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CONTENT

EDIBLE FILMS AND COATINGS – PRODUCTION AND APPLICATION

REVIEW ARTICLE

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ABSTRACT

Edible films and coatings are active packaging systems used in food production that are gaining increasing attention. In addition to food protection and shelf-life extension, the advantage of edible films is their safety for consumption, unlike other packaging materials that pose environmental issues.

Basic film forming components are proteins, lipids and/or polysaccharides. Plasticizers, antimicrobials, antioxidants, colours and/or aroma are often added. Recent research studies are mainly focused on the application of natural antimicrobials in edible films for meat and minimally processed vegetable application.

The aim of this review is to give an overview of most important knowledge in edible film and coating applications in the food industry.

Keywords: edible films, active packaging, production, application

INTRODUCTION

One of the major food industry challenges is prolongation of shelf-life, esp. when dealing with fresh produce. At the same time, consumers request maximum safety with a minimum impact on food itself and environmental issues pose a challenge for the packaging of such products.

These issues raised the interest in edible films and coatings. In the 1980s this theme became widely popular, although edible films were used in China as early as 12th and 13th century[1,2]. To this day edible films and coatings are extensively researched, but, nevertheless, they are still a very popular and contemporary scientific theme.

The basic definition of edible films and coatings is " a thin layer of material which can be consumed and provides a barrier to moisture, oxygen and solute movement for the food"[3]. Films are produced as free-standing active packaging material, while coatings are casted directly on the product[1,4]. They are used for fresh, minimally processed, processed food, as flavour encapsulating agents, carriers for antimicrobial components[1], and are usually produced from proteins, lipids and carbohydrates. Plasticizers facilitate film formation, and antimicrobial components, vitamins, antioxidants, aromas, colours etc. can be added to improve their properties as active packaging.

Both films and coatings have to be safe for consumption, biodegradable, they should not affect sensory properties of products and must ensure food quality and safety preservation. Mechanical properties (tensile strength and elongation at break), physico-chemical characteristics (moisture content, solubility, swelling, glass transition temperature...), barrier properties (gas and water vapour permeability) as well as biological activity (antioxidant and antimicrobial properties) highly determine the applicability of edible films.

BASIC COMPONENTS OF EDIBLE FILMS AND COATINGS

Proteins

Film forming properties of a number of proteins, both of animal (gelatin, casein, whey proteins) and plant origin (zein, gluten, soy protein) have been extensively researched over the past three decades.

Physical and chemical properties of protein films are influenced by amino acid composition, electrostatic charge, amphyphylic properties, as well as secondary, tertiary and quaternary structure changes due to pressure, heat, irradiation, mechanical damage, acid, alkali, salt, metal ion, enzyme action etc[1,5].

Mechanical properties of protein films are satisfying. They are good O_2, CO_2, N_2 and aroma barriers and have good organoleptic properties. However, due to a mainly hydrophilic character, these films are often a poor barrier to water migration[5,6].

Gelatin

Gelatin is produced by partial acid or alkali hydrolysis of collagen at high temperatures in the presence of water. This protein has a random configuration of polypeptide chains in aqueous solutions and gives flexible, strong films impermeable for O_2[1].

Sobral et al. extensively researched bovine hide and pigskin gelatin. They reported that drying conditions highly influence film forming properties of gelatin films – if drastic, these conditions (time, temperature, RH) can destabilize film forming solution and cause exudation of plasticizer[7]. When the drying temperature approached the melting temperature of the film, exudation of plasticizers occurred and drying kinetics decreased with the increase of air temperature[8]. As other protein films, gelatin films have poor water vapour barrier (WVB) properties. However, this drawback can overcome surfactant addition, such as lecithin[9]. Marine gelatin is extensively researched over the past years as an alternative to bovine gelatin films. Tuna fish gelatin films had lower puncture force than mammalian gelatin films, but better WVB properties[10,11]. Similar results were reported for brownstripe red snapper and bigeye snapper gelatin films[12]. Atlantic halibut gelatin films are transparent, weakly coloured and highly extensible[13] and good film forming properties showed squid gelatin as well[14].

Casein

Casein molecules have a random coil nature and an ability to form numerous hydrogen bonds and therefore easily form transparent, flexible, tasteless films from aqueous solutions without further treatment[1]. Electrostatic interactions and hydrophobic forces are also involved in film formation. When casted without plasticizer, casein films tend to be brittle, but this issue can be addressed by application of plasticizer, such as glycerol[15].

Due to a high number of polar groups casein films excellently adhere to different substrates and prevent migration of O_2, CO_2 and aromas[16]. Sodium caseinate shows good water vapour barrier properties and thermal stability, however, this property is lost in films, due to plasticizer application. Addition of fatty acids to film forming solution increases a number of hydrophobic groups and decreases WVP of obtained film, but, at the same time negatively influences texture properties[17].

Whey proteins

When carefully processed, whey proteins form transparent, tasteless films similar to casein ones. Unlike casein, whey proteins require heating to form intermolecular disulphide bonds[1]. Cross-linking can be induced by transglutaminase as well, however, a heating method is more cost-effective. Mechanical properties and WVP of whey protein films are highly influenced by plasticizers and addition of lipids[18,19].

Whey protein isolate (WPI) films plasticized with glycerol are transparent, smooth, flexible and homogenous. WVP of these films is proportional to WPI concentration and RH gradient[20].

Zein

Zein is a hydrophobic protein found in maize, obtained as a by-product of the bioethanol and oil industry. It is traditionally used as a coating material in the confectionary industry[21]. Zein films are prepared from ethanol or isopropanol solution at high temperatures, with plasticizer addition[1,5]. Physic-chemical properties of alcoholic zein solutions are highly influenced by a concentration of alcohol, which, in turn, affects film properties[22].

WVP of zein films is similar or lower than other protein films and can be reduced by thermal denaturation of protein, whereas O_2 and CO_2 permeability are higher than whey protein films[5]. Film brittleness is reduced by glycerol addition, whereas polyphenols increase its flexibility[21].

Gluten

Wheat gluten films are homogenous, transparent, strong and good water barriers. Since gluten is insoluble in water, film casting requires a complex solvent mixture and acidic or alkali conditions to brake inter- and intramolecular disulphide bonds. Most often, KOH, NaOH or NH_4OH and acetic, citric, propionic or phosphoric acid is used[5]. Alkaline-produced films are yellow and have unpleasant taste, whereas films produced in acid conditions show problems in protein dispersion. Gliadin and glutenin dispersion in gluten films can be enhanced by ultrasound treatment[23].

The rheological properties of gluten films can be altered from smooth to rubber like by high pressure treatment[24]. WVP is similar to other protein films. It is highly dependent on plasticizer used and increases with temperature and RH increase. At low RH and low temperatures O_2 and CO_2 permeability is significantly lower compared to other biopolymer and synthetic films. However, gas permeability also increases with temperature increase[5].

Films made of gliadin and glutenin fractions of gluten have also been studied. Glutenin formed stronger films with better barrier properties than both gliadin and gluten films, while gliadin films had better optical properties. Thermal treatment highly influenced film properties: in 55 – 75 °C range films became stronger, less extensible, and less permeable for water vapour. Above 70 °C glutenin films disintegrated, and yellow-brown coloration and darkening occured[25].

Lipids

Lipid films were the first edible films. The practice of dipping oranges and lemons into wax dates back to the 12th and 13th century in China and in 16th century England fruit was “larded”[1]. Today, waxes are commonly used coatings for fresh fruits and vegetables[1].

Generally, lipids form flexible, cohesive films which are excellent water vapour barriers due to their insolubility in water. Polar resin films are good barriers for O_2, CO_2 and ethylene. Current research articles are mainly dealing with the investigation of lipid application in bilayer and multilayer films, where they are applied to reduce WVP of polysaccharide and protein films[1].

Polysaccharides

Cellulose and its derivatives

Due to a large number of intra-molecular hydrogen bonds cellulose is water insoluble. Etherification of cellulose results in formation of water soluble ethers: methylcellulose (MC), hydroxypropylmethylcellulose (HPMC) and hydroxypropylcellulose (HPC) which have good film forming properties. MC, HPMC and HPC films are prepared from water or ethanol solutions. They are flexible, transparent, tasteless and odour-free[1,5]. WVP of these films is highly influenced by the hydrophobic: hydrophilic ratio of film components. Water-soluble plasticizers and active components increase WVP and decrease tensile strength of CMC films[26].

Starch and derivatives

To produce an edible film, the starch suspension should be rapidly heated to 50 °C (for starches with less than 40% amylose) – 155 °C (high amylose starches). If the film is prepared, the suspension is casted on a flat, smooth surface and carefully cooled. If the cooling process is too rapid, cracks and crevices will form, and over-dried films become brittle[5].

Starch based edible films are transparent or translucent, tasteless and odour-free. Tensile strength and flexibility of starch films are determined by macromolecular chain mobility in the amorphous phase, amylose: amylopectin ratio, plasticizer and water content. These films are excellent O_2 barriers and good barriers for CO_2. WVP is similar to protein based films[5]. Tapioca starch-based films have a low water permeability[27], which is further decreased by surfactant addition[28] and cross-linking with glutaraldehyde[29].

When polyols are used as single plasticizers in high concentrations, separation of polyols from starch films occur. However, binary polyol mixtures can be used in concentrations up to 40% without difficulties in film handling or stickiness appearance, but the negative effect of polyol addition on water sorption and WVP was not inhibited when binary mixtures were used[30]. Research of Hernandez et al. showed that glycerol, most often used polyol plasticizer slows down starch film digestibility[31], showing its potential in dietetic formulations.

EDIBLE FILM AND COATING APPLICATIONS

Fruits and vegetables

Application of edible films and coating to fresh fruits and vegetables is based on the establishment of a controlled or modified atmosphere. When considering the edible film application for fresh fruits and vegetables, special attention has to be paid to:

- water vapour permeability values – to decrease the water loss from fruit,
- O_2 permeability values – to reduce the production of ethylene,
- CO_2 permeability values – to ensure a high CO_2 content in the atmosphere around the fruit and retard microorganism growth,
- mechanical properties – to ensure the integrity and durability of the film[32].

Edible films improved the shelf-life of numerous fruits and vegetables by retarding moisture loss, O_2 permeation to the surface, and inhibition of microorganisms. For instance, gluten film and bilayer film made from gluten and lipids improved firmness retention of strawberries and delayed decay, with better results when bilayer films were used. However, bilayer films were unacceptable to consumers[33]. CMC and MC coatings successfully inhibited cell-wall degrading enzymes on strawberry surface[34] and chitosan films retarded their weight loss[35].

Shelf-life of minimally processed fruits and vegetables can also be significantly improved, which is shown in researches on minimally processed carrot, where chitosan coating reduced surface whiteness during storage[36]. Chitosan coatings slow down the polyphenoloxidase activity and prevent browning of pumpkin and sodium alginate coating reduced browning of fresh-cut pear[37]. Chitosan film incorporated with rosemary and olive extracts prevented browning of minimally processed squash[38].

If antimicrobial agents are added to films, they are potent inhibitors of mycotoxigenic moulds[26], *S. aureus, E. coli*[39] etc.

Fish and meat products

Gennadios et al. reviewed edible coating application on seafood, poultry and meat[40]. Since then, many research articles have dealt with the preservation of meat and fish products using edible films and coatings. Here, only a few examples will be given.

Itturiaga et al. incorporated natural extracts into gelatin and MC films to minimally processed fish and successfully maintained antimicrobial activity against *L. monocytogenes, P. fluorescens and A. hydrophylla* for as long as one month[41]. Gelatin-based and chitosan-gelatin films incorporated with oregano and rosemary extracts inhibited microbial growth and reduced lipid oxidation of cold smoked sardines[42].

Chitosan-sunflower oil films reduced metmyoglobin content in pork meat hamburgers and chitosan films reduced microbial development[43]. Soy protein edible films incorporated with oregano and thyme extracts inhibited the development of *E. coli* and *S. aureus* on fresh ground beef during refrigerated storage[44]. WPI edible coatings incorporated with oregano and clove essential oils doubled the storage time of chicken breasts[45].

Pectin-based antimicrobial film showed promise in the shelf-life prolongation of ready-to-eat turkey in research of Jiang et al.[46].

Oil-fried products

Deep fat fried products are very appealing to consumers due to a soft, moist interior covered with crispy crust, but can contain up to 50% fat. Coating potato strips with MC and HPMC edible coatings reduced oil uptake during frying by 35 – 40% without a significant influence on texture properties[47], and gellan gum and guar gum coatings also showed promising results[48]. Even better results were achieved by coating mashed potato balls with these films – fat uptake reduction was app. 60 – 80%[49], and dough discs absorbed app. 30% less fat during frying when coated with MC[50].

CONCLUDING REMARKS

This short overview of literature gave basic insight into the scientific research on edible films and coatings. However, the subject is wide enough and all scientific aspects of it have not been reviewed in this article. The authors kindly advise reading other review articles for full coverage of the subject.

Nevertheless, there are still many areas for further research of edible films and coatings. Alternative bases for film development could be investigated, esp. usage of food industry by-products. The influence of films and coatings on sensory properties and consumer acceptance have not been researched to the extent where it can be concluded without any doubt that they will not significantly influence consumers' perception of the products. WVP permeability is still an issue when films and coatings are applied to fruit and vegetables, although bi-layer films did improve barrier properties. Film and coating in industrial conditions should also be considered in the researches.

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APPLICATION OF FLUORESCENCE SPECTROSCOPY FOR AUTHENTICATION OF THE BOTANICAL ORIGIN OF HONEY

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

Honey is a remarkably complex natural liquid that is reported to contain at least 181 substances. The composition of honey is variable and depends primarily on the floral source; however, certain external factors also play a role, such as seasonal and environmental factors and processing. Honey is a supersaturated solution of sugars, of which fructose (38%) and glucose (31%) are the main contributors. A wide range of minor constituents is also present in honey, many of which are known to have antioxidant properties. These include phenolic acids and flavonoids, certain enzymes (glucose oxidase, catalase), and amino acids and proteins. As polyphenols and aromatic amino acids are strong fluorophores, fluorescence spectroscopy should be helpful in authenticating the botanical origin of honey.

In this preliminary study, the application of fluorescence spectroscopy for authentication of the botanical origin of acacia, meadow and honeydew honey was investigated.

Two different fluorescence emission scans were recorded between 350 nm and 650 nm, with excitation wavelengths of 340 and 440 nm. In further experiment, the spectrofluorimetric method „3D excitation increment measurement“ was performed. This method presented an easy way of obtaining the complete spectra of all fluorescent components in honey. The fluorescence spectra were recorded between 330 nm and 650 nm and between 410 nm and 700 nm, respectively.

When excited at 340 nm, the emission spectra of acacia honey showed a fluorescence intensity peak at 440 nm, while other types of honey showed no significant fluorescence intensity. When excited at 440 nm, the emission spectra of all honey types showed fluorescence intensity peaks between 497 nm and 535 nm.

Our results further show that the fluorescence spectra obtained by 3D excitation increment measurements are characteristic for a particular type of honey.

Our results indicate that fluorescence spectroscopy may be used as a useful method for rapid authentication of the botanical origin of honey.

Keywords: honey, botanical origin, authenticity, fluorescence spectroscopy, 3D excitation increment measurement

INTRODUCTION

The authenticity of food is one of the most important issues in the area of food safety and quality control. Regulatory authorities, food processors, retailers and consumers are interested in the origin and quality of honey[1,2,3,4].

A number of new analytical techniques combined with multivariate data analysis have been proposed for the determination of the botanical origin of honey. They are, for example, based on physical and chemical measurands determined during quality control of honey[5,6] or the former combined with the determination of mineral content[7] as well as carbohydrate composition[8], amino acid composition[9], mass spectrometry or metal oxide semiconductor based gas sensors[10,11], differential scanning calorimetry[12], pyrolysis mass spectrometry[13], Raman[14] and near infrared spectroscopy[15].

To estimate the botanical origin of honey it is necessary to use a number of methods of analysis, which requires a lot of time and money. Therefore, there is a need to develop new methods that wil allow fast, reproducible and inexpensive determination of its botanical and geographical origin[16,17]. The spectroscopic techniques probably present the most promising approach. They only require very little or no sample preparation, no harmful reagents, but are fast, allow getting a fingerprint of the overall chemical composition of honey, and show the ruggedness and the excellent repeatability of physical methods. It is known that honey contains numerous fluorophores such as polyphenols[18,19,20,21] and amino acids[22,23]. By studying the fluorescence spectroscopic properties of honey samples, a relatively fast and reliable method for authentication of honey and determination of its botanical origin can be developed[24].

MATERIALS AND METHODS

The study included 45 different samples of natural honey: 11 acacia, 25 meadow and 9 honeydew. Samples were collected from individual producers from Bosnia and Herzegovina.

Fluorescence spectra of honey samples were recorded using a spectrofluorimeter RF-5301 PC (Shimadzu, Japan).

Honey samples that were used for recording fluorescence spectra were prepared in the following manner: in order to dissolve eventually formed crystals, 20 g of each sample of honey was incubated for 8 hours in a water bath at 40 ° C, cooled to room temperature and then used for recording fluorescence spectra in a 1 cm quartz cell.

Fluorescence emission spectra from 350 nm to 650 nm were recorded after excitation of the honey samples at 340 nm. Fluorescence emission spectra from 447 nm to 650 nm were recorded after excitation of the honey samples at 440 nm. In further experiment, the spectrofluorimetric method 3D Excitation increment measurement was used. The fluorescence emission spectra were recorded between 330 nm and 650 nm and between 410 nm and 700 nm, respectively. The 3D excitation measurement was performed to collect several 2D excitation spectra in fixed time intervals. All recorded 2D spectra were collected in a 3D data object by using software Panorama fluorescence 1.1 (LabCognition, Analytical Software GmbH & Co. KG). The sampling interval was 1.0 nm with the 10.0 nm excitation increment for each fluorescence spectra between 330 nm and 650 nm and between 410 nm and 700 nm, respectively. The entrance and exit slits for the excitation light-beam were both 1.5 nm.

RESULTS AND DISCUSSION

When excited at 340 nm, the emission spectra of acacia honey showed fluorescence intensity peak at 440 nm, while honeydew and meadow honey showed no significant fluorescence intensity (Figure 1).

When excited at 440 nm, the emission spectra of all honey types showed fluorescence intensity peaks between 497 nm and 535 nm (Figure 2). These broad bands are primarily due to flavins[25] .

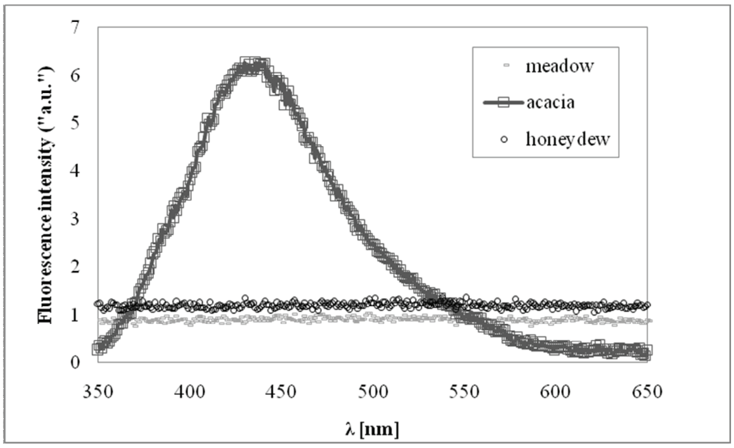

Figure 1. The fluorescence emission spectra of three different honey types recorded between 350 nm and 650 nm with an excitation wavelength of 340 nm in a 1 cm cell at 25 °C

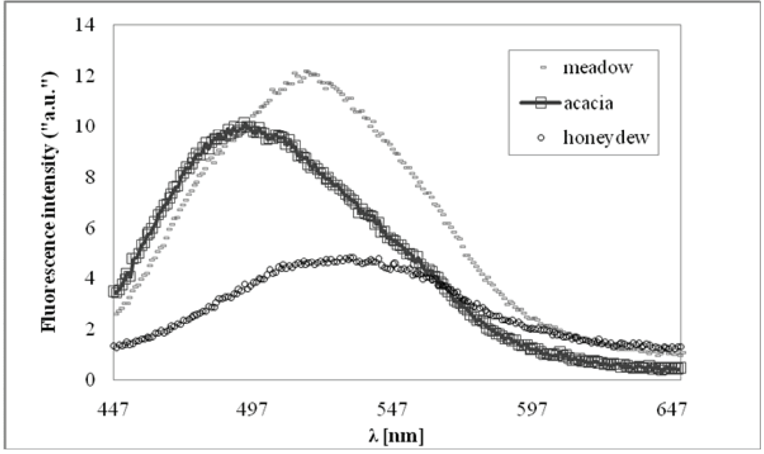

Figure 2. The fluorescence emission spectra of three different honey types recorded between 447 nm and 650 nm with an excitation wavelength of 440 nm in a 1 cm cell at 25°C

The results of 3D excitation increment measurement from 330 nm to 650 nm for all analysed honey types are presented in Figure 3. The 2D spectrum of emission maxima obtained for acacia honey (Figure 3A) is significantly different when compared to meadow and honeydew spectra (Figure 3B, C). Acacia 2D spectra showed two maxima, y. between 330 nm and 460 nm and between 460 nm and 650 nm of excitation, respectively. The first maximum, observed between 330 nm and 460 nm in acacia, is absent in meadow and honeydew 2D spectra. At excitation of 555 nm, all honey types showed the maximum of fluorescence emission intensit.

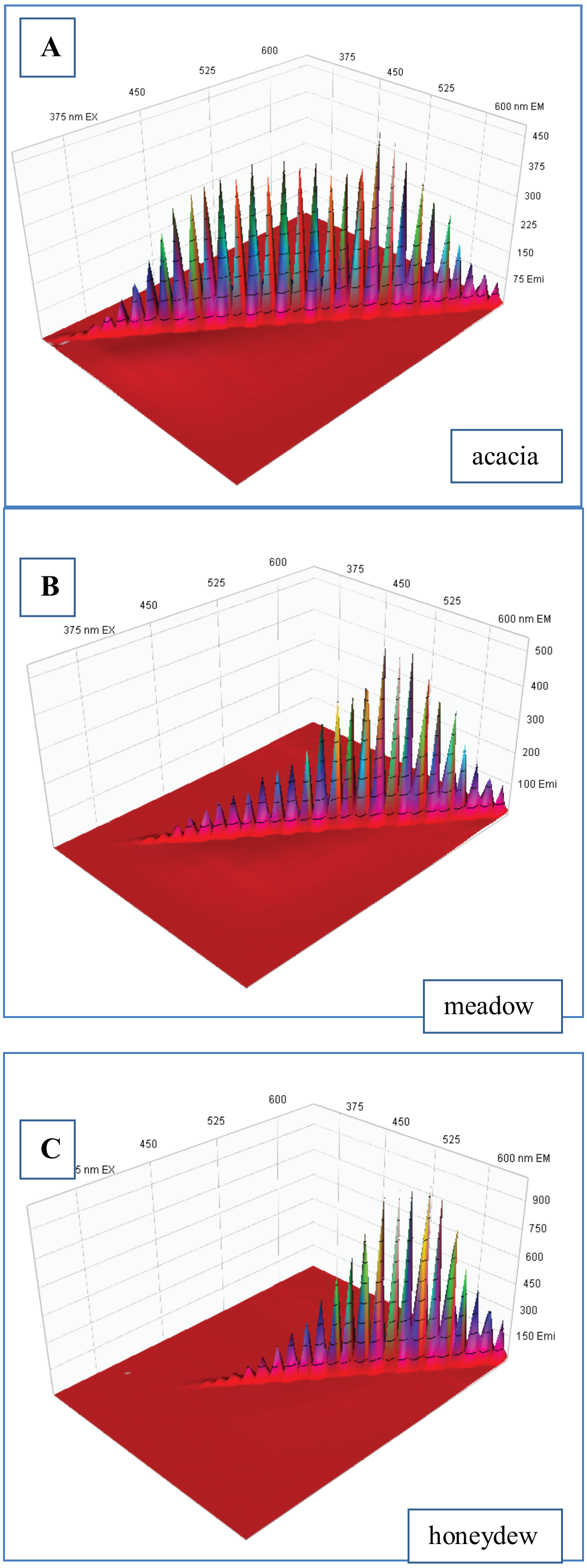

Figure 3. The fluorescence spectra of different honey types recorded between 330 nm and 650 nm by the spectrofluorimetric method 3D Excitation Increment Measurement in 1 cm cell at 25°C: A) acacia; B) meadow and C) honeydew

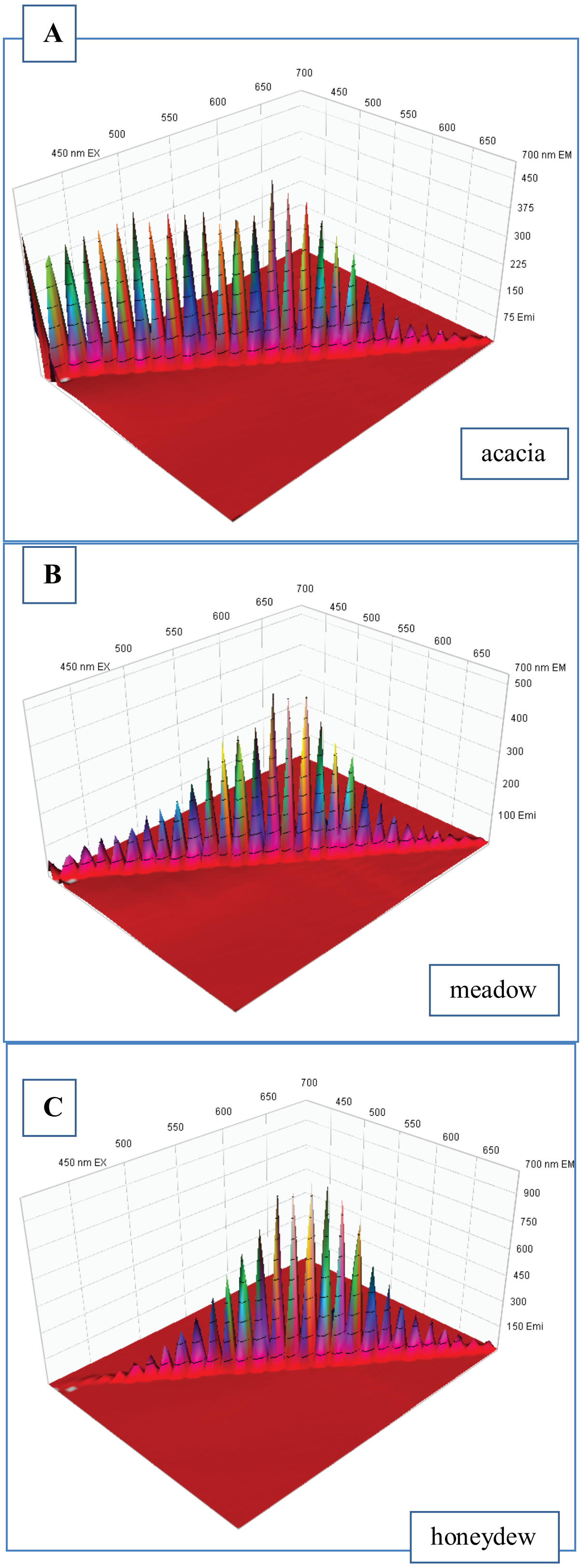

Figure 4. The fluorescence spectra of different honey types recorded between 410 nm and 700 nm by the spectrofluorimetric method 3D Excitation Increment Measurement in 1 cm cell at 25°C: A) acacia; B) meadow and C) honeydew

The results of 3D excitation increment measurement from 410 nm to 700 nm for all analysed honey types are presented in Figure 4. 2D spectra of emission maxima measurements of acacia (Figure 4A) is significantly different when compared to the meadow and honeydew spectra (Figure 4B,C). Acacia 2D spectra showed two maxima again, between 410 nm and 460 nm and between 460 nm and 700 nm of excitation, respectively. The first maxima, observed between 410 nm and 460 nm in acacia, is absent in meadow and honeydew 2D spectra. At excitation of 555 nm, all honey types showed the maximum of emission measurements. 2D spectra between 650 nm and 700 nm were the same for all honey types (Figure 4A, B, C).

Given that the acacia honey is unifloral, it was expected that this type of honey is easier to identify compared to the multifloral types of honey studied, because it contains a smaller number of different fluorescent components. Our results showed that in both application modes of 3D excitation increment measurement, acacia honey had characteristic spectra.

CONCLUSION

Our results indicate that fluorescence spectroscopy may be used as a useful method for rapid authenticating of the botanical origin of honey. This method requires a simple protocol of sample preparation and does not need harmful reagents. Also, the method is very fast, reproducible and reliable.

Fluorescence spectra obtained by a 3D excitation increment measurement method showed that this spectrofluorimetric application can be used for identification of acacia honey. This study opens new possibilities of research of the potential of fluorescence spectroscopy and identification of botanical origin of honey, especially of monofloral types.

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APPLICABILITY OF ULTRAFILTRATION MEMBRANES FOR CUTTING-OIL TREATMENT OF WATER

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

One of the most progressive ways of oily wastewater cleaning is the use of ultrafiltration (UF) membranes, but the main disadvantage of the process is membrane fouling, which is caused by deposition and adsorption of the emulsified oil droplets on the membrane surfaces, which results in substantial decline in permeate flux with operation time and consequently limits its wide application. The membrane surface property plays a crucial role in determining the fouling resistance property of membranes. It is widely accepted that the more hydrophilic the membrane surface is, the better the antifouling ability it exhibits.[1] Also, electrostatic phenomena, often quantified by the membrane zeta potential, influence permeate flux and the fouling behaviour.[2]

In this research UF of cutting-oil by three different polymeric membranes (regenerated cellulose (RC), polyacrylonitrile (PAN) and polyetherimide (PEI)) is presented. The membrane flux (J) in dependence of time (t) was studied. Surface and structural properties of membrane are very important for their permeability, which defines the capacity of filtration device. Therefore, physical parameters as contact angle and streaming potential of membranes were analysed. Considering the results of these measurements the applicability of polymeric UF membranes and their surface behaviour were determined.

The tests showed that among three different UF membrane materials, regenerated cellulose showed the best performance in terms of the water flux and fouling tendency, due to its good hydrophilicity.

Keywords: ultrafiltration, polymeric membranes, oily wastewater, fouling, contact angle, zeta potential

INTRODUCTION

Cutting-oil emulsions are used as lubricants and coolants specifically designed for the metal-working processes. Used metal cutting-oils cause high levels of contamination and rancid odours due to the presence of emulsifiers, corrosion inhibitors, extreme pressure agents, biocides, antifoam compounds, etc., therefore their treatment and final disposal must be handled carefully. The upcoming tightening legislation related to metal-working fluids disposal stimulates the research and development of new materials and processes which would allow the safe disposal of cutting-oils.

Membrane filtration has arisen during past decades as clean and effective technology in the treatment of oily wastewaters offering high removal efficiencies of oil, low energy consumption, and a compact design compared to robust space consuming traditional processes.[3-6] However, the major drawback in membrane processes remains the decline in permeate flux with time, mainly because of concentration polarization, membrane fouling due to surfactant or oil adsorption on the pore walls, gel layer formation or pore blocking by oil droplets.

The proper choice of membrane material plays a crucial role in membrane fouling prevention. Hydrophobic membrane material is often used for ultrafiltration membrane. Its properties exhibit excellent chemical and thermal stability but, because of its hydrophobic properties in nature, fouling is the critical problem. For example, free oils can coat hydrophobic membranes resulting in poor flux. Hydrophilic membranes preferentially attract water rather than oil, resulting in much higher flux.[7] Furthermore, surface charge characteristics of polymeric membranes, which are most commonly described through surface zeta potentials determined from electro-kinetic measurements, are important in the sense of interactions between membranes and oil drops in emulsion.[8]

In this work the main objective was to study the effect of membrane surface properties, such as hydrophobicity and zeta potential of membranes, on the cutting-oil emulsion ultrafiltration performance.

MATERIALS AND METHODS

Model solution

The model solution was prepared by mixing commercial cutting-oil (Die-lubric 7050) and water for 10 min at about 500 rpm. The major part of cutting-oil is represented by the polysiloxane emulsion and small fraction of non-ionic emulsifier. The volume percentage of oil in water was 0,5 % (v/v), i.e. 70 mg/L of oil concentration. pH of the model solution was 7. The cutting-oil characteristics are listed in Table 1.

Table 1. Properties of the commercial cutting-oil (Die-Lubric 7050) used for the model solution

Parameter	Value
Colour	White
pH (20°C, 100%)	9 – 9,5
Viscosity (20°C), cSt.	1,8
Density (20°C), g/mL	0,994
Solubility in water	Fully miscible

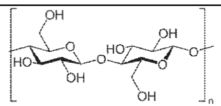
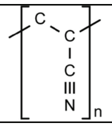
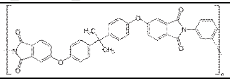
The particle size/oil-droplets size distributions and zeta potentials measurements were of 0,5 % model solution conducted using the Zetasizer Nano ZS instrument (Malvern Instruments, UK) equipped with dynamic light scattering and laser Doppler micro-electrophoresis.

Ultrafiltration

The filtration experiments were performed in a dead-end batch ultrafiltration cell Milipore model 820 of 200 mL capacity and with a membrane area of 28,7 cm^2. An electromagnetic bar was used to stir the solution at speed 250 rpm. The experiments were carried out at room temperature (around 25°C) and with operating pressure 3 bar using nitrogen gas.

Three flat sheet membranes (regenerated cellulose (RC), polyacrylonitrile (PAN) and polyetherimide (PEI)) were selected for testing. The membrane characteristics are summarized in Table 2.

Table 2. Characteristics of membranes used

Membrane material	**Nominal MWCO (kDa)**	**Ph**	**Chemical structure**
regenerated cellulose (RC)	30	1-11	
polyacrylonitrile (PAN)	15	2-10	
polyetherimide (PEI)	16	2-10	

Membranes were prepared for use according to the manufacturer's instructions. Prior to the experiments the initial water flux, J_{wi}, of each membrane was measured with MilliQ water. The emulsion flux through the membrane, J, was determined by collecting and measuring the permeate volume over a certain time. Flux reduction (FR) during ultrafiltration was calculated using (Eq. 1):

$$FR = J_f/J_i, \qquad \text{(Eq.1)}$$

where J_f is final constant permeate flux and J_i initial permeate flux.

After each experiment with cutting-oil emulsion, the membranes were washed with distilled water for 30 min to remove labile solutes, and the final water flux, $J_{wf;}$ was then determined. Membrane fouling tendency (*MFT*) was quantified using (Eq.2):

$$MFT = 1 - J_{wf}/J_{wi}. \qquad \text{(Eq.2)}$$

The regeneration of fouled membrane was examined with 1M NaOH solution, which was found to be very efficient.

Contact angle measurements

The hydrophilicity or wettability of fresh membranes was determined by using the contact angle technique. The contact angles were measured with the goniometer KRÜSS DSA 100 using the sessile drop method. In this test a ~20 µL drop of MilliQ water was placed onto the dried membrane surface with a microsyringe, and the air–water–surface contact angle was measured using software "Drope Shape Analysis" within 5 s. The contact angle measurements were performed in triplicate using separate pieces of membrane.

Zeta potential measurements

The zeta potential was determined through electro-kinetic measurements and indicates the status regarding surface charges along the interface between solids and liquids.[9] The evaluation of electrical charges on the surface of membranes was performed using an Anton Paar (SurPASS) zeta potential meter from pH 2 to 10. Zeta potentials were calculated from the measured streaming potentials using the Helmholtz–Smoluchowski equation.[10]

RESULTS

Model solution

From Figure 1 it could be seen that the particle size/oil-droplets size distributions and zeta potentials measurements of 0,5 % model solution were 253,3 nm and -21,7mV, respectively.

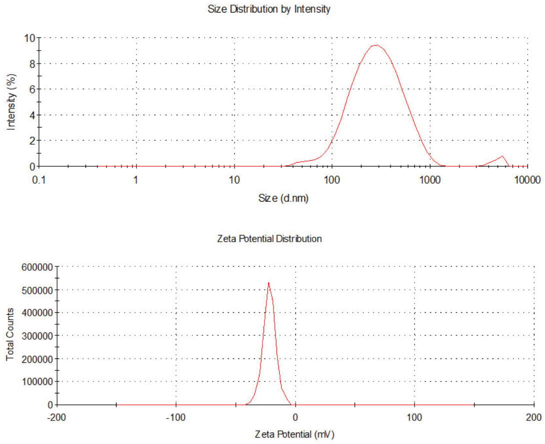


Figure 1. Particle size and zeta potential distribution in 0,5 % (v/v) model solution

Ultrafiltration

Ultrafiltration was done on RC, PAN and PEI membranes at operating pressure of 3 bar. The measurements of water fluxes before and after UF of 0,5 % (v/v) cutting-oil emulsion and emulsion steady permeate flux are gathered in Table 3.

Table 3. Water fluxes before and after UF of 0,5 % (v/v) cutting-oil emulsion, membrane fouling tendency and emulsion permeate flux reduction

Membrane material	**J_{wi} (L/(m^{2}hbar))***	**J_{wf} (L/(m^{2}hbar))***	***MFT***	**J_i (L/(m^{2}hbar))***	**J_f (L/(m^{2}hbar))***	***FR***
regenerated cellulose (RC)	210	181	0,14	57	27	0,47
polyacrylonitrile (PAN)	214	80	0,63	39	22	0,56
polyetherimide (PEI)	39	8	0,80	21	12	0,57

* at 3 bar, 25°C, stirred cell (250 rmp).

Pure water fluxes were measured to study the permeability of the fresh membranes. It can be seen from Table 3 that the pure water fluxes J_{wi}, of fresh RC and PAN membrane were about 5 times higher than that of PEI membrane.

The filtration of the feed emulsion containing 0,5 % (v/v) of cutting oil-in-water ran for 3 hours each time, and then stable, i.e. constant permeate flux was measured (Figure 2). Significant flux reduction occurred with all three membranes during ultrafiltration of emulsion. *FR* value determined by using (Eq. 1) was 0,47 for RC membrane. Higher values for PAN and PEI membranes were obtained 0,56 and 0,57 respectively.

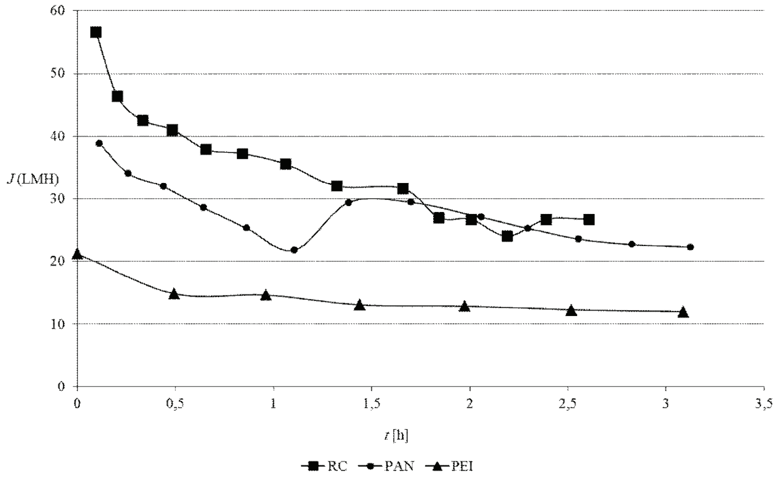


Figure 2. J as a function of t of RC, PAN and PEI membrane

After the experiments with cutting-oil emulsion, membranes were washed with distilled water, and was denoted as the final water flux, $J_{wf;}$. Fouling tendency was characterized by the ratio J_{wf}/J_{wi}. From Table 2 it could be seen that the least affected was the RC membrane; the water flux recovery was 86 %.

On Figure 3 is well seen the difference in flux reductions and fouling tendencies between three membranes. Despite the fact that the difference between the permeate flux reductions (*FR*) of different membranes is insignificant, there is a large difference in membrane fouling tendency (*MFT*). The reason is in some other important membrane properties, such as hydrophobicity and membrane surface zeta potential, which plays a key role in determining the membrane performance.

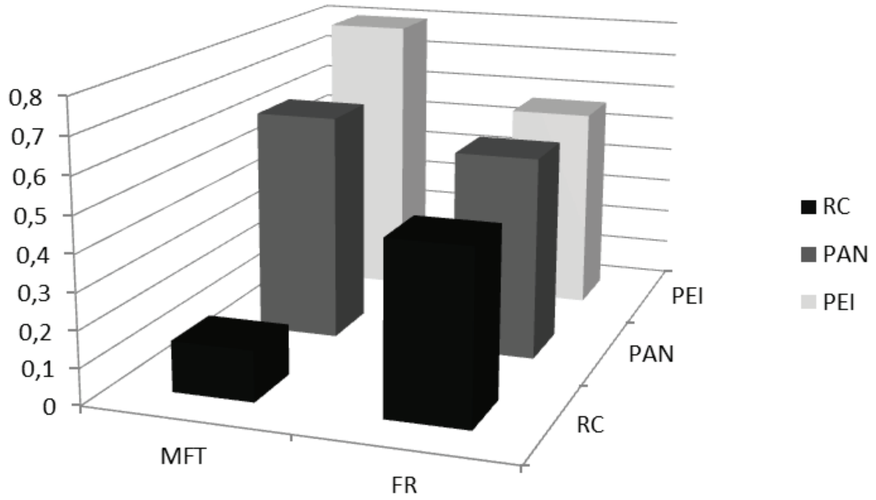


Figure 3. Membrane fouling tendency and flux reduction for RC, PAN and PEI membrane

Contact angle measurements

In the case of oil-in-water separation, it is widely recognized that hydrophilic materials are less sensitive to adsorption compared to hydrophobic ones.[11] The measurements of membranes contact angles (Figure 4) correspond to water flux measurements. RC membrane had the lowest contact angle of 54,2±1,0°, corresponding to the highest hydrophilicity. PEI membrane had the highest contact angle of 80,6±0,9° corresponding to the lowest hydrophilicity. The contact angle was 61,3±2,0° for PAN membrane. Hydrophobic solutes, such as cutting-oil, have tendency to adsorb on hydrophobic membranes, resulting in poor flux. On the other hand, hydrophilic membranes preferentially attract water rather than oil, resulting in much higher water flux. As a result of the low fouling tendency and the higher permeate flux the RC membrane appears to be better choice than the PAN and PEI membranes for application of treatment of cutting-oils. PAN and PEI membranes are less hydrophilic than the RC membrane, so they were easily wetted with surfactants present in the oily emulsion and fouled.[12]

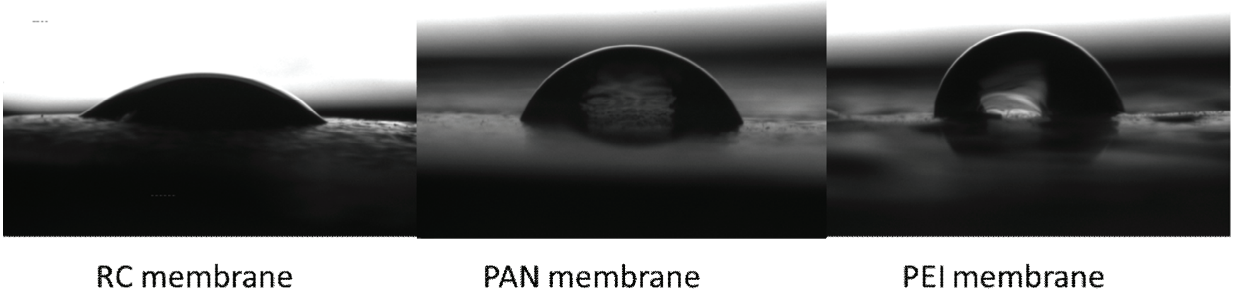


Figure 4. Contact angles of RC, PAN and PEI membrane

Zeta potential measurements

The zeta potentials of fresh membranes as a function of pH are shown in Figure 5. All membranes showed the same trend, an increase of negative charges in relation to pH in the range from 2 to 10. The main differences between the membranes are in relation to the intensity values of negative charges present on its surface. At pH 7, i.e pH of the cutting-oil emulsion, zeta potential of the RC membrane is -24 mV, of the PAN membrane -103 mV and of the PEI membrane -85 mV.

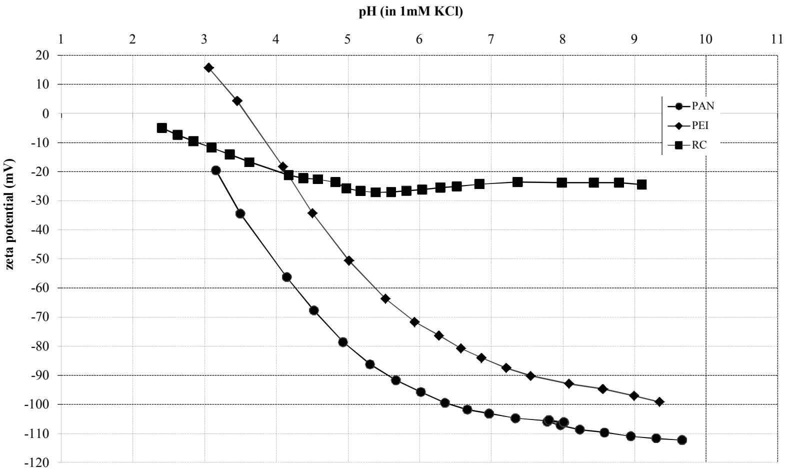


Figure 5. Zeta potentials obtained from zeta-potential measurements for fresh PAN, PEI and RC membrane

The zeta potential of the emulsion droplets was measured at -21 mV. The oil droplets tend to disperse at high charge density, resulting in a cake consisting of fine particles. As a consequence, in case of the PAN and PEI membrane the cake resistance increased and the permeate flux decreased. These observations are in line with those obtained by Zhao.13

CONCLUSION

In this work, the separation of oil from cutting-oil emulsion with RC, PAN and PEI ultrafiltration membranes dead-end filtration system has been investigated. Our tests showed that among three different UF membrane materials, regenerated cellulose showed the best performance in terms of the water flux and fouling tendency, due to its high hydrophilicity and lower charge density in comparison with PAN and PEI, which prevented a cake formation of fine particles on the membrane surface.

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VALIDATION OF DISTILLATION METHOD FOR DETERMINATION OF SO_2 IN BEER

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

Sulphur dioxide (SO_2), as produced in beer during fermentation, is harmful in high concentrations whereby the maximum allowed concentration in Slovenia is 20 mg/L. The purpose of this study was the implementation and validation of the distillation method, which is the officially recognised and prescribed method for sulphur dioxide determination in beer in Slovenia and the EU. This method was also compared with the spectrophotometric DTNB method that was previously used for routine analysis. Our research work on the distillation method proved that the method is suitable for SO_2 determination in beer: optimal distillation time was 20 min using 150mL of sample. The average recovery at the concentration of 9.7mg/L was 100.0 % and 85.9 % at 15.3mg/L. The average repeatability of the method (n=16) was 3.1%, whereby no outliers were detected. Practical application of the method was tested on 25 different beer samples (lager - light, dark, low alcohol, non-alcohol, a mixture of beer and other beverages). For the majority of real samples the measured concentrations of SO_2 where within the range from 1.0 to 2.5mg/L.

Keywords: sulphur dioxide, beer, distillation, validation, DTNB.

INTRODUCTION

Traditionally, and according to the still valid Bavarian “Reinheitsgebot” law from 1516, beer is a beverage that is made only from barley malt, hops and water. Nevertheless, apart from the basic raw materials, substitutes for barley in the form of various non-malted cereals are used. In European and American countries, maize grits are more commonly used whilst non-malted cereal and rice in Asia. In addition to these commonly used cereals non-malted barley is also often used[1]. Besides water, ethanol and carbon dioxide, beer contains several other compounds, such as phenols, higher aromatic alcohols, esters, inorganic species, sulphur dioxide etc. Sulphur dioxide (SO_2) a product of fermentation, is a natural antioxidant but in beer it has often been recognised as an off flavour. It is present in different chemical forms; they are all strongly pH dependent, whereby the pH of beer[2] is around 4.1±0.3. Synthesis and also the concentration of SO_2 are directly influenced by metabolism and yeast activity; if the yeast growth is intensive and under optimal environmental conditions, negligible amounts of SO_2 are produced. By reducing the yeast activity more SO_2 is produced whereby the beer’s »character« changes. Besides the fermentation process parameters, the selection of yeast regarding its vitality and low presence of oxygen can increase the final SO_2 concentration in beer. The concentration of SO_2 is usually controlled at the end of the brewing process, whereby the maximum allowed concentration is 20mg/l in the EU[3-6] and 25mg/l in the USA[7]. For determination of SO_2 in beer the EBC (European Brewery Convention) recommended: distillation[4], enzymatic[5] or *p*-rosaniline[6] methods. Besides these methods flow injection analysis[8,9] (FIA), ion chromatography[10] (IC), gas chromatography[11] (GC), voltammetry[12-14] and chronopotentiometry[2] are also described in the literature. Our research work was focused on the implementation and validation of the distillation method for routine analysis, whereby the distillation method was also compared to the spectrophotometric - DTNB method, that was previously used as the standard method for SO_2 determination in beer.

MATERIAL AND METHODS

DTNB method

After acidification of the beer, the SO_2 was transported within a nitrogen atmosphere to the DTNB reagent. The reaction between the 5,5 –dithiobis (2-nitrobenzoic acid) and SO_3^{2-} anions is presented in Figure 1, all other experimental parameters and details are available in the literature[3,15].

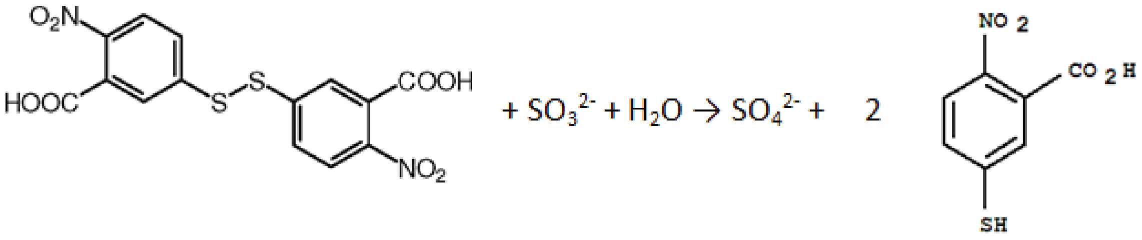

Figure 1. Reaction between 5,5-dithiobis(2-nitrobenzoic acid) and SO_3^{2-}

Distillation method

In the first step the solution of H_2O_2 was neutralised using 0.01MNaOH. After the addition of beer to H_2O_2, the present SO_2 was converted to H_2SO_4 within a nitrogen atmosphere. Finally, determination of H_2SO_4 was performed volumetrically using NaOH as a standard reagent (Figure 2). All other experimental parameters with details are available in the literature[4,15].

$$SO_2 + H_2O_2 \rightarrow SO_3 + H_2O$$

$$SO_3 + H_2O \rightarrow H_2SO_4$$

$$H_2SO_4 + 2NaOH \rightarrow Na_2SO_4 + 2H_2O$$

Figure 2. Reactions between H_2O_2 and SO_2 and volumetric determination of H_2SO_4 using NaOH

RESULTS AND DISCUSSION

The distillation method is also known as the Monier-Williams method[16]. This is a simple, efficient and inexpensive method but has, in practice, several modifications using the same chemical principle – SO_2 reaction with H_2O_2 within a nitrogen atmosphere, distillation, and volumetric determination of formed H_2SO_4 using NaOH as a reagent.

The optimisation of distillation time is therefore crucial and was studied within the range from 5 min to 25 min. From Figure 3 it is evident that the concentration of SO_2 increased intensively during the first interval - up to 10 min but was almost constant after 15 min. After 20 min the determined SO_2 concentration varied at around 1%.

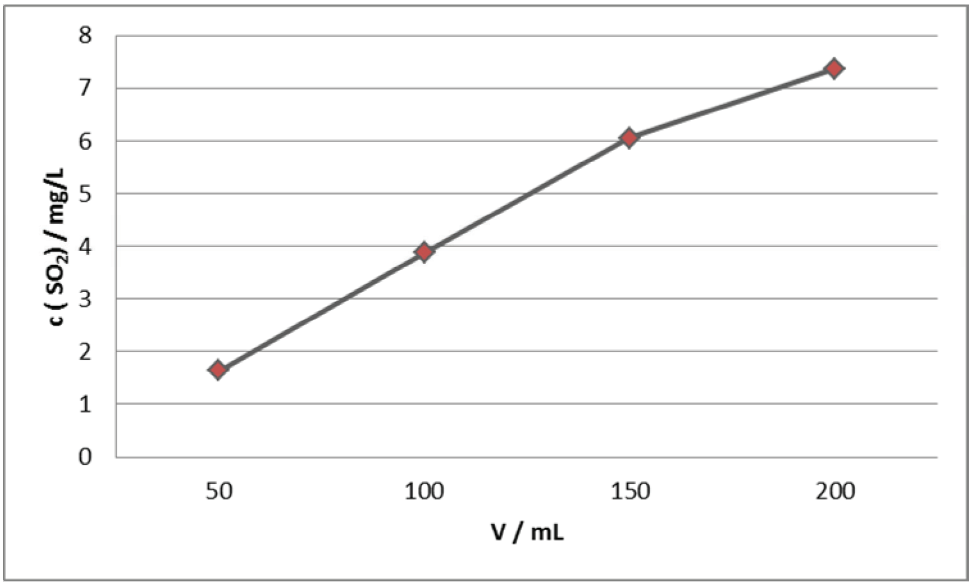

Figure 3. Optimisation of distillation time

Linearity - volume sample versus determined SO_2 concentration was studied as the next step of optimising the distillation method. The volume of beer was increased from 50mL to 200 mL, using 50mL steps, whereby the concentrations and volumes of all other reagents were constant. Beyond 150 mL of sample (Figure 4) the determined SO_2 concentration slightly decreased. In all other experiments we used 150mL of samples for analysis but the sample volume can be increased, when low concentrations of SO_2 are expected.

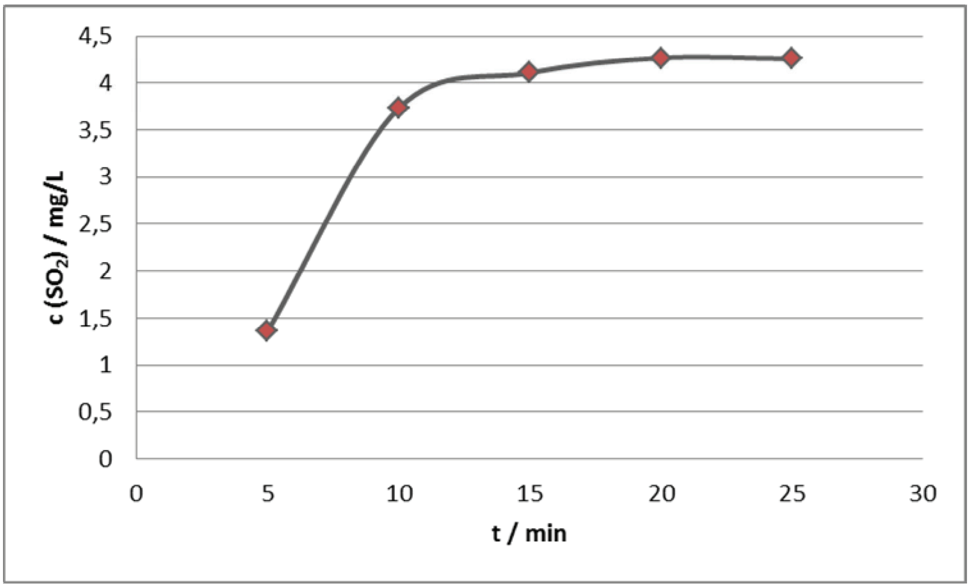

Figure 4. Distillation method – linearity

Typical validation parameters (recovery, repeatability, accuracy, reproducibility, outliers) were studied in the next step. The average repeatability of the method (n=16) was 3.1%, whereby no outliers were detected when the Grubbs' test was performed (Table 1).

Table 1. Reproducibility of measurements

Measurement	SO_2 concentration day 1 (mg/L)	SO_2 concentration day 2 (mg/L)
1	3,97	4,13
2	4,16	4,16
3	4,03	4,16
4	4,16	4,19
5	4,29	4,19
6	4,54	4,22
7	4,35	4,26
8	4,29	4,29
Average	4,22	4,20
s	0,18	0,05
∑s	0,13	
RSD_i (%)	3,11	
$F_{calculated}$	0,12	
F_{table}	4,60	
$t_{calculated}$	0,35	
t_{table}	2,14	

Using significance testing (F and t-tests - Table 1) it was confirmed that reproducibility is acceptable; no significant difference was observed between measurements at the significance level of 0.05. The average recovery at the SO_2 concentration of 9.7mg/L was 100.0% and 85.9 % at the level of 15.3mg/L. The number of significant decimal places was also evaluated; all the results on real samples are therefore reported using one significant digit (Table 2).

Table 2. Concentration of SO_2 determined with the distillation and DTNB method

Sample No.	**Sample**	**DTNB method (mg/L SO_2)**		**Distillation method (mg/L SO_2)**	
1	Lager beer	2.0	2.3	1.3	1.3
2	Lager beer	4.6	5.1	3.6	3.6
3	Lager beer	4.0	4.5	2.6	2.6
4	Lager beer	0.8	0.7	0.3	0.2
5	Lager beer	0.8	0.6	0.3	0.3
6	Lager beer	2.5	2.5	1.2	1.2
7	Lager beer	5.1	5.2	4.2	4.3
8	Lager beer	2.3	2.3	1.6	1.5
9	Lager beer	2.2	2.1	1.0	1.1
10	Lager beer	1.8	1.9	< 0.2	< 0.2
11	Lager beer	1.9	1.8	1.2	1.2
12	Dark beer	1.9	1.9	1.0	1.0
13	Dark beer	1.1	1.1	< 0.2	< 0.2
14	Dark beer	1.3	1.2	< 0.2	< 0.2
15	Dark beer	1.1	1.1	0.3	0.3
16	Dark beer	2.3	2.3	1.9	1.9
17	Non-alcoholic beer	1.8	2.0	1.1	1.1
18	Non-alcoholic beer	1.1	0.9	< 0.2	< 0.2
19	Non-alcoholic beer	1.1	1.0	0.3	0.3
20	Non-alcoholic beer	1.1	0.9	< 0.2	< 0.2
21	Beer-based mixed drink	0.9	0.9	< 0.2	< 0.2
22	Beer-based mixed drink	0.8	0.8	< 0.2	< 0.2
23	Beer-based mixed drink	2.0	1.9	2.0	2.1
24	Beer-based mixed drink	2.1	2.4	2.0	2.0
25	Beer-based mixed drink	1.5	1.5	1.7	1.7

CONCLUSIONS

When comparing the proposed distillation method with *p*-rosaniline or the DTNB method, where formed coloured complexes with SO_2 are spectrometrically determined (at 550 nm and 435 nm), the reagent consumption and time of analysis was significantly decreased.

Another comparison between the distillation and the DTNB method on 25 real beer samples (lager-light, dark, low alcohol, non-alcohol, a mixture of beer and extracts) was also made (Table 2). The analyses were performed in duplicates and for the majority of samples the measured SO_2 concentrations were within the range from 1.0 to 2.5 mg/L, which is several times lower than the allowed concentration (20 mg/L).

Finally, our research work and validation on the distillation method proved that this method is simple, efficient and inexpensive and can therefore be used for daily routine analysis of SO_2 in beer.

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INFLUENCE OF LIGAND STRUCTURE ON THE EFFICIENCY OF PB AND CD IONS TRANSPORT THROUGH LIQUID ORGANIC MEMBRANES

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

Methods for determination of heavy metal content in natural resources (water, soil, air) and also in industrial raw materials and products are increasingly occupying the attention of researchers. Recently used methods for determination of metal cations are generally based on the principle of „molecular recognition“ and include complexation of metal cations with suitable ligands, followed by their removal in the form of coordination compounds. Efficiency of complexation expressed through the stability of formed complexes is determined by compatibility between cations and ligands. The parameters that define the ligand nature are: the number and type of electron-donor atoms, the structure of molecules (macrocyclic or open-chained), polarity, hydrophilic-lipophilic balance, etc. Useful technique for successful separation and preconcentration of metal cations is the transport through liquid organic membranes. The process involves extraction, diffusion and re-extraction of metal ions. Efficiency of this process depends on the choice of: ligands, organic solvents-liquid membranes, counter-ions and stripping agents.

In this paper, the influence of ligand structure on the efficiency of Pb and Cd ions transport through liquid organic membranes, was investigated. Two type of ligands: macrocyclic (18-crown-6 and dibenzo-18-crown-6), and acyclic (nonionic surfactant Triton X-100) were used. Organic solvents: dichloromethane and 1,2-dichloroethane were used as the liquid membranes. Experiments included application of homemade “transport cell” which enables transport of metal ions from source aqueous phase through organic membrane layer to receiving aqueous phase. Concentrations of transported Pb and Cd ions were determined by atomic absorption spectrometry. Gained results showed higher transport efficiency in experiments with macrocyclic ligands compared to acyclic. It was also showed that substituents in macrocyclic structure decrease the efficiency of transport in both type of organic solvents.

Key words: Pb and Cd metal cations, ligands, complexation, liquid membrane transport

INTRODUCTION

Among the numerous techniques for efficient removal of metal cations from natural resources, based on an investigation of metal ion complexation with suitable ligands, the transport through liquid organic membranes has lately attracted particular attention of researchers.[1,2] Since the interactions between metal cations and ligands ("host-guest" interactions) depend on many factors, the resulting stability of the formed complex can be increased by varying of experimental conditions during transport experiments, e.g. during complex formation in organic solvent as a membrane. One of the most important factor influencing the complex formation is the compatibility between "the host" (ligand molecule) and "the guest" (metal ion). Also, the temperature and other factors such as type of solvent, type of counter ion, presence of coexisting species, etc., have a certain influence on the complex stability.

Polyether ligands are among the most suitable host molecules for many metal ions. Oxygen atoms in their structure (as electron donors) enables the formation of a coordination-covalent bonds with metal ions. Also, the amphiphility of their molecules provides the ability of their implementation in polar and nonpolar solvents. Crown ethers have a specific macrocyclic structure which consists of the polyether chain forming "the crown" with a hydrophilic cavity and a hydrophobic surface. The macrocyclic structure of these ligands provides high stability of formed complexes with metal ions due to the "macrocyclic effect".[3] Modeling of structural characteristics (the ring size, the type and number of donor atoms, different substituents, etc.) can increase or decrease their affinity and selectivity toward certain metal ions. Furthermore, there are polyether compounds with an open-chain structure of their molecules which can also be used as ligands for metal ions, such as the surface active agents (nonionic type). These surfactant molecules can interact with metal ions as ligands, as well as the solubilisation agents in a form of micellar aggregates. Fig.1. shows structures of these amphiphilic molecules used as ligands in the experimental part of this paper.

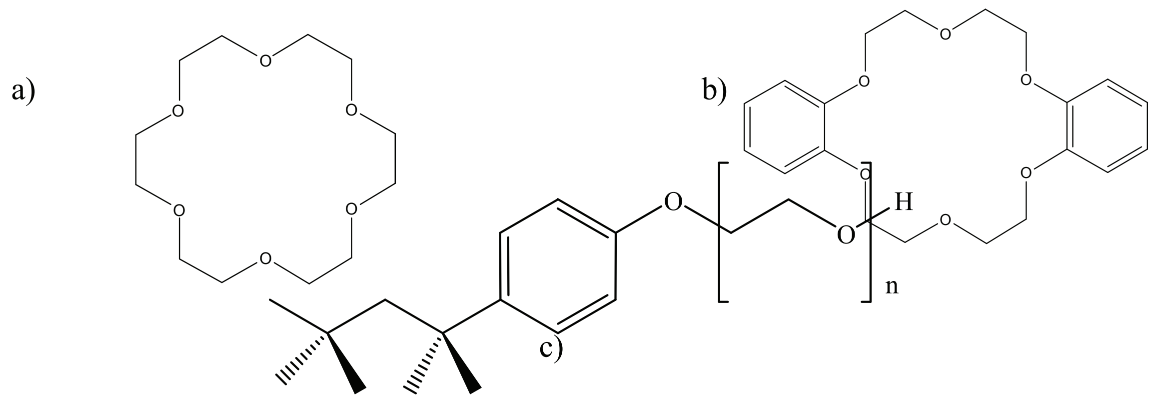

Figure 1. Structures of polyether ligands: macrocyclic "crown ethers" (a,b) and open-chained surfactant molecule (c)

Understanding of the metal-ligand interactions inside formed complexes is the basis for implementation of these systems in many separation techniques (extraction, chromatography, electrophoresis), as well as in determination methods (spectrometry, conductometry, polarography, voltametry).[4] An interesting application of these systems has already been demonstrated in transport experiments for selective removal of toxic metal ions from natural resources.[5] One of the most efficient type of transport is based on the implementation of the "bulk liquid membrane" (BLM) system, which is relatively simple and includes a combination of three processes: extraction, diffusion and re-extraction of analyte.[6] Theoretical models for this type of transport were formulated already in 1973 by Reusch and Cussler[7] for the simple BLM systems, but they can also be used for an explanation in more complicated systems. Since the macrocyclic ligands are the "carriers" of metal ions during transport experiments, they need to have adequate values of complex formation constants, proper solubility in the membrane matrix, and also required lipophilicity in order to prevent "leakage" outside the membrane in a solution of the analyte. These facts indicate the importance of the choice of ligands used in these systems. Since the transport occurs between two aqueous phases through the organic phase, the driving force is a gradient of metal ion concentration.[8] In this paper, the effects of different ligands, macrocyclic and open-chained, are presented as metal ion transport efficiency, expressed through the metal ion concentration in the aqueous phases of system, and measured with an atomic absorption spectrometric (AAS) technique.

EXPERIMENTAL PART

Chemicals

- Standard Cd(II) solution (1000 mg/L), ($Cd(NO_3)_2$ in 0,5M HNO_3), Merck
- Standard Pb(II) solution (1000 mg/L), ($Pb(NO_3)_2$ in 0,5M HNO_3), Merck
- $C_{12}H_{24}O_6$ (18-crown-6); 99%, ACROS OGANICS, (18C6)
- $C_{20}H_{24}O_6$ (dibenzo-18-crown-6); >99%, TCI, (DB18C6)
- $C_6H_3N_3O_7$ (picric acid)
- Nonionic surfactant: $C_{14}H_{22}O(C_2H_4O)_{9,5}$ Triton X-100, p.a. Sigma-Aldrich
- Organic solvents:
  CH_2Cl_2, dichloromethane; p.a. Kemika (DCM)
  $C_2H_4Cl_2$, 1,2-dichloroethane; p.a., Kemika (1,2-DCE)

- CH_3COOH, acetic acid; purris. p.a., Fluka
- $NaOH$, sodium hydroxide; g.r., Merck
- $Na_2S_2O_3$, sodium thiosulphate; purrum.p.a. Sigma-Aldrich

Transport procedure

The procedure involved a cylindric glass cell – the "transport cell" (Fig.2.), with an inner diameter of 5 cm, which in its centre contained a glass tube (inner diameter 2cm). The central tube provided separation between two aqueous phases. Source phase, SP (inside the tube) consisted of a 10mL buffer solution (CH_3COOH/CH_3COONa) at pH = 5, and contained a mixture of the metal ions ($1\cdot10^{-3}$M) and counter ion, picrate ($1\cdot10^{-3}$M). Receiving phase, RP (outside the tube) consisted of a 30 mL solution of suitable stripping agent, thiosulphate ($1\cdot10^{-1}$M). The membrane phase (MP) contained 50 mL of suitable ligand ($1\cdot10^{-3}$M) dissolved in nonpolar solvent; it is the layer that lies beneath the aqueous phases and that connects them. The membrane phase was magnetically stirred by a Teflon-coated magnetic bar at a rate of 20 rpm. Under these conditions contact surfaces between the membrane and two aqueous phases remained flat and well defined.

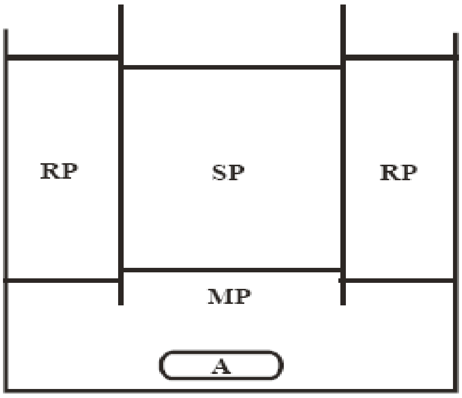

Figure 2. "Transport cell" used for the experiment: SP-source phase; RP-receiving phase; MP-membrane phase; A-magnetic stirrer

Apparatus and measurements

Measurements of metal ions concentration were performed with Atomic Absorption Spectrometer Perkin Elmer 3110, at wavelengths for Pb^{2+} and Cd^{2+}: 261,40 nm and 326,10 nm, respectively. Both aqueous phases were measured along with a series of standard solutions which were prepared similarly, in order to convert the atomic absorption signals to concentration units. Measuremets were carried out during transport procedure: every hour in the first 4 hours of transport, and also after 24 hours.

RESULTS AND DISCUSSION

In previous investigations, authors[9,10] assumed that the most influential process during transport experiments was the metal ions release from the complex in the membrane phase to the receiving aqueous phase through the contact surface between the two phases. Based on this assumption, authors[10] also proposed the possible mechanism for metal ion transport (Fig.3). In order to better understood every step involved in the transport process, numerous experiments were carried out in presented work; different compositions of the aqueous phases and also the membrane phase were included. The obtained results showed that under described experimental conditions a higher efficiency of Cd^{2+} and Pb^{2+} ion transport was achieved with 18C6 as a carrier in the membrane phase, comparing to DB18C6 (Fig. 4). It was assumed that the presence of two substituents in the DB18C6 molecule reduced the flexibility of this ligand and therefore the complex formation with metal ions decreased. Consequently, metal ions uptake from the source phase and also their releasing into the receiving phase is lower, which was also assumed by other authors in similar investigations.[11]

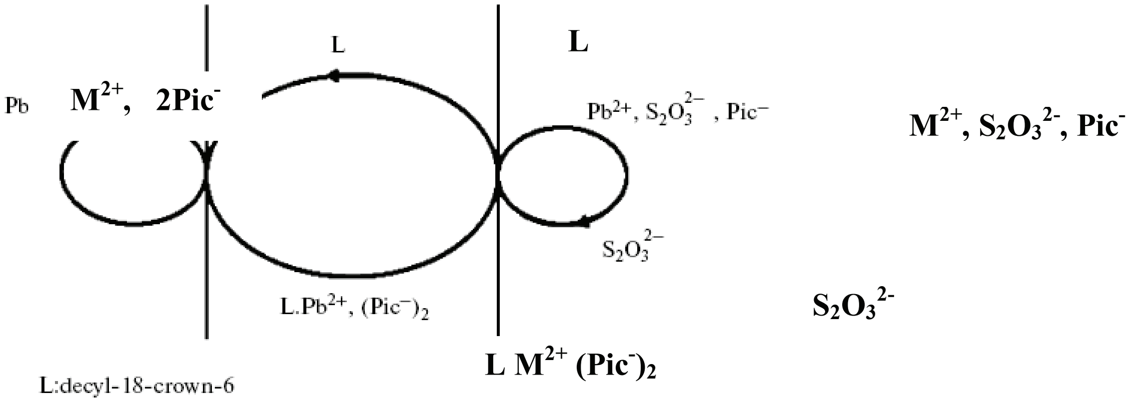

Figure 3. Proposed mechanism of metal ion transport through organic liquid membrane containing dissolved ligand (L), from source aqueous phase (which contains: metal cations, M^{2+} and counter ions picrate, Pic$^-$) to receiving aqueous phase (contains stripping agent: thiosulphate ions)

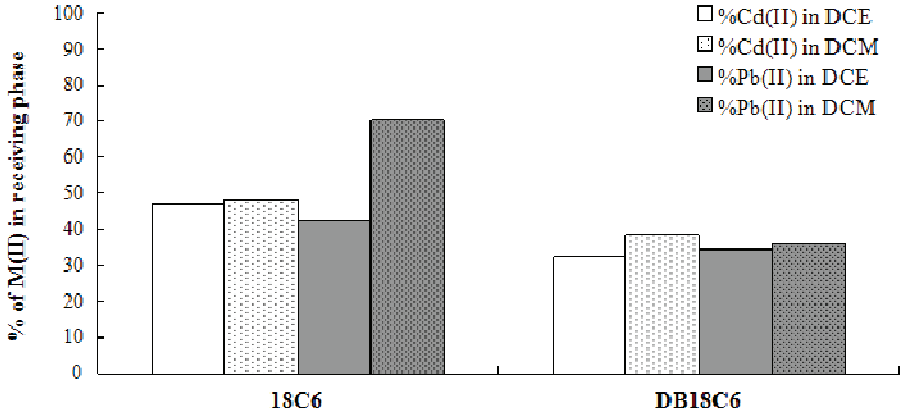

Figure 4. Comparison of transport efficiency using different "carriers" in liquid organic membrane (source phase: $1 \cdot 10^{-3}$M Pic$^-$ + $1 \cdot 10^{-3}$M Pb^{2+} or Cd^{2+}; receiving phase: 0,10 M $S_2O_3^{2-}$; membrane phase: $1 \cdot 10^{-3}$M crown ether)

The effect of acyclic ligand was examined through the experiment where nonionic surfactant Triton X-100 was used as ligand in the membrane. Measurements were carried out every hour during the first 4 hours of transport. The results showed that there was no obvious transport of metal ions into the receiving phase (Fig. 5). Based on decreasing concentration of cations in the source phase, it was clear that metal ions uptake from the source phase occurs, but not their releasing into the receiving phase.

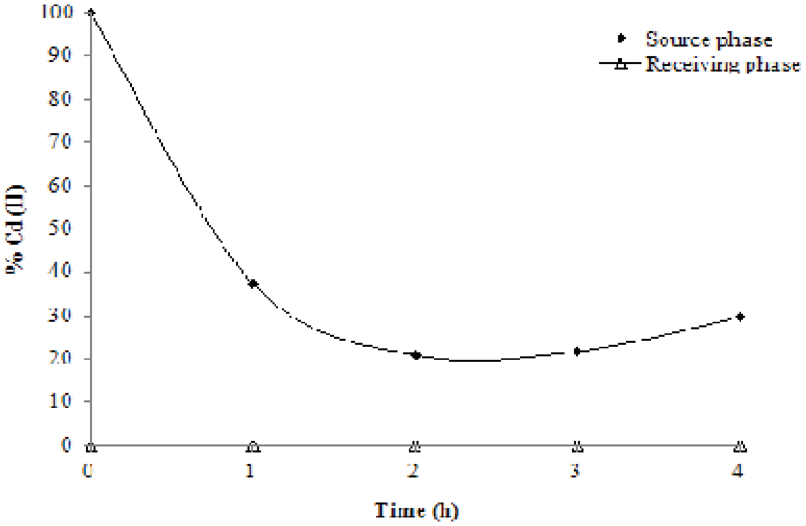

Figure 5. Efficiency of Cd^{2+} *ions transport through liquid organic membrane, during first 4 hours of the experiment using DCM as membrane solvent (source phase:* $1 \cdot 10^{-3}$ *M* Pic^{-} + $1 \cdot 10^{-3}$ *M* Cd^{2+}*; receiving phase: 0,10 M* $S_2O_3^{2-}$ *; membrane phase:* $1 \cdot 10^{-3}$ *M TX-100)*

This assumes possible interactions between metal ions and nonionic surfactants in membrane phase, but those interactions obviously do not provide the required equilibrium between complexation and decomplexation which is crucial of transport processes. Here we concluded that formed aggregates of surfactant molecules in the membrane phase probably “incorporate” metal ions in their structure, preventing their release into the receiving phase. These results are in accordance with investigations[12] of interactions between Cd^{2+} ions and nonionic surfactant HEGMDE (hexaethylene glycole monodecyl ether), using the EMS method (Electrospray Mass Spectrometry). Authors proposed the possible structure (Fig. 6.) which implies that a HEGMDE molecule is similar to the crown ether and provides interactions between six oxygen atoms in the polyether chain and Cd^{2+} ions which results in coiled conformation.[12]

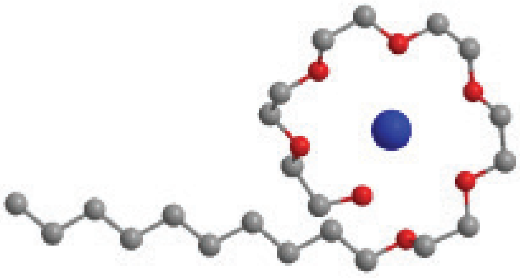

Figure 6. Proposed structure of the compound that resulted from interaction between Cd^{2+} *ions and HEGMDE molecule*[12]

When the membrane contains both ligands: nonionic surfactant and crown ether, transport efficiency decreases compared to systems that only contain crown ether (Fig.7). The assumed incorporation of the formed complex in micellar structure of surfactant in the membrane is also indicated in the presented work.

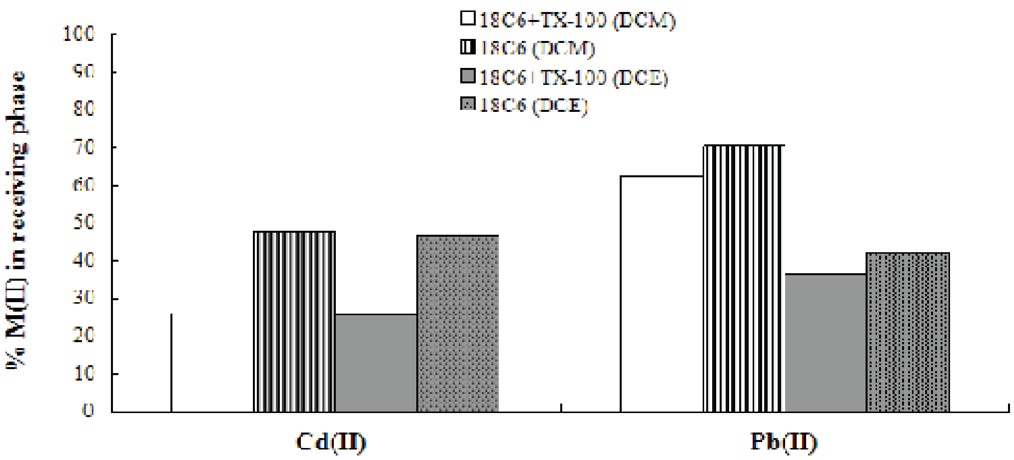

Figure 7. Influence of nonionic surfactant TX-100 added in membrane phase on transport efficiency (source phase: 1 ·10^{-3}M Pic^{-} + 1·10^{-3}M Pb^{2+} or Cd^{2+}(II); receiving phase: 0,10 M $S_2O_3^{2-}$; membrane phase: 1·10^{-3}M 18C6 ± 1·10^{-3}M TX-100)

CONCLUSIONS

- Macrocyclic ligands crown ethers are more suitable “host” molecules for complexation of Pb^{2+} and Cd^{2+} ions compared to nonionic surfactants (with open chains) due to the "macrocyclic effect" which provides higher stability of formed complexes
- Nonionic surfactant TX-100 is not appropriate ligand: the transport of metal ions through the liquid membrane is not possible because the required interactions (complexation/decomplexation) do not occur in the absence of macrocyclic ligands
- Presence of nonionic surfactant TX-100 with crown ether in the membrane decreases transport efficiency due to incorporation of formed complexes into the micellar aggregates which prevents decomplexation and metal ions release from the membrane into the receiving phase
- 18-crown-6 is more suitable for complexation of Pb^{2+} and Cd^{2+} ions compared to dibenzo-18-crown-6 due to its greater flexibility: two benzo substituents in the DB18C6 molecule make the structure more rigid and reduce the possibility of access for metal ions

LIST OF SIGNS AND ABBREVIATIONS

18C6 – 18-crown-6
AAS – Atomic Absorption Spectrometry
BLM – Bulk Liquid Membrane
DB18C6 – Dibenzo-18-crown-6
DCE – Dichloroethane
DCM – Dichloromethane
M^{2+} - Metal cation
MP - Membrane Phase
Pic^{-} - Picrate ion
RP - Receiving Phase
SP - Source Phase

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PREDICTING THE BALLISTIC STRENGTH OF ARAMID FIBER COMPOSITES BY IMPLEMENTING FULL FACTORIAL EXPERIMENTAL DESIGN

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

The purpose of the study is to predict the ballistic strength of hard aramid fiber/phenolic ballistic composites by implementing the full factorial experimental design. When designing ballistic composites two major factors are the most important: the ballistic strength and the weight of the protection. The ultimate target is to achieve the required ballistic strength with the lowest possible weight of protection. The hard ballistic aramid/phenolic composites were made by open mold high pressure, high-temperature compression of prepreg made of plain woven aramid fibre fabric and polyvinyl butyral modified phenolic resin. The preparation of the composites was done by applying the 2^2 full factorial experimental design. The areal weight of the composites was taken to be the first factor and the second – fibre/resin ratio. The first factor low and high levels were chosen to be 2 kg/m^2 and 9 kg/m^2, respectively and for the second factor – 80/20 and 50/50, respectively. The first-order linear model to approximate the response i.e. the ballistic strength of the composites within the study domain (2 – 9) kg/m^2 x (80/20 – 50/50) ratio was used. The influence of each individual factor on the response function was established, as well as the interaction of the two factors. It was found out that the estimated first-degree regression equation with interaction gives a very good approximation of the experimental results of the ballistic strength of composites within the study domain.

Key words: aramid fibre, ballistic composites, factorial design, regression equation, V5

INTRODUCTION

Since the beginning of armed conflict, armour has played a significant role in the protection of warriors. In present-day conflicts, armour has unarguably saved countless lives. Over the course of history—and especially in modern times—the introduction of new materials and improvements in the materials already used to construct armour have led to better protection and a reduction in the weight of armour. Body armour, for example, has progressed from the leather skins of antiquity through the flak jackets of World War II to today’s highly sophisticated designs that exploit ceramic plates and polymeric fibres to protect a person against direct strikes from armour-piercing projectiles and fragments of explosive devices. The advances in vehicle armour capabilities have similarly been driven by new materials.

The ever increasing needs for safety and security are driving the demand for armour solutions capable of countering present and future threats. But optimal protection needs to be achieved without compromising practical constraints such as weight and cost reductions. One of the “new” materials which is widely used in the last three decades is an aramid fibre.

The aramid fibre is a crystalline molecule that consists of long molecular chains that are highly oriented and show strong intermolecular chain bonding in the para position, as shown in Figure 1.

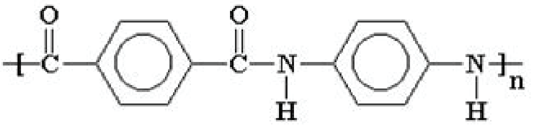

Figure 1. Chemical structure of para-aramid

It is made from the reaction of para-phenylenediamine (PPD) and molten terephthaloyl chloride.

The resounding characteristic of the aramid fibre is its remarkable strength. This very strong fibre has made its biggest impact in the ballistics defence where it is used in bulletproof vests and helmets. It is stronger than fibreglass and five times stronger than steel on a kilogram-for-kilogram comparison. Aramid fibre molecules are ordered in long parallel chains, and the key structural feat is the benzene aromatic ring[1,2] that has a radial orientation which gives the molecule a symmetric and highly ordered structure that forms rod-like structures with a simple repeating backbone. This creates an extremely strong structure with little weak points and flaws. It is one of the strongest man-made fibres. Its high elongation at break, high modulus and high strength make it the ideal reinforcement solution for reducing weight and for combating increasing threats[3,4].

Very high strength of aramid fibres is an essential factor in the energy absorbing mechanism needed to defeat dynamic ballistic impact or to mitigate blast. This makes aramid fibres the material of choice for:

- Ballistic vests and helmets
- Blast panels that protect against land mines
- Engineered ballistics panels (either stand-alone or as part of a combined solution)
- Spall liners

Because of their high strength/weight ratio aramid fibres are widely used for personal ballistic vests or as reinforcements for composites for personal protection[5, 6, 7].

By combining fibres with an appropriate resin matrix system – typically phenolic – essential mechanical and physical properties can be engineered into the composite.

EXPERIMENTAL PROCEDURE

Experimental composite plates were made by impregnation of aramid fibre fabric with thermosetting phenolic resin modified with polyvinyl butyral. Intrinsically brittle phenolic resin is modified for flexibility which better contributes to the kinetic energy absorption of the high-speed bullet and fragment impact and its dispersion in adjacent layers. As reinforcement plain woven aramid fibre fabric was used with the areal weight of 435 g/m^2, finished with a phenolic resin compatible coupling agent. The composites i.e. laminates were produced by open-mold compression at high pressure and a temperature of 155 ^{o}C within 150 minutes for fully curing i.e. cross-linking of the resin. No post-curing treatment was done.

During the impregnation several factors were observed (speed of impregnation, resin viscosity, metering rolls gap in the impregnating machine) so that the required resin pick-up and its content in the prepreg were achieved.

The areal weight of the composites was adjusted simply by adding more prepreg layers in the press packet from the lowest to the highest area weight in accordance to the experimental design.

In the 2^2 full factorial experimental design (FFED) the areal weight of the composite was taken to be the first factor and the second factor was taken to be the fibre/resin ratio. For the first factor the low and the high levels are 2 kg/m^2 and 9 kg/m^2, respectively, and for the second factor – 80/20 and 50/50, respectively. Within this relatively narrow areal weight region, which is of importance only for panels for personal ballistic protection, linear dependence of ballistic strength vs. areal weight was assumed. That is why the first-degree model with interactions was used to predict the response i.e. the ballistic strength of the composites within the study domain (2 – 9) kg/m^2 x (80/20 – 50/50) fibre/resin ratio.

The full factorial experimental design allows making a mathematical modelling of the investigated process in the study domain in the vicinity of a chosen experimental point[8,9,10]. To cover the whole study domain, for the areal weight of the composites the experimental point 5,5 ± 3,5 kg/m^2 was chosen, and for the resin content , the experimental point 35 ± 15 % (which corresponds to previously defined levels for fibre/resin ratios).

All tests were done with a standard 1.1g chisel-nosed fragment simulating projectile which is non-deformable, made of quenched and tempered steel with a flat rectangular tip. The ballistic limit velocities, V50, were calculated in accordance to the STANAG 2920 calculation method. V50 value presents 50% probability of penetration i.e. of non-penetration and is a statistical method developed by US military. In accordance to the FFED procedure 4 (2^2) trails are needed, i.e. all possible combinations of the variables are tested.

Table 1. Coding convention of the variables

	Areal weight, kg/m^2	**Resin content, %**
Zero level, $x_i=0$	5.5	35
Interval of variation	3.5	15
High level, $x_i=+1$	9	50
Low level, $x_i=-1$	2	20
Code	x_1	x_2

The coding of the variables was done in accordance to Table I.

RESULTS AND DISCUSSION

The test results are presented in Table II together with the experimental matrix

Table 2. Experimental matrix with results

Trials	x_1	x_2	x_1x_2	Aramid composite
				V_{50}, (m/s)
1	-1	-1	+1	238.9
2	1	-1	-1	557.0
3	-1	1	-1	217.4
4	1	1	+1	504.4
-1 Level	2 kg/m^2	20 %	-	-
+1 Level	9 kg/m^2	50 %	-	-

By implementing the 2^2 full factorial experimental design it was found out that response function with coded variables, y_k, was:

$$\boldsymbol{y}_k = 379,43 + 151,26\boldsymbol{x}_1 - 18,53\boldsymbol{x}_2 - \\ -7,78\boldsymbol{x}_1\boldsymbol{x}_2 \tag{1}$$

and in engineering variables, y_n:

$$y_n = 156,42 + 48,40x_1 - 0,42x_2 - \\ -0,15x_1x_2 \tag{2}$$

In the FFED the term x_1x_2 is the interaction between factors which also might have influence on the response, in our case *V50* value.

Analyzing the regression equation it can be found out that the main positive contribution to the *V50* is given by the areal weight of the composites i.e. *V50* is directly proportional to the areal weight of the composites. On the other hand, the resin content of the composite has an inversely proportional effect on ballistic strength which means, the higher the resin content, the lower the ballistic strength. The interaction of the two factors, with a coefficient of -0.15, has a slightly negative effect on the ballistic strength which is of secondary order compared to the influence of areal weight and resin content.

To validate the equation, theoretically calculated results are compared with experimental values for composites with areal weight of 2, 3, 4, 5, 6, 7, 8 and 9 kg/m^2 and constant resin content of 35%.

The results are presented in Figure 2.

This comparison can be done with any other value for the resin content as long as it is within the study domain.

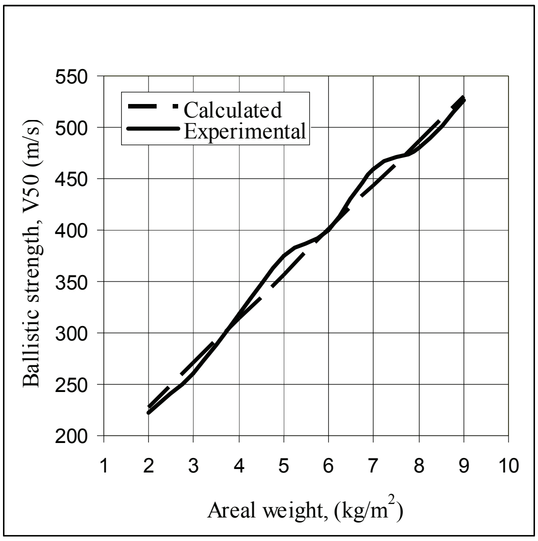

Figure 2. Ballistic strength vs. areal weight of composites

As it can be seen from Figure 2 there is a very good match between calculated and the experimental values. All calculated values are placed in a straight line which is in accordance with the assumed model of the experiment and are in close proximity of the experimental data.

How can the regression equation (2) be used?

a) For a given request for the ballistic strength, by substitution of y_n the areal weight of the composites can be calculated.

b) For a given weight limit (x_1 factor) y_n can be calculated.

In both above cases the resin content (x_2 factor) has to be 20% for the most favourable outcome.

CONCLUSION

Although, for the wide range, the ballistic strength is not a linear function of the areal weight of the composites[11,12,13], if the study domain is precisely established (narrow enough), the 2^2 full factorial experimental design can be applied, to give a good approximation of the experimental data.

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IDENTIFICATION OF BIOACTIVE COMPOUNDS IN AQUEOUS EXTRACTS OF CULTIVATED STRAWBERRIES' LEAVES (*Fragariae folium*) USING UHPLC-MS METHOD

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

In this study two types of dry leaves of cultivated strawberries (zenga and colonnettes strawberries) were used. Aqueous extracts were obtained by maceration in hot water at room temperature for 45 minutes. Higher content of total extractive matter has been obtained from the leaves of zenga species. The following compounds have been identified from the extract of colonnettes strawberry leaves using the HPLC–DAD–HESI–MS analysis: rutin, coumaroyl-galloyl-glucopyranoside, hydroxyphenylethyl-coumaroyl-glucopyranoside, a derivate of hydroxyphenylethyl-coumaroyl-glucopyranoside, chlorogenic acid and *p*-coumaroylhexose. In the extract of zenga type, beside the mentioned compounds, it has been identified and pelargonidin-3-diglucoside. The presence of rutin and chlorogenic acid is important, because they posses antioxidant and anticancer properties, and their content was determined in extracts. The content of both compounds was higher in the extract of zenga species (3 g/100g of dry extract for chlorogenic acid, and 2.61 g/100 g of dry extract for rutin). The presence of phenolic compounds in the extracts suggests their possible application as natural antioxidants.

Keywords: strawberries, extraction, bioactive compounds, UHPLC-MS method

INTRODUCTION

MATERIALS AND METHODS

Reagents

Acetonitrile and water were purchased from Fisher Chemical (LC-MS and HPLC grade, respectively). Rutin and chlorogenic acid standards were purchased from Sigma-Aldrich (Germany). Formic acid was purchased from Carlo Erba (France).

Plant material

Strawberries (*Fragaria ananasa* L.) were grown during 2012-2013 in a plastic tunnel (2.5 m high), covered with a polyethylene film (0.15 mm thick; Ginegar Plastic Products-Ltd.). Zenga (Zenga zengana) and colonnettes (Mont Everest) strawberries were cultivated in an experimental garden located in the village Badince near Leskovac, in the Southeast of Serbia.

The strawberries leaves were harvested in May 2013. The plant material was dried in the shady, cool place and stored in paper bags at room temperature.

Extraction

The measured quantity (2.0 g) of chopped plant material was extracted by maceration with boiling water (100 ml). Maceration was performed for 45 minutes at room temperature. The extract was separated by filtering under a weak vacuum. The content of the extractive matter (dry extract) was determined on a SCALTEC SMO 01 apparatus (Scaltec Instruments, Germany) at 105°C. The yield of the extractive matter was calculated on the basis of the dry residue content.

Determination of plant extract yield

The yield of evaporated dried extracts based on dry weight basis was calculated from the following equation 1:

$$\textit{Yield (g/100 g of plant material)} = (W_1 \times 100) / W_2 \quad (1)$$

where W_1 is the weight of the extract after the solvent evaporation and W_2 is the weight of the plant material.

LCQ Orbitrap mass analysis

All experiments were performed by using a Thermo Scientific liquid chromatography system (UHPLC) connected to LCQ Fleet Ion Trap Mass Spectrometer (Thermo Fisher Scientific, San Jose, California, USA) with heated electro spray ionization (HESI). Xcalibur (version 2.2 SP1.48) and LCQ (version 2.7.0.1103 SP1) software were used for an instrument control, data acquisition and data analysis. Separations were performed on a Hypersil gold C18 (50 × 2.1 mm, 1.9 µm) from Thermo Fisher Scientific.

The mobile phase consisted of (A) water + 0.1% formic acid and (B) acetonitrile + 0.1% formic acid. A linear gradient program at flow rate of 0.350 mL/min was used 0–2 min from 10 to 20% (B), 2–4.5 min from 20 to 90% (B), 4.5–4.8 min 90% (B), 4.8–4.9 min from 90 to 10% (B), 4.9–12.0 min 10% (B). The injection volume was 8 µL and temperature column of 25 ºC. Spectral data from all peaks were recorded at 230, 280, 360 and 510 nm. The mass spectrometer was operated in negative mode. The HESI-source parameters were as follows: source voltage 4.5 kV, capillary voltage –31.00 V, tube lens voltage –85.40 V, capillary temperature 300 ºC, sheath and auxiliary gas flow (N_2) 32 and 12 (arbitrary units). MS spectra were acquired by full range acquisition covering 150–700 m/z. For fragmentation study, a data dependant scan was performed by deploying the collision-induced dissociation (CID). The normalized collision energy of the collision-induced dissociation (CID) cell was set at 30 eV.

RESULTS AND DISCUSSION

In previous studies several solvents were used for extraction of compounds from foods including water, methanol, aqueous acetone, ethanol and ethyl acetate[12,13]. In this investigation we wanted to compare the composition of two types of strawberries species and in extraction of both species water was used as solvent. The yields of the total extractive matter (dry extract) obtained from the zenga and colonnettes strawberries per 100 g were 28.55 and 25.87 g, respectively. As it could be seen the higher content of total extractive matter has been obtained from the leaves of zenga strawberry (9.4% higher in compared to colonnettes strawberry). If we take into account that both species were cultivated under identical conditions, the difference in yields is a consequence of using different cultivated species of strawberries.

LCQ Orbitrap mass analysis of strawberries extracts

Based on previous studies it is known that berry fruits contain a wide variety of phenolics including hydroxybenzoic and hydroxycinnamic acid derivatives, anthocyanins, flavonols, flavanols, condensed tannins (proanthocyanidins) and hydrolyzable tannins[12]. In order to identify the composition of two strawberries extracts (zenga and colonnettes strawberries) the HPLC–DAD–HESI–MS methods were used. The compounds were identified based on retention times, UV-VIS absorption spectrum and mass spectra by matching their molecular ions obtained by LC–HESI–MS and LC–MS/MS methods with the theoretical molecular weights from literature data. In addition, rutin and chlorogenic acid were confirmed by standard reference.

Figures 1 and **2** show the HPLC–DAD chromatograms of the zenga strawberry and colonnettes strawberry extracts at 280 nm, respectively. The identified compounds from the both strawberries extracts, labelled as peaks 1–8, following the elution orders in the HPLC–DAD chromatograms, are shown in **Tables 1** and **2**. Based on **Figures 1** and **2,** it can be concluded that in both strawberries extracts similar compounds were identified. The first peak was identified as rutin, quercetin-3-*O*-rutinoside and it was identified in both extracts at retention times 4.23 and 4.20 min (**Table 1** and **2**). MS/MS fragmentation of the $[M–H]^-$ ion at 609 *m/z* shown a fragment ion at 254 *m/z* which represents a deprotonated molecule of the glycoside and ion at 301 *m/z* that corresponds to the deprotonated aglycone $[M–H]^-$. The presence of rutin in the strawberry was previously reported by Seeram and collaborators (2006) and it was expected. The second peak (t_R = 4.72, **Figure 1**) had the $[M–H]^-$ ion at 477.10 *m/z*, and it is identified as coumaroyl-galloyl-glucopyranoside. Based on its fragments on 163 and 145 *m/z* which are characteristic for coumaric acid (**Table 1**), it can be concluded that this compound contains a gallic acid moiety together with coumaroyl glycoside. The loss of 163 *m/z* in MS/MS fragmentation indicates the release of hexose from the compound, which could indicate that the two phenolic acid moieties are directly linked to each other. The fragmentation pathway of this compound detected in strawberries was also described in a previous study by

Hanhineva and collaborators (2009)[14]. The presence of coumaroyl-galloyl-glucopyranoside is also detected and in the second extract at t_R = 4.71 min (**Figure 2**). The results from MS/MS fragmentation of peaks which are labelled as **3**, **3*a*** and **3*b*** (**Table 1** and **2**), indicate that isomers of 2-(4-hydroxyphenyl)ethyl-coumaroylglucopyranoside are present in both extracts. The MS/MS analysis showed the characteristic fragmentation of coumaroyl hexose for all three compounds. The ion at 307 *m/z* represents a coumaroyl hexose moiety with the loss of water and phenylpropanoid residue. In addition, the *m/z* signals of 163 and 145 correspond to the coumaroyl moiety after breakage of the ester bond with sugar, and after an additional loss of a water molecule. Pelargonidin-3-diglucoside detected only in the first extract and it is labelled as peak **5**. It had the $[M–H]^-$ ion at 595 *m/z* and fragment ions at 433 and 271 *m/z*. The obtained ion at 271 *m/z* due to the loss of 324 amu corresponds to two glucose moieties. The fact that the ion at 271 *m/z* is the most abundant one in MS/MS fragmentation and indicates that the two glucose moieties are linked to each other i.e. it is a diglucoside derivative of pelargonidin. Glycosides of pelargonidin belong to a group of anthocyanins and they are responsible for some the favorable properties of strawberries (Seeram et al., 2006). Chlorogenic acid is identified as peak **6** in both extracts (t_R = 7.25 and 7.28 min) with the $[M–H]^-$ ion at 353 *m/z.* The MS/MS spectrum of compounds in both extracts showed the fragment ion at 191 *m/z* as the base peak and the intense ion at 179 *m/z*. Peaks **7** (t_R = 6.81 and 7.51 min) with the $[M–H]^-$ ion at 325 *m/z* was identified as *p*-coumaroylhexose in both extracts. MS/MS fragmentation of the $[M–H]^-$ ion resulting ions at 265, 187, 163 and 145 *m/z* which indicate the loss of hexose. The presence of *p*-coumaroylhexose in the strawberry has also been reported by Kajdžanoska, Gjamovski and Stefova (2010)[15]. Peaks **4** and **8** in the first extract (t_R = 6.81 and 7.51 min), as well as peaks **4**, **4*a*** and **5** (t_R = 6.52, 7.65 and 6.85 min) in the second extract are identified as derivates of hydroxyphenylethyl-coumaroyl-glucopyranosides. This is suggested based on their MS/MS fragmentation and the matching with fragment ions at 163 and 145 *m/z* which are also present in MS/MS fragmentation of hydroxyphenylethyl-coumaroyl-glucopyranoside.

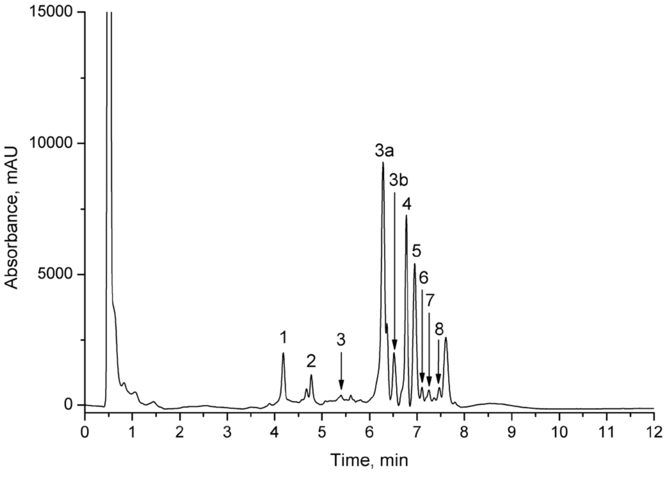

Figure 1. UHPLC chromatogram of extract from zenga strawberry leaves detected at 280 nm

Table 1. Identified products of zenga strawberry extract

Peak, no.	**t_R, min**	**m/z [M-H]$^-$**	**MS/MS**	**Compound**
1	4.23	609.5	301.20, 254.96	Rutin
2	4.72	477.2	417.11, 331.08, 163.04, 145.04	Coumaroyl-galloyl-glucopyranoside
3, 3a, 3b	5.45, 6.35, 6.61	445.3	307.06, 247.06, 163.01, 145.02	Hydroxyphenylethyl-coumaroyl-glucopyranoside
4	6.81	265.01	235.1, 163.03	derivate of Hydroxyphenylethyl-coumaroyl-glucopyranoside
5	6.87	595.3	433.01, 271.4	Pelargonidin-3-diglucoside
6	7.25	353.2	191.3, 179.2	Chlorogenic acid
7	7.34	325.3	265.03, 187.02, 163.05, 145.01	*p*-coumaroylhexose
8	7.51	293.3	265.07, 163.03	derivate of Hydroxyphenylethyl-coumaroyl-glucopyranoside

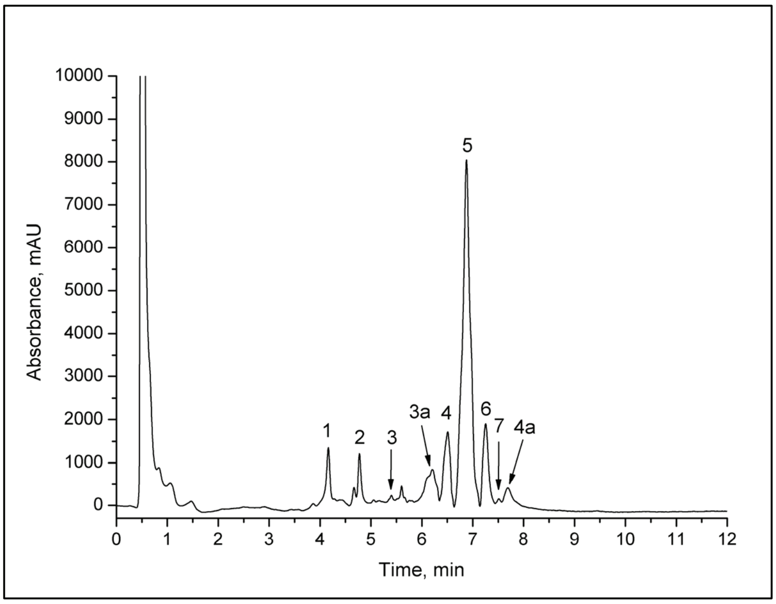

Figure 2. UHPLC chromatogram of extract from colonnettes strawberry leaves detected at 280 nm

Table 2. Identified products of colonnettes strawberry extract

Peak, no.	**t_R, min**	**m/z [M-H]$^-$**	**MS/MS**	**Compound**
1	4.20	609.5	301.20, 254.96	Rutin
2	4.71	477.2	417.11, 331.08, 163.04, 145.04	Coumaroyl-galloyl-glucopyranoside
3, 3a	5.45, 6.35	445.3	307.06, 265.07, 247.06	Hydroxyphenylethyl-coumaroyl-glucopyranoside
4, 4a	6.52, 7.65	293.3	265.07, 235.12, 163.01	derivate of Hydroxyphenylethyl-coumaroyl-glucopyranoside
5	6.85	265.01	235.1, 163.03	derivate of Hydroxyphenylethyl-coumaroyl-glucopyranoside
6	7.28	353.2	191.3, 179.2	Chlorogenic acid
7	7.49	325.3	265.03, 187.02, 163.05, 145.01	*p*-coumaroylhexose

Content of compounds with biological activity

It is known that chlorogenic acid and rutin belong to a polyphenol class, which represent bioactive compounds that have antioxidant, anticancerogenic, antimicrobial and anti-inflammatory properties[7,16,17]. Based on this we determined the content of these two components.

Table 3. The yields of the total extractive matter and the content of rutin and chlorogenic acid in both species of strawberries leaves

		Strawberry species	
		Zenga	**colonnettes**
Total extractive matter (g/100 g of plant material)		28.55	25.87
Chlorogenic acid, g/100 g of dry extract		3.0	2.53
Regression equation[1]	R^2		
$y=18.2574x+0.0355$	0.9985		
Rutin, g/100 g of dry extract		2.61	1.73
Regression equation[1]	R^2		
$y=20.7361x+0.0325$	0.9983		

[1] $y = ax+b$; where x is concentration in mg/mL, and y is area under curve at the selected wavelength.

The content of the chlorogenic acid and rutin obtained from two analyzed species of strawberries was determined and represented per 100g of dry extract (**Table 3**). Based on these results it can be concluded that higher content was in zenga species for both determined compounds. These results can be explained by the fact that zenga showed higher yields of total extractive matter, compared with colonnettes species.

CONCLUSIONS

In conclusion, we have identified the major phenolic compounds in both strawberry species by using the HPLC–DAD–ESI–MS methods. Both strawberries species showed similar composition, except pelargonidin-3-diglucoside, which was identified only in zenga strawberries. The yield of the total extractive matter (dry extract), the content of the chlorogenic acid and rutin was higher in zenga strawberries. The results obtained analyzing the mentioned extracts give good basis for future investigations of their biological activity, as well as for a preparation of products based on these extracts.

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APPLICATION OF THE WASTE WATER RUNOFF INDEX FOR THE PART OF SARAJEVO CANTON

PROFESSIONAL PAPER

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ABSTRACT

This paper discusses the importance of establishing a practical, relevant monitoring application index runoff wastewater (hereinafter index BDE). Applied empirical indicator of dependence associated key performance indicators in a number/index useful for comparison in time and space calculated towards the part of the Sarajevo Canton, which belongs to the Bosnia river basin for the period from 2004 to 2013. This indicator can be useful for the annual assessment with the aim of reducing specific water consumption as a long-term strategic commitment to the concept of sustainable water resources management. On the basis of the proposed method is a simple way to give a quantitative assessment of urban sustainability of water supply and sewerage. Presented indicate a negative development indicators tend to fall where for 2004 was 0,16, for the 2013 0,13 index points. The indicators used in the process of integrated water resources management can be more narrowly oriented to the specific goals of water management and used for research and comparison: water quality, spatial and temporal variations and availability of using water, the efficiency of water use efficiency of water delivery etc. Model conceptual framework of the pressure - state - response " PSR ", for its own purposes developed OECD countries with a view to assessing the ecological sustainable development. When using this framework, the selected indicators have to reflect the pressures on the environment, the state of the environment and the response (reaction) society to perceived environmental problems.

Keywords: monitoring, wastewater, runoff index population.

INTRODUCTION

Based on the DPSIR framework is based on the standard typology indicators of the European Environment Agency (EEA) , which is developed the so-called. CORE set of indicators. The main purpose of CORE set of indicators is to provide practical and stable base for reports to the EEA , and improve the quality and geographical coverage of the data flow.

The indicators used in the water sector in Bosnia and Herzegovina were the ones that reflect the qualitative and quantitative characteristics of water and in some cases the efficiency of water use. Problems related to water are handled only engineering solutions (Supply Management). Traditional indicators are measured achievements in different areas - sectors (social, environmental and economy), but independent from each other, without going into the analysis of relationship and interaction between these field on each other. Each sector has been measured by "their" indicators, and on the basis that only brought conclusions about the level of achievement in this area. Multidimensional indicators began to establish and used before nineties Ages. Characteristics this framework stems from the analytical framework - the aggregate indicator as a whole is expressed a number that gives a complete picture of a given issue, where each Component of which he consists reflects the theme for themselves within these issues and thus give the possibility to review the impact of the individual topics in the overall issues reflected in the index.

MATERIAL AND METHODS

The methodology used primarily is determined by the characteristics of the issues engaged , unifying , analyzing and structuring data and the evaluation of the application of indicators, on the other hand provide a logical starting point for further planning and implementation of protection.

A well-known procedure for assessment of sustainability is the Bellagio principles from 1997 (International Institute for Sustainable Development 1997). The Bellagio principles suggest that the assessment of progress toward sustainable development should be based on (1) a clear vision of sustainable development and goals that define that vision, (2) a holistic perspective, (3) essential elements, (4) adequate scope, (5) practical focus, (6) openness, (7) effective communication, (8) broad participation, (9) ongoing assessment, and (10) institutional capacity. A last revision of the principles was made by IISD in 2009 where the importance of acknowledging differences in perspectives and the selection of which societal perspectives (narratives) to base the communication on is more explicitly addressed.

Another well-known procedure for assessment of sustainability is the PICABUE framework (Mitchell, May and McDonald 1995). The acronym PICABUE refers to Principles of sustainability, Issues of concern, Construction of indicators, Augmenting indicators (to represent strong sustainability), addressing Boundary issues (for spatial scale), developing Uncertainty indicators, and Evaluating indicators.

This indicator with respect to protecting the quality of water flow takes into account the amount of spilled wastewater from sewage systems agglomeration into the recipient basin and puts them in relation with the relevant fluxes to the total population, as well as demographic load. The amount of spilled wastewater from sewage systems in the watershed streams in relation to the total population in the catchment area is a measure of the load water flow and is referred to as Qww / P, where Qww amount of spilled waste water in m3/y, and P population:

$$DE = \underline{Qww} \quad (m^3/pop.y) \qquad (1)$$

In addition to this key indicators relevant indicator can be considered as average perennial runoff catchment expressed per capita (IR, IR95). Thus, the availability of (wealth) water resources obtained as the ratio of mean multiannual discharge basin per capita per year and expresses the relationship:

$$IR = \frac{Qr}{P} \quad (m3/pop.y) \qquad (2)$$

where the average monthly Qr (multi-year) discharge in m3/y, and P population. Availability (wealth) of water resources can be expressed as the ratio of the minimum monthly flow medium basin 95% No per capita per year and expressed by the relationship where the average monthly Qr (multi-year) discharge in m3/y, and P population.

$$IR95 = \frac{Qr\ 95}{P} \quad (m3/stan.god.) \qquad (3)$$

where Q95 minimum mean monthly discharge basin 95% No in m3 / y., and P population. For calculating aggregate indicators which key indicators combine interrelationship of population, the amount of spilled wastewater and relevant discharge water recipient, adopted a methodological approach that is based on principal border factor:

$$index = \frac{\text{bearing capacity - Critical load}}{\text{Average available water resources - load capacity}} \qquad (4)$$

Using expressions (1), (2) and (3) the key performance indicators DE, IR IR95%, using the principles of limit factor (4) created the aggregate indicator is defined by the following legality:

$$indexBDE=\frac{\log IR95 - \log DE}{\log IR - \log IR95} \qquad (5)$$

Mathematical discussion of this indicator shows that the index value the more minor (index BDE <1) if the amount of wastewater spilled into the waterway over time increases. That is, the value of the index decreases if the critical load / amount of spilled wastewater (DE, m3 / pop.y) approaching carrying capacity / relevant minimum flows for a recipient (IR95% m3 / pop.y).

RESULTS

Ecological indicators of sustainable development reflect all causal connections. Thus, the primary driving force is population growth that has an impact on the environment (Figure 1). According to the Federal Bureau of Statistics (hereinafter FOS), the population of the Canton grows at an annual average of 0.50%. The highest population concentration is in the municipality of Novi Grad, which has a population of about 29,00 %. Of the total population, 87.90% is urban population, and 12.10% suburban population.

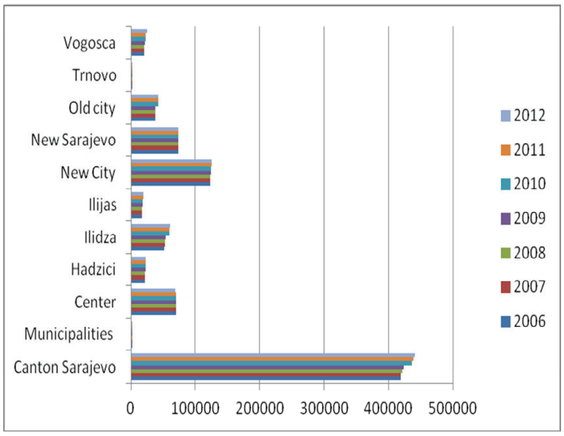

Figure 1. Changes in population

Source: Djurdjevic D. (2004.-2012.) Statistical Yearbook - demographic statistic Sarajevo: FOS ISSN 1512-5106 p. 132 ("featuring the author")

The Millennium Development Goals Indicators currently consist of 48 indicators linked to the eight goals derived from the United Nations Millennium Declaration. Report of the UN Human Development-MRC 2008/2009th; indicators of pressure on space and natural resources is growing at a rate of 0.50% of Canton compared to 50,00% worldwide. Indicator access to safe drinking water to 85,00% of Canton, 97,00% of Bosnia and Herzegovina, 100,00% of the EU. Indicator of improved access to sanitation to 75,00% of Canton, 95,00% of BiH, 100,00% of the EU and Croatia. The current coverage is concentrated in the municipal centers. Definitions of common indicators used in BiH does not give the option of comparing and positioning in relation to other countries .
Estimation of the necessary amount of drinking water is done by expected demographic development and estimated consumption elements (Figure 2).

Source: Federal Water Strategy, 2008; capacity of groundwater Sarajevo fields exhausted the existing abstraction of 82,00%.

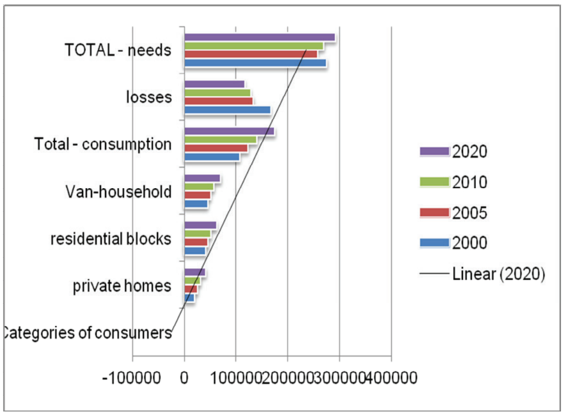

Figure 2. Estimates of the total water demand

Additional quantities of 200-400 l/sec. provided from the intake of river Bosnia. Specific consumption of households 120-300, 303 BiH, 150-200 l/pop/Day EU. Industry 64 Canton Sarajevo, 45 l/pop/day BiH. Planned consumption of 167 l/pop/ Day, and losses of 40,00% indicates a deficit of future water requirements. Connecting local water and putting them under constant control, thus providing a sufficient amount of the general level of security of the population's health. Average specific consumption of drinking water in households and institutions, and in industrial production is 120 and 64 liter/resident/day respectively. Reduced activity as well as the rational use of water for industry has a positive impact on the quality of surface water.

According to the authors Water Poverty Index (WPI) established three categories of water consumption in households are: insufficient amounts of water for the population (below 50 liters / capita / day), excessive water use (over 150l / capita / day), and the category of efficient use of water (50 liters / capita / day / - 150 liters / capita / day).

Falkenmark Water Stress Indicator (FWSI), according to Falkenmark - in, the availability (Figure 3). of water is greater than 1700 m^3 /pop/ Y, is defined as the limit above that the water shortage occurs only occasionally or locally. Affordability of water below this level is considered the lack of water, and depending on the available quantities causes various degrees of restrictions on people and development. In areas where the availability of water above 1000 m3/pop./ Year, it is considered lack of water is such that it represents a constraint on economic development, human health, and standard life, and in case of availability of water below 500 m3/pop/Year, lack of water is considered essential limits for life. Dry Season Flow Index (DSFI), based Falkenmark - these definitions , the river basin is exposed to water stress if there is to available to less than 1 700 m3/pop/Yr, and watersheds that have water with amounts ranging between 1700 m3/pop/Year and 4000 m3/pop/Year are considered to be adequately supplied.

Pressure is a direct result of human activity, the amount of spilled wastewater from sewage systems

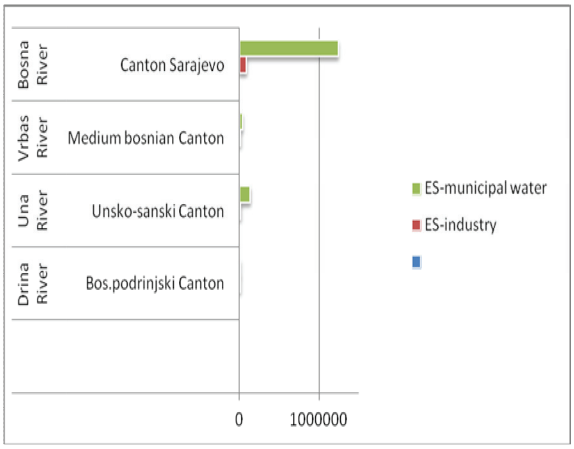

Figure 3. Overview of the total pollution load caused by residents and industries divided into sub-basins
Source: Bijedić A. et al. (2009) Draft: Water Management Strategy FBiH. Sarajevo: 207-225 ("featuring the author ")

The quality of the Bosnia River is seriously affected and the major cause of pollution as a concentrated source of pollution is municipal wastewater discharged by the residents. The effect of industrial pollution expressed as an equivalent number of inhabitants is 86 245. The adverse impact of surface water pollution on human health is potentially multiple which is reflected in an impact on the quality of drinking water resources, water for irrigation, recreation, etc.

State indicators (Figure 4) indicate the current state of the environment, the quality of water recipient as a result of spilled wastewater.

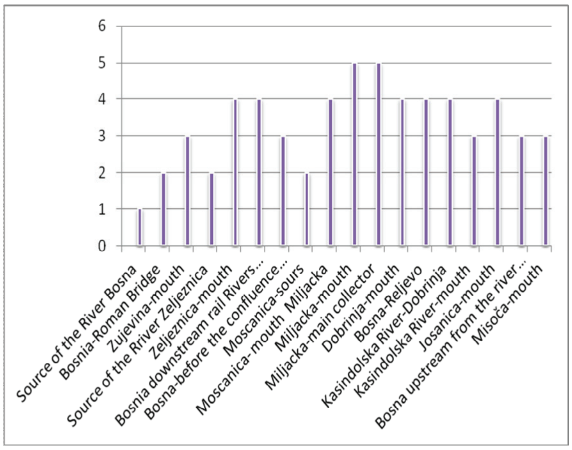

Figure 4. Estimation of the total quality class
Source: sn (2006-2009) Laboratory findings and monthly reports from Public Health Institute of the Sarajevo Canton ("featuring the author")

The MRC report 2009: BiH in relation to its neighbors Croatia, Serbia, Bulgaria and Romania represents an upstream country. Without built systems to protect water quality limits the use of downstream users. Specific pollutants are: nitrogen, phosphorus, fats and detergents. Average measured values mg P/l in the basin Bosnia 0.1 (limits 0.01) BOD 0.16 $O_2/\ m^2$ (limits 0.16). River basin management plans in addition to turning the general public cross-border cooperation. Report EEIA, 2009.; 80,00% of the rivers of Bosnia and Herzegovina in the class IV and V (weak and poor quality), 10,00% of rivers in Class III (moderate quality). The estimated quality degradation requires development of more sophisticated tools for sustainable management. Bearing in mind the EU directive and the United States experience necessary to develop Surveying and assessing the contribution of pollutants , form an integral plan for the preservation of water resources (define maximum permitted load The quality of water evaluation was done using a compulsory group indicators for evaluating the overall ecological function of water such as physic-chemical (temperature, odor, turbidity, conductivity, chlorides, volatile residue, suspended solids, pH, dissolved oxygen, oxygen saturation, biological oxygen demand, chemical oxygen demand, ammonia, nitrates, nitrites, phosphates and microbiological indicators. The analysis determined the following microbiological indicators total coliforms, number of viable germ of water at 37^0C and 22^0C, Fecal coliforms and Fecal streptococci seeds in 100 ml of water. Relevant parameters for assessing the quality of surface waters as "the waters of the laws on the categorization of watercourses" (Official Gazette of BiH No.70/06) Water Act ("Official Gazette of BiH", No. 18/98). The increased values of the group indicators point to changes in the quality of water, i.e. watercourses mostly polluted by organic matter. for each pollutant).

The measurement results of microbiological and physic-chemical indicators of water quality of the river Bosnia in the period from 2004 to 2012, indicate the anthropological impact on the quality of the river Bosnia in Sarajevo field. In Roman bridge noticed poorer quality than the required (II. Classes instead I), the decline is more pronounced going from the mouth of Railways (III. class II instead) Over Zujevina river (III. class II instead) to Miljacka river that collects the majority of municipal wastewater Sarajevo (V class III instead), and the inlet of the river Bosnia degrade its quality in the downstream (V class III instead).

Impact is a result of changes in the quality of the environment, pressures on people, ecosystems and social systems. The quality of water supply can be judged by the epidemiological situation related to diseases whose causes may be present in contaminated water, and these are in first place the intestinal infectious diseases (Figure 5).

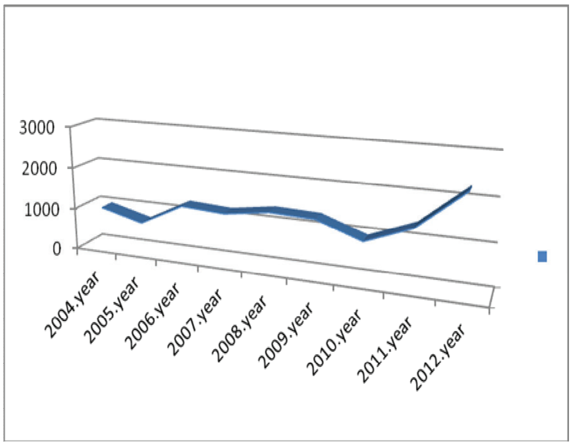

Figure 5. Number of the affected with intestinal infectious diseases per year
Source: Preljević A. Žilić A. (2004-2012) Bulletin on infectious diseases in Sarajevo. Department of Public Health K. S. ("Featuring Aurora")

A total of 13,424 patients with intestinal infectious diseases were registered at the Canton of Sarajevo from 2004 to 2012. Acute enterocolitis is the most common disease. Most patients were in the age group of 25-49 years i.e. the active population, which can be related with nutrition in public buildings.

Indicator "mortality of children under 5 years old," which is an indicator of health, depending on the degree of access to clean water, people, is not representative and relevant to the Sarajevo Canton, considering that the unavailability water or poor water quality is not the cause of child mortality.

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Degradation of water quality of the Bosnia River is present immediately after the Bosnia source area. Although wastewater is collected through a network of city sewers, it flows into the Bosnia River without prior treatment. In addition to the amount of spilled wastewater into the recipients, the index methodology included the average flow and minimum mean monthly flow of 95,00%. The index for the period 2004-2012 was calculated using appropriate values of the estimated population, amount of discharged wastewater and relevant flow in the river basin (Figure 6).

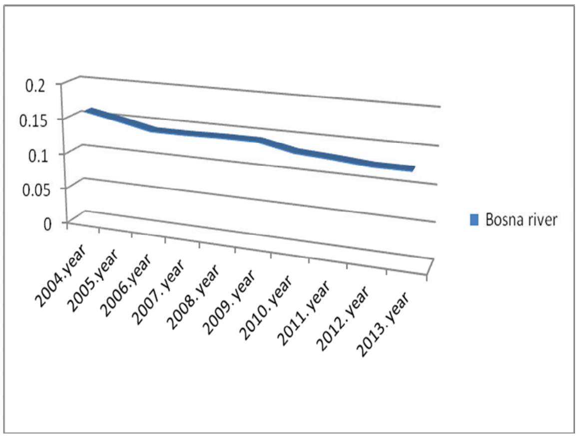

Figure 6. Wastewater runoff index

Over the period of eight years (2004. to 2012) the index reflected the negative development trend. Although all macroeconomic indicators pointed to an economic production lag, water consumption in the industry was not reduced in comparison to the volume of production. The Bosnia River, in the upper course after the source in Sarajevo field, receives flow from its right tributaries Željeznica, Dobrinja and Miljacka River, and the left tributary of the Zujevina River. The catchment area of the Bosnia River is equal to the affluent catchment area and so the nature of the flow depends on their characteristics.

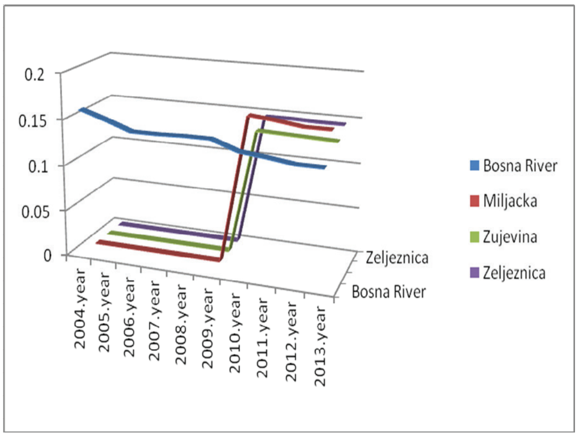

Figure 7. Calculated index values in the area of three main tributaries of the Bosnia River

The distinctiveness of the catchment area is that the largest number inhabitants in the rural areas lack sewage systems, and collection and final disposal of wastewater is based on a great number of direct discharges or improperly made cesspools. According to the overall pollution, it can be classified as diffuse pollution since wastewater is discharged into the soil as a recipient. The dangers of this type of wastewater discharges are contaminated–groundwater resources. In essence, both kinds of wastewater discharges are uncontrolled, unsustainable and contrary to the principles of sanitation of a village, while consequences can only be estimated.

In the catchment area of the Bosnia River about 60 direct polluters have been registered while other economic organizations drain their wastewater into sewers. Industrial wastewater is treated in primary and secondary treatment processes, about 15,00% and 1,00% respectively. Recirculation and reuse of wastewater is done only in 5,00% of the buildings.

Certain guidelines and recommendations adopted at the global level are not binding however they have the strength and guidance of internationally accepted rules of conduct. It is well known that the implementation of the obligations arising from the Directive requires huge investment funds, therefore well prepared data will be of great importance for the negotiations with the Commission. The obtained data are measurable, and systematic monitoring provides clear relations between a degree of implementation of the measures and effects of changes in surface water quality.

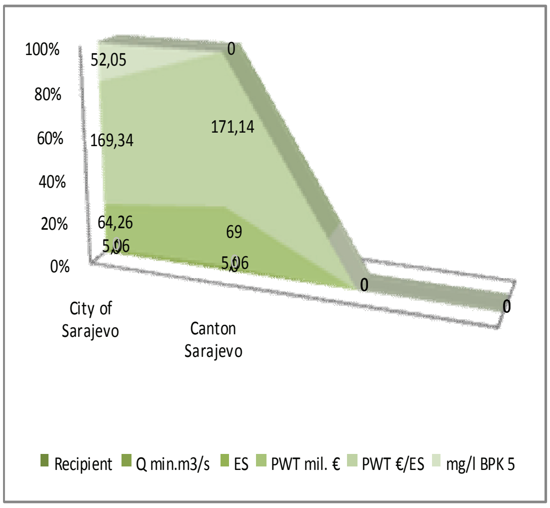

Figure 8. Indicators reaction - water protection

Source: International policies and support countries in Southeast Europe, 2008: The investment cost of a wastewater treatment plant of € 69.00 million will provide benefits of 12.86 million €/year to the inhabitants of the Canton. By implementing the EU Water Framework Directive, the total economic gain for the people of Bosnia and Herzegovina amounts to 389 million €/year, and per capita 53-78 million €/year; qualitative drinking water improvement for 55,00% inhabitants in Bosnia and Herzegovina; the quality of surface water of 12.85 to 33.35 million €/year; the total value over 20 years is 117-172 million €/year.

DISCUSSION

The weakness of the calculation methodology BDE 's is the fact that there is no standard against which to evaluate the calculated index value. The methodology is based the methodology the calculated HDI (aggregated indicators) and provides only the definition of the relative position areas with respect to one another.

The results of wastewater runoff index suggest a negative development trend of the indicator from the viewpoint of the established criteria of sustainability. The increase of approximately 0.98% of spilled municipal wastewater into the watercourses of the part Sarajevo Canton is a result of highly specific consumption and misuse of water in households and industry. The numerical value of the indicator for 2013 corresponds to the amount of spilled sewage of 1557m^3 per day, 56,352 m^3 per year, which is more for 4m^3/day or 100 m^3/year than in 2012.

Veljkovic N. Indicators sustainable development and management of water resurs.2006.; Demographic indices shows South Morava river (1980 2006), and the Bosnia River Basin (2004 to 20012) indicate a negative development tendencies trend. High specific energy consumption 120-300l / ft. / Day and for purposes of water use caused an increase in wastewater spilled from 35% in the South Morava river basin, sub-basin of 0.90% in Bosnia. Percentage of population connected to waste water treatment plants in the EU from 35 to 80% , 16% of Serbia, Bosnia and Herzegovina 3% , Canton 1 % . Optimistic projections for 2023.g. spilled wastewater from 5% to HP, the index BDE = 0.697 equivalent 23 277m^3 / year.

The main purpose of the indicators of sustainable development is to ensure a stable and practical basis for reporting to the European Environment Agency, improving quality and geographic coverage of data flow. The applied index helps better understanding of complex environmental problems and provides quantitative information in a simple and straightforward manner. Calculation of the index at the local community level is useful for improving knowledge and awareness of the local population and their involvement in a transparent decision-making process.

CONCLUSION

A healthy environment is a basic precondition for quality life and the preservation of human health. Percentage population coverage by public water supply is high (94,83% of households in the Sarajevo Canton have drinking water available in the house). Diseases in which the agent is water-transferred are limited and mostly occur small water supply systems that are not under constant supervision of public health service public. Health institutions communicated epidemiological data, but no data on direct regarding environmental factors and human health. Feel a lack of targeted research in specific cases of environmental pollution and the impacts of these pollution has on human health.

The largest number countries (almost all EU countries and Croatia) have 100% access to drinking water and sanitation.

Specific water consumption in households in the Sarajevo Canton falls into the category excessive (inefficient) water use , defined according to the authors and WPI.

During the period from 2004 to 2012. showed no significant changes in the concentration of organic matter in the rivers, as evidenced by the BPK_5 and ammonia (NH_4). These values indicate that the condition rivers generally good considering the oxygen content in the water and saturation (saturation) water oxygen . However, the Bosnia river is much more polluted than other rivers. Biochemical oxygen demand BOD_5 denotes period-to-day biochemical oxygen demand, which is required for the biological degradation of organic matter. High levels of BOD_5 indicates the organic contamination that causes decreases in oxygen and harmful effects on aquatic ecosystems. In general, the main causes of organic pollution are untreated municipal and industrial wastewater.

Although the general condition of the discharge and treatment of wastewater from public sewerage system in the Sarajevo Canton unsatisfactory, little progress has been made in increasing the number of connections to the system Public Sewer and very little progress in terms of the relationship between the annual amounts of purified water and the total volume of waste water. The largest percentage of the total volume of wastewater consists of wastewater from households. The number of people who are connected to the sewage system is higher in urban areas. Percentage of population living in agglomerations (> 2000 EBS) which is connected to the sewer system is estimated at 46% at the state level (Water Policy in BiH, 2011).

Problems in the area of water management that indicate the unsustainability of the system are: inadequately drainage and wastewater treatment, pollution of rivers downstream from urban agglomerations and industrial areas, high water losses in public water supply system and inadequate tariff policy.

In order to achieve the necessary conditions for the effective implementation of EU directives in relation to the water sector, it is necessary to prepare general plans for water supply, sewerage and wastewater treatment systems, the characterization of river basins as a first step in developing management plans river basins, effectively handful cling debts, improve coordination and harmonization of environmental statistics.

If the pollution load caused by the inhabitants and pollution from the industry are compared, it can be concluded that the pollution load from industry is slightly ahead in terms of the total pollution that reaches the water bodies of the Sarajevo Canton. The main pollutants are suspended organic matter, nitrogen and phosphate compounds. The great microbial load can have negative impacts on human health, environmental contamination and reduction of potable water resources. The absence of laws which define responsibilities and tasks is just part of the problem in the implementation of activities and measures concerning the protection of waters. The unreliable register of industrial pollutants and data on their pollution load was overcome by using a data quality effluent test.

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THE COGNITIVE GRAPHICS APPLICATIONS IN REAL TIME PROCESS CONTROL OF COMPLEX SYSTEMS

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

The real time process control of complex technological process requires special human- machine interface which will support the decision making process efficiently. One of prospective solution is use of cognitive graphics, which presents the most important data collected from the technological process in form which is close to the human. The presentation of process data using cognitive graphics can be easily understand by operator providing fast and efficient decision making. In article the use of cognitive graphics for presentation of process data from different technological proces is explained and discussed. The cognitive graphics application is not limited to technological processes but can successfully used as support of decision making in other fields too.

Keywords: complex system, process control, cognitive graphics, human machine interface,

The modern technological processes are becoming more complex and faster. Because humans are still making decisions in most cases it is extremely important that presentation of data collected in the technological process has to be efficient, easy to understand, providing clear basis on which operators can make correct decisions. However in most cases the presentation of data is to complex in form, which is not simple so that it is very difficult for operators to understand the situation, what is going on in the process and which decision has to be making to control process efficiently. Cognitive graphic image is a model interpretation of the state of some object, situation, and phenomena. Graphic image is constructed using special graphic tools. Object can be any entity for which the model can be synthesized. Cognitive model of the image is based on three elements, what can be presented in the form:

$$M=\langle P,R,I\rangle$$

where:
P is a set of concepts in the domain M.
R is a given set of relations in M, which are using for construction of the figures (images),
and I is the set of the interpretation of figures (images).
The set M is in the image presented by the linked set of graphics elements. R and I present rules.
A linguistic means of describing graphic images have to be:

- Graphemes (graphics primitives)
- Rules of construction, joints, overlays, position and other operations, the synthesis of graphic (geometric) shapes of the image;
- Rules of cognitive (cognitive) interpretation of graphic (geometric) shapes, images.

In this approach the graphic (geometric) figure - is any nonempty set of points. Such a definition has several advantages:

- It does not restrict the class of shapes (points, lines, graphs, charts, surfaces, bodies, etc.).

Under this definition it is possible to operate with objects (shapes) of any dimension and in the spaces with any number of dimensions;
To describe the relationship between the points forming the shape can be used in a variety of formal (mathematical and "not quite") and informal apparatus. For example: algebra, calculus, graph theory, logic, semiotics, etc. These apparatus provide the appropriate methods, tools and technologies.
Such an understanding of graphical tools brings them closer to the semiotic system and the formal language with its own alphabet (dictionary of graphemes) and syntax.

Cognitive tasks of computer graphics systems, information support of decision-making

Well-known specialist in artificial intelligence, D.A. Pospelov, in the preface to [1] and [2] formulated three main tasks of cognitive computer graphics (CGC) in decision support systems based on the figurative representation.

The first task is to create models of knowledge representation, which represent the objects specific to logical thinking, and images-paintings, handling creative thinking.

The second task is to visualize the human knowledge, which cannot be described by the text.

Third - finding ways to transition from the observed images-paintings to the formulation of some hypotheses about the mechanisms and processes that are hidden behind the dynamics of the observed patterns.

In terms of information technology supporting decision-making to these three tasks CGC should be added the fourth. It should ensure the creation of conditions for the development of the operator’s intuition and creativity by constructing a graphical user interface based on the methods of cognitive graphics.

The development of computer information systems supporting decision-making is usually based on the first two tasks of cognitive graphics. Usually user interface present the data in a familiar character-digital form.The large set of such data presented to the operator, could not be used for decision making.

A clear understanding of the third and fourth tasks of cognitive graphics allows formulating additional requirements as to the actual graphics, as well as to the appropriate software and methodological support.

Among them are:

- The adequacy of the studied objects or processes used engineering methods and techniques of teaching;
- Accessibility for the perception of users;
- Convenience for the analysis of qualitative regularities of distribution parameters;
- Aesthetic appeal, the speed of image formation.

Charts are often used in mathematical studies in economics, physics and other exact sciences.

Schemes are already more advanced forms than graphics, and include objects and relations between them. In various branches of the concept of the scheme is expressed in different terms: in the radio industry - concept, in the computer industry - a flowchart algorithms, Petri nets, in the design - drawings, in operative management - a mimic in linguistics - the semantic network in math - graphs.

Users should also be able to choose the type of image. The fact is that the same information can be displayed in graphical form in different ways. Results of special studies of these types of graphical display of information indicate that each person by virtue of their individual, personal perception of their own evaluates the effectiveness of a particular type of image, and the evaluation of different people may differ materially. Therefore, computer decision support system should have a set of different ways to graphically display information. This will allow each human operator to select the most suitable for him the type of image, or to use a variety of graphic patterns for the analysis of the object and control.

In addition, there is a need to provide user interface designers and information systems to manage the image - to vary its size, colour, position of the observer, the number and position of light sources, the degree of contrast can be represented by variables, etc. All of these features of a graphical interface not only allows designers to choose suitable forms of graphic images, but also introduce gaming and research components of the work, naturally prompting management professionals to a deep and comprehensive analysis of the properties of the studied objects and processes.

Imaginative ways of presentation of situations

As noted above, for solving the decision problem situation can be represented in various forms:

- in numeric form, as an integral sign;
- in a symbolic form - the text description of the situation;
- in graphical form - charts, diagrams, pictographs and drawings.

We will discuss some of graphical forms

Graph - a collection of pixels that are displayed in the coordinate space - the easiest way to display the situation.

Schemes as well as the graphics are used to assess the situation (assessment object for understanding the technological, organizational or business process, to display the sequence of actions, etc.)

Pictographs. Basic idea is to represent individual units of observation in the form of certain graphic objects, with the values of variables are associated with certain characteristics or parameters of these objects (usually one observation = one object). This correspondence is such that the overall appearance of the objects varies as a function of the configuration values. Thus, the observer can identify unique for each configuration of values of visual characteristics of objects. There are variety pictographs: Pie charts, rays, polygons, profiles, columns, "Chernoff Faces" [3].

Cognitive images. Cognitive images are a graphical representation of the correlations between the variables of state of the system. Correlation dependences form a cognitive knowledge base. Cognitive images for decision support systems have such an important property as the unique interpretation to any human operator in a specific area [4].

Cognitive graphics in the presentation and analysis of images

Given the above the role of visual images, whether to include in the composition of information systems, shaped the various elements of visualization, which will improve the efficiency of the perception of the results of the intellectual system human operator.

This is even more justified for figurative representation, since all forms of coding and transmission of knowledge cognitive symbols (KCS) to be recognized as the most ancient.

One might think that using the KCS will be a condition of submission is difficult or long-explainable in words. And subjectivity of cognitive graphics help you.

Graphic image, which is based on the notion of cognition, must initiate the intellectual processes and not only the generation of new knowledge, but also solve problems in weakly structured domains of knowledge. For example, the thought process is activated when solving problems about the idea of abstract paintings, which is a super semi-structured domain. Inclusion in the Knowledge Base visualization elements can be with a special interest taken by the operator. At the same time, a visual representation of the output parameters requires "tuning” of knowledge base for a particular user, given its individual and experienced representation, and the ability to create imaginative ideas or analytic-synthetic activity of the brain depending on the predominance of right or left hemisphere of brain.

We can say that we in this case will focus on "direct stimulation of" right-brain activity in the brain of a computer user's cognitive graphics. Presenting expert image can be purposefully generate (activate) or another associative strings of images (to start the process creative thinking).

In cognitive systems we need to develop interfaces that use both natural language and graphics for the transfer of certain content. It is interesting to develop semantic models for the interpretation of graphical expressions that contain a variety of perceptual (tactile) characteristics associated with the depicted object.

To some extent, human imagination can meet the 'spatial' cognitive schema, or map. And if they are regarded as the world picture of a level of abstraction that can describe and provide the knowledge base of decision support systems as independent units, not only as a basic semantic categories (symptoms, facts, events, etc.) related relations, it would come closer to the vision of the world's human operator.

Tasmo-Bit has developed cognitive models and practical applications cognitive graphics in the intelligent information system "Cogra [5, 6], which was successfully used for monitor and support of decision making system in complex energy production and gas transport systems in Russian Federation.

Different figurative representation in Cogra provide a convolution of a large amount of data and their representation in accustomed to human ways.

For example as a cognitive image of the state of the reactor plant in the system Cogra is used rotating globe. State variables globe are parallels, meridians, the axis of the poles and the equator and the rotational speed of the globe relative to the axis of the poles, the spots on the globe.

State variables globe are determined based on the measured data stored in the database, or by calculation or by treating the cognitive model of knowledge. State variables are generated every second. Depending on processes occurring in the reactor, the globe may be changed:

- speed;
- shape by pulling from the poles or equator;
- the position of the parallels;
- the position of the meridians;
- color; cover its surface with white spots. All configuration changes of shape Globe are described in the Manual for use of this figure. Operator-technologist, manager are trained in the actions to be taken in one or another problem situations.

The cognitive graphics approach can be used in several different areas like medical diagnostics, monitoring of complex project progress, monitoring of learning process with the aim to improve their realization.

Because more and more of described process are using web application (Internet or Intranet) the use of Scalable Vector Graphics [7] for generation of cognitive images can present significant improvement in the application of cognitive graphics because of following facts:

- the quality of images will be better
- images can be easily generated
- There are more application using mobile devices where the use of SVG present significant advantage [8].
- Accessibility of SVG.

We believe that use of SVG for generation of cognitive images will be very successful approach in the future.

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