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C O N T E N T

MODIFICATION OF POTATO STARCH

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

Application of native starch in food production is highly limited due to retrogradation, instability in acid conditions and at high temperatures etc. To overcome these problems and extend starch application, native starch is modified by chemical, physical and enzymatic procedures.
The aim of this research was to prepare different modified starches from commercial native potato starch by acetylating (with acetanhydride), cross-linking (with sodium tripolyphosphate, STPP) and combination of these procedures, and to determine their rheological and thermophysical properties.
The results showed that all modified starches had lower gelatinisation temperature and lower gelatinisation and retrogradation enthalpy than native starch. Dual modification with higher proportion of cross-linking reagent resulted in 50%-decrease of gelatinisation enthalpy compared to native starch. Acetylated and acetylated cross-linked starch had lower peak viscosity, whereas cross-linked starch had higher peak viscosity than native counterpart.
While acetylating resulted in increase of % breakdown, other applied modifications resulted in its decrease.
Swelling power and solubility increased by acetylating and decreased proportionally to increase of cross-linking reagent both by cross-linking and dual modification.

Keywords:*starch, potato, acetylating, cross-linking, rheological and thermophysical properties*

INTRODUCTION

Starch is a carbohydrate with major application in food production, where it is used as thickening, stabilising, gelling, adsorption, film forming agent, for binding aroma compounds etc. (Babić et al., 2009a; Šubarić et al., 2007). Chemically, starch is mixture of two polysaccharides: linear amylose, where glucose units are bound by α-l→4 glycosidic linkages and amylopectin, where glucose molecules are linked by α-l→4 (95%) and i α-l→6 (5%) glycosidic bonds. In nature, starch is found in form of granules with different shapes and sizes, depending on plant origin. Starch granules are semi-crystalline, i.e. contain amorphous and pseudo-crystalline regions (Tester et al., 2004).

Application of native starch in food industry is limited due to retrogradation and instability in acid conditions, resulting in syneresisandunstabletexture, gelatinisation limitations,thermaldegradationetc.Toimprov e or achieve specific functional properties of starch, different modification procedures are used (esterification, cross-linking, oxidation, pregelatinisation etc.) (Babić et al., 2009b). Gelatinisation, retrogradation, viscosity and melting are the mostimportantpropertiesforstarchapplicatio n which can be influenced by modification.

Acetylation is esterification of starch molecules by acetyl group resulting in formation of starch acetate (Babić et al., 2007). Proportion of substituted groups in starch chain is often expressed as degree of substitution (DS), defined as a number of substituent moles per number of anhydroglucose moles. Acetylation results in decrease of gelatinisation temperature and retrogradation, with more pronounced changes with increase of DS (Babić et al., 2009b).

Starch has two types of hydroxyl groups: primary at C-6 atom and secondary at 2ndand 3rd C-atom. Both –OH types can react with multifunctional reagents giving cross-linked starches. The aim of cross-linking is to produce starch with reduced swelling capacity to limit gelatinisation during cooking. Cross-linking reduces loss of solubles from the granule and enhances starch granule. Granule swelling is limited and their volume proportion in water phase is reduced, which decreases viscosity (Cui, 2005).

The aim of this research was to prepare differentmodifiedstarchesfrom commercial native potato starch by acetylating (with acetanhydride), cross-linking (with sodium tripolyphosphate, STPP) and combination of these procedures, and to determine their rheological and thermophysical properties.

MATERIALS AND METHODS

Materials used: commercial potato starch, Agrana, Austria; acetanhydride,“Kemika”, Zagreb, Croatia; sodium tripolyphosphate,Agros Organic, USA; sodium hydroxide,“Kemika”,Zagreb,Croatia; HCl,“Kemika”, Zagreb, Croatia; Na_2SO_4, Sigma-Aldrich, Germany.

Acetylation of potato starch (*K*) with acetanhydride (*AA*) was performed as follows:native starch (100 g, dry basis) was dispersed in distilled water (225 ml) and stirred for 0.5 h at 25°C. NaOH (0.45 mol/dm^3) solution was used to adjust the suspension pH to 8.0. Acetic anhydride was added drop-wise at 4, 6 and 8% levels (based on dry weight of starch) while maintaining constant stirring and the pH within the range of 8.0-8.4 using NaOH solution (0.45 mol/dm^3). The reaction was allowed to proceed for 10 min after the completion of aceticanhydride addition. The pH of the starch suspension was then adjusted to 4.5 with 1 N HCl and filtered through a Buchner funnel. Starch cake was reslurried in distilled water (200 ml) and filtered again (the slurring and filtering steps were repeated twice), and then air-dried to achieve ca. 86 % dry matter.

Cross-linking was performed as described by Lim &Seib (1993) by addition of 2.5% or 5% sodium tripolyphosphate (dry matter based).

Dual modified starches were prepared first by acetylation with 8% acetanhydride, drying to less than 14 % water content and than cross-linking by addition of 2.5% or 5% sodium tripolyphosphate (dry matter based).

Thermophysical properties (gelatinisation and retrogradation) of starch were analysed by differential scanning calorimeter (DSC) Mettler-Toledo DSC model 822^e as described by Babić et al. (2009a).

Rheological properties of native and modified starches were determined as described by Ačkar et al. (2010).Swelling power (KB) and solubility (IT) weredetermined as described by Mandala &Bayas (2004).

RESULTS AND DISCUSSION

DSC gelatinisation and retrogradationparameters of native and modified potato starches are shown in Table 1. All modified starches (acetylated, cross-linked and dual modified) had lower onset gelatinisation temperatures and gelatinisation enthalpy than native starch, where acetylated starch had lowest onset temperature (59.5°C) and dual modified starch *K+AA+STPP 5 %*lowest gelatinisation enthalpy (8.84 J/g). The same observations were made for maize starch, and for acetylated tapioca and maize starch by Xu et al. (2004) and by Babić et al. (2007, 2009b) respectively.

Acetyl groups, bound by acetylation, are bulkier than –OH groups, and weaken H-bonds between amylose and amylopectin moleculesin granule. Therefore, less energy is required to gelatinise starch, whichisreflectedindecreaseofgelatinisation temperatures and enthalpy (Babić et al., 2009b; Rutenberg&Solarek, 1984). In addition to formation of cross-linked products,STPPmodificationofstarchresults in formation of monostarch phosphate (starch ester) where substituted phosphate groups act similarly to acetic groups. Therefore, these modificates have lower gelatinisation temperatures and enthalpies,too(Lim&Seib, 1993; Fennema, 2008). Based on results, one can conclude that substitution reactions prevailed during modification of starch with STPP.

DSC retrogradation parameters of native and modified starch are shown in Table 2. During cooling, starch/water system spontaneously passes to the state with lower energy. Molecular interactions (starch chains bonding by H-bonds) after cooling gelatinised paste are called retrogradation. Retrogradation enthalpy of native starch was 7.69 J/gafter 7-day storage at 4°C and 8.16 J/g after 14-day storage at the same temperature. Acetylation, cross-linking and dual modification lowered retrogradation enthalpies of potato starch. After 7 days, retrogradationenthalpiesofmodifiedstarches increased in the following order: *K+AA+STPP5%*(2,52 J/g) <*K+AA+STPP 2,5 %* (2,79 J/g) <*K+STPP 2,5 %* (6,01 J/g) <*K+STPP 5 %* (6,17 J/g) <*K+AA* (6,5 J/g); and after 14 days:*K+AA+STPP 5 %* (5,46 J/g) <*K+AA+STPP 2,5 %* (6,74 J/g) <*K+STPP 2,5 %* (6,9 J/g) <*K+STPP 5 %* (7,0 J/g) <*K+AA* (7,34 J/g). Retrogradation was reduced due to steric hindrance of bonding melted starch molecules due to bulky substituent groups (Babić et al., 2007; Fennema, 2008).

Rheological parameters of native and modified potato starches are shown in Table3.Peakviscosity(maximumviscosity of starch paste after starch gelatinisation) of native starch was 1621 BU. Cooling starch pastes to 50°C results in viscosity increase due to starch retrogradation.

Acetylated starch paste had lower peak viscosity than native starch (1608 BU). In addition,viscositiesatallmeasuredtemperatures were lower than native starch, while % breakdown practically equalled to nativestarch(K=69,28%; K+AA=70,02 %).

Cross-linking increased starch paste viscosity compared to native starch at all measured temperatures, where increase was proportional to amount reagent used. Peak viscosity of K+STPP 2.5% was 2041 BU, K+STPP 5%=1925 BU, at 50°C K+STPP 2.5% =1811 BU, i.e. K+STPP 5%=2496 BU. As earlier mentioned, modification of starch with STPP results not only in cross-linking but in ester

formationwhich is supported by lower gelatinisation temperature and higher peak viscosity (Lim &Seib, 1993).

Table 1*DSC gelatinisation parameters of native and modified potato starches (35% suspensions)*

Sample	T_o[ºC]	T_p[ºC]	T_e[ºC]	ΔT[ºC]	ΔH_g[J/g]
K	62,0	65,9	72,5	10,5	16,08
K+AA	59,5	63,6	70,2	10,7	15,42
K+STPP 2,5 %	60,9	64,8	71,0	10,1	13,83
K+STPP 5 %	61,1	65,2	71,8	10,7	15,08
K+AA+STPP 2,5 %	60,2	63,2	71,2	11,1	9,78
K+AA+STPP 5 %	60,6	63,9	72,5	11,9	8,84

DSC gelatinisation parameters: T_o, onset temperature; T_p, peak temperature; T_e, endset temperature; ΔT, gelatinisation (T_e- T_o); ΔH_g, gelatinisation enthalpy. K, potato; AA acetanhydride; STPP, sodiumtripolyphosphate

Peak viscosity of K+STPP 2.5% was 2041 BU, K+STPP 5%=1925 BU, at 50°C K+STPP 2.5% =1811 BU, i.e. K+STPP 5%=2496 BU. As earlier mentioned, modification of starch with STPP results not only in cross-linking but in ester formationwhich is supported by lower gelatinisation temperature and higher peak viscosity (Lim &Seib, 1993).

Dual modified (acetylated cross-linked) starches *K+AA+STPP* 2.5 % and *K+AA+STPP 5 %* had lower peak viscosities compared to native, acetylated and cross-linked starches. Dual modified starch with 5% STPP had lower peak viscosity (665 BU) than the sample with 2.5 % STPP (1453 BU).

Percent breakdown of starch pastes is a measure of stability of pastes during shearing at high temperatures – lower the value, more stable the paste. Native potato starch had 69.28% breakdown.

Table 2*DSC retrogradation parameters of native and modified potato starch after 7 and 14 days of storage at 4 ºC*

Sample (after 7-day-storage)	T_o[ºC]	T_p[ºC]	T_e[ºC]	ΔH_r[J/g]
K	41,4	55,8	71,3	7,69
K+AA	43,0	59,4	72,1	6,50
K+STPP 2,5 %	41,6	54,6	71,9	6,01
K+STPP 5 %	41,8	54,3	71,5	6,17
K+AA+STPP 2,5 %	42,0	51,2	60,4	2,79
K+AA+STPP 5 %	40,1	52,0	60,7	2,52
Sample (after 14- day-storage)	T_o[ºC]	T_p[ºC]	T_e[ºC]	ΔH_r[J/g]
K	41,3	55,4	72,4	8,16
K+AA	41,3	57,2	73,0	7,34
K+STPP 2,5 %	41,9	54,9	72,2	6,90
K+STPP 5 %	42,2	55,9	72,3	7,00
K+AA+STPP 2,5 %	42,6	54,6	71,8	6,74
K+AA+STPP 5 %	40,1	52,0	61,3	5,46

DSC retrogradation parameters: T_o, onset temperature; T_p, peak temperature; T_e, endset temperature; ΔH_r, retrogradation enthalpy. K, potato; AA acetanhydride; STPP, sodiumtripolyphosphate

Cross - linking significantly decreased % breakdown proportionally to the increase ofreagentamount.%breakdown increased in the following order: *K+AA+STPP 5 %* (3.01) <*K +STPP 5 %* (3.64) <*K+STPP 2,5 %* (44.2) < *K +AA+STPP 2,5 %* (59.8) < K (69.28) <*K+AA* (70.02).

Table 3*Rheological parameters of native and modified potato starch*

Sample	*K*	*K+AA*	*K+STPP 2,5 %*	*K +STPP 5 %*	*K +AA+STPP 2,5 %*	*K+AA+STP P 5 %*
Pasting temperature [°C]	68,2	61,05	63,1	63,2	61,65	69,8
Peak viscosity [BU]	1621	1608	2041	1925	1453	665
Viscosity at 92 °C [BU]	844	787	1587	1784	1011	664
After 15 minat 92 °C[BU]	498	482	1139	1855	594,5	645
Viscosity at 50 °C [BU]	1041	842	1811	2496	982	947,5
After 15 minat 50 °C[BU]	891	690	1452	2124	794,5	843
% Breakdown	69,28	70,02	44,2	3,64	59,08	3,01

K, potato; AA acetanhydride; STPP, sodiumtripolyphosphate

Swelling power (KB) and solubility (IT) of native and modified potato starches are shown in Figure 1. Potato starch has significantly higher swelling power than other native starches. According to Hermansson & Svegmark (1996), volume of potato starch granules increases 100 times during swelling, while for grain starches this increase is merely 30 times due to lipids surrounding starch granule. Acetylated starch had higher KB and IT than native because acetylation weakens intermolecularbondsbetweenstarchmolecules (Rutenberg & Solarek, 1984). Cross-linking decreased KB and IT proportionally to the amount of reagent.

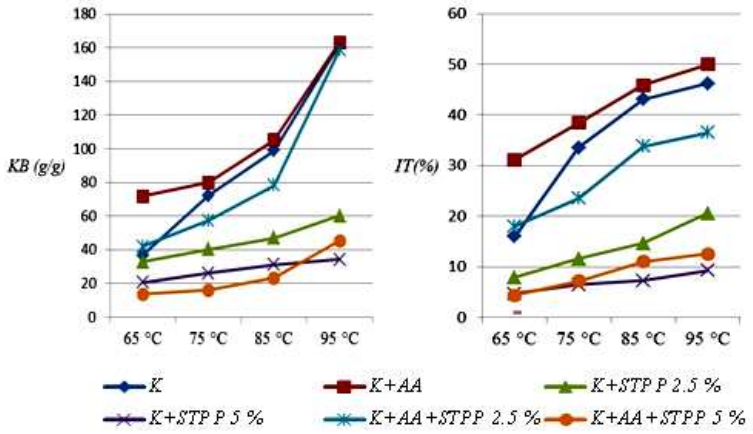

Figure 1*Swelling power and solubility of native and modified potato starch*

Dual modified starches had significantly different values of KB and IT, dependingoncross-linkingreagentconcentration (STPP), where sample modified with lower concentration of STPP (2.5%) had significantly higher KB and IT compared to sample *K+AA+STPP 5 %.*

CONCLUSION

Acetylated potato starch gelatinised at lower temperatures and had lower gelatinisation enthalpy and retrogradation enthalpy after 7 and 14 days of storage compared to native starch. Acetylation decreased viscosity of potato starch paste and increased swelling power and solubility.

Cross-linking with STPP resulted in lower gelatinisation temperatures, gelatinisation and retrogradation enthalpy and swelling power and solubility, but increased viscosity and stability of starch pastes during shearing at high temperatures.

Dual modification (acetylation and cross-linking) resulted in decrease of all measured parameters: gelatinisation temperature, gelatinisation enthalpy, retrogradation enthalpy, paste viscosity, % breakdown, KB and IT.

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MILK PRODUCTION SITUATION IN THE TUZLA CANTON

PROFESSIONAL PAPER

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ABSTRACT

The aim of this study is to determine the state of the production and purchase of milk in Tuzla Canton, and on the basis of the received information to provide guidelines for improving milk production in this area.
Milk production is the most important part of livestock production and it is of strategic importance for the development of agriculture in Tuzla and Bosnia and Herzegovina.
The financial support for agricultural production from the Federation of Bosnia and Herzegovina that has been accomplished in the area of TK, for milk production accounts for 33.40%.
The survey covered all municipalities of Tuzla canton, with the number of dairy cows, and milk production. On the basis of data on the average milk production it can be concluded that the production of milk per milking cow is still at a very low level. In 2010 TK area had 6106 manufacturers, who together had 8454 dairy cows. Total amount of milk purchased in Tuzla Canton in 2010. is 18,689,153 liters of milk.
They also made comparisons with the production in the neighboring countries and the EU.

Keywords: *milk, dairy cattle, production*

INTRODUCTION

Milk production is the most important part of livestock production and is of strategic importance for the development of agriculture in Tuzla and Bosnia and Herzegovina.The financial support for agricultural production from the Federation of Bosnia and Herzegovina that has been accomplished in the area of TK, for milk production accounts for 33.40%. Milk is primarily food, but also the raw material that has great potential, because out of it we can get a large number of products in the form of sour milk, yogurt, cheese, cream, butter and other products. The main problems faced by farming and farming production in Tuzla Canton, as well as in Bosnia and Herzegovina,are small production, excessive imports, inadequate structure of production, low price of milk and a very low manufacturer standard. Some of them are literally on the edge of existence.

Lack of financial resources is a major obstacle to agricultural producers. Seen as long-term, farming concept could be practically the main direction in increasing milk production, because the standard of quality, which requires the European Union, is impossible or difficult to achieve without a larger share of farm production in the total milk production in the country. Therefore, it is necessary to take adequate measuresto improve the racial composition, health, nutrition and care of livestock, as well as using the new biotechnologies in manufacturing. Only in 2010 18,689,153 liter of fresh raw milk was purchased,

although average price of milk was 0.50 km / liter, the total value of milk production was 9,344,576.50 KM.

Official data available from the Ministry of Agriculture Water and Forestry, Canton and the Federation, as well as the data ofthe Federal Statistical Office were collected.

RESULTATS

Table 1*Milk purchases by the municipalities of Tuzla Canton*

Municipality	2007	%	2008	%	2009	%	2010	%	No. cow
Banovići	751,785	4.46	743,463	3.74	538,068	3.04	525,352	2.81	210
Čelić	621,679	3.69	786,650	3.96	637,374	3.60	731,242	3.91	377
Doboj istok	238,705	1.42	378,453	1.91	495,691	2.80	489,013	2.61	370
Gračanica	2,420,621	14.38	3,077,995	15.50	2,722,107	15.38	2,303,369	12.32	1344
Gradačac	4,951,586	29.41	5,925,294	29.85	4,550,936	25.71	4,284,795	22.93	1978
Kalesija	3,445,145	20.46	3,323,925	16.74	3,725,158	21.04	5,012,250	26.82	1773
Kladanj	0	0	0	0	47,878	0.27	38,967	0.21	0
Lukavac	124,570	0.74	253,505	1.28	252,820	1.43	283,614	1.52	111
Sapna	467,280	2.77	622,600	3.14	490,573	2.77	481,570	2.58	391
Srebrenik	543,996	3.23	564,434	2.84	674,759	3.81	510,250	2.73	300
Teočak	146,815	0.87	221,829	1.12	120,537	0.68	129,902	0.70	126
Tuzla	124,569	0.74	296,619	1.49	645,057	3.64	454,457	2.43	211
Živinice	3,001,525	17.82	3,658,350	18.43	2,802,350	15.83	3,444,372	18.43	1,263
Tuzla Canton	16,838,276	100	19,853.117	100	17,703,308	100	18,689,153	100	
Federation BiH	97,484,000		113.697,000		107,272,000		99,950,000		
TK/Federation %	17.27		17.46		16,50		18.70		
No. milk cow					10,723				8,454

Source: Ministry of agriculture, water and forestry Federation B&H Ministry of agriculture, forestry and water Tuzla Canton

80 requirements for achieving Financial Support submitted from 20 buyers for 4395 manufacturers, 9 application filed bythetwobuyersfor1711 manufacturers.1637 milk producers do not fulfill the quota for incentives (1,500 liters quarterly) but were included in incentives overcooperatives (organizers of purchase).Total number ofmilk producers-6106. Average of repurchased milk is 3061 liters by the producer.

The total number of dairy cows in 2010 is 8454, which amounts to an average of 1.38 dairy cows per producer.

Average milk production per milking cow in 2010 is 2211 liters.

Average milk production per milking cow in 2009 is 1651 liter.

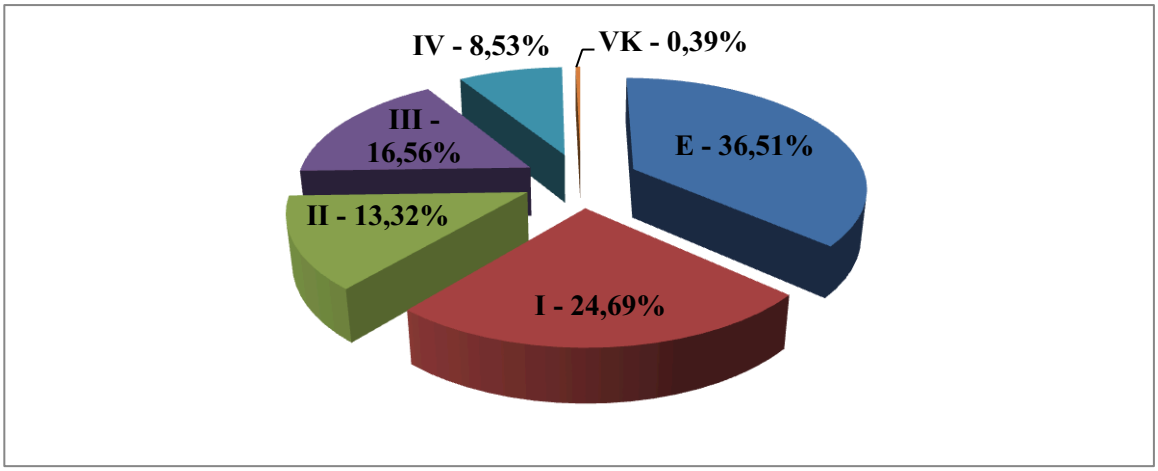
Table 2*Value of milk production in the Tuzla Canton*

Name	Value KM
Milk production	9.344.576,50
Incentives for raw milk	2.990.264,68
Incentives for heifers	764.100,00
Incentives for dairy cows Tuzla Canton	929.400,00
TOTAL	14.028.341,18

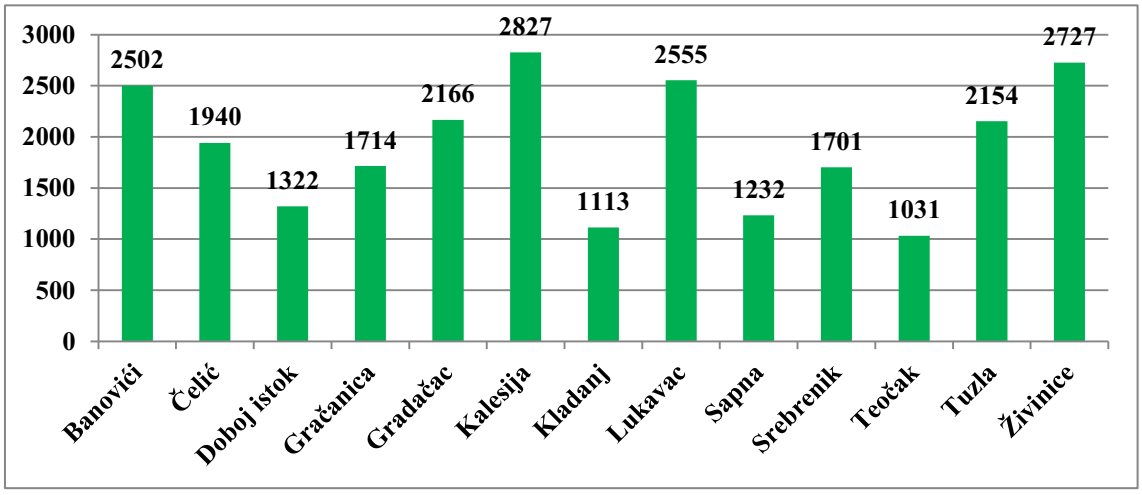
Source: Ministry of agriculture, water and forestry Federation B&H, Ministry of agriculture, forestry and water Tuzla Canton, Agricultural Institute Tuzla

The main causes of the relatively low milk production per cow are next:

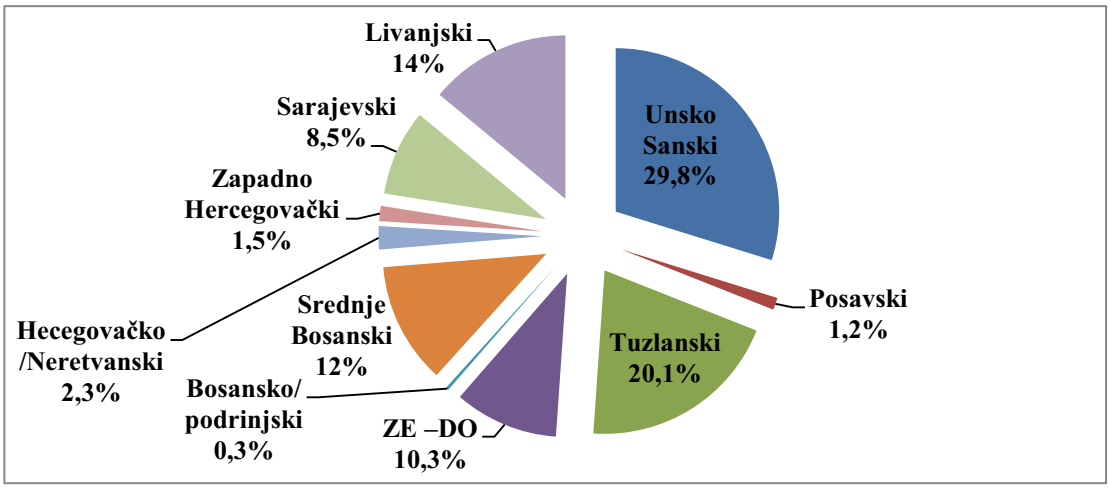
- A large part of the milk and beef producers generates revenues only for ensuring existence. This production is often they additional occupation.
- Poor organization of labor on farms.
- Lack of modern machinery for soil preparation, sowing and preserving quality forage.
- Old barns and equipment.
- The lack of arable land for the provision of high-quality roughage. State owned land is getting hard to lease.
- Lackof necessary funds for further investmentandmodernizationofproduction.
- Lack of education among milk producers in terms of quality of food and balanced diets in order to increase the quantity and quality of raw milk.
- Insufficient use of new technologies in breeding of dairy cows.
- Most farms function as a social category, not a business system. Milk production is their tradition, a way of life, and less the business activity.
- A low level of utilization of the production potential by cow, usually because of inadequate nutrition and housing. No appropriate choice of breed as the means of production, appropriate intensity of production possible.
- A very inadequate producer structures considering on the size of his property. Most of the households possess 1 to 3 ha of agricultural area.
- Milk production is mostly dislocated on the family farms with a small herd of cows (up to 5 cows).
- There is a small number of credit and developmentally capable manufacturers who can develop the production of milk.
- Insufficient or small investment in milk production through incentives and compensation from the authorities.
- No regular payment of incentives by the government.
- Utilization of agricultural extension and advisory services for the development of milk production.



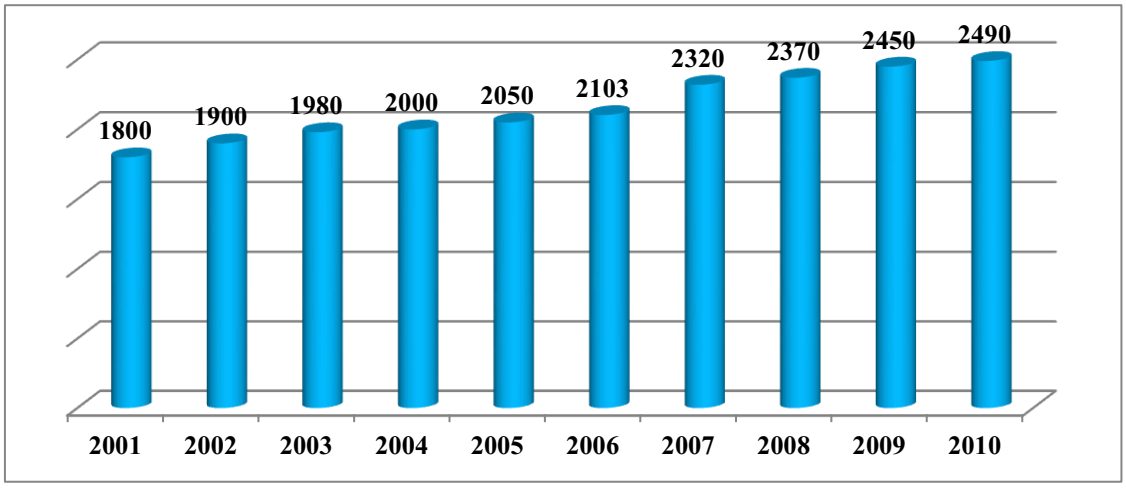
Graph 1*Raw milk quality classes in Tuzla Canton, 2010*
(Source: Veterinary Institute Tuzla)



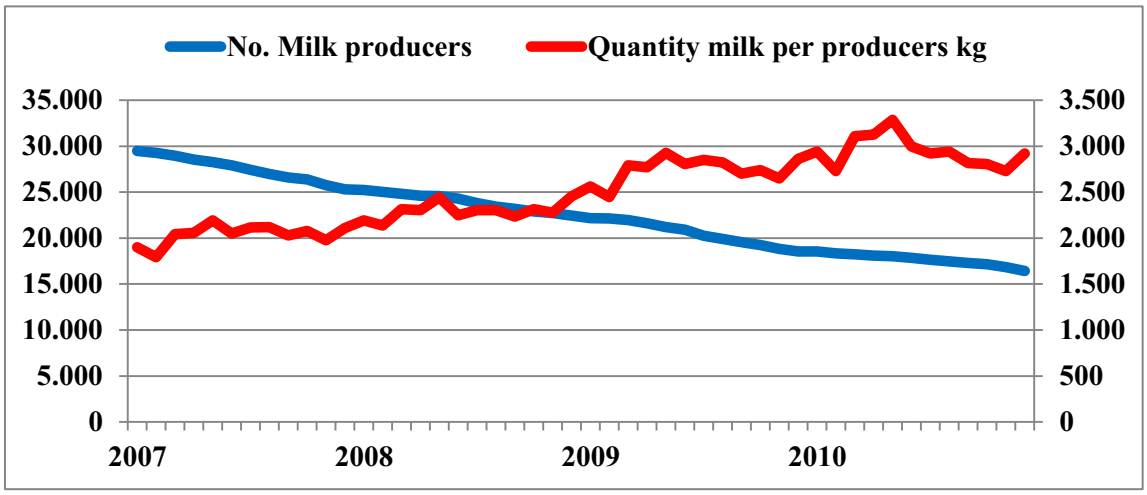
Graph 2*Milk productions per cow by municipalities in Tuzla Canton (litre) in 2010*



Graph 3 *Milk purchases by cantons in the Federation BH in 2010*

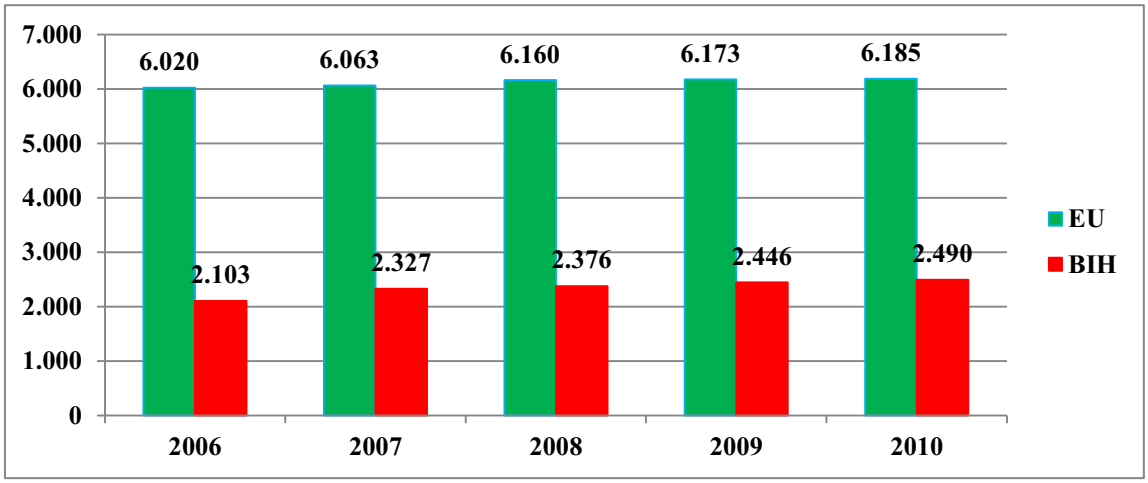


Graph 4 *Milk productions per cow in Bosnia and Herzegovina (litre)*
(Source: Federal institute of statistics Sarajevo)

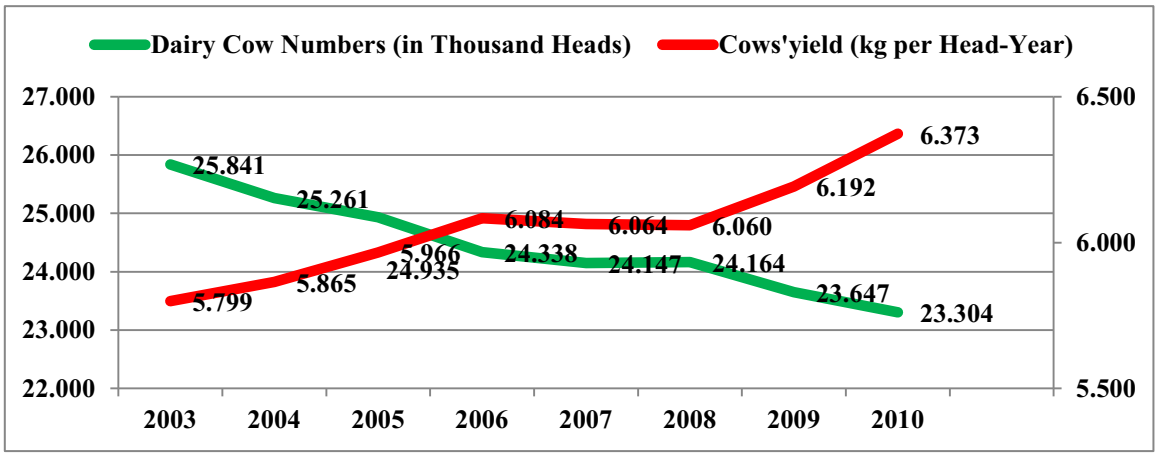


Graph 5 *Number milk producers and milk production per farm in Croatia*
(Source: HPA - Croatian Agricultural Agency)

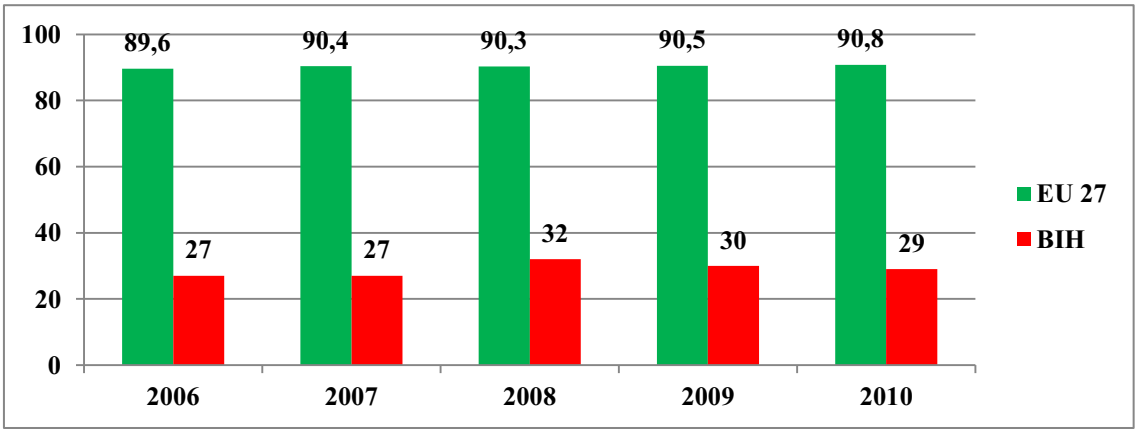
Situation in milk production in Croatia points to the process of reducing the number producer of milk and increase milk production per farm. It is a process makes bigger farms that must happen in and Bosnia and Herzegovina.



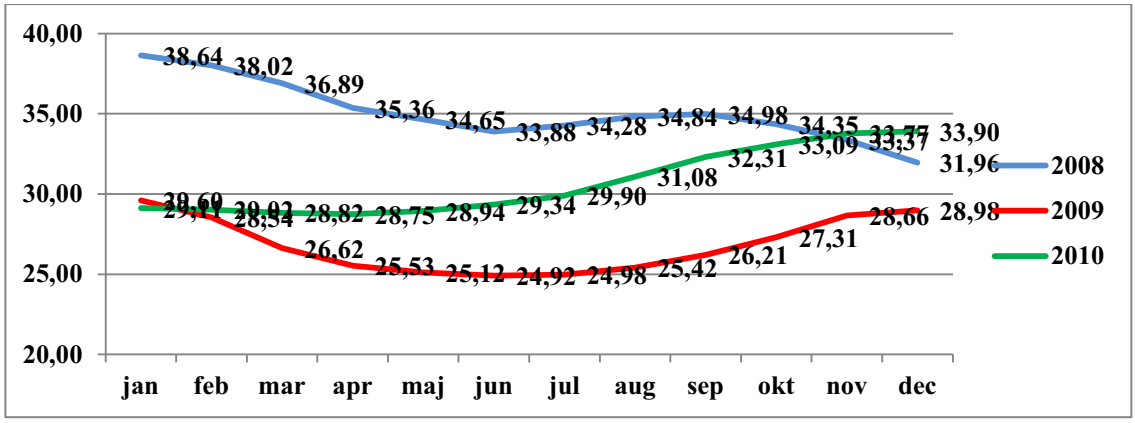
Graph 6 *Milk productions per cow litre/year in BH and EU 27, from 2006 to 2010*
(Source: European Dairy Assosiation, Federal institute of statistics)



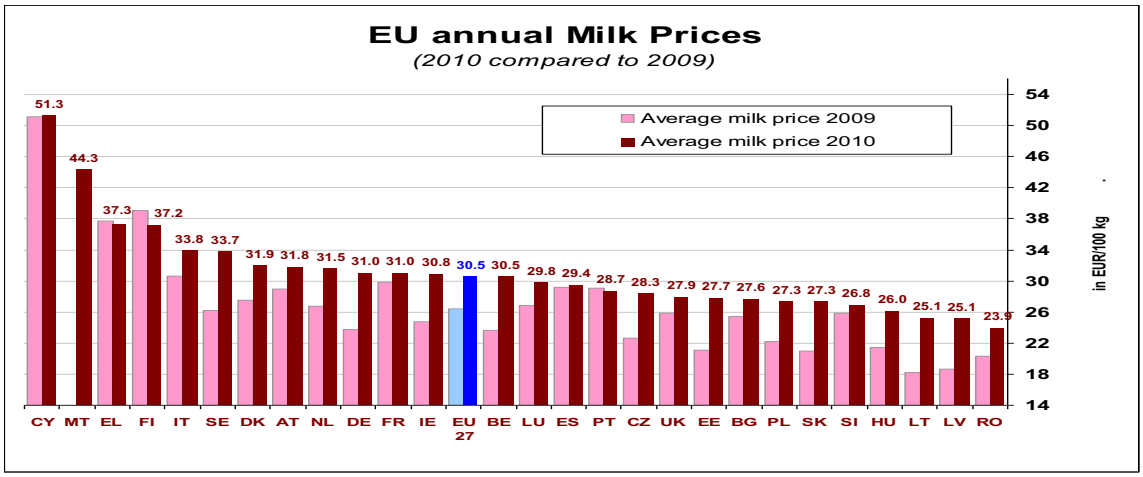
Graph 7 *No. of dairy cows and milk production per cow in EU 27*
(Source: Milk Management Committee Statistics EU)



Graph 8 *Milk purchases in EU 27 and BH, % from total production 2006 – 2010*
(Source: DG Agri –EU, Milk Management Committee Statistics EU)



Graph 9 *Raw milk purchasing prices in EU from 2008 until 2010, Euro/100 kg*
(Source: Milk Management Committee Statistics EU., LTO – Nederland International Milk price comparation)



Graph 10 *Average milk price EU 27*
(Source: LTO – Nederland International Milk price comparation

CONCLUSION

In order to complete revitalization and development of livestock production, following measures are proposed:

- Work in the field of adoption of positive law regulations in hereditary and land policy, which would stop the further reduction of agricultural areas and achieve consolidation of farms.
- Adoption of active credit lines at the local and state level, raisethe investment in the modernization and expansionof existing production capacity, as well as construction of new large specialized farms.
- With increased intensity introduce young dairy cattle and mixed breed cows which will directly affect the increase in production levels.
- Maximuminvolvementofprofessionalss houldsignificantlyimprove the production of quality forage as the most important component in the diets of dairy animal.
- Cooperate with the expert teams of local higher education institutions to implement continuous education of milk producers, which will indirectly affect the quality and quantity of production.

Milk production should be primarily directed towards cheaper and better quality of products. This is possible with new

technologies, especially in the way of storing and handling of animals, nutrition, hygiene, etc.

Giving the priority to systems that make maximum use of cheaper inputs - forage off the lawn and arable crop rotations. Intensity of milk production per cow should be confronted to profit per unit of capital investment. Support to milk development sholud be on the voluminous stern.

Milk production on smaller farms shouldgraduallytakeonthecharacteristics - organic, indigenous and healthier production.

The program development of milk production in Tuzla Canton, and BiH should be based on three categories of producers:

- Small dairy herd, with an average of 5 dairy cows, have no motive or a precondition for business growth.
- Modern conventional production systems adapted to small farms with an average of 15-20 dairy cows in the herd. It is estimated that these farms could makean average production of 4500-5000 liters of milk per cow per year.
- The system of specialized family dairy farms with 40 to 50 cows in the herd. On these farms it could be made production of 5000-6000 liters of milk per cow per year.
- System of large commercial dairy farms (with more than 100 cows in the herd), which could provide more than 6,000 liters of milk per cow per year.

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9. Veterinary Institute Tuzla.
10. HPA – Croatian Agricultural Agency

ROLE OF TECHNOLOGY AS A BASIS OF CLEANER PRODUCTION

PROFESSIONAL PAPER

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ABSTRACT

The present study describes the general cleaner production aims, which correspond to the prevention criteria of the IPPC-Directive. It presents cleaner production practices and technologies, and examines the methods of successful application of cleaner production practices in companies which seek to realize ecological targets.
Minimization of waste and reductions in material and energy inputs are the most important environmental aims. Sustainable technological development and innovations do not automatically lead to total reduction of environmental burden of industrial production. However, technological innovation is an important factor and seems to play a central role in the long-term initiation of cleaner production. Sustainable technology is usually connected with the design and analysis of complex, integrated management systems and sustainable development, and it is a central target in environmental science and growth of global economies. Environmental improvement of a company's strategy by application of the idea of cleaner production linked with sustainable technologies leads to produce environmentally friendly products and leads to increase the position of company on the market.

Key words: theoretical fundamentals of cleaner production, clean technology, sustainable development

INTRODUCTION

The industrial engineering consumes materials and is dependent on a continuous supply of them. Increasing population and living standards cause the consumption rate to grow. Finding ways to use materials more efficiently is a prerequisite for a sustainable future. Recent global attention to the issues and challenges of sustainable development is forcing industries to conduct self-assessments to identify where they stand within the framework for sustainability, and more importantly, to identify opportunities, strategies and technologies that support achieving this goal. Design for environmental sustainability is the long-term view: that of adaptation to a lifestyle that meets present needs without compromising the needs of future generations. The time-scale is measured in decades or centuries and the adaptation required is much greater.

Environmental technology (abbreviated as *envirotech*) or green technology (abbreviated as *greentech*) or clean technology (abbreviated as *cleantech*) is the application ofenvironmental science and green chemistry to conserve thenatural environment and resources, and to curb the negative impacts of human involvement. Sustainable development is the core of *environmental technologies*.

Sustainable engineering is the process of using energy and resources at a rate that does not compromise the natural environment, or the ability of future generations to meet their own needs.

This is the time when people try to reach sustainable development through achievement of zero landfill status, minimize storm water discharge and

pollutant loadings into protected waters of the state, reduce energy consumption, andattempttocreateself-supportinginfrastructures.

Considering the costs of energy, inefficiency of generating waste, it is the sustainable development that we need to focus on in the future. Many of the critical environmental problems we face today are related to water, energy food security and waste. These involve low tech solutions which are available now and can be applied immediately; information on these technologies can be distributed broadly using electronic networks. These are four specific activities in support of sustainable development.

1. Re-address engineering responsibilities by incorporating sustainable development principles into the codes of ethics of engineering organizations throughout the world.
2. Incorporation of long term environmental impacts and costs into the analysis of alternative solutions being considered.
3. Information exchange is a very important part of sustainable development and technological innovation.
4. Near term solutions to critical global environmental issues such as fresh water and global climate change exist for application in both developed and developing countries and for all regions of the world. These solutions can be put in service in one to three year timeframe by engineers, business leaders and government policymakers.

1. The material life cycle and criteria for assessment and energy

The material life cycle is shown in Figure 1, drawn from the Earth's resources, which are processed to give materials. These are manufactured into products that are used and, at the end of their lives, discarded, a fraction perhaps entering a recycling loop, the rest committed to incineration or landfill. Energy and materials are consumed at each point in this cycle (we shall call them "phases") with an associated CO_2, SO_x, NO_x and other emissions and gaseous, liquid and solid waste, collectively, called environmental "stressors". These are assessed by the technique of life cycle analysis (LCA). A rigorous LCA examines the life cycle of a product and assesses in detail the eco-impact created by one or more of its phases of life, cataloging and quantifying the stressors. This requires information for the life history of the product at a level of precision that is only available after the product has been manufactured and used. It is a tool for the evaluation and comparison of existing products, rather than one that guides the design of those that are new. A full LCA is time consuming and expensive, and it cannot cope with the problem that 80% of the environmental burden of a product is determined in the early stages of design, when many decisions are still fluid. This has led to the development of more approximate "streamline" LCA methods that seek to combine acceptable cost with sufficient accuracy to guide decision-making, the choice of materials being one of these decisions. But even then there is a problem: a designer, seeking to cope with many interdependent decisions that any design involves, inevitably finds it hard to know how best to use data of this type. How are CO_2 and SO_2 emissions balanced against resource depletion, toxicity or ease of recycling?

There is an international agreement: the Kyoto Protocol of 1997 binding the developed nations that signed it to

progressively reduce carbon emissions, i.e. CO_2. At the national level the focus is more on reducing energy consumption,but since this and CO_2 production are closely related, they are nearly equivalent. Thus, there is certain logic in basing design decisions on energy consumption or CO_2 generation (Fig. 1).

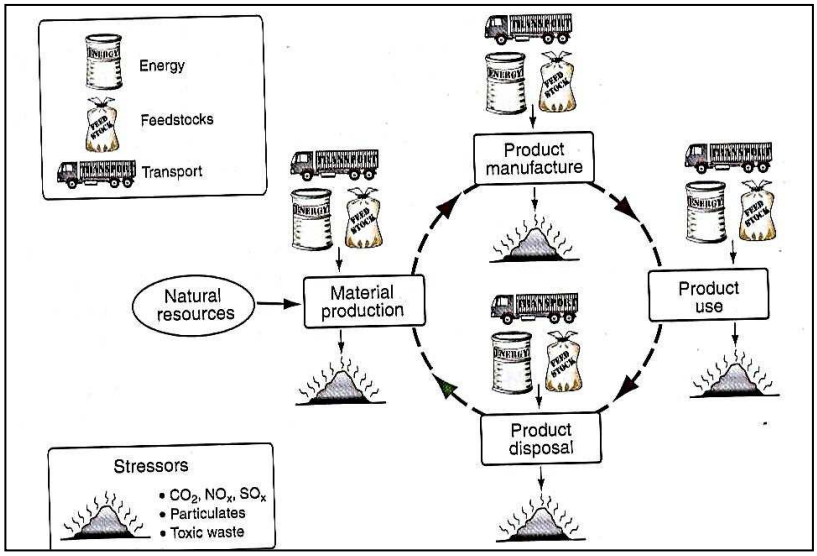

Figure 1. Ore and feedstock are mined and processed to yield a material. This is manufactured into a product that is used and, at the end of its life, discarded or recycled. Energy and materials are consumed in each phase, generating waste heat and solid, liquid and gaseous emissions. The material life cycle. (B. Allenby, 2004)

2. Sustainable technology andnatural environment

There are a lot of possibilities to reduce the environmental burden of industrial production. For example, optimization of the environmental performance through goodhousekeeping,totalqualitymanage-ment, application of end-of-pipe techniques, recycling of wastes, nonrene-wableproducts substitution or adaptation to clean technologicalinnovations. Clean technology is the most important factor for economic growth of industries and it seems to play a main role not only in the idea of cleaner production, but also in sustainable development. The development of clean technology seems to be the main factor of a company's strategy. Each of the companies, which wants to reach the competitive position on the market and wants to be environmentallyfriendly should compile the strategy of technology.The risk of initiation of a strategy of technology may be limited across accumulating, processing and using in decision making process, on information about techniques, products, machines, capital and human resources and environmental parameters.The basic actions of preparation of the strategy of technology contains a recognition of all using technologies in company and an identification of allcomponents of technology (Fig.2), which are the object of scientific investigations.

Analyzing of all components of technology is very important. It helps in the selection of suitable techniques of production, which should guarantee established productivity, quality of realized processes and allows to manufacture ecological products. The initiation of the new technology is very expensive process, however in along period of time, technology is one of main factors which influences quality of products.

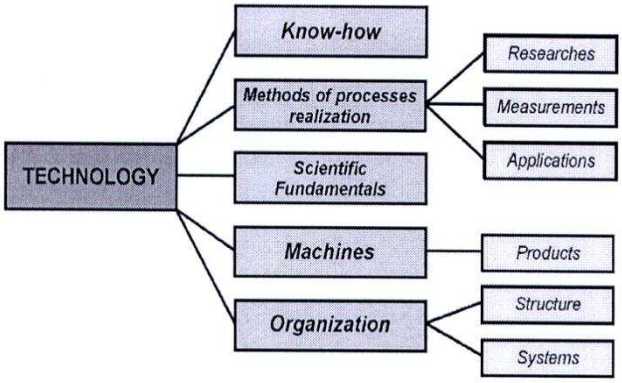

Figure 2. Components of technology (W. Shramm et al., 1998)

The better quality of products causes not only the growth of competitiveness, but what is more, it influences the productivity of process, as a result that the modern technologies influence shortening the duration of the production cycle and increasing the number of products.

Inractice, a technology and realization of technological processes isin the exact relationship from elements of working and natural environments. Steering of technological processes cannot be realized without consideration of all settings in company processes and external environment (Fig.3).

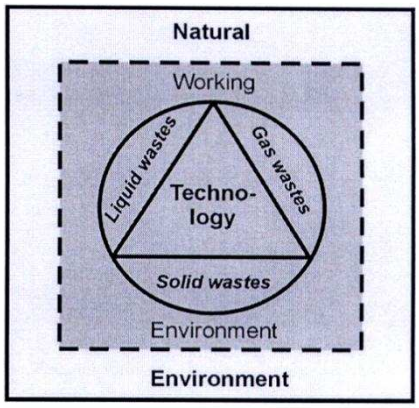

Figure 3. Technology and natural environment (F. A. Vollenbroek, 2002)

Because of the fact that the process technologies should be carried out from a cleaner production point of view, the development of sustainable technology should be based on the general cleaner production aims. The technological process, which is based on clean technology should tend to reduce or minimize the amount of:

- resources consumed;
- waste and emissions generated;
- hazards of the waste and emissions generated (mainly by usage substitution of input materials);
- risk of accident or malfunction.

The mentioned purposes essentially correspond to the prevention criteria of theIPPC-Directive (Integrated Pollution Prevention and Control). According to the

above ecological targets, companies should applythefollowingtechnologicalinnovations:

- auxiliary technology, which includes all the supporting technologies to monitor and control the existing production process and all the logistics and technological infrastructure;
- end-of-pipe technology,which can be defined as all techniques added at the end of the existing processes to decrease the amount of environmentally harmful emissions;
- in-process technology, which includes improvement and application of the existing technology - changes are integrated within the process hardware of the existing production steps;
- newtechnology, which includes a new production process principle or a new technical plant design.

Generally, most companies, when implementing changes in theirproduction process, apply the first three stages of technological innovations: auxiliary technology, end-of-pipe technology or in-process technology. However, introducing a new (sustainable) technology brings the best profits.

Cleaner production is defined as the continuous application of an integrated preventative environmental strategy to processes and products to reduce risks to humans and the environment. As for production process, cleaner production includes conserving raw materials and energy, eliminating toxic raw materials and reducing the quantity and toxicity of all wastes.

Successfulapplicationofcleaner production in companies depends on property management, maintenance, adequateinfrastructure and training of people. The transfer of cleaner production practices should be realized by:

- technological capacity (ability to adapt clean technologies),
- training capacity (ability to train and educate various groups of people about the ideas of cleaner production),
- institutional capacity (ability to network and co-operate among different stakeholders),
- government capacity (ability to prepare andimplement policies in different policy fields).

Technological capacity is one of the most important methods of applying the idea of cleanerproduction.Environmentaltechnolog y is usually connected with the design and analysis of complex, integrated management systems and sustainable development in the areas of:

- role of thedesign in the operation of environmental technology, control of integrated environmental systems,
- role of computer methods in the operation and control of environmental systems,
- education and training requirements to provide efficient operation and maintenance of complex environmental systems in the range of clean technology.

The successful promotion of idea of cleaner production and environmentally sound technologies is necessary to:

- built business strengths of company,
- connect the business and environmental advantages of sustainable technology,
- initiate long-term investments in the technology transfer and development,
- existence of government assistance and support mechanisms.

However, cleaner production and sustainable technologies will not be efficient without environmental management systems, which are the framework, set by top management of company.

CONCLUSION

Minimization of waste and emissions and reductions in material and energy inputs are the most important environmental aims. Sustainabletechnologicaldevelopment and innovations do not automatically lead to total reduction of environmental burden of industrialproduction.However,technological innovation is an important factor and seems to play a central role in the long-term initiation of cleaner production.

Environmental improvement of acompany's strategy by applicationof the idea of cleaner production linked with sustainable technologies leads to produce environmentally friendly products and moreover leads to increase the position of company on the market. Cleaner products must be given an essentially stronger meaning in the future because of the necessary transition to sustainable economy and development.

Sustainable development and idea of cleaner production is a central target in environmental science and plays a key role in the growth of global economies. Therefore, modern industrial and manufacturing companies should apply technologies designed to minimize pollution and use of finite resources. These technologies tend to improve the global environment and human life.

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SURFACE MODIFICATION OF POLY (VINYLIDENE FLUORIDE) TO MINIMISE PROTEIN ADSORPTION

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

A method for grafting PEG onto modified poly (vinylidene fluoride) (PVDF) films was developed to produce a biocompatible surface coating. Surfaces covered with polyethylene glycol (PEG) have been shown to be biocompatible because PEG yields nonimmunogenicity, nonantigenicity and protein rejection. Due to the fact that PVDF does not possess functional groups which allow a surface modification, the first step was to create amino groups on the PVDF surface by chemical vapour deposition (CVD) polymerisation of 4-amino[2.2]-paracyclophane. These surface amines reacted with fomyl-terminated PEG´s with various molecular weights in order to obtain varying chain density of PEG.
All modification steps were verified by means of X-ray photoelectron spectroscopy (XPS), attenuated total reflection infrared spectroscopy (IR-ATR) and contact angle measurements. The influence of the chain length on lysozyme repellence was investigated by means of surface-MALDI-ToF mass spectrometry. Lysozyme adsorption was significantly suppressed on the PVDF surface modified with PEG 5000.

Keywords: Protein adsorption, CVD, PEG, PVDF, XPS, Surface-MALDI-ToF-MS

INTRODUCTION

In biomaterial research there is a strong interest in new materials, which combines the required mechanical properties with improved biocompatibility. Instead of developing new biomaterials, surface coating or surface modification presents a way to preserve the mechanical properties of established materials and to improve the surface biocompatibility[1]. An effective approach for developing a clinically applicable biomaterial is to modify the surface of a material that already exhibits excellent biofunctionality and bulk properties.
The aim of biomaterial research is to biologically functionalize implant surfaces which interact with the body so that the biological system will not be disturbed, and at the same time the correct adhesion and differentiation of the cells will be stimulated. Many synthetic surfaces adsorb proteins when exposed to blood or other protein-containing solutions[2]. These interface-related phenomena often result in a number of severe clinical complications such as implant-associated infections or thrombus formation on blood-contacting artificial surfaces and are therefore crucial for biocompatibility of the synthetic material. The rate and amount of adsorbed protein are dependent on the physico-chemical properties of the polymeric surface such as wettability and surface charge density. Therefore surface modification procedures are developed to create ultrathin protein-repellent nano-

structured and biologically functionalized interfaces on the implant surfaces.

Grafting of poly (ethylene glycol) (PEG) is a common strategy for reducing the non-specific adsorption of biomolecules on surfaces[3-5]. Several theories – both qualitative and quantitative – have been proposed to explain the effectiveness of PEG as a component of protein-resistant surfaces[6-9]. The protein rejecting capability of PEG-containing coatings depends on a range of parameters, including PEG molecular weight, interfacial PEG chain density, polymer chain architecture, etc, whereas the interfacial graft density has proved to be the most crucial property of a PEG layer for minimizing protein adsorption[9-11].

Poly (vinylidene fluoride) (PVDF) has been used as a biomaterial due to its nontoxicity, extraordinary durability and chemical stability[12]. Grafting of PEG onto the surface of PVDF should improve the biocompatibility of this material. Due to the fact that PVDF does not possess functional groups which allow a surface modification, creation of functional groups onto PVDF surface is required. The coating of materials by chemical vapour deposition (CVD) polymerisation process using functionalised paracyclophane enables the introduction of functional groups (e.g. amino groups) in a single solvent-free step. The CVD polymerisation is already used for biomaterial applications and seems to be an ideal method for the coating of metal surfaces[13]. The coating of polymer surfaces by CVD is relatively new method.

The understanding of the interaction which takes place between the modified PVDF surface and the components of the biological system is an important requirement of biomaterial development. Therefore all modification steps were verified by means of X-ray photoelectron spectroscopy (XPS), attenuated total reflection infrared spectroscopy (IR-ATR) and contact angle measurements. Protein adsorption studies were carried out by means of surface MALDI-ToF-MS.

The present study involves the development of a bioactive PVDF-surface which minimise protein adsorption and subsequent cell adhesion. In the present study the effect of the chain density of PEG grafted onto modified PVDF surfaces on minimization of protein adsorption and cell adhesion was studied. Lysozime was chosen as model protein. This protein is abudant in a number of secretion such as tear, saliva, human milk and mucus.

The PEG graft-modified PVDF films can be used directly as a biomaterial with a nonfouling surface while still bearing the excellent bulk properties of PVDF.

MATERIALS AND METHODS

2.1. Polymer and chemicals

PVDF was purchased from Solvay Adv. Polym (France). Phosphate-buffered saline (PBS) was obtained from Merck (Germany). Sodium cyanoborohydride were purchased from Fluka (Germany). Methoxy-terminated formyl-PEG (M-PEG, molecular weight 5000 and 30000) were purchased from Shearwater Polymers. Lysozyme was obtained from Sigma-Aldrich (Germany).

2.2. Chemical functionalisation

CVD polymerisation was carried out in a self-designed installation, which allows high freedom in selecting the process parameters. The monomer 4-Amino[2.2]-paracyclophane was synthesized from [2.2]-paracyclophane by the method described elsewhere[14]. After the procedure a PVDF-surface is coated with poly [*o*-amino-*p*-

xylylene-*co*-*p*-xylylene] (PVDF-amino-ppx).

M-PEG was grafted onto aminated surface by reductive amination, using $NaCNBH_3$ as the reducing agent for the intermediate Schiff base. The grafting was performed under marginal solvation conditions (´cloud point`). 1 mg/ml M-PEG was dissolved in 0.1 M sodium phosphate buffer at pH 6.3 containing 11% (w/v) K_2SO_4. 3 $mgml^{-1}$ $NaCHBH_3$ were added prior to the immersion of the aminated PVDF substrates. The reaction was carried out at 60°C for 8 hours.

2.3. Protein adsorption

PVDF and PVDF-modified surfaces were incubated in 1 mg/ml lysozyme in PBS for 1 hour at 37°C. The samples were rinsed 3x with water and analysed immediately with MALDI-ToF-MS.

2.4. Physical and chemical surface characterization

All X-ray photoelectron spectra (XPS) were recorded on an X-Probe™ 206 spectrometer (Surface Science Instruments, Mountain View, CA). An aluminium anode producing AlK_α X-rays at 1486.6 eV was used as an X-ray source. The binding energies are referenced to hydrocarbon at 285.0 eV.

IR spectroscopic investigations were carried out with a Nicolet 710 SXR Fourier transform infrared (FTIR) spectrometer. Spectra were recorded in the attenuated total reflection mode (ATR) using a Ge-crystal.

Contact angles were measured using the captive bubble method with pure water at room temperature on a G40 system (Krüss, Hamburg, Germany).

Surface-MALDI-ToF-mass spectra were obtained using a BRUKER BIFLEX™ III MALDI time-of-flight mass spectrometer (Bruker-Franzen Analytik GmbH, Bremen, Germany) equipped with a nitrogen laser (337 nm wavelenght and 3 ns pulse width). In present experiments a small sample piece cut from PVDF modified films was placed onto the stainless steel MALDI sample holder. Sinapinic acid in a 0.1% solution of trifluoroacetic acid in acetonitrile/water was applied onto the sample surface and the solvent was left to evaporate before the sample holder was inserted into the spectrometer.

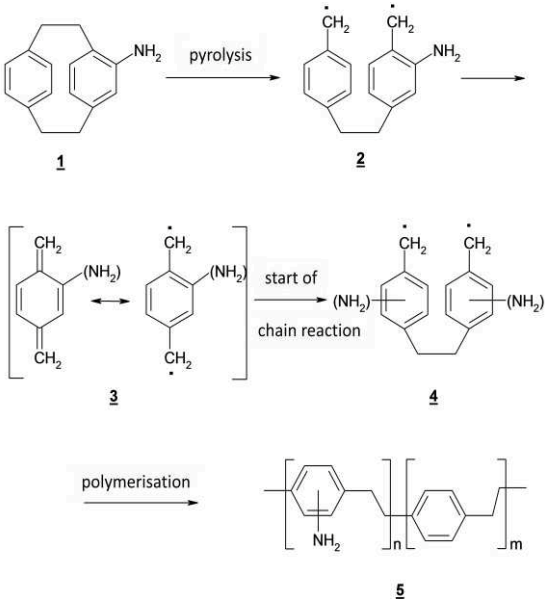
RESULTS AND DISCUSSION

3.1. Surface modification

Chemical vapour deposition (CVD) of 4-Amino-[2.2]-paracyclophane is used as a method to introduce amino functional groups on PVDF surface. Scheme 1 illustrates individual reaction steps which lead to creation of PVDF-amino-ppx surface.

During the pyrolysis at >600°C, a homolytic cleavage of one of pcp-bridges of amino-pcp **1** occurs and biradical **2** is formed. Those biradicals are cleaved further to high reactive monomers **3**. Reactive species recombine on the cooled sample surface which is the start of chain polymerisation reaction that in subsequent process leads to formation of Amino-ppx **5**.

The hydrophilicity of the surfaces, monitored by the contact angle is expected to be significantly reduced for the modified surfaces.



Scheme 1. *Reaction steps during CVD polymerisation which lead to creation of amino-ppx surface*

The „captive bubble“ contact angle of untreated PVDF averages 72° indicating the hydrophobic nature of the material. After modification with 4-Amino [2.2]-paracyclophane, (PVDF-amino-ppx) surface shows a contact angle of 54° having a moderate hydrophobic character. Grafting of PEGs to the PVDF-amino-ppx surfaces leads to an increase in wettability with water (contact angles less than 20°). There is no detectable influence of the PEG length on the wettability.

The modification of the PVDF surface was studied qualitatively by the IR-ATR spectra. Figure 3 shows the IR-spectra of PVDF and PVDF-amino-ppx.

The IR-ATR spectrum of PVDF-amino-ppx (Figure 3-2) shows characteristic absorption bands attributed to ppx-scafold vibration with a strongest band (C=C)- vibration at 1520 cm^{-1}. Also, characteristic absorption bands attributed to primary amino groups are present at 3445 and 3362 cm^{-1}, a NH-deformation vibration at 1623 cm^{-1} and a CN-valence vibration at 1292 cm^{-1}. The band at 812 cm^{-1} is attributed to aromatic CH- deformation vibration. The grafting of PEG is not detected by IR-ATR.

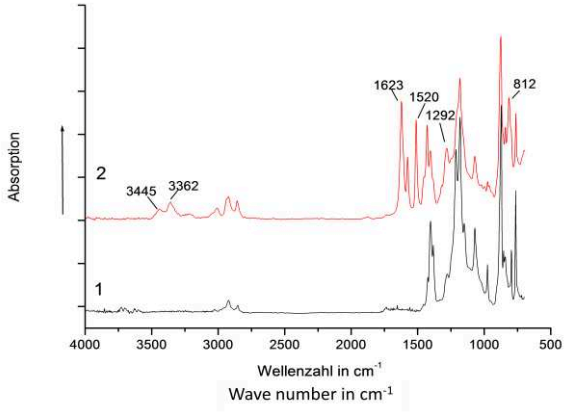


Figure 3. *Overlay of IR-ATR spectra: (1) unmodified PVDF, (2) PVDF-amino-ppx*

Quantitative information about the chemical composition of the outermost (10 nm) surface layer of the unmodified and modified PVDF surfaces has been obtained by means of XPS. All modifications steps were reflected in clear alterations of the surface element composition. XPS data showing the elemental composition as well as the different carbon species are listed in Table 1.

Theoretical ratio of carbon to fluorine in PVDF sample ($-CH_2-CF_2-$) should be 1:1. Measured value is 46.9-atom % carbon to 49.8-atom % fluorine, also oxygen and nitrogen as contaminant are detected. Theoretical ratio in high resolution C1s spectra for PVDF for ($\underline{C}H_2-CF_2$)-carbons pecies at 286,5 eV and for ($CH_2-\underline{C}F_2$)-carbon species at 290.9 eV should be 1:1. Results of XPS measurement showed the ration of 23,4 atom-% to 20,3 atom-%. Remaining 3.2 atom-% belongs to aliphatic carbon (C-H, C-C) at 285.0 eV. The XPS spectrum of the amino-ppx on PVDF shows increase in nitrogen content after coating to 5,3 atom % nitrogen due to introduction of amino groups, whereas fluorine disappeared (Table 1).

Table 1. *Untreated and modified PVDF surfaces characterized by means of XPS for elemental composition and binding energy*

Surface	**Carbon (C1s)** atom-%				**O1s** atom-%	**N1s** atom-%	**F1s** atom-%
		Binding energy (eV)			Binding energy (eV)		
		285.0eV	286.5eV	290.9eV	532.1eV	399.7eV	688.0eV
		C-H,C-C	C-O,C-N CH_2-CF_2	CF_2-CH_2	C-O	C-N	C-F_2
1. PVDF	46,9	3,2	23,4	20,3	2,4	0,9	49,8
2. PVDF-amino-ppx	89,5	83,7	5,8	-	5,2	5,3	-
3. +mPEG 5000	71,8	18,6	53,2	-	27,1	1,2	-
4. +mPEG 30000	75,8	32,3	43,5	-	22,2	2,0	-

The intensity of C-C photoline from aromatic ring in amino-ppx surface increases obviously. The peak at 286.5 eV is attributed to the C-N component instead of CH_2-CF_2 because no fluorine was detected on PVDF-amino-ppx surface. At the same time the C1s peak of virgin PVDF at 290.1 eV has disappeared. The peak at 286.5 eV is an indicator for amino groups present on the surface. After introduction of amino groups onto PVDF surface, the modified PVDF surface is suitable for grafting of PEG-chains as a protein repellent surface. The efficiency of grafting the M-PEG chains onto the PVDF-amino-ppx surface was derived from the ratio of the XPS intensities of the ether carbon atom (originating from PEG) and alkyl carbon atom (originating from PVDF-amino-ppx). Deconvolution of the C1s-XPS of the PVDF-amino-ppx-PEG 5000 surface shows two contributions with respective binding energies of 285.0 eV and 286.5 eV. The dominating 286.5 eV contribution from the ether carbon atom of M-PEG 5000 indicates a successful binding reaction between M-PEG 5000 and the amino-ppx coated surface (C-O/C-C 2.86). Pure PEG samples exhibit a single C1s peak centred at 286.5 eV. As shown in Table 1, the grafting of the M-PEG 30 000 was less successful than that of M-PEG 5000 (C-O / C-C 1.35).

The XPS data of Table 1 also show that both M-PEG coatings invariably are thinner than the information depth as none of the analyses matches the theoretical composition of pure PEG (a single C-O peak and no N from underlaying amino-ppx layer). Thus, XPS represent superpositions from the M-PEG coatings and the underlying layers.

The ability of M-PEG layers to repel *in vitro* the lysozyme was assessed by surface-MALDI-ToF mass spectrometry. Surface-MALDI-ToF-MS detects small amounts of adsorbed material[15] but is difficult to quantify.

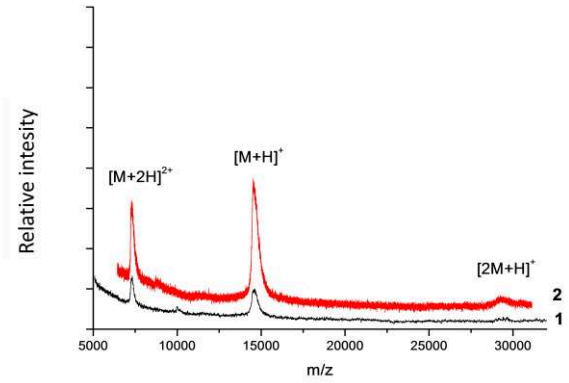


Figure 4. *Surface-MALDI-ToF mass spectrum recorded of (1) PVDF and (2) PVDF-amino-ppx surfaces after exposure to lysozyme solution. The observed peaks are indicative of selective adsorption of lysozyme*

Lysozyme is a relatively small protein with a net positive charge at pH 7.4 (physiological condition). Amino groups from amino-ppx surface are positively charged at pH 7.4[14]. Lysozyme uptake into positively charged layers should be reduced due to charge repulsion effects. However, Figure 4 shows that modification of PVDF with amino-ppx layer grafting yields a surface that strongly adsorbs lysozyme. The density of amino groups on the ppx-layer is not high and a relatively small protein such as lysozime can adsorb between amino groups on the hydrophobic ppx-surface. The peaks observed at m/z values of 14229 and 7072 are assigned to the protonated molecular ion $(M+H)^+$ and the doubly charged molecular ion $(M+2H)^{2+}$ of lysozyme. The reason why the m/z value of the $(M+2H)^{2+}$ ion is not exactly half that of the $(M+H)^+$ ion is that introduction of a sample of finite thickness leads to an indeterminate shift in the mass scale[16].

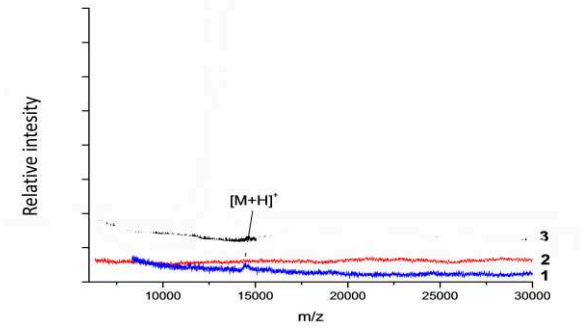


Figure 5. *Surface-MALDI-ToF mass spectrum recorded of (1) PVDF-amino-ppx-PEG 30000 and (2) PVDF-amino-ppx-PEG 5000 coating after exposure to lysozyme solution*

Figure 5 reproduces a Surface–MALDI-ToF mass spectrum recorded on the PVDF-amino-ppx-PEG ´cloud point´ grafted coating after immersion for 1 h in the 1 $mgml^{-1}$ lysozyme solution in PBS pH 7.4. As expected, no Surface-MALDI signals were detected for the M-PEG MW 5000 coating. For M-PEG MW 30000 there is still some evidence of protein adsorption, indicating that grafting conditions are not sufficient to obtain protein repellancy.

CONCLUSION

In the present study PEG chains with different length were grafted onto PVDF modified surfaces. The aim was to achieve minimization of protein adsorption. A coating was fabricated with different length of the PEG chain by covalent coupling of methoxylated PEGs on the amine surface. Elevated temperature and a high salt content were used to produce the marginal solvation conditions (´cloud point`) required for the highest PEG coverage on the surface[17]. The results show that the PEG surface coverage on amine interlayers depends on the ethylene oxide chain length

and that the protein resistance of M-PEG coatings varies with the degree of coverage. The XPS data show considerably higher PEG coverage when M-PEG 5000 (shorter chain) is used for grafting. The M-PEG 30000 (longer chain) coating was found to adsorb measurable amounts of lysozyme from a PBS solution, whereas M-PEG 5000 was found to inhibit protein adsorption.

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Tečnosti za hlađenje i zaštitu motora
Maziva i tečnosti za industrijska postrojenja
Ulja za obradu metala
Mazive masti
Bazna ulja Grupa II i Grupa III
Bijeljeni parafini
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LISTERIA MONOCYTOGENES IN FOOD AND WATER ENVIRONMENT OF TUZLA

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

Listeria monocytogenes (*L. monocytogenes*) is a pathogenic bacterium, the causative agent of oflisteriosis, a disease in humans and animals. Listeriosis belongs to diseases with most common deadly outcome estimated at about 30%. Routes for spread of infections are numerous and insufficiently researched. It has been isolated almost from all environments and all food categories, and there is more and more evidence that contamination of food products originates from environmental sources. The objectives of this research were to determine participation of *L. monocytogenes* speciesin four categoriesof food(milk and dairy products, meat and meat products, vegetables and fruit), which are sold in the area of municipality of Tuzla, determine the presence of the mentioned bacterium in water environment (the Jala river and lake of Modrac) and test the serologic characteristics and antibiotic sensitivity of the isolated strains. Of total 210 food and water samples *L. monocytogenes* were isolated from 6.66% samples of meat and meat products and from 36.4% samples of water, while it was not isolated from milk, dairy products, fruit and vegetables.*L. monocytogenes* isolates belonged to the most common causes of human listeriosis. Serotype 1/2a was proportionally distributed in food (meat and meat products) and water. In samples of water, serotypes 1/2b and 4b were isolated while in food samples serotype 1/2c was isolated as well. All isolates of *L. monocytogenes* are sensitive to chloramphenicol, erythromycin, gentamicin,ampicillin, penicillin G, streptomycin, tetracycline, trimethoprim-sulfametoxazole andvancomycin, and are resistant to ceftriaxone. Since Bosnia and Herzegovina is one of the few countries in which legally binding provisions of food control for the presence of *L. monocytogenes*still do not exist, results of this research might make a contribution to a procedure of introduction of legal obligation of food control to this pathogen.

Key words: *L. monocytogenes, serotypes, food, water, antibiotics.*

INTRODUCTION

Listeria monocytogenes (*L. monocytogenes*) is a pathogenic bacterium, the causing agent of listeriosis, a disease in humans and animals. Routes for spread of infections are numerous and insufficiently researched. It has been isolated almost from all environments and all food categories, and there is more and more evidence that contamination of food products originates from environmental sources. Ability of propagation at refrigeration temperatures (+4°C) makes this microorganism pose a big problem in food industry, and a potential health threat[1]. Interest of world public in this bacterium has not ceased since the epidemics in South California in 1985, caused by consumption of contaminated food[2].*L. monocytogenes* is, in approximately 1% cases a causative agentof alimentary infections[3], so listeriosis is, as a food-borne disease, underestimated againstsalmonellosisandcampylobac-teriosis. However, it is important to emphasise that listeriosis is classified in

diseases with most common deadly outcome estimated at about 30% [4].

Of thirteen known serotypes within this type, the most common causative agents of human listerioses are: 1/2a, 1/2b and 4b. Within *L. monocytogenes* serotypes a big diversity with extremely variable pathogenic potential is present. While some strains of *L. monocytogenes* are very pathogenic and sometimes deadly for humans and animals, the others are relatively avirulent and do not cause a huge damage in the host.

Listeriosis therapy is based on use of penicillin or ampicillin, separately or in combination with aminoglycosides, or for persons allergic to β-lactamic antibiotics on use of trimethoprim associated with one sulfonamide[5].4

Phenomena *L. monocytogenes*resistanceto antibiotics are very rare, however, in the past years the strains resistant to ampicillin, tetracycline,trimethoprim-sulfamethoxa-zole, gentamicin, methicillin, streptomycin, and erythromycin[6] have been frequent. Although a few researches conducted in our country have shown a high contamination of raw meat with *L. monocytogenes*[7], Bosnia and Herzegovina is still one of the few countries in which legal obligations of food control to this pathogen still do not exist. The main intentions of this research referred to:

a) Determining the presence of *L. monocytogenes*species in four categories of food (milk and dairy products, meat and meat products, vegetables and fruit), which are sold in the area of municipality of Tuzla

b) Determiningthe presence of *L. monocytogenes*species in water environment (the Jala river and Modrac lake) in the area of municipality of Tuzla

c) Determining the presence of *L. monocytogenes*species in relation to other species from the genus *Listeria*;

d) Testing the antigenic characteristics of the isolated strains of *L. monocytogenes*that would offer us an insight into their serotype attachment and possible link with strains from water environment and potential health threat, and

e) Testing the sensitivity of the isolated strains of *L. monocytogenes*to antibiotics that are most frequently used in therapy in medicineandveterinarymedicine(penicillin, ampicillin, tetracycline, erythromycin, gentamicin, streptomycin,trimethoprim-sulfametoxazole,chloramphenicol,vancomycin and ceftriaxone).

MATERIAL

Microbiological analyses to presence of *Listeria* spp.was conducted on 210 samples, thereof 180 food samples and 30 water samples. All samples were collected in September and October of 2010. Samples of milk and dairy products (n=60), fruit (n=30) and vegetables (n=30) were collected from street and market vendors, while meat and meat products (n=60) were sampled in butcher shops of the municipality of Tuzla. Samples of surface waters were collected on 15 localities of the Jala River with tributaries and 15 localities of Modrac Lake with tributaries. Designations of samples are shown in Figure 1 and Figure 2. Water was sampled into sterile bottles of 1l.

METHODS

Samples for analyses were transported to a laboratory in the refrigerator with ice and were kept in a refrigirator at temperature of

+4°C until processing. All samples were analysed within 6 hours from the moment of sampling.

Isolation of *Listeria* spp. from food.For isolation of listeria, a horizontal method was used for detection and enumeration of *Listeria monocytogenes*, ISO 11290-1:1996/Amd.1:2004 (E)[8].

Isolation of *Listeria* spp. from water.From each sample of water 100 ml was filtered through sterile filters with pores of 0,45 μm (Millipor), which were thereupon aseptically transferred into 90 ml ½ Fraser broth and incubated at 30°C/24 hours, and then 0,1 ml of inoculum was transferred into test tubes with 10 ml of Fraser broth. Incubation of innoculated medium lasted up to 48 hours at 35°C. Screening on selective hardsubstrate,identificationandconfirmation was performed the same way as with food analyses.

Serotypization of *L. monocytogenes*isolates. Phenotypic subtypization of *L. monocytogenes*was carried out with use of antiserum against somatic (O) antigens and flagellar (H) antigens according to producer's instructions (Denka Seiken, Tokyo, Japan).

Testing the sensitivity of *L. monocytogenes*to antibiotics

Testing was performed according to recommendations of Clinical and Laboratory Standard Institute (CLSI) with disk-diffusion method[9,10]. Mueller Hinton (MH) agar was used, and zones of inhibition (diameters) were interpreted according to the CLSI standards for stafilococi, due to lack of specific standards for *Listeria* spp. In antibiogram test, 10 antibiotics, which are often used in human and veterinary medicine, were used. The following antibiotics were used (Liofilchem, Italy): chloramphenicol 30μg,ceftriaxone 30μg, erythromycin 15μg, gentamicin10μg,ampicillin 10μg, penicillin G 10IU, streptomycin 10μg, tetracycline 30μg, trimethoprim-sulfametoxazole 1,25/23,75μg,vancomycin 30μg.

RESULTS AND DISCUSSION

Isolated strains of *L. monocytogenes*had identical microscopic picture of gram positive, asporogenic rods distributed separately, in pairs, in form of the letter V or more of them parallel. Macroscopic aspect of the colonies of all isolates of *L. monocytogenes*grown on the selective substrate was also identical. On Oxford agar, after 24 hours typical colonies increased for listeria; small, diameter of 1mm, surrounded by gray and black aureole. After 48 hours, the colonies got dark, sometimes with greenish glow, and were approximately 2 mm in diameter with black aureole and recessed centre. Isolated strains of *L. monocytogenes*were motile, which manifested in typical increase on BHI+0,25% agar after incubation at 25°C from 24 to 48 hours. All isolates of *L. monocytogenes*were catalase-positive and oxidase negative. They had the same biochemical profile in the tests of Microgen Listeria-ID System, in fact they showed a positive reaction to esculin, arabitol, rhamnose, trehalose, methyl -D-glucose, methyl -D-mannose and hemolysis of sheep erythrocytes; and negative to mannitol, xylose, ribose, tagatose and glukose-1-phosphate. Reaction of hemolysis was confirmed also on blood agar, on which small colonies were formed with a narrow zone of β hemolysis.

Meat and meat products

Incidence of listeria in analized food and water samples is shown in Table 1. Bacteria

from the genus *Listeria* were isolated in 90% (27/30) of raw meat samples (minced meat, ćevapčići and sausages). *L. monocytogenes*was isolated in 6,66% (2/30), and *L. innocua*in 83,33% (25/30) of samples. The similar data ware obtained by researchers in Turkey (5%)[11], Egypt[12] (6%) and Slovenia[13] (6,81%).Researches in Italy[14] showed incidence of *L. monocytogenes*in beef in 5,4% of the samples, and in minced meat and sausages 4,5%. Significantly higher incidences were depicted by several researchers. In his research in the area of BiH, Lončarević stated incidence of *L. monocytogenes* of 20%[15], whileHodžić and Hukić[7] isolated *L. monocytogenes*in 34,3% samples of raw meat from the area of North East Bosnia. Bunčićisolated *L. monocytogenes*in 69% of samples of minced meat in Yugoslavia[16]. In Spain, *L. monocytogenes*was isolated in *34,9%[17],* in Belgium42,1%[18] and in Great Britain from 20% to 40% [19] samples of raw meat. The highest incidence of *Listeria* spp. in raw meat of 100% was reported in Canada with 77% samples positive to *L. monocytogenes*[19].Results of our research showed the presence of *Listeria* spp. in finished meat products (dried meat and soudjouk) in 33,33% (10/30) samples, and *L. welshimeri*in 6,66% (2/30) samples and *L.innocua*in 20% (6/30) samples were alsoisolated(Table 1).

Our data are similar to incidence that was determined in finished meat products in Ankara, where it was 5% (2/40), and *L. innocua* was isolated in 5% (1/20) and *L. grayi* in 5% (1/20) samples[20]. There are so many reviews in the literature that describe presence of *L. monocytogenes* in meat and meat products in higher percentage than the one that was determined in our research.*L. monocytogenes* was isolated in 28.2% samples (sausages) in Italy[21]and in 19% samples of sausages in Yugoslavia[16]. In samples of soudjouk in Turkey *Listeria* spp. were isolated in 21% (63/300) of which *L. monocytogenes* was represented with 11.6% (35/300) samples [22].

Serotypes of *L. monocytogenes* 4b, 1/2a, 1/2b and 1/2c have been isolated in more than 98% of clinical cases of human listerioses[19]. In our research, serotype 1/2a was dominant in 75%, while serotype 1/2c was represented in 25% samples. Serotypes 1/2a and 1/2c were dominant in researches in the area of North East Bosnia [23]in 41.8% and 30.9% samplesrespectively. Serotypes 4b and 1/2b were isolated in lower percentage (5.5% and 9.1% samplesrespectively). Dominance of serotype 1/2a in meat and meat products was also shown by the research in Ankara[24], in which all strains of *L. monocytogenes* belonged to this serotype, as well as by the study in Italy[23] in which serotypes 1/2a, 1/2c and 3a dominated. In comparison to the mentioned studies in which serotypes 1/2a and 1/2c dominated, in Japan[25] 1/2b (41%) and 1/2a (33%)were dominant, while 1/2c and 4b were isolated in 12% and 10% samples of minced meatrespectively. Regardless of differences in incidence, all serotypes isolated in our research and the mentioned researches represent a potential health threat.

The most frequent species in our research was *L. innocua.* In products of raw meat, it was isolated in 83.3% samples, while in finished meat products it was isolated in 20% samples. Presence of any species from the genus *Listeria* may bean indicator of bad hygiene, which can also favour survival of *L. mononcytogenes.* Contamination of meat with *L. monocytogenes*usually happens after slaughter of animals and may

originate from animals, workers' hands, equipment and tools that are used[13]. *L. monocytogenes* can attach and survive on various contact work surfaces due to biofilm forming ability [26]. Furthermore, during the process of transformation of raw meat into a meat product *L. monocytogenes* may be inserted, which depends on personal and general hygienic measures and process parameters. Direct evidence to this is the research of contamination of work surfaces in butcher shops in the area of Tuzla city[27].

Milk and dairy products

In domestic literature there are no data on researches of presence of listeria in raw milk, cheese and cream, while foreign literatureis full of data on incidence of listeria in milk and dairy products. The data from different parts of the world often differ essentially. Results of our research did not show the presence of listeria in samples of raw milk, cheeses and cream.Listeria species in raw milk were not been isolated neither by researchers from Brazil and Italy in their studies. The other group of authors from Brazil[28]states that from total of 12.7% samples of raw milk and from 0.9% samples of pasteurized milk, bacteria from the genus *Listeria*were isolated. In research of Benić[29]in Croatia bacteria from the genus *Listeria*were isolated in 0.8% samples of raw milk. The other group of authors[2] from that country reports on incidence of listeria in samples of fresh cheese of 6.66% (4/60),and in cream samples of 10% (6/60). *L. monocytogenes*was isolated only in 1.66% (1/60) cream samples, while in the cheese samples no isolation of *L. monocytogenes*was found. Pintadoet al. reported on incidence of *L. monocytogenes* (29%) and other *Listeria* spp. (*L.inoccua i L. seeligeri*) (75%) in soft cheese produced ofraw milk in Portugal[30]. Considering that research was conducted at the beginning of autumn and that researchers in Ireland [31]determined seasonal variations for spring, summer and winter; and that all dishes (packings) in which milk and dairy products were sold did not satisfy visually the basic hygiene standards, such results of our research cannot be attributed with certainty either to seasonal variations or high hygiene standards, which are applied in households in the area of the Tuzla region.

Fruit

Microbiological analysis of fruit for presence of bacteria from the genus *Listeria* indicated presence of the mentioned bacteria in 13.34% (4/30) samples. The only representative- *L. innocua*was isolated in 40% (4/10) samples of fresh strawberries. In samples of fresh grapes and fresh plums no isolation of *Listeria* spp. was found (Table 1).A rare connection between consumption of fruit and listeriosis and the fact that most fruit grows above the ground, thereby is not the subject of frequent contacts with the ground or with listeria contaminated with feces, lead to an assumption that incidence of listeria in fruit can be low or lower than the one noticed in raw vegetables [32]. Results of our research are in favour of this assumption. Plums and grapevines grow at significantly larger distance from the ground, as a potential source of contamination, in relation to strawberries that grow and ripen on the ground or very near the ground, accordingly a considerably lower incidence of listeria is expected in/on them than the strawberries. The research showed a high contamination of strawberries with listeria of 40%, which werecontaminated either through the soil, or during other manipulations with strawberries up to a final consumer:

picking, cleaning, transportation, storage etc. Some types of fruits are a good medium for listeria growth and propagation, while the other types are unfavourable for certain acid fruit juices. Acid medium decreases growth and survival of *L.monocytogenes*on many citrus fruits andnuts. Listeria was not isolated in grapes and plums as the fruits with lower pH, while they were isolated in strawberries, which have somewhat higher values of pH. Results of Flesset al. suggest that*L. monocytogenes* is able to survive, but not to grow on surface of fresh, untouched or sliced strawberries during expected shelf life of fresh fruit[33]. Natural organic acids in plums, first of all, the apple acid in combination with a series of phytochemicals, are connected with control of microrgnisms growth [34].Extract of plum (3%) in raw meat is more than 90% efficient in prevention of growth of main food pathogens among which is also *Listeria*[35].Researches of Rhodesashowed that commercial juice of red grapes has a strong inhibitory effect on *L.monocytogenes*(number of bacteria of *L. monocytogenes*reduced by 6 log within 10minutes)[36].

Vegetables

First incidences of listeriosis were associated with consumption of raw vegetables (cabbage salad)[37].In our research, listeria were isolated in 3.33% (1/30) samples ofvegetables and *L. seeligeri*was isolated in 10% samples of cauliflower (1/10) (Table 1).In the USA, no isolation of *Listeria* spp. was found in 92 samples of cauliflower. The mentioned research included also 92 cabbage samples and 92 lettuce samples in which *Listeria* spp. were isolated in2.2% and 1.1% samplesrespectively. *L. monocytogenes*(1.1%) and *L. seeligeri* (1,1%) were isolated in cabbage samples, while *L. innocua* (1,1%) was isolated in lettuce [38]. Meloniet al. [39]reported on 2% and 24% contamination of vegetables and products of vegetables with *L. Monocytogenes*and*Listeria s*pprespectively. Also, Vitas and Garcia-Jalon[17] described 1.8% and 10.4% contamination of vegetables and products of vegetables with *L. Monocytogenes*and*Listeria* spp.respectively (*L. seeligeri, L. inoccua*and *L. welshimeri*).Mongeand Arias-Echandireported on 20% and Soriano et al. on 10% lettuce samples contaminated with *L. monocytogenes,*while Kaneko et.al. did not isolate a single *L. monocytogenes.* Similar results were obtained by Thunberg at al. who did not isolate *Listeria* spp. in 12 lettuce samples and 10 caulliflower samples [40]. On the basis of results ofWiedmannet al. who state that *L.seeligeri*is more often isolated from the ground than*L.innocua*or*L.monocytogenes*[19], we assume that contamination of caulliflower in our research most probably occurred through the soil. However, as there are no convincing proofs that the ground is a real reservoir for*L.monocytogenes*, our understanding of ecology of soil for other *Listeria*spp. is also very limited.

Although the analysed types of vegetables generally show a low incidence of*L.monocytogenes*and *Listeria*spp., contamination of vegetables with*L.monocytogenes*may be a source of infection forhuman listeriosis because these foods are mainly eaten raw or mixed with other vegetables in salads.

Water of the Jala River and Modrac Lake

All samples of the Jala River and tributaries, in our research, were positive to*Listeria* spp.,where incidence of*L.*

*monocytogenes*was recorded with 60% (Table 1). Similar results were recorded in California where *L. monocytogenes*was isolated in 62% samples of river andcoastal sea waters[19]. Incidence of *L. monocytogenes*in samples of water of Modrac Lake was 13.33%. Analysis of water from a river and a lake in Greece showed a relatively lower incidence of *L.monocytogenes*[19]than 4%. From the South Nation River in Canada, 10% (32) samples were positive to *L. monocytogenes*[41]. *Listeria*spp. were also found in most of the treated waters (84.4%) and raw sludge (89.2%) from six French urban facilities for waste water treatment and one composting plant [42]. In the mentioned study in Canada, *L. monocytogenes*isolates belonged to serotypes 1/2a and 3a (50%) and 4b, 4d and 4e (32%)[41]. In the
research in France, dominant serotypes were 4b/4e (49.5%), and 1/2a with 33.6%, 1/2b with 33.6% and other serotypes among which incidence of 1/2c was 0.93%[42]. In our research, in water of the Jala River and tributaries and ModracLake and tributaries, 63.6% of isolates belonged to serotype 4b, while serotypes 1/2a and 1/2b were present in 27.3% and in 9.1% samples respectively.

The research and the mentioned studies proved the presence of*L. monocytogenes*strains, which are indicated as the most frequent causative agents of listeriosis associated with food (4b,1/2a and 1/2b).

As the area of Tuzla city is densely populated, a high rate of isolation of listeria in our work reflects the fact that the Jala River receives numerous sewage outfalls.

Most of suburban and rural settlements do not have a constructed sewer network, which is supported by the fact that all tributaries of the Jala River contribute to its contamination. There is no facility for waste water treatment in the city. High incidence of*L. monocytogenes*in the Jala River indicates that spread of this pathogen is also possible along the course of the Spreča River into which the Jala empties. Since the Jala and Spreča rivers flow through the urban andagricultural areas, presence of*L. monocytogenes*can represent a manifold risk for appearance of listeriosis, because these waters are often used for watering the crops and for livestock watering. In addition, Modrac lake water is used for fishing, swimming and sports on water, thus representing a potential source for spreading the pathogen to wildlife, and through it indirectelly to humans as well.

Table 1. *Incidence of* listeria *in food and samples of water*

Type of sample		Number of samples	Number of isolated *Listeria* spp. (%)	Number of isolates			
				L. monocytogenes (%)	*L. innocua* (%)	*L. welshimeri* (%)	*L. seeligeri* (%)
Raw meat	Minced meat	10	9 (90)	2 (20)	7 (70)	-	-
	Ćevapčići	10	10 (100)	-	10 (100)	-	-
	Raw soudjouk	10	8 (80)	-	8 (80)	-	-
Finished meat products	Dried meat	15	5 (33,33)	1 (6,66)	3	1	-
	Soudjouk-Bosnian beef sausages	15	5 (33,33)	1 (6,66)	3	1	-
Milk and dairy products	Raw milk	30	-	-	-	-	-
	Fresh cheese	15	-	-	-	-	-
	Cream	15	-	-	-	-	-
Fruit	Strawberries	10	4 (40)	-	4	-	-
	Grapes	10	-	-	-	-	-
	Plums	10	-	-	-	-	-
Vegetables	Cabbage	10	-	-	-	-	-
	Lettuce	10	-	-	-	-	-
	Cauliflower	10	1 (10)	-	-	-	1
Water	The Jala river	9	9 (100)	3 (33,33)	6	-	-
	Tributaries of the Jala river	6	6 (100)	6 (100)	-	-	-
	Lake of Modrac	10	1 (10)	1 (10)	-	-	-
	Tributaries of Modrac	5	4 (80)	1 (20)	2	1	
Total		**210**	**62 (29,5)**	**15 (7,14)**	**43**	**3**	**1**

Serologic characteristics of *L. monocytogenes*isolates

*L. monocytogenes*isolates belonged to strains 1/2a, 1/2b, 1/2c and 4b (Table 2). Fifty three percent of isolates belonged to serogroup1 and 46.66% to serogroup 4. Serotypes 1/2a and 4b were dominant in 40% and 46.66% of positive samplesrespectively. Serotype 1/2a was proportionally distributed in food (meat and meat products) and in water. Serotypes 1/2b and 4b were isolated in samples of water, while serotype 1/2c was isolated in food.

Table 2.*Distribution of L.* monocytogenes *serotypes isolated from food and water*

SAMPLES	No. of isolates of serogroup 1 (%)	No. of isolates of serogroup 4 (%)	No. of serotypes (%)			
			1/2a	1/2b	1/2c	4b
Raw meat	2 (13,33)	-	2(13,33)	-	-	-
Finished meat product	2 (13,33)	-	1(6,66)	-	1(6,66)	-
Water of the Jala river and tributaries	4 (26,66)	5 (33,33)	3 (20)	1(6,66)	-	5(33,33)
Water of Modrac lake and tributaries	-	2 (13,33)	-	-	-	2(13,33)
Total	8 (53,33)	7 (46,66)	6 (40)	1(6,66)	1(6,66)	7(46,66)

Since a connection was determined by serotypization between *L. monocytogenes*isolated in food and water (serotype 1/2a), the most probably it is about fecal-oral cycle of the circulation of listeria between food-environment, and human-animal.

CONCLUSION

Of total 210 analysed samples of food and water *Listeria* spp. were the most numerous in samples of meat, meat products and samples of water. Detection of *Listeria* spp. in meat and meat products is of particular interest, because the consumers are often exposed to risk through these products.

*L. monocytogenes*isolates belonged to the most common causative agents of human listeriosis: 1/2a, 1/2b, 1/2c and 4b. Serotype 1/2a and serotype 4b were dominant in 40% and 46.66% positive samplesrespectively. *L. monocytogenes*serotypes 1/2b and 4b were isolated only in samples of water, while serotype 1/2c was isolated only in food samples. Serotype 1/2a was isolated both in food (meat and meat products) and in water.

Incidence of listeria in the Jala River and tributaries, and Modrac Lake and tributaries was relatively high, and it should be emphasized that there is a real threat for health of humans and animals from the presence of the mentioned pathogen in water.For defining the importance of rivers and lakes as sources of human and animal listeriosis further researches are necessary. Application of waste water treatment with process optimization, are the main guidelines for reduction of spread of listeria in the environment, and the possible, additional introduction to human or animal food chain.

As resistance to antibiotics has reached a critical stage in human and veterinary medicine,furthermonitoringofantimicrobial resistence of this pathogen is important from the aspect of providing more effective treatment of human listeriosis.

Considering that listeria were not isolated in raw milk, cream and cheese, researches of this type should be continued in order to determine whether incidence and possible variations of *Listeria* spp. can be attributed to seasons of the year, cattle feeding method or microflora of milk itself, and bacteriocins in milk.

*L. monocytogenes*was not isolated in samples of fruit and vegetables.The fact that *L. monocytogenes*can be present in any ecologic niche warns to a possibility of outbreak of sporadic cases of listeriosis associated with unusual types of plant food until now - fruit and vegetables - which also includes the types analysed in our research.

As Bosnia and Herzegovina is one of the few countries in which any legally binding provisions of food inspection for testing the presence of *L. monocytogenes*still do not exist, the results of this paper might give a contribution to the procedure of introduction of legal obligation for food control to this pathogen.

ACKNOWLEDGMENTS

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REPORTS ON THE CONFERENCE "ICGTec2012" 28 - 30 MAY, 2012.

In June, 2011. the University of Tuzla was visited by representatives of the University of Malaysia - Pahang, with the aim of establishing cooperation between these two universities. The workshop covered opportunities for future collaboration on joint research projects, possibilities of further development of cooperation through the exchange of students and teaching staff of the university, as well as the organization of the international conference which would brought together scientists and experts of European and Asian countries.

Mentioned visit resulted in an International conference on green technology and global ecosystems for sustainable development "ICGTec2012", which was organized in collaboration of the Center for Research and land resources management (CERRM) Malaysia, University Malaysia Pahang (UMP) and the University of Tuzla. The conference took place from 28-30 May, 2012., at the Faculty of Technology, University of Tuzla.

The objectives of this event were: the exchange of information and ideas among scientists and experts, not only from Bosnia and Herzegovina and Malaysia but also international participants from other European and Asian countries, on the latest research and innovations in the field of green technology, expanding the idea of green technology in Bosnia and Herzegovina for the purpose of its application as a basic framework for the development of the country, developing continuous cooperation among researchers within and outside Bosnia and Herzegovina, in order to efficient exchange and promotion of the latest research and innovation.

The conference was attended by forty-eight exhibitors from Malaysia, Iran, Iraq, Bangladesh, Mexico, Algeria, India, New Zealand, Libya, Croatia and Bosnia and Herzegovina, and more than a hundred teachers, students and experts from practice. Topics of presentations were related to the following areas: green technology and green energy, sustainable construction and infrastructure, climate changes, ecosystems, sustainable community development and environmental protection and sustainable management of water and waste. The special significance of the conference was the presentation of the latest developments and innovations in the field of water quality monitoring via system of sensor analyzers, about which the preliminary presentation was held by prof.dr. Hyunook Kim of the University of Seoul, Republic of Korea.

A large number of conference participants had the opportunity to exchange knowledge and experience in the field of their own research with colleagues from other countries, which created the preconditions for the realization of future cooperation and potential scientific research ideas.

Among the guests of the conference were Minister of Education and Science of the Federation of Bosnia and Herzegovina Damir Mašić, Mayor of Tuzla Jasmin Imamovic and Minister of Education, Science, Culture and Sports of Tuzla Canton prof. dr. Nadja Avdibašić-Vukadinovic.

The ceremonial event of the Conference was the signing of the Protocol on

cooperation between the University of Pahang in Malaysia, the Federal Ministry of Education and Science of Bosnia and Herzegovina and the Ministry of Education, Science, Culture and Sports of Tuzla Canton, in order to increase the participation of Bosnia and Herzegovina in international projects and intensifying cooperation between BiH and Malaysia, because of the possibility of a large number of interesting researches of interest to both countries

International Conference on green technology and global ecosystems for sustainable development 2012th resulted in the signing of a Memorandum of Understanding (MOU) between the University Malaysia Pahang and the University of Tuzla, by which are formally framed ways of cooperation through the exchange of students, teachers and staff, recognition of study programs and the development of joint study programs, and the launch of joint scientific research and education projects.

On the final day of the conference there was a meeting between the president of the conference, Dr Faizal Wan Wan Ishak and vice chancellor for academic research work at the University of Tuzla, prof.dr. Naser Prljača in order to define topics of interest for Bosnia and Herzegovina, which would be explored in future joint research projects of Malaysia and Bosnia and Herzegovina.

Scientific and technical papers of presenters at the end of the Conference were printed in the Proceedings of the Conference: "INTERNATIONAL CONFERENCE ON GREEN TECHNOLOGY & Ecosystem for Global Sustainable Development 2012", Publisher: University of Pahang, Malaysia, November 2012th.

REPORTS ON THE 7th SCIENTIFIC SYMPOSIUM ABOUT PRODUCTION AND PROCESSING OF FOOD WITH INTERNATIONAL PARTICIPATION, 30. AUGUSTE, 2012."agroTECH 2012"

Faculty of Technology, University of Tuzla is already seven years organized the Symposium about production and processing of food "agroTECH"". Symposium held at the International Fair of Agriculture and Food Industry in Gradacac. Co-organizers of this Symposium are Agricultural - Food faculty, University of Sarajevo and Ministry of Agriculture of Tuzla Canton. Symposium" agroTECH" has already a significant tradition in Bosnia and Bercegovini and throughout the region.

This year, in the 39th International Fair of Agriculture and Food Industry in Gradacac held the 7th Symposium on the production and processing of food "agroTECH 2012". During of Symposium was held Round table with current issues for farmers and food industry, and has presented a total of 14 scientific and professional papers in the field of agriculture, food processing and quality control of food products.

At the round table topics were presented:" Map the value in use of land as a factor of regional planning and rural development"," Sources and lending conditions in agriculture", "Cultivation of new species from small fruit" and topic" Typical autochthonous food products as a basis for improving rural livelihoods".

Round table topics included the issue of agricultural producers in Bosnia and Herzegovina and the region. Highlighted the aim of determing the land to demonstrate the importance of planning methods and the use of land resources, and the importance of specific insurance needs of credit conditions for farmers. Presented are the typical autochthonous food products in Bosnia and Herzegovina from the perspective of the possibilities of improving the quality of life in rural areas. Typical autochthonous food products are represented as the ability to creative production and one of the options to reduce poverty in rural areas.

Speakers at the Round table were professors from the Agricultural- food faculty University of Sarajevo and Faculty of Technology University of Tuzla. The discussion was attended by many representatives of agricultural organizations and farmers have expressed significant interest in these topics and highlight the importance of these issues for the development and promotion of domestic production.

In the scientific and technical part of Symposium,participants from the University of Osijek, University of Zagreb, University of Novi Sad, Institute of Field and Vegetable Crops in Novi Sad, University of Mostar, University of Sarajevo, University of Tuzla, Agricultural Institute of Tuzla, and other institutions, as well as NGOs, presented their work and their experiences.

Faculty of Technology in Tuzla will continue affirmation of Symposium "agroTECH" and collaboration with Universities and other institutions in Bosnia and Herzegovina and abroad, and to promote new advances in agriculture, food production and processing, and to provide new professional knowledge experts in the field of agriculture and food processing.

REPORTS ON THE 2nd SCIENTIFIC SYMPOSIUM WITH INTERNATIONAL PARTICIPATION, 08 - 10 NOVEMBER, 2012. ''ENVIRONMENTAL AND TOURISM POTENTIALS''

For three years in a row Faculty of Technology Tuzla organized a Symposium titled ''Environmental and tourism potentials''.

During this three years, Symposium is evaluated and transformed from Conference on tourism, catering industry, and environmental potentials to the terms under which it held today.

Wherewith we are led?

Today, everything is connected with the environment, the problem of reducing pollution up to the possibility of utilization of wastes into useful purposes. Today, waste management is a hot topic in academic communities.

That protection of the environment, reducing pollution on the one hand and tourism on the other hand, can not be considered separately from the food industry, chemical and agricultural production, as a major source of pollution, we thought that Faculta of Technology Tuzla which has existed for 53 years, during which evolved into a respected institution of higher-education with four of study programs and eight orientations is the right place for one such Symposium.

Our goal is that, in accordance with we on the Faculty of Technology be involved in, through scientific and research work, make a contribution for the development and advancement of industry not only in Bosnia and Herzegovina but also beyond, opening up new questions, pointing to the current problems and possible solutions.

It we can only through, the organization of such events, exchange of new knowledge and information with those who deal with this issue.

This year, we had the distinguished speakers from Korea, Croatia, Macedonia and Slovenia who recognized the importance of the topic and responded to the call. No less importantly, the participation of distinguished colleagues from the industry. Moreover, their experiences, the problems that they meet every day, are invaluable in solving problems.

During the first day of the Symposium topics were focused on the possibility of utilization of wastes into useful purposes and the ways in which it affects the development of tourism, while the topics of the second day was focused on specific practices in the ways of utilization of waste and its management.

The general conclusion of the Symposium was that it was necessary to work intensively on developing awareness on waste and its disposal.

Highlighting areas with great tourist or authentic potential is the way that a country becomes recognizable and attractive for both tourists and investors.

Bosnia and Herzegovina has a large space with enormous natural resources, cultural, historical and traditional values, with opportunities to develop authentic tourism and a lot of space for the development of tourism, which is based on a natural and healthy environment, and healthy food.

Organization of this Symposium would not be possible without the full understanding and support of our industrial partners

Global Ispat Koksne Industry, Sisecam Soda Lukavac, Solana Tuzla Inc. (Saltworks d.d. Tuzla), Cement factory Lukavac, TUV Adria.

Scientific and proffessional papers of lecturers at the conference will be printed in the Journal of Acta Tehnologica issued by Faculty of Technology, University in Tuzla.

INSTRUCTIONS FOR AUTHORS OF PAPERS

1. The manuscript which is to be submitted to the Editorial Board should be written in English, in two columns with double spacing on one side of A4 paper, with all margins of 2.54 cm (1 "), font Times New Roman 12 pt. The work will be sent in electronic form, prepared solely using word processing program Microsoft Word, ending with the 2003 versions. The file should be named as follows: TA_last name of first author _first word of title.doc. The extension must match the image format (tif, pcx, jpg, png ....). Images should have a resolution of min. 300 dpi and should be prepared so that they can be printed well in B / W technique. Each individual image should not be greater than one third of A4 format. Image labels should be written below the picture.
2. The size of the article (text, along with summaries, pictures and drawings and with a list of literature references, not counting titles and signatures, as well as information about authors) should be limited to 6 pages (two illustrations correspond to approximately one page). An exception can be negotiated with the editorial board, and to receive a larger volume of work if the content and quality justifies it.
3. Abstracts should be attached to the manuscripts:
• Summary (synopsis) of the maximum volume of a one printed site. It must explain the purpose of the paper, and must include the important data and conclusions, as well as keywords. This summary should be entered in the manuscript right after the header of the article.
4. The paper should contain the full official address, phone and e-mail address of all authors (on a separate sheet). Emphasize the correspondence author, with whom will the editorial board consult.
5. The title of the article should be specific and informative, in order to better determine the content of the paper. It is desirable to be as short as possible.
6. Text should be clear, concise, and grammatically correct without spelling errors, written in third person (impersonal).
7. Papers should be written with the assumption that readers know the discussed subject. Thus in (a short) introduction should briefly be stated only what is necessary for understanding of the text.
8. Experimental technique and device should be described in detail only if they deviate significantly from the description already published in the literature; for the known techniques and devices only source of necessary information is provided.
9. Tables and diagrams should be drawn and described so that they are understandable without reading the text. The same data should not be placed at the tables and diagrams, except in exceptional cases. The author will then give its reasons, and its validity is subject to final assessment of Editorial board and its reviewers.
10. Tables that contain a lot of data, where not all are necessary for understanding of the text should be reduced to the minimum. If desired by author, the editor will store complete tables in the archives and enable to interested readers an insight into the archive. This should be stated as a note with the abbreviated table.
11. Symbols of physical values should be written cursive, and unit of measure with vertical letters, eg. V, m, p, t, T, but: m3, kg, Pa, °C, K.
12. Formulas and equations should be typed, if possible, in one line (use a oblique fractional line instead of horizontal).

Indexes, at top and bottom, should be written clearly. Avoid upper indexes so that they would not replace with numerical exponents. All special characters (Greek letters, etc.) that can cause confusion, authors should explain separately.

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

14. References cited should be selective rather than extensive, except when it comes to review article. Literature citations should be enclosed on a separate sheet of paper and they should be numbered in the order they appear in the text. The numbers of citations are written to the text as a superscript. If the original literature was not available to the authors, they should cite by the source from which the quotation was taken.

Abbreviations for magazines must be in strict accordance with the abbreviations that are alleged by the Chemical Abstract.

Example of citing journals:

1. J. J. Sangiovanni, A. S. Kesten, Chem. Eng. Sci. 26 (1971) 533.

Example of citing patents:

2. J. Ehrenfreund (Ciba Geigy A. -G.), Eur. Pat. Appl. 22748, 21 Jan 1981; C. A. 95 (1981) 7078b.

Example of book citation:

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

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

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