H_{16}O_3N^{+} + H^{+} \quad (1)$$

where H^+ ion is formed[11].

The dependence of the absorbance of the complex as a function of wavelength was investigated in the pH range from 2.50 to 5.00. The positions of absorption maxima are independent of pH, indicating the formation of only one type of complex at these pH values.

The dependence of the absorbance of the complex on pH was investigated. The absorbances of 0.002 mol L^{-1} $UO_2(NO_3)_2$, 0.040 mol L^{-1} orciprenaline and the mixture (containing their concentrations the same as in the single solutions) were measured. The measurements were made at different pH values, for three ionic strength values (I= 0.085 mol L^{-1}; 0.110 mol L^{-1}; 0.135 mol L^{-1}) and at 360.4 nm. For each ionic strength, three curves A = f(pH) were obtained: for solutions $UO_2(NO_3)_2$, orciprenaline and their mixture. By subtracting relevant absorbancies of the solution $UO_2(NO_3)_2$ and orciprenaline from

their mixture, the fourth curve ΔA = f(pH) was obtained in Figure 6. It represents the change of the complex absorbance with pH.

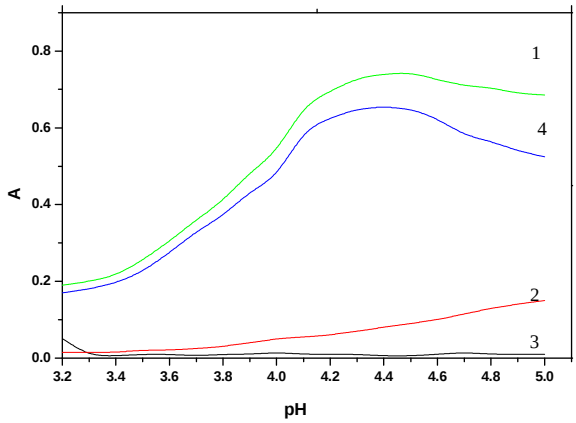


Figure 6: Dependence of the absorbance on pH: 1- mixture 0.002 mol L^{-1} $UO_2(NO_3)_2$ and 0.040 mol L^{-1} orciprenaline; 2- 0.002 mol L^{-1} $UO_2(NO_3)_2$; 3 - 0.040 mol L^{-1} orciprenaline; 4 - ΔA = f (pH).

Stability Constant of the Complex

For the calculation of the stability constant of the complex, β_1, Bjerrum′ s method was used (equations 2-3)[12], combining with the equations (4) - (6)[12-17]. The highest complex concentration which matches the values A_{max} and pH ≈ 4.4 is on the maximum of curve ΔA = f(pH) (Fig. 6). Since the overall concentration of orciprenaline $[C_{11}H_{17}O_3N]_0$, i.e. $[HL]_0$ in the mixture is 20 times higher than UO_2^{2+} ions concentration, it can be considered that the most of the UO_2^{2+} ions were bound into the complex, i.e. the concentration of the complex equaled the overall concentration of uranyl ions, $[UO_2^{2+}]_0$, ($[Complex] \approx [UO_2^{2+}]_0$). Therefore, the molar absorptivity of the complex was calculated from the equation:

$$a = \frac{A_{max}}{[UO_2^{2+}]_0} \qquad (2)$$

It was not possible to calculate concentration of the complex, [Complex], from A_{max} using the Bjerrum′ s method, because the concentration of UO_2^{2+} ion at that point will be $[UO_2^{2+}] = 0$, and it is not possible in equilibrium reactions. The stability constant of the complex $UO_2C_{11}H_{16}O_3N^+$ was determined at pH 3.90.

The concentrations of the complex, UO_2^{2+}, and $C_{11}H_{16}O_3N^-$ ($[L^-]$) were calculated at pH= 3.90 from the following equations:

$$[Complex] = \frac{A}{a} \qquad (3)$$

$$[UO_2^{2+}]_0 = [UO_2^{2+}] + [Complex] \qquad (4)$$

$$[HL]_0 = [HL] + [L^-] + [Complex] \qquad (5)$$

$$k_{dl} = \frac{[H^+][L^-]}{[HL]} \qquad (6)$$

k_{d1} is the first dissociation constant of orciprenaline in water solution (Moffat**,** 1986**)**. The stability constant β_1 of the complex:

$$\beta_1 = \frac{[Complex]}{[UO_2^{2+}][L^-]} \qquad (7)$$

was calculated for three different ionic strengths (Table 1).

Table 1. The concentration stability constants, β_1, of UO_2^{2+} - orciprenaline complex at different ionic strength values, pH=3.90, T = 298 K

I (mol L^{-1})	β_1	logβ_1
0.085	$5.70 \cdot 10^6$	6.76
0.110	$2.92 \cdot 10^6$	6.47
0.135	$2.02 \cdot 10^6$	6.30

The thermodynamic stability constant of the complex was determined by extrapolation from the diagram $\log\beta_1 = f(I^{1/2})$, as shown in Figure 7, and it's value is $\log\beta_1^0 = 7.52$. The thermodynamic parameter ΔG_1^0 for the reaction of forming the complex at room temperature (25^0C) was calculated:

$$\Delta G_1^0 = -RT\ln\beta_1^0 = -42.9 \text{ kJ/mol} \qquad (8)$$

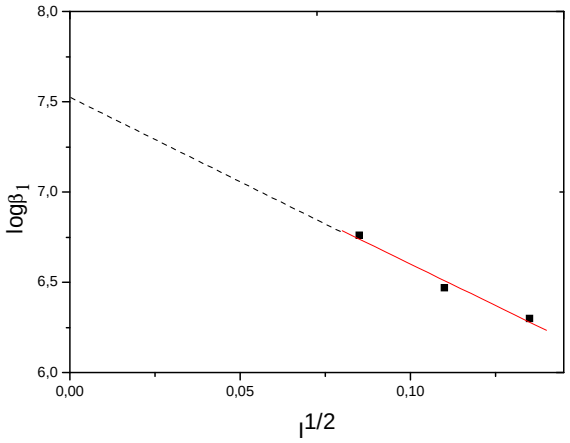


Figure 7: Dependence of UO_2(II)-orciprenaline complex stability constants, β_1, on ionic strength I, T=298K.

Determination of orciprenaline – Validation study
Linearity

The relatively high value of the stability constant of the complex uranyl (II)-orciprenaline enables the quantititative determination of orciprenaline in aqueous media and pharmaceutical preparations using the complex. The calibration curve method was used, requiring prepared solutions containing a constant concentration of $UO_2(NO_3)_2$ (0.025 mol L^{-1}) and different concentrations of orciprenaline at pH = 3.90. Linear dependence absorbance at 360.4 nm of the complex on the concentration of orciprenaline was obtained in the interval 0.0020 - 0.0245 mol L^{-1}. Water was used as the blank.

The regression equation:

$$A = 19.73\,c + 0.0175; \qquad r = 0.99983$$

was calculated, where A is absorbance and c is concentration in mol L^{-1}, Figure 8. The net absorbance of the complex was obtained by subtracting the absorbance due to unreacted UO_2^{2+} from the total absorbance. The good linearity of the calibration curve and negligible scatter of experimental points are represented by the high correlation coefficient.

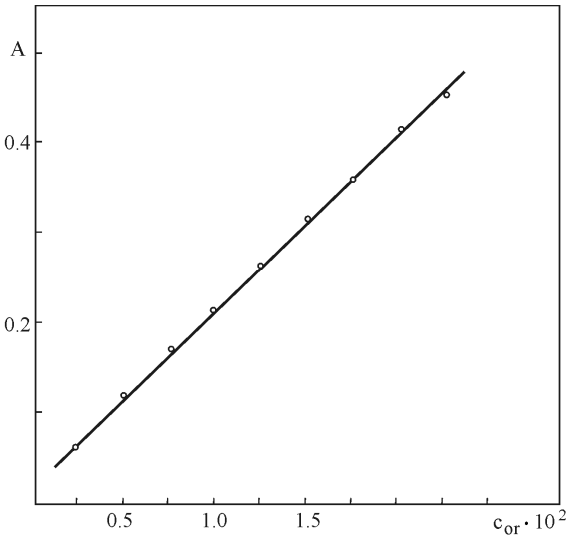


Figure 8: Calibration curve.

LOQ (Limit of Quantification) and LOD (Limit of Detection)

The limit of detection (LOD) was calculated by establishing the minimum

level at which orciprenaline can be detected, and it was 0.00052 mol L^{-1}.

The limit of quantification (LOQ) was determined by establishing the lowest concentration that can be measured with acceptable accuracy and precision[18,19]. Orciprenaline can be quantified at a concentration of 0.00174 mol L^{-1}.

Precision

The accuracy of the method was determined for three different orciprenaline concentra-tions (Table 2). The repeatability of the method is fairly high indicated by low values of SD. The results obtained by the proposed procedure indicate that the method is precise for the determination of orciprenaline in water solutions and pharmaceutical preparations. The results are shown in Table 2.

Table 2: The spectrophotometric determination of orciprenaline in aqueous solutions and determination of orciprenaline in pharmaceutical preparations

Taken (mol L^{-1})	Found (mol L^{-1})	Recovery (%)	SD	CV (%)
0.00500	0.00500	100.00	$1.01 \cdot 10^{-4}$	2.02
0.01250	0.01243	99.44	$1.43 \cdot 10^{-4}$	1.15
0.02000	0.02002	100.20	$1.72 \cdot 10^{-4}$	0.86
Alupent tablet 0.0200 g/tbl	0.01986	99.30	$1.39 \cdot 10^{-4}$	0.71

Robustness

The robustness of the method was shown by the capacity to remain unaffected by small variation method parameters (temperature, reagent's concentration, pH, ionic strength).

Interference

According to the fact that UO_2^{2+} ion makes complexes with other compounds (like 3-hydroxyflavone[11], hesperidin[14], phenyleph-rine[16] and rutin[17]), where these complexes show great absorption ability in the spectral domain of investigating the complex UO_2^{2+}-orciprenaline, it is necessary to exclude the presence of these compounds in the investigated aqueous solutions. On the other hand, the influence of commonly used excipients (colloidal silicon dioxide, cornstarch, dibasic calcium phosphate, lactose, magnesium stearate) in pharmace-utical dosage forms of orciprenaline (tablets) did not interfere with the complexing reaction and its measurement.

Analytical application

From the Table 2 it can be seen that results are accurate and reproducible, and that the proposed spectrophotometric method is suitable for the rapid determination of orciprenaline in pharmaceutical dosage forms (tablets).

CONCLUSIONS

The thermodynamic stability constant at 25^0 C of the UO_2(II)-orciprenaline complex $\log\beta_1^0$=7.52 and the thermodynamic parameter, thermodynamic potential, ΔG_1^0= - $RT\ln\beta_1^0$ = -42.9 kJ/mol indicate the process of complex formation is spontaneous at the examined temperature.
Since absorbance of the complex at 360.4 nm is a linear function of orciprenaline concentration without high excess of UO_2^{2+} ions, the spectrophotometric measurements can be used for quantitative determination of orciprenaline in aqueous solutions without the presence of interfering compounds, and it can be determined in pharmaceutical dosage forms.

ACKNOWLEDGEMENTS

This work was partly supported by the Ministry for Science and Environmental Protection of the Republic of Serbia, Project No 172043.

REFERENCES

1. A.C. Moffat, J.V. Jackson, M.S. Moss, *Clarke´s Isolation and Identification of Drugs*, The Pharmaceutical Press: London, 1986.
2. E. Mutschler, H. Dernardorf, *Drug Actions*, Medipharm Scientific Publishers: Stuttgart, 1995.
3. F.A. El-Yazbi, M.H. Abdel-Hay, M.A. Korany, *IL Farmaco Ed. Pr*., 40 (1985) 50.
4. Y. Lv, R. Zhao, Z. Zhu, X. Xu, X. Zhang, *Luminiscence*, 20 (2005) 298.
5. S. Tanabe, T. Togawa, K. Kawanabe, *Analytical Sciences.*, 5 (1989) 513.
6. D. Malešev, D. Veselinović, Z. Radović, L. Pavun, *Arh. Farm*., 3-4 (1995) 85.
7. L. Pavun, D. Malešev, D. Veselinović, *Arh. Farm*., 53 (2003) 27.
8. Vogel, *Quantitative Inorganic Analysis*; Longmans, Green and Co: London-New York – Toronto, 1951.
9. J. Yoe, A. Jones, *Ind. Eng. Chem. Anal. Ed.*, 16 (1944) 111.
10. H. Irving, T. Pierce, *J. Chem. Soc.* (1959) 2565.
11. D. Malešev, Z. Radović, M. Jelikić-Stankov, *Monatsh. Chem.*, 121 (1990) 455.
12. J. Inczédy, *Analytical Applications of Complex Equilibria*, Horword and Willy, New York, 1976.
13. D. Malešev, Z. Radović, M. Jelikić Stankov, *Spec. Letters.*, 26 (1993) 1985.
14. V. Kuntić, M. Kosanić, D. Malešev, Z. Radović, U. Mioč, *J. Serb. Chem. Soc*., 63 (1998) 565.
15. V. Kuntić, D. Malešev, *J. Serb. Chem. Soc*., 72 (2007) 921.
16. L. Pavun, D. Malešev, D. Veselinović, *J. Serb. Chem. Soc.*, 72 (2007) 799.
17. V. Kuntić, D. Malešev, Z. Radović, M. Kosanić, *J. Agric. Food Chem,*. 46 (1998) 5139.
18. *Validation of analytical procedures: Methodology* ICH Guideline Q2B, Federal Register 62, No 96 27463., 1997.
19. J.N. Miller, J.C. Miller In *Statistics and Chemometrics for Analytical Chemistry*, 5^{th} ed., Pearson Education Ltd: London, 2005.

MODIFICATION OF BARLEY STARCH WITH MIXTURES OF ORGANIC DICARBOXYLIC ACIDS AND ACETANHYDRIDE

ORIGINAL SCIENTIFIC PAPER

Đurđica Ačkar[1], Drago Šubarić[1], Jurislav Babić[1], Ana Šoštarec[1], Dijana Miličević[2]

[1]*University of J. J. Strossmayer in Osijek, Faculty of Food Technology Osijek, F. Kuhača 20, 31000 Osijek, Croatia, tel: 00385 31 224 300*
[2] *University of Tuzla, Faculty of Technology, Tuzla*

ABSTRACT

The aim of this research was isolation of starch from barley cultivar *Barun* and its modification with mixtures: adipic acid/acetanhydride and azelaic acid/acetanhydride (5%) and characterization of properties of native and modified starches, important for starch application in food industry. Thermophysical and pasting characteristics, swelling power, solubility and resistant starch content of native and modified starches were determined.
The results showed that modified starches had lower gelatinization enthalpy, higher peak temperature and lower tendency to retrograde than the native starch. Modified starch pastes had lower gelatinization temperature, higher peak viscosity, higher hot and cold paste viscosities, lower breakdown and higher setback values. Swelling power and solubility of modified starches were higher (except SOL (%) of BSAD and BSAZ at 65°C) than of the native starch in temperature range 65-85° C, but lower at 95° C. Modifications decreased resistant starch content of starch.

Key words: barley starch, adipic acid, azelaic acid, modification

INTRODUCTION

Starch is a major component of barley grain and, although it has not been studied to the same extent as corn, wheat or rice, it has recently gained a lot of interest due to its potential to replace other common starches in food and industrial applications[1,2].
Cross-linking stabilizes granules and strengthens relatively tender starch. Pastes from cross-linked starches are more viscous and less likely to breakdown with extended cooking times, increased acid content or severe agitation[3]. Epichlorohydrin (EPI), phosphoryl chloride (POCl3), sodium trimetaphosphate (STMP), sodium tripoly phosphate (STPP), a mixture of adipic acid and acetic anhydride, and vinyl chloride are the main cross-linking agents used to give cross-linked, food grade starches[4,5]. Adipic acid reacts with starch and produces mainly cross-linked starches as well as mono-derivatives[6,7,8].
Starch-carboxylic complexes of oxalic, malonic and succinic acids were also studied[9], as well as octenyl succinic anhydride modified starches[10].
The present paper studies the chemical modification of barley starch (cultivar *Barun*) with the mixtures of adipic acid/ acetanhydride and azelaic acid/

acetanhydride (5%). Gelatinisation, retro-gradetion and pasting characteristics, swelling power, solubility and *in vitro* resistant starch content of native and modified starches (properties important for starch application in food industry) were determined.

MATERIALS AND METHODS

Barley (cultivar *Barun*, harvest 2007) was locally provided by Agricultural Institute Osijek. Acetic anhydride, Kemika, Croatia, was of analytical grade, and adipic acid and azelaic acid, Fluka, Germany were for synthesis. All other chemicals used were of analytical grade.

Starch isolation

Alkaline extraction of starch from barley cultivar *Barun* was performed according to the method of Ackar et al. (2010)[11].

Starch modification

Starch (100 g d.m.) was modified with mixture of acetanhydride and adipic or were determined in triplicate, following a method of Babić et al. (2007)[16].

Degree of substitution (DS) was determined titrimetically, following a method of Ogawa et al. (1999)[18].
Since organic acids were used in amounts significantly smaller than acetanhydride, DS is expressed through acetyl substituents and calculated as follows:

$$DS=(\%Acetyl \times 162)/(4300-(42\times\%Acetyl))$$

Where:

$$\%Acetyl=(V_b-V_s)\times N_{HCl}\times 0.043\times 100/W$$

V_b = ml 0.2 M HCl used to titrate blank;

azelaic acid (5 %, d.w.b.) according to method of Ackar et al. (2010)[11]. Dry matter content was determined in dried starch by oven drying (130 °C/90 min).

Characterization of native and modified starches

Chemical composition of native starch was analyzed. Protein content was determined by Kjeldahl method ISO 3188[12], ash was determined by ISO 5809 method[13], crude fibres were determined by Scharrer-Kürschner[14] method and lipid content was determined by Soxhlet method[15].

Gelatinisation and retrogradation properties of native and modified starches were analyzed using differential scanning calorimeter DSC 822e (Mettler Toledo) equipped with STARe software following a method of Babić et al., 2007[16]. Pasting properties of starches (7% db, 100 g total weight) were determined using Micro Visco-Analyser (Model 803202, Brabender Gmbh & Co KG, Duisburg, Germany) following method of Ackar et al. (2010)[17]. Swelling power (SP) and solubility (SOL)
V_s= ml of 0.2 M HCl used to titrate sample;
N_{HCl} = normality of HCl used for titration;
W = the sample weight (g).

Resistant starch content was determined by AOAC 2002.02 method, using Megazyme enzymatic kit[19].
The experimental data were analyzed by an analysis of variance (ANOVA) and Fisher's least significant difference (LSD) with significance defined at $P<0.05$. All statistical analyses were carried out using software program STATISTICA 7 (StatSoft, Inc, USA).

RESULTS AND DISCUSSION

The isolated starch had high purity (Table 1). It contained no proteins and ash and crude fiber content were lower than determined for starch isolated in researches of Li et al. (2001)[20], Soares et al. (2007)[21] and Verwimp et al. (2004)[22].

Since in some of these researches protein content was very low, one can assume that traces of proteins could be present in starch isolated in this research, but in quantities that are not detectable by the applied method.

Table 1. Chemical composition of native starch isolated from barley (cultivar Barun).

Proteins [% d.m.]	Ash [% d.m.]	Crude fiber [% d.m.]	Lipids [% d.m.]
Not detected	0.20	0.09	0.65

Lipid content was, however, somewhat higher than determined in the above mentioned researches.

The onset temperature (T_o) of gelatinization (Table 2) for native barley starch was 58.33 °C, peak temperature (T_p) was 61.66 °C and endset temperature (T_e) was 65.42 °C.

It has been reported that normal barley starches gelatinize at T_o 52.0-61.4 °C, T_p 58.1-65.3 °C and T_e 62.7-74.4 °C[23]. The enthalpy of gelatinization determined for native barley starch in this research (7.46 J/g) was, however, lower than the values (11.4-14.2 J/g) reported in the above mentioned researches. However, Tester (1997) reported that gelatinisation enthalpy of normal barley starch averages between 7.8 – 10.6 J/g, depending on growth conditions. High growth temperatures facilitate amylopectin crystallization and to some extent increase gelatinisation enthalpy[24], which could explain differences in ΔH_{gel} values between this and other researches.

Table 2. DSC gelatinization and retrogradation properties of native (NBS) and modified barley starches. Starches were modified with 5% mixtures of adipic acid/acetanhydride (BAD) and azelaic acid/acetanhydride (BAZ), respectively.

	T_o[ºC]	T_p[ºC]	T_e[ºC]	ΔH[J/g]
Gelatinization (n=3)				
NBS	58.33±0.15	61.66±0.15	65.42±0.06	7.46±0.08
BSAD	58.83±0.25	62.42±0.07	66.10±0.11	7.35±0.10
BSAZ	58.17±0.15	61.77±0.12	65.43±0.06	7.23±0.11
Retrogradation after 7 days at 4ºC (n=3)				
NBS	41.33±0.45	51.61±0.12	61.28±0.41	4.63±0.16
BSAD	42.79±0.12	62.83±0.17	60.73±0.15	3.31±0.17
BSAZ	43.47±0.25	53.30±0.20	60.67±0.36	2.69±0.16
Retrogradation after 14 days at 4ºC (n=3)				
NBS	42.99±0.21	50.60±0.19	61.50±0.12	5.75±0.06
BSAD	43.33±0.37	52.97±0.40	61.30±0.20	3.79±0.03
BSAZ	42.86±0.21	52.53±0.25	60.67±0.35	3.43±0.21

T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔH, gelatinization/retrogradation enthalpy. Values are means ±SD of triplicate.

The enthalpy of gelatinization (ΔH_{gel}) decreased after modification of barley starch with the mixtures of adipic acid/ acetanhydride and azelaic acid/ acetanhydride (Table 2). Kim et al. (2008) also observed a decrease of gelatinization enthalpy due to modification with glutaric acid[25]. Substituent groups cause steric hindrance between starch chains and less energy is needed for hydration of starch molecules. The DSC gelatinization temperatures (T_o, T_p and T_e) were similar for all investigated samples.

In this study, the retrogradation character-ristics of native and modified barley starch gels (35% db) were observed after 7 and 14 days of storage at 4ºC (Table 2). Starch molecule recrystallization occurs in a less ordered manner in stored starch

gels than in native starches. In view of this, less heat is needed to regelatinize stored starch gels. This explains the observation of retrogradation endotherms at temperature range below those for gelatinization.

Modification considerably diminished the retrogradation of starch gels with lowest values for BSAZ starch. The crystallinity of starch granules is disrupted during chemical modification[26], and this leads to a greater degree of separation between the outer branches of adjacent amylopectin chain clusters in modified starches compared to those in native starches.

Significant differences were observed among the native and modified barley starches in their behaviour during heating and cooling in excess of water (Table 3). A drop in pasting temperature (BSAD=65.1ºC, BSAZ= 65.45ºC and NBS=72.65ºC) and rise in peak viscosity (BSAD=556 BU, BSAZ= 498.5 BU and NBS=270 BU) were observed in modified starches.

Table 3. Pasting properties of native (NBS) and modified barley starches (7%, w/w, db). Starches were modified with 5% mixtures of adipic acid/acetanhydride (BAD) and azelaic acid/acetanhydride (BAZ), respectively.

	NBS	**BSAD**	**BSAZ**
Pasting temperature [ºC]	72.65±0.08	65.1±0.8	65.45±0.2
V_{max} [BU]	270±2.8	556±5.6	498.5±2.2
Viscosity at 92ºC [BU]	180.5±3.5	487.5±4.9	425.5±6.4
Hold 20 min at 92ºC [BU]	242±1.4	522.5±4.9	492±2.8
Viscosity at 50ºC [BU]	439±3.5	965.5±7.8	923.5±3.5
Hold 20 min at 50ºC [BU]	369.5±4.2	766.5±2.1	709.5±2.6
Breakdown	28±1.4	33.5±0.7	6.5±0.8
Setback	197±4.2	443±2.8	431.5±6.3

V_{max}, maximum viscosity; Breakdown= V_{max}-viscosity at 92ºC after 20 min; Setback=viscosity at 92ºC after 20 min - viscosity at 50ºC before holding. Values are means ±SD of triplicate.

Acetylation influences interactions between starch chains by steric hindrance, altering starch hydrophilicity and hydrogen bonding and resulting in a lower gelatinization temperature and greater swelling of granules, the latter resulting in an increased peak viscosity[27,28].

All starch samples showed an increase in viscosity during cooling to 50°C, with respect to the holding period at 92°C. This increase is indicative of the tendency of starch to retrogradation. BSAD starch had highest viscosity at 50°C (965.5 BU), followed by BSAZ starch (923.5 BU),

while it was lowest for NBS starch (439 BU). All investigated starches had very low breakdown values: BSAZ (6.5), NBS (28) and BSAD (33.5). The setback viscosities of the modified starches were greater than that of the NBS starch. Hoover and Vasanthan (1992) explained that setback values reflect the extent of water immobilization around the charged centres of starch components and those of free and helically complexed lipid molecules, rather than starch paste retrogradation.

The association of water molecules with these charged centres would decrease the effective water concentration in the continuous phase, resulting in a viscosity rise during the cooling cycle[29].

The SP and SOL of all investigated starches differed significantly at all measured temperatures (Table 4).

BSAZ starch had the highest values of SP and SOL at 75 and 85°C (at 95°C NBS starch had the highest values of SP and SOL, but the lowest at 65, 75 and 85°C). The increase in swelling power at „lower temperatures“ may be due to weakening of intermolecular hydrogen bond due to introduction of substituent groups. The similar behaviour was reported for succinylation of canavalia starch[30].
Native barley starch had higher resistant starch (RS) content compared to both modified barley starches (Table 5).

Table 4. Swelling power (SP) and solubility (SOL) of native and modified barley starches (n =3). Starches were modified with 5% mixtures of adipic acid/acetanhydride (BAD) and azelaic acid/acetanhydride (BAZ), respectively.

Sample	65 ºC	75 ºC	85 ºC	95 ºC
	SP [g/g]			
NBS	7.21±0.21	9.44±0.12	10.79±0.24	23.49±0.26
BSAD	10.04±0.18	9.92±0.17	12.15±0.09	20.29±0.10
BSAZ	9.77±0.36	10.11±0.19	12.53±0.01	20.67±0.65
SOL [%]				
NBS	5.60±0.12	4.50±0.14	11.0±0.05	40.0±0.24
BSAD	5.33±0.77	6.11±0.22	12.49±0.15	26.73±0.14
BSAZ	4.93±0.28	6.69±0.31	13.18±0.19	25.37±0.37

Values are means ±SD of triplicate.

Among modified starches, BSAD starch had 0.62% RS, while BSAZ had 0.55% RS.

Lower values of total starch content can be consequence of enzyme inhibition by changes in starch structure.

Table 5. Degree of substitution (DS) and resistant starch content of native and modified barley starches (n = 3). Starches were modified with 5% mixtures of adipic acid/acetanhydride (BAD) and azelaic acid/acetanhydride (BAZ), respectively.

	DS	***NRS* (%)**	***RS* (%)**
NBS		70.1±0.05	0.72±0.05
BSAD	0.08	89.7±0.38	0.62±0.04
BSAZ	0.08	62.6±0.05	0.55±0.02

NRS-not resistant starch; RS-resistant starch; Values are means ±SD of triplicate

CONCLUSIONS

Barley starch modified with adipic acetic mixed anhydride and azelaic acetic mixed anhydride could be useful in preparation of food where resistance to shearing at high temperatures and towards retrogradation are needed.

REFERENCES

1. S. You, M. S. Izydorczyk, *Carb. Polym.* 69 (2007) 489.
2. A. Altan, K. L. McCarthy, M. Maskan, *J. Food Eng.* 92 () 377.
3. L. Kaur, J. Singh, N. Singh, *J Sci. Food. Agric.* 86 (2006) 1945.
4. S. Wattanchant, K. Muhammad, D. Hashim, R. A. Rahman *Food Chem.* 80 (2003) 463.
5. J. Singh, L. Kaur, O. J. McCarthy, *Food Hydrocoll.* 21 (2007) 1.
6. F-X. Luo, Q. Huang, X. Fu, L-X. Zhang, S. Yu, *Food Chem.* 115 (2009) 563.
7. O. B. Wurtzburg (1960). Preparation of starch derivatives. US Patent Nr 2,935,510.
8. S. Mali, M. V. E. Grossmann, *Lebensm. Wiss. U.-Technol.* 34 (2001) 384.
9. J.K. John, K. C. M. Raja, *Carb. Polym*. 39 (1999) 181.
10. R. Hui, C. Qi-he, F. Ming-liang, X. Quiong, H. Guo-qing, *Food Chem.* (2009) 81.
11. Đ. Ačkar, D. Šubarić, J. Babić, A. Jozinović, M. Kopjar, B. Miličević, *Proceedings 45th Croatian and 5th International Symposium on Agriculture* (2010) 647.
12. ISO 3188:1978 - Starches and derived products - Determination of nitrogen content by the Kjeldahl method - Titrimetric method. International Organisation for Standardization.

ACKNOWLEDGEMENTS

This work is a result of the scientific project "Development of new modified starches and their application in food industry" supported by the Ministry of Science, Education and Sports of the Republic of Croatia.

13. ISO 5809:1982 – Starches and derived products – Determination of sulphated ash. International Organisation for Standardization.
14. J. Nawrot, J.R. Warchalewski, D. Piasecka-Kwiatkowska, A. Niewiada, M. Gawlak, S.T. Grundas, J. Fornal, *9th International Working Conference on Stored Product Protection Proceedings* (2006) 400-407.
15. Soxhlet extraction method with diethyl ether AOAC Method 920.39.
16. J. Babić, D. Šubarić, Đ. Ačkar, D. Kovačević, V. Piližota, M. Kopjar, *DLR* 103 (2007) 580.
17. Đ. Ačkar, J. Babić, D. Šubarić, M. Kopjar, B. Miličević. *Carb Polym* 81 (2010) 76.
18. K. Ogawa, I. Hirai, C. Shimasaki, T. Yoshimura, S. Ono, S. Rengakuji, Y. Nakamura, I. Yamazaki, *Chem Soc Japan*, 72 (1999) 2785.
19. AOAC official method 2002.02 Resistant starch in starch and plant materials. Official methods of analysis of the AOAC international (18th ed.). Gaithersburg, Maryland: AOAC International.
20. J. H. Li, T. Vasanthan, B. Rossnagel, R. Hoover, *Food Chem.* 74 (2001) 395.
21. R.M.D. Soares, A. de Francisco, P. Rayas-Duarte, V. Soldi, *J. Food Qual.* 30 (2007) 357.

22. T. Verwimp, G. E. Vandeputte, K. Marrant, J. A. Delcour, *J. Cereal Sci.* 39 (2004) 85.
23. T. Vasanthan, R. Hoover, Barley starch: production, properties and uses. In: J. BeMiller, and R. Whistrler (ed), Starch Chemistry and Technology (3rd ed.), Academic Press, 2009.
24. R. F. Tester, *Int. J. Biol. Macromol.* 21 (1997) 37.
25. M. J. Kim, S. J. Choi, S. I. Shin, M. R. Sohn, C. J. Lee, Y. Kim, W. I. Cho, T. W. Moon, *Carb. Polym.* 74 (2008) 787.
26. N. Saroja, T. R. Shamala, R. N. Tharanathan, *Process. Biochem.* 36 (2000) 119.
27. H. Liu, H. Corke, *Starch/Stärke* 51 (1999) 249.
28. N. Singh, L. Kaur, *J. Sci. Food Agric.* 84 (2004) 1241.
29. R. Hoover, T. Vasanthan, *Carbohydr. Polym.* 19 (1992) 285.
30. D. Bentacur-Ancona, E. Garcia-Cervera, E. Canizares-Hernadez, L. Chef-Guerrero, *Starch/Starke* 54 (2002) 540.


Iz tuzlanske mljekare!
DAR PRIRODE
www.ppm.ba
MLIJEKO I MLIJEČNI PROIZVODI
Mlijeko i mliječni proizvodi poznati su kao namirnice važne za održanje zdravlja. Pogotovo mlijeku se već stoljećima pripisuju brojna preventivna i terapijska djelovanja. Najvažniji je izvor kalcija, a odrasla osoba može podmiriti svoje potrebe za kalcijem već sa tri velike čaše mlijeka dnevno. Mliječni napitci sadrže važne minerale, proteine i vitamine, a fermentirani mliječni proizvodi sadrže vrijedne bioaktivne sastojke – probiotike koji se brinu za zdravu crijevnu mikrofloru. U grupu mliječnih proizvoda, koji nastaju procesom fermentacije mlijeka spadaju: svježi sir, pavlaka, mileram, jogurt, kefir, mliječni i sirni namazi, kiselo mlijeko, te razni drugi mliječni proizvodi.

THE POSSIBILITY OF USING SLUDGE FROM WASTE WATER TREATMENT PROCESS IN THE CELLULOSE AND PAPER INDUSTRY TO PRODUCE COMPOST

SCIENTIFIC PAPERS

Dragan Pelemiš[1], Vahida Selimbašić[2], Snježana Hodžić[3], Vedran Stuhli[2], Tonči Iličić[1]

[1]*„Inproz“ Institut za zaštitu i projektovanje d.o.o. Tuzla*
[2]*Tehnološki fakultet Tuzla*
[3]*Prirodno-matematički fakultet u Tuzli*

ABSTRACT

The treatment of sludge obtained from a process of waste water treatment depends on treatment itself and final disposal and can cost up to one third of the total expenses of waste water treatment. The use of sludg as well as the use of other manures produced from secondary raw materials in agriculture has been subject of controversial discussions for many years. The main aim of this study was to determine physico-chemical and microbiological characteristics of sludge, and based on the quality of sludge to determine whether it is possible to use it in the composting process and in agriculture.

This study suggests that the composting is one of the possible solutions of sludge treatment in “Natron-Hayat” factory. After treating and conditioning of sludge, it can be used in the compost production or in agriculture.

It is recommended to use the sludge obtained from the secondary tank or surplus sludge after the biological waste water treatment for the compost production. The daily amount of the sludge is estimated to 10 tons.

Key words: sludge, composting, agriculture, treatment

INTRODUCTION

Pulp and paper industry is one of the biggest water consumers in technological process and also one of the biggest polluters considering the amount and composition of waste water.

In “Natron-Hayat” factory, waste water (technological and sanitary) is directed through a sewage collector to a waste water treatment plant where a process with activated sludge is applied. Organic matter is removed from waste water using aerobic microorganisms. By aeration of sludge mixture and waste water, exchange of substrates and metabolic products with required amount of oxygen for the life of microorganisms is provided. After the aeration, sludge is separated from refined effluent in a tank.

Since sludge is subject to further decomposition and often contains patho-gens, it is necessary to make it innocuous for environment and reduce its volume before its use or final disposal.

In “Natron-Hayat” factory, sludge is burned in its own boiler and the possibility of composting this waste arises as an alternative.

The actual purpose of this process is to turn biodegradable organic waste into more stable form as well as to destroy pathogens.Sludge obtained from a waste water treatment plant of Pulp and Paper

Industry contains valuable organic ingredients.

Sludge composting can be successfully done if adequate conditions are created (aeration, moisture, C/N ratio etc.), especially in controlled processes.[1] It is also suitable for composting with manure.[2]

Sludge from industrial plants can be used in agriculture, only if its composition is similar to sludge from utility plants.[3]

Significant dryness can be accomplished by composting as well, especially in a case of wet substrates such as municipal and industrial sludge.

Sludge issues can be solved in an ecologically suitable way by composting, which should be used as a primary option whenever its quality allows.

The main aim of this process was to determine sludge quality by physico-chemical and microbiological analysis as well as a possibility of its use in the composting process and in agriculture. The obtained indicators were compared with requirements defined by domestic and foreign regulations from this area.

MATERIALS AND METHODS

Materials

Testing and characterization were performed on sludge which is generated by filtering waste waters of Pulp and Paper Industry "Natron-Hayat" d.o.o., Maglaj.

The sludge from a primary tank (sample 1), secondary tank (sample 2) and filter presses (sample 3) was used as the material.

Sample 1 was taken directly before a sludge tank. Sludge from the secondary tank was sampled before a sludge thickener, while the primary and secondary sludge mixture was taken directly from the filter presses (sample 3).

Microbial surfaces used for isolation and identification were manufactured by "*Lifilchem"*, Italy.

The fresh samples were poured into clean, sterile containers and transported to a laboratory at $+4^0$ C.

Methods

Methodology for analyzing the physical characteristics of sludge

Analysis of physical properties of sludge included the determination of moisture content and ash content or loss on ignition. These determinations were executed using the methods defined by the standards BAS EN 12880:2003 i BAS EN 12879:2003.

Methodology for analyzing the chemical characteristics of sludge

The following chemical properties of sludge were analyzed:

- pH-value,
- electrical conductivity,
- total carbon,
- total nitrogen,
- C/N ratio,
- total P, K, Na and
- concentration of heavy metals (Cd, Cr, Cu, Hg, Ni, Pb, Zn, Mo, As, Co, Mn).

The pH-value was determined directly from the samples 1 and 2 on pH-meter "Sartorius" with combined electrode. Electrometric measurement of the pH-value of the sludge from the filter presses was performed in 10 g filtrate of the fresh sample mixed on a rotary mixer for 1 hour

in 100 ml deionized water (1:10 w-v sample: water).

The electrical conductivity of the sludge from the primary and secondary tank was measured directly with a conduct meter "Hach" HQ40d, and of the sludge from the filter presses was measured in a prepared extract with 20 g of fresh sample mixed on the rotary mixer for 1 hour in 100 ml deionized water (1:5 w-v sample: water).

The total carbon was determined by destruction of the sample by wet procedure at 135° C.

The total nitrogen was determined by distillation of sludge with the template of boric acid. Retitration of the resulting ammonium dihydrogen borate was done with the standard solution of sulfuric acid.

The C/N ratio was calculated using the content of total carbon and total nitrogen data.

The total phosphorus concentration in a basic solution was determined with the spectrophotometric ammonium-molybdate method (BAS ISO 6878-2002 EN 1189). "CARY-VARIAN 50" spectrometer was used.

The basic solution of sludge sample for determination of microelements and toxic heavy metals was prepared by destroying dry sample with aqua regia digestion.

The total potassium and sodium concentration was determined by the atomic absorption spectrometry - firing technique (VARIAN AA 240 FS), with specific lamps and standards (MBH Analytical LTD).

The heavy metals content was determined on atomic absorption spectrometry VARIAN AA 240 FS with graphite atomizer GTA 120, by all three techniques: firing technique (atomization in flame), graphite furnace (electro thermal atomization) and VGA (hydride technique).

The mercury concentration was determination on "Leco"USA, typ: AMA-254 device.

Methodology for microbiological analysis

Microbiological analysis was conducted to determine the presence of coliforms and streptococci of fecal origin, as the indicators of fecal contamination. The microbiological researches included the qualitative analysis of all three samples.

250 ml of each sample was taken into sterile glass bottles and transported in a portable refrigerator to the laboratory where they were sown on growth media within 24 hours.

Sediment and supernatant were used for the microbiological analysis.

50 µl supernatant of each sample was inoculated on growth media. Also, 50 µl of the sediment of each sample was suspended into 1 cm^3 of physiological solution, and then sown on growth media.

The planted growth media for detection of bacteria were incubated in a thermostat at 37ºC/48 hours. The growth media for detection of fungi (yeasts and molds) were incubated in the thermostat at 28-30ºC/72 hours.

The following growth media were used:

- Andrade lactose peptone water, endo agar – the differential medium for cultivation of enterobacteria (coliforms),
- Azide dextrose broth, potassium telurit agar – for the cultivation of streptococci of the faecal origin,
- Sabouraud agar – the medium for cultivation of fungi,

- Media for examining the biochemical properties of bacteria (lactose, maltose, saccharose, urea, citrate, indole, manit, H_2S,polychrom,Vogues Proskauer/methyl red, phenylalanine deaminase and catalase test),
- Blood agar – the non-selective culture medium,
- SS agar – the selective medium for *Salmonella/Shigella spp. cultivation*

Typical colonies grown on the culture medium were identified by the standard microbiological methods.

Native preparation was made from each sample, while sediments and supernatant were observed. The native preparation was made so that a drop of the examined sample was put on a clean glass slide, covered with a cover glass and observed with the microscope using 10x, 20x and 100x magnifying lenses.
Microscope "Boeco" Germany was used.

Table 1. Physical properties of paper mill sludge

Examined quality indicators	Unit	Sample 1	Sample 2	Sample 3
Moisture content	%	85,80	94,36	83,64
Ash content at 550 °C	%	13,51	25,38	23,26
Anneal loss at 550 °C	%	86,49	74,62	76,74
Anneal loss at 1100 °C	%	2,30	4,73	3,98

RESULTS AND DISCUSSION

Physical properties of paper mill sludge

The basic caracteristics of the sludge from the primary tank (sample 1), sacondary tank (sample 2) and filter presses (sample 3) are shown in Table 1.

All samples of sludge contain the high percentage of water, especcially the sludge from the secondary tank (94,36%), because the content of dry matter is mainly of organic origin.

Therefore, it is necessary to dry sludge mass with an additional treatment and to reduce moisture content to 40-60% after the removal of moisture on the filter presses and befor the beginning of composting.

The ash content in samples 2 and 3 is approximately the same (25,38% and 23,26%), while in sample 1 is slightly lower (13,51%). During composting, the ash part increases due to rapid degradation of organic matter, which is the most intensive during the first month while the further increase of ash concetration is very slow.

Chemical properties of paper mill sludge

The highest pH-value (6,97) was measured in the sludge from the filter presses (sample 3), while the lowest value (6,64)

was measured in the secondary tank (sample 2). The pH value of the primary tank was between these two values (6,74). The optimum pH value for most of bacteria is 6-7,5, and for fungi and actinomycetes is between 5,5 and 8,0[5]. According to the results, we can conclude that the pH value of the activated sludge is neutral, and that its value does not have to be adjusted before composting.

Table 2. Chemical properties of paper mill sludge

Examined quality indicators	Unit	Sample 1	Sample 2	Sample 3
pH		6,74	6,64	6,97
Elecrtrical conductivity	μS/cm	635	593	228
Total C	g/kg	329	282	292
Total N	g/kg	0,938	11,55	10,84
Total P	g/kg	0,19	0,51	0,32
Na	g/kg	0,87	2,55	0,91
K	g/kg	0,41	1,56	0,72
C/N ratio		350:1	24,4:1	27:1

The measured electro conductivity ranges from 228 μS/cm (sample 3) to 635 μS/cm (sample 1), which is far below the value that Allison[6] indicated as phytotoxic (4000 μS/cm). High electro conductivity reduces accessibility of water and adoption of nutrients, and its low value may indicate low fertility because of low potassium, calcium and magnesium.

The analyzed sludge samples do not have electrocondu-ctivity that would cause phytotoxic effect. During the initial phase of composting, electroconductivity is increased although the intensity of changes depends on the type of compost material. Also, in some composts its decline after initial period of composting has been determined which can be associated with humification and low ion concetration.[7]
The total carbon concetration is the highest (32,9%) in the primary sludge (sample 1), and the minimum concetration is in the sludge from the secondary tank (28,2%). The carbon content in the sludge from the filter presses is between these values (29,2%).

In sample 1 (primary sludge), there is not enough nitrogen because it is not present in waste water. Therefore, urea is added before biological waste water treatment in aeartion pools. This explains the presence of nitrogen in samples 2 and 3.

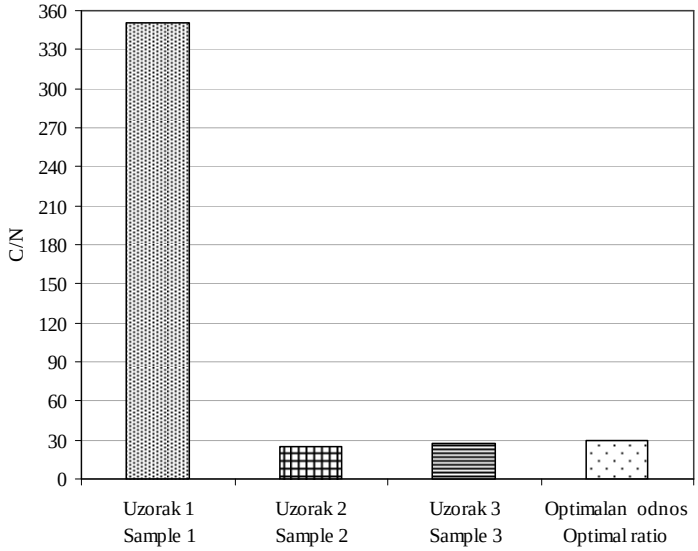


Figure 1. Carbon-Nitrogen ratio in the paper mill sludge samples

C/N ratio is a significant indicator of compost stability, and information on stability is completed with other indicators such as respiration, pH, volume density, organic matter reduction and self-heating.[8,9] Since microorganisms use 30 carbon parts on each nitrogen part for their needs, so the optimal C/N ratio in compost mass is 30:1.

The primary sludge is characterized with the high C-N ratio (350:1) which is typical of this kind of waste water.[10]

The C/N ratio in samples 2 and 3 was near optimum (24,4:1; 27:1) and represents a good basis for composting processes (Figure 1). According to Jackson at al.[11], the phosphorus and potassium optimum content compared to carbon and nitrogen is C/N/P/K=30/1/0,5/0,1. The obtained P, K and Na concentration values in the sludge samples are low.

They are the highest in sample 2, i.e. the sludge from the secondary tank.

A lack of phosphorus is shown, which is 10-26 times less than the optimum. There is enough potassium in sample 2, while in samples 1 and 3 its deficit ranges from 28% (sample 3) to 59% (sample 1).

During the formation of the compost mass it is neccessary to add those nutrients, primarly phosphorus. Basic phosphates can be added.

Proportion of sodium is low, which is one of the benefits of sludge. Absolute mass of alkali and alkaline-earth metals in sludge cannot be increased during the composting process, although increase of the percentage share in dry matter can be expected since organic matter is lost while the proportion of ash containing these elements is increased by the composting process.

Table 3. The content of heavy metals in paper mill sludge

Heavy metals	Unit	Sample 1	Sample 2	Sample 3	Concentration allowed[12]
Cadmium (Cd)	mg/kg	1,0	0,95	0,70	5
Chromium (Cr)	mg/kg	4,5	6,3	4,6	500
Cuprum (Cu)	mg/kg	38,2	26,2	30,8	500
Mercury (Hg)	mg/kg	0,123	0,120	0,153	5
Nickel (Ni)	mg/kg	9,5	14,1	8,7	80
Plumb (Pb)	mg/kg	14,5	17,0	19,0	500
Zinc (Zn)	mg/kg	115	130	105	2000
Molybdenum (Mo)	mg/kg	0,72	0,54	0,38	20
Arsenic (As)	mg/kg	0,42	0,36	0,24	20
Cobalt (Co)	mg/kg	1,6	2,65	3,2	100
Manganese (Mn)	mg/kg	103,5	79,5	95,5	-

Heavy metals concetrations

The results of testing the presence of heavy metals in the sludge samples are shown in Table 3.

By its own quality, sludge meets the requirements for the use in agriculture defined by Directive EZ 86/278/EEZ Appendix 1 B[13] and regulations of some European countries relating to heavy metals limiting values. The measured concetrations of all metals are far below value limit prescribed by Regulation on determining allowable quantity of hazardous substances in soil and methods of their testing ("Official Gazette F BiH", 72-09) [12].

According to this Regulation, the allowable manganese concetrations are not determined because the element is not marked as potentially toxic. Its presence can be considered positive propertie of sludge since manganese is an essential trace element. The measured heavy metal concentrations provide the use of sludge as an independent substrate without mixing with soil, as a component in substrates preparation, as a soil conditioner and organic fertilizer.

Microbiological analysis of sludge

Coccoid and rod shaped bacteria were observed in all samples in supernatant and sediment by the analysis of the native prerparations of the sludge from the wastewater treatment plants.

In the sediment, freeswimming and sessile protists were not identified. The results of the quantitative microbiological analysis are showned in Tables 4, 5 and 6.

Table 4. Microbiological analysis – sample 1

Identified microorganisms	Sediment	Supernatant
Coliformn bacterias	*Enterobacter* sp. *Escherichia coli*	*Enterobacter* sp. *Escherichia coli*
Fecal streptococci	*Streptococcus faecalis*	*Streptococcus faecalis*
Salmonella types	Not isolated	Not isolated
Antrakoidi	*Bacillus* sp	*Bacillus* sp
Molds	*Aspergillus* sp.	*Aspergillus* sp.
Yeasts	Not isolated	Not isolated

Table 5. Microbiological analysis – sample 2

Identified microorganisms	Sediment	Supernatant
Coliformn bacterias	*Enterobacter* sp. *Citrobacter* sp.	*Enterobacter* sp. *Escherichia coli*
Fecal streptococci	*Streptococcus faecalis*	*Streptococcus faecalis*
Salmonella types	Not isolated	Not isolated
Antrakoidi	*Bacillus* sp.	*Bacillus* sp.
Molds	*Aspergillus* sp.	Not isolated
Yeasts	Not isolated	Not isolated

Table 6. Microbiological analysis – sample 3

Identified microorganisms	Sediment	Supernatant
Coliformn bacterias	*Enterobacter* sp. *Citrobacter* sp. *Escherichia coli*	*Enterobacter* sp. *Escherichia coli* *Citrobacter* sp. *Proteus* sp.
Fecal streptococci	*Streptococcus faecalis*	*Streptococcus faecalis*
Salmonella types	Not isolated	Not isolated
Antrakoidi	*Bacillus* sp.	*Bacillus* sp.
Molds	*Aspergillus* sp.	*Aspergillus* sp.
Yeasts	Not isolated	Not isolated

Bacteria isolated by this microbiological slugde analysis probably come from the sewage of the plants, because the sewage treatment and waste water treatment are performed in the same plant.

Since the indicators of fecal contamination were identified, we should be alert to the danger of pathogenic microorganisms during the sludge disposal.

Disposal of this kind of the sluge into enviroment could cause the pathogens spread, pollution of surface and undergro-und waters, and toxic effect on humans, animals and plants. In factory "Natron-

Hayat", the sludge obtained by waste water treatment is burnt in its own boiler avoiding the danger of microbiolo-gical pollution. From this point of view, the composting process could be the alternative to sludge disposal because it represents the effective way of destroying bacteria and other microorganisms.

During the thermophilic phase, temperature can reach up to 80^0 C in the core of the compost pile which enables completion of the quality compost hygieni-zation. Also, favorable temperature that lasts long enough inside the compost pile should prevail in order to efficiently destroy the pathogenic microorganisms.

At the compost pile temperature of 65^0 C, the majority of pathogenic microorganisms dies in a few days, but if the temperature is lower than 55^0 C, the process of destroying the pathogens extends and lasts for a few weeks or even months. In agriculture, the use of the compost obtained from this sludge is only allowed if all the sanitary rules and regulation are obeyed and if the compost is checked, in terms of the existence of pathogenic microorganisms.

CONCLUSIONS

Based on the results of the analysed physical, chemical and microbiological features of the sludge from waste water treatment plants of Pulp and Paper Industry "Natron-Hayat" d.o.o., Maglaj, it can be concluded:

- Sludge contains a high percentage of water, especially the sludge from the secondary tank (94,36 %). In order to use this sludge in the composting process, it is necessary to reduce its moisture to acceptable limits (40-60%).
- Sludge has a significant buffering value because of its almost neutral reaction, so before the beginning of the composting process it is not necessary to adjust its pH-value.
- The neutral pH-value of sludge makes it optimal for the accessibility of nitrogen, phospho-rus, potassium and copper and especially it is suitable since it reduces the transfer of heavy metals into the food chain and its phytotoxicity.
- Electrical conductivity is not too high to cause phytotoxicity, howe-ver it is not too low either which would indicate poor accessibility of nutrients.
- The ratio of carbon and nitrogen in samples 2 and 3 is ideal and represents a good basis for develo-pment of composting process.
- The satisfactory share of macro and micro elements was found.
- The concentracions of heavy metals are way under allowed values defined by Regulations on determi-ning allowable quantity of hazardous substances in soil and methods of their testing ("Official Gazette F BiH", 72/09) as well as by EU Regulations (86/278/EEC).
- The indicators of fecal contamina-tion *Streptococcus faecalis*, coliform bacteria as well as the coliform bacteria of fecal origin (*Escherichia coli*) were identified by the microbiological analysis of the sludge samples.

- Anaerobic spore-forming bacteria and apergillus mold were also isolated in the samples.
- The significant difference in quantitative structure of microbiological population of supernatant and sediment was not determined.
- The quality of sludge provides its use as raw material for the compost production.

The composting is one of the possible solutions of the sludge disposal in the factory of "Natron-Hayat". After the treatment and conditioning of sludge, it can be used in the compost production and also in agriculture. The final product can be used in crop cultivation, as a soil improver, and also for improving the fertility of soil.

For compost production, the use of the slugde obtained from the secondary tank, that is, the surplus sludge after the biological waster water treatment is recommended. The daily amount of this sludge is estimated to about 10 tons.

REFERENCES:

1. H.Dinel, T.Marche, M.Schnitzer, T.Pare, P.Champagne, Co-composting of Paper Mill Sludge and Hardwood Sawdust Under Two Types of In-Vessel Processes, Journal of Environmental Science and Health (2004) Vol B39, No. 1, pp. 139-151,
2. Degremont, Tehnika prečišćavanja voda, Građevinska knjiga, Beograd, 1976, pp. 409.
3. Pravilnik o životinjskom otpadu i drugim neopasnim materijalima prirodnog porijekla koji se mogu koristiti u poljoprivredne svrhe ("Sl. novine F BiH", br. 08/08).
4. B.Poček, Metode za bakteriološki, virusološki i parazitološki pregled vode za piće, Beograd, 1990, pp. 628-640.
5. W.H.Thompson, Test Methods for the Examination of Composting and Compost, The United States Composting Council Research and Education Foundation, The United States Department of Agriculture, 2001.
6. F.E.Allison, Soil Organic Matter and Its Role in Crop Production. Elsevier, Amsterdam, 1973.
7. S.M.Tiquia, N.F.Y.Tam, Co-composting of spent pig litter and sludge with forced-aeration, Bioresource Technology, 2000, 72: 1-7.
8. D.A.Iannotti, T.Pang, B.L.Toth, D.L.Elwell, H.M.Keener, H.A.J. Hoitink, A quantitative respirometric method for monitoring compost stability. Compost Sci. Utilization 1 (1993) 52-65.
9. E.Epstein, The science of composting, Technomic Publishing Company, Lancaster, Pennsylvania, 1997, 487.
10. K.C.Das, E.W.Tollner, T.G.Tornabene, Windrow Composting of Paper Mill By-Products: Scale-Up and Seasonal Effects, Compost Science & Utilization, (2002) Vol. 10, No 4, 347-355.
11. M.J Jackson, M.A. Line, Composting pulp and paper mill sludge - effect of temperature and nutrient addition method, Compost science & Utilization. JG Press, Emmaus, Pennsylvania, 1997.
12. Pravilnik o utvrđivanju dozvoljenih količina štetnih i opasnih materija u zemljištu i metode njihovog ispitivanja ("Sl. novine F BiH", br. 72/09).

13. Council Direktive of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture (86/278/EEC), Official Journal OJ L 181, 8.7.1986.
14. N. Mirecki, Kompostiranje, NVO "Proizvodnja Zdrave Hrane", Nikšić, (2007) 1-15.

INFLUENCE VARIETY AND MULCHING LAND ON MASS HEAD AND CONTENTS VITAMIN C BY LETTUCE

ORIGINAL SCIENTIFIC PAPER

Aleksandra Govedarica-Lučić[1], Vesna Milić[1]

[1]*Faculty of Agriculture, East Sarajevo*

ABSTRACT

Lettuce, a leaf vegetable, is an essential ingredient in everyday meals. It is abundant in minerals, vitamins and other biologically active substances; therefore it is very important in human nutrition. Yield and chemical composition of lettuce (*Lactuca sativa l.*) vary depending on a cultivar and environment. Considering the above, the aim of the research was to test cultivar effects and soil mulching (non-mulching, polyethylene foil, agrotextile, polyethylene folio + agrotextile) on yield and amount of vitamin C in lettuce.

The experiment was conducted by a random bloc system repeating it four times in experimental the field of Faculty of Agriculture East Sarajevo.

The research included three cultivars of lettuce: Archimedes, Santoro and Kibou. The maximal lettuce head weight was observed in cultivar Santoro grown on the agrotextile (623.86 g), while the smallest heads were those of Archimedes (257.71 g) grown on soil without mulch.

The minimal level of vitamin C of 21.40 mg/100g was observed in Santoro cultivar grown on the black polyethylene foil, while the maximal level of vitamin C was in all three lettuce cultivars in the variant of PE foil + agrotextile (Archimedes 35.80 mg/100g, Santoro 41.10 mg/100g, Kibou 42.30 mg/100g).

Key words: lettuce, cultivar, vitamin C, soil mulching

INTRODUCTION

Lettuce is a plant of short vegetation grown in gardens, fields or greenhouses as one of the leading cultivated crops. It belongs to a group of green-orange vegetables characterized by high content of biologically significant substances (minerals, vitamins, organic compounds with antioxidative effect).

Lately, lettuce can be bought on a market throughout the year. A direct soil mulching allows an earlier crop for 10-15 days. Additionally the agrotextile cover allows greater head weight.

Use of appropriate materials for mulching the land and the rational use of fertilizers and pesticides can be achieved with reasonable yields meet the quality standards without adverse impact on the environment**.** According to *Vogel-in (1996)* cultivation of lettuce using mulching as well as agrotextile cover is nowadays a mechanized process.

Providing that crop yield and chemical composition of lettuce (Lactuca sativa L.) vary depending on a cultivar and environment, the aim of our work was to test cultivar effects and agro-technical measures on head weight and level of vitamin C in lettuce grown in winter.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse without additional heating in the experimental field of Faculty of Agriculture of Eastern Sarajevo.

The experiment was conducted by the random bloc system repeating it four times on a 2.4 m^2 (0.3 x 8 m) experimental parcel with three beds, one bed for each cultivar.
Nursery plants were grown in containers with 40 openings (10 x 4) of dimension h =4,0cm, vol=70cc, in the greenhouse without additional heating. They were sown at the beginning of September using coated seed. The containers were filled with Klasmann substrate.

The 25 days old nursery plants were transplanted in the beds at 20 cm distance between plants, and 30 cm between the beds creating an area of nearly150 000 plants/ha.

As for irrigation, we used a system "drop by drop" which was placed together with mulch.
We tested two factors: mulching and cultivar.

The variants of mulching used in the study were:

1. control plot
2. polyethylene foil
3. agrotextile
4. combination of polyethylene foil and agrotextile

The three cultivars of lettuce used in the study were:

1. Archimedes RZ
2. Santoro RZ
3. Kibou RZ

Harvest was carried out in December. On each sample plot, we have taken for 5 plants with a variety of chemical analysis.Chemical analysis of plant material and of vitamin C was done at the Chemical Faculty in Novi Sad, according to standard methods implemented by scientific institutions.

The obtained data were analyzed using analysis of variance. All grades of significance were derived based on the F test and LSD test for materiality threshold of 5% and 1%.

RESULTS AND DISCUSSION

The results of examining the cultivar effects and soil mulching on lettuce head weight during a winter production are shown in Table 1.
The maximal lettuce head weight among the examined varieties and mulching variants was achieved in cultivar Santoro (623.86 g) by using the agrotextile. However, the minimal weight was observed in cultivar Archimedes (257.71 g) using the control plot.

The results shown in Table 1 suggest that the mulching influenced the crop yield, i.e. the differences in values of lettuce head weight were observed.

The heaviest lettuce heads were observed (623.86 g) in the agrotextile variant. Combined application of the polyethylene foil and agrotextile increased a head weight and crop yield in contrast to the control,

which was in accordance with the research results *(Djurovka at.al 2009, Patil 2001, Balalic 2004).*

The weight differences in lettuce heads between the control and agrotextile were statistically very significant.

The observed differences in the lettuce head weight among the examined varieties were statistically significant. The statistically very significant greater lettuce head weight was observed in Santoro cultivar in contrast to Archimedes and Kibou.

Table 1 Average lettuce head weight (g)

Mulching	Cultivar			Average
	Archimedes	Santoro	Kibou	
Control	257,71	441,74	289,05	329,50
polyethylene foil	303,66	458,87	280,49	347,67
polyethylene foil + agro textile	258,45	433,81	363,40	351,89
agro textile	305,00	623,86	478,78	469,21
Average	281,20	489,57	352,93	374,56

LSD	A	B	AxB
0.05	92,11	79,76	173,75
0.01	123,12	106,62	213,24

Vitamin C Content

Vitamin C is an essential nutrient for humans. The cultivars of lettuce containing increased vitamin C content and anthocyans are good sources of antioxidants especially in winter when consumption of fresh vegetable is decreased.

Vitamin C content is shown in Table 2.
The results of cultivar effects on vitamin C content are in accordance with the research *(Vogela, 1996; Siomons, 2002)* which suggested that vitamin C content
ranged from 31.50 mg/100g to 37.25 mg/100g depending on a cultivar.

Table 2 Vitamin C content (mg/100g)

Mulching	Cultivar			Average
	Archimedes	Santoro	Kibou	
Control	28,30	22,50	38,70	29,83
polyethylene foil	34,40	21,40	36,80	30,86
polyethylene foil + agro textile	35,80	41,10	42,30	39,73
agro textile	35,30	41,00	31,20	35,83
Average	33,45	31,50	37,25	34,06

Lazic at.al (1994) concluded in their study that different factors can influence vitamin C content, among which the cultivar as the main one.

The analyses of vitamin C content regarding production showed that all ways of production were significantly different.

The highest content of vitamin C was observed in the mulching variant combining the agrotextile and polyethylene foil (39.73 mg/100g), while the control variant had significantly lower vitamin C content (29.83 mg/100g).

CONCLUSION

Based on the results obtained analyzing the cultivar effects and mulching on lettuce head weight and vitamin C content, it can be concluded:

- The heaviest lettuce heads were observed in Santoro cultivar grown on the agrotextile.
- Lettuce head weights greatly varied depending on the way of production, where the best variant was the agrotextile.
- Agrotextile application additionally increases an earlier crop.
- Vitamin C content depends on a series of factors (cultivar, length of vegetation, soil fertilization), where the most dominant factor is cultivar.

LITERATURE

1. Balalić I. (2004): Effects of production method and substrate on yield and quality of lettuce (Lactuca sativa L.). Master Degree Thesis. Novi Sad.
2. Đurovka M., Rašković V., Marinković D., Perlaki Z., (2009): Influence of mulching, cover agrotekstilom and low tunnels, the earlier arrival of watermelon. XIV consulting on Biotechnology, Faculty of Cacak.
3. Đurovka M., Bajkin A., Lazić B., Ilin Ž. (1996):The effects of mulching and direct cover for earliness and yield vegetable. Review works, Institute for Arable and vegetable.Novi Sad. Notebook 25, p. 467-474.
4. Lazić B., Marković V., Đurovka M., Ilin Z. (1994): Interdependence contents nitrate, more vitamins C and beta karotena by salads, and cauliflower.Contermporary agriculture, XL II (Early issue),143 -147.
5. Patil S. (2001): Effect of surface applied mulch on growth, yield and weed infestation in pepper (*Capsicum annum L.*). Procc.Simp, Poster presentations, PP1/04/A-3.
6. Siomos A.S., Papadopoulou, Dogras C.C. and N.D. Niklis (2002). Quality of romaine and leaf lettuce at harvest and during storage. Acta Hort.579,641-646.
7. Vogel G.(1996): Handbuch des speziellen Gemüsebaues,Asteracea, 45-83(Eugen Ulmer Verlag, unter Mitarb.von H.D.Hartmann, K.Krahnstoever, Stuttgart).

TECHNOLOGICAL NOTES

Process Equipment for Treatment of Contaminated Soil and Sludge Waste

In the context of hazardous waste treatment under the POEO Act, it is imperative that the responsible person uses proper process plant and equipment competently to conduct the treatment. This approach would help assure that the favourable treatment results achieved at the laboratory or pilot scale trials can be repeated consistently at the full scale treatment and high treatment standards are maintained at all times.

Industry may practise either or both of the following methods in the immobilisation treatment of contaminated soil and sludge waste:

1. Chemical fixation: Chemical reagents are used to convert the target contaminants contained in the waste to a chemically stable form(s) suitable for landfill disposal.
2. Stabilisation/solidification: Cement and/or pozzolans reagents are used to transform the waste into a stable monolithic substance suitable for landfill disposal.

Unlike washed and clean aggregates used in cement concrete, contaminated soil and sludge waste including river sediments can be very heterogeneous with a mixture of materials of different particle size distributions, shapes, densities and surface properties (eg clayey and plastic). Such dissimilar characteristics and rheological properties can compromise the immobilisation treatment.

The responsible person must use a properly designed and engineered treatment plant that is adequately equipped with automatic or semi automatic control in respect of waste and chemical reagents handling. Avoid or prohibit any manual operation which is prone to human error and may be unreliable.

The mechanical mixer functions as the chemical reactor of the immobilisation treatment process. It should be equipped with a stationary mixing compartment and an agitator fitted with heavy duty mixing paddles/blades; and it can perform the following mixing duties:

- Provide positive agitation/stirring of the mix and achieve rigorous mixing e.g. turbulent flow within the mixing compartment.
- Adequately handle homogeneous and heterogeneous solids including soil, aggregates and sludges, and materials exhibiting plastic properties e.g. silt and clay.
- Capable of achieving a homogenous mix within minutes of mixing.

As a matter of OEH policy the tumbler type mixer e.g. small DIY rotating concrete mixer, rotating mixer mounted on a delivery truck, rotary hoe or bull dozer are not acceptable mixing devices for the immobilisation treatment of contaminated soil and sludge waste. Such machineries cannot discharge the above mixing duties for processing hazardous waste or sludge.

The following types of mixer (Perry, Chemical Engineers Handbook, McGraw Hill) could attain the above mixing duties and are considered suitable for the purposes of the immobilisation treatment of contaminated soil or sludge waste. They are commonly used in industry operations.

1. Pug mill mixer.
2. Paddle type mixer including Ribbon mixer and Turbine/Pan mixer.

However, the choice of mixer is a waste specific issue and the responsible person should conduct a test run before adopting the equipment for full scale treatment. OEH

would consider and approve other types of mechanical mixing device on merits.

Cement-based Solidification/Stabilisation Treatment of Organic Chemical Contaminants in Waste

The cement-based Solidification / Stabilisation (S/S) treatment is a viable waste immobilisation treatment process for certain contaminants. This technique essentially involves the use of a binding reagent to micro encapsulate the contaminants with or without the addition of chemical additives and the principal binder used is Ordinary Portland Cement (OPC).

Contaminants (toxic or non toxic) in waste are often not compatible with the cement hydration reactions and can adversely affect the setting of the mix. One should engage a specialist consultant/contractor before conducting a S/S treatment.

This advisory note only deals with the treatment of semi-volatile organic contaminants in contaminated soil or waste sludge. Further advice may be sought from OEH regarding the S/S immobilisation treatment.

Cement & Additives in S/S Treatment

When ordinary cement is mixed with water, its chemical constituents undergo 6 principal hydration reactions. The initial pH of the mix rises quickly to a pH of 12 (alkali). With a proper control of the water to cement ratio, quantities of additives and the use of suitable mixing equipment, the treated waste would set and attain unconfined compressive strength (UCS) greater than 1 MPa within 7 days.

The actual compositions of commercial OPCs can vary from type to type. Supplementary cementitious materials (SCMs) commonly used to enhance the properties of concretes include industrial by-products such as pulverised fly ash (PFA) from coal combustion, blast furnace slag (BFS) from iron making and condensed silica fume (CSF) from the ferrosilicon industry. These by-products can have widely variable chemical compositions depending on the production source. For example, CSF-OPC blend is used in making high strength concrete, whilst PFA has performance-enhancing effects on the OPC mix in terms of worka-bility, pumpability, strength, shrinkage, and permeability. PFA can also serve as a sorbent for some metals and organic chemical compounds in waste treatment. BFS has its own source of lime (CaO) and silicate compounds. It can react with water in the presence of calcium hydroxide to form cementitious substances. Other SCMs include silica fume, granulated furnace slag, metakaolin (eg calcined clay). Incorporate SCMs into the cement mix when the modified mix benefits the S/S treatment process.

In treating hydrocarbon contaminated wastes, chemical additives such as surfactants can, amongst other things, greatly improve the mixing performance and setting of the mix. Seek advice of the specialist chemical supplier.

The Cement-based S/S Treatment Process

The contaminants present in the waste can interfere with the S/S treatment in many ways. These include:

- Inhibits the hydration process by preventing the water molecules from reacting with the silicate compounds.
- Slows down or prevents the formation of water-containing calcium silicate crystalline compounds (silicate gel).
- Reacts with the calcium hydroxide to form undesirable complexes.
- Prevents the coating of the silicate gel on the aggregates and hence the setting of the mix.

- Forms weak mineral complexes which do not bind well with the silicate gel.
- Retards the cement set and the mix takes much longer time to cure.
- Interferes with cement hydration due to incomplete immobilisation of contaminants.
- Excessive water associated with the contaminants or waste can cause problems in setting.

It is imperative to identify and speciate all contaminants (organic and inorganic) present in the waste. Use favourable lab scale/pilot scale trial results only as a guide to test and optimise the mix formulation for the full scale treatment. Use larger quantities of binder/reagents to allow for the less than ideal mixing efficiency at full scale treatment (Jeffrey Means et al. 1995).

The preparation of samples and UCS test method should be based in accordance with OEH's requirement as per the General Immobilisation Approval 2004/14 for coal tar impacted soil from former gas work sites. Mixing is a critical element of any chemical fixation and S/S treatment process (J Connors, 1990, J Connors, Portland Cement Association, 1997). The mechanical mixer functions as a chemical reactor. Design and engineer the full scale S/S treatment in accordance with the draft OEH policy for Mixing Plant and Equipment for Immobilisation Treatment, Oct 2007. For example, the following types of mixer may be commissioned for the full scale S/S treatment:

Pug mill type mixer. Paddle type mixer including Ribbon mixer and Turbine/Pan mixer.

Treatment of Organic Hydrocarbons

Organic contaminants known to affect the cement S/S treatment. These can affect the cement setting performance even at relatively low concentrations. The cement based S/S treatment is not suitable for fixing Volatile organic chemicals (VOCs) because these chemicals are capable of escaping into the environment under ambient conditions. Contaminated soil, waste sludge and residues containing semi-VOCs may be suitable for treatment by the cement-based S/S method.

Semi VOCs means any organic chemical compound based on carbon chains or rings with a vapour pressure less than 2mm of mercury (0.27 kPa) at 25^{o} C and 101.3 kPa, and has a boiling point of greater than 100^{o} C at 101.3 kPa. The cement treated waste must meet the following UCS criteria.

The European 'Atex' Directives 94/9/EC and 999/92/EC exhibit a lack of differentiation between explosive gas mixtures and explosive dust clouds. Consequently, some very substantial differences between gases/vapours and dusts have either been neglected or addressed inadequately.

A new approach for standardization of design of electrical apparatuses for 'explosive atmospheres' has been adopted by the International Electrotechnical Commission (IEC) as well as in the European Union (CEN and Cenelec). A central concern is to combine and align standards for combustible dusts with the corresponding ones for combustible gases/vapours.

The European 'Atex' Directives 94/9/EC [1] and 1999/92/EC [2] appear to be a main source of inspiration in this effort, due to the lack of differentiation between explosive gas mixtures and explosive dust clouds in the directives. Consequently, in producing the new aligned/combined standards some very substantial differences

between gases/vapours and dusts have either been neglected or addressed inadequately.

Daimler's declared policy is one of integrated environmental protection

Our objectives are to use the natural resources which form the basis of our existence on this planet sparingly and in a manner which takes the requirements of both nature and humanity into consideration. You too can help to protect the environment by operating your vehicle in an environmentally-responsible manner.
Fuel consumption and the rate of engine, transmission, brake and tyre wear depend on the following factors:

- operating conditions of your vehicle
- your personal driving style

You can influence both factors.
You should therefore bear the following in mind. Operating conditions:

- avoid short trips, as these increase fuel consumption.
- observe the correct tyre pressure.
- do not carry any unnecessary weight in the vehicle.
- monitor the vehicle's fuel consumption.
- remove the roof rack once you no longer need it.
- a regularly serviced vehicle will contribute to environmental protection. You should therefore adhere to the service intervals.
- always have maintenance work carried out at a qualified specialist workshop, e.g. a Mercedes-Benz Service Centre.

Personal driving style:

- do not depress the accelerator pedal when starting the engine.
- do not warm up the engine when the vehicle is stationary.
- drive carefully, maintaining a safe distance to the vehicle in front.
- avoid frequent, sudden acceleration.
- change gear in good time and use each gear only up to $^{2}/_{3}$ of its maximum engine speed.

Composting Fish Waste from the Aquaculture Industry

Composting is simple, effective, and transforms the wastes generated on aquaculture farms into a valuable on-farm resource. Previously, disposal of processing wastes (e.g. heads, frames and offal) and fish mortalities generated on-farm has been by pit burial.
Composting wastes is consistent with best practice environmental management and is now a condition of Environment Protection Authority Victoria (EPA) waste discharge licenses.
A practical and effective method of composting fish wastes generated by the local salmonid aquaculture industry has been developed at Alexandra in the Goulburn Valley and has been adopted by several local trout farms. This Aquaculture Note is a summary of the farm's combined experiences and recommendations.
http://www.eps.manchester.ac.uk/about-us/news/?archive=twelvemonths&id=6387

Carbon calculator adds up to first prize

A carbon calculator software tool developed by chemical engineers at The University of Manchester clinched the Outstanding Achievement in Chemical and Process Engineering prize at IChemE’s annual innovation and excellence awards.
Professor Adisa Azapagic, from the School of Chemical Engineering and Analytical

Science, won the award for a software tool called CCaLC that helps companies measure and improve their carbon footprint at minimum cost.

The free tool has been developed specifically for industry, taking a lifecycle approach applicable to many sectors and supply chains.

It has already been downloaded by more than 500 organisations across the world. The award, sponsored by Rowan House, recognised CCaLC as the best entry across all categories.

The tool has been used to calculate the carbon footprint of a huge range of things. One astounding finding using the calculator found that supermarket lamb curry ready-made meals eaten in the UK amounted to an annual carbon footprint equivalent to 5,500 car trips around the world or 140 million car miles.

Professor Azapagic found that the fast food meal generates the equivalent of 4.3 kg of carbon dioxide emissions per person.

The £1m project is led by Adisa Azapagic, Professor of Sustainable Chemical Engineering, and funded by organisations including the Engineering and Physical Research Council, and the Natural Environment Research Council.

Professor Adisa Azapagic said: "I am delighted that CCaLC has won the top IChemE prize. Being a chemical engineer, this award is important to me as it recognises the 'outstanding contribution to chemical and process engineering". "CCaLC has been developed by a team of chemical engineers and I am particularly pleased that their hard work has been recognised by this prestigious award.

"I am particularly pleased that the top IChemE prize was awarded to a project related to sustainability - this further reinforces the commitment of both the IChemE and the chemical engineering profession to sustainable development."

http://gigaom.com/cleantech/electric-motor-maker-remy-files-for-ipo/

Following Tesla Motor's successful IPO last year, more hybrid and electric car tech makers are looking to land on the public markets. On Friday, electric motor maker Remy International filed for an IPO for up to $100 million. The company's principal products are starters, alternators and hybrid and electric motors. Remy sells its electric motors to companies like Mercedes, GM, Allison Transmission and electric car startup Aptera. The company also received a $60 million grant from the Department of Energy to further develop its electric motor technology. Remy calls itself the "leading non-OEM producer of hybrid electric motors in North America." For the year ending December 31 2010, Remy generated sales of $1.10 billion and net income of $21.19 million. That's substantial growth from the year ending December 31 2009, when Remy generated sales of $910.74 million and net income of $14.06 million. Remy has been in business for more than a century and was a division of GM for 75 years, but was spun out in 1994. Remy is one of the rare companies we've written about that's shooting for an IPO and has a solid growing and profitable business. That's because it's not a risky, early-stage technology, like other IPO cadidates Amyris, Solyndra or even stand-alone EV maker Tesla. Starters, alternators and hybrid motors have been on the market for years. The only early stage area of Remy's business is electric motors for all-electric cars, like Aptera's. Aptera has seemed to drop off the radar as it struggled to move

into commercialization and bet on receiving a DOE loan. Remy says only 3 percent of its sales in 2010 were from hybrid electric motors. Remy also faced a recent issue with Italian auto parts maker Tecnomatic, which sued Remy, accusing it of stealing Tecnomatic's tech to apply for the DOE grant. Remy says it will use the proceeds of the funds from the potential IPO to pay off debt, and potentially acquire companies. Remy plans to list under the symbol RMYI.

http://www.infoniac.com/health-fitness/alcohol-kills-faster-than-ecstasy.html

Alcohol kills faster than ecstasy

A new drug list was revised by Britain's drug experts to reveal that alcohol and tobacco put your health at greater risk than such dangerous drugs as LSD, cannabis and ecstasy. Experts conducted a new all-compassing research with the government top advisory committee involved to make a shocking conclusion on the harm of regular tobacco and alcohol. A modern classification of drugs doesn't reflect the real situation on harmful substances. Thus more than 20 drugs were tested on their social and individual impact and results lead to revision of ABC system. A team of 29 psychiatrists took part in the the study to classify all drugs into nine groups that were analyzed on the physical harm, addiction and social danger. Another group consisted of 16 experts did the research in several fields including chemistry, pharmacology, psychiatry and legal field to say a final word on drug ranking. According to this new drug list, alcohol is named A drug. It slightly outruns heroin and cocaine. Tobacco was placed ninth and fell within a category B drug, after amphetamine. Such dangerous drugs as cannabis, LSD, ecstasy were left far near the end of the drug list.

This latest study raised the question that government's drug policy was not paying enough attention to modern state of affairs and didn't take notice of the scientific findings. There should be no surprise that ecstasy drug was placed almost at the end of the list. One person a day dies from acute alcohol poisoning in comparison to 10 people a year who die from ecstasy overdose. A new classification brings to light many issues concerning drug policy with a more thoughtful approach and corresponding legal response. Drug and alchohol abuse is more common than we think. Learn more about alchool or opiate addiction by visiting a trusted detox site. A drug addiction is usually a very difficult segment of one's life, but with the proper care, it can be beat.

INSTRUCTIONS FOR AUTHORS OF PAPERS

1. The manuscript which is to be submitted to the Editorial Board should be written in two columns with double spacing on one side of A4 paper, with all margins of 2.54 cm (1 "), font Times New Roman 12 pt. Thework will be sent in electronic form, prepared solely using word processing program Microsoft Word, ending with the 2003 versions. The file should be named as follows: TA_last name of first author _first word of title.doc. Pictures should not be fitted into the text, but rather in electronic form, stored in each separate file. Image files should be named on following way: first TA_last name of first author_picture_number of picture. Appropriate extension. The extension must match the image format (tif, pcx, jpg, png ....). Images should have a resolution of min. 300 dpi and should be prepared so that they can be printed well in B / W technique. Each individual image should not be greater than one third of A4 format.
Image labels should be written below the picture.

2. Titles and signatures placed bellow drawings and diagrams should be submitted in a separate file (in English), which should be named as follows: TA_last name of first author_title.doc.

3. The size of the article (text, along with summaries, pictures and drawings and with a list of literature references, not counting titles and signatures, as well as information about authors) should be limited to 6 pages (two illustrations correspond to approximately one page). An exception can be negotiated with the editorial board, and to receive a larger volume of work if the content and quality justifies it.

4. For successful inclusion of articles published on BCS languages in international information flows, parts of the manuscript should be written in the language of the article, as well as in English, namely: the text in tables, figures, diagrams and drawings, their titles, and signatures, and a list of abbreviations and marks.

5. Abstracts should be attached to the manuscripts:
• Summary (synopsis) in the language of article of the maximum volume of a one printed site. It must explain the purpose of the paper, and must include the important data and conclusions, as well as keywords. This summary should be entered in the manuscript right after the header of the article.
• The same summary in English (summary) with keywords (descriptors - keywords).

6. The paper should contain the full official address, phone and e-mail address of all authors (on a separate sheet). Emphasize the correspondence author, with whom will the editorial board consult.

7. The title of the article should be specific and informative, in order to better determine the content of the paper. It is desirable to be as short as possible. The title should be cited on one of the BCS languages and in English.

8. Text should be clear, concise, and grammatically correct without spelling errors, written in third person (impersonal).

9. Papers should be written with the assumption that readers know the discussed subject. Thus in (a short) introduction should briefly be stated only what is necessary for understanding of the text.

10. Experimental technique and device should be described in detail only if they deviate significantly from the description already published in the literature; for the known techniques and devices only source of necessary information is provided.

11. Tables and diagrams should be drawn and described so that they are understandable without reading the text. The same data should not be placed at the tables and diagrams, except in exceptional cases. The author will then give its reasons, and its validity is subject to final assessment of Editorial board and its reviewers.

12. Tables that contain a lot of data, where not all are necessary for understanding of the text should be reduced to the minimum. If desired by author, the editor will store complete tables in the archives and enable to interested readers an insight into the archive. This should be stated as a note with the abbreviated table.

13. Symbols of physical values should be written cursive, and unit of measure with vertical letters, eg. *V, m, p, t, T,* but: m3, kg, Pa, °C, K.

14. Formulas and equations should be typed, if possible, in one line (use a oblique fractional line instead of horizontal). Indexes, at top and bottom, should be written clearly. Avoid upper indexes so that they would not replace with numerical exponents. All special characters (Greek letters, etc.) that can cause confusion, authors should explain separately.

15. In the paper should be used sizes and units of measurement in accordance with International System of Units (SI). For specific nomenclature a list of all used labels and definitions should be add in the language of the article and on the English language.

16. References cited should be selective rather than extensive, except when it comes to review article. Literature citations should be enclosed on a separate sheet of paper and they should be numbered in the order they appear in the text. The numbers of citations are written to the text as a superscript. If the original literature was not available to the authors, they should cite by the source from which the quotation was taken. Abbreviations for magazines must be in strict accordance with the abbreviations that are alleged by the Chemical Abstract.

Example of citing journals:
1. J. J. Sangiovanni, A. S. Kesten, Chem. Eng. Sci. 26 (1971) 533. Example of citing patents:
2. J. Ehrenfreund (Ciba Geigy A. -G.), Eur. Pat. Appl. 22748, 21 Jan 1981; C. A. 95 (1981) 7078b.

Example of book citation:
3. W. Mehl, J. M. Hale, Insulator Reactions, in: P. Delahay and C. W. Tobias (ed.), Advances in Electrochemistry and Electrochemical Engineering. Vol. 6, Interscience Publ., New York, 1967, pp. 399-458.

17. In the corrective prints only the author can correct the error text. For possible changes in the text (additions, etc.), the author bears the cost.