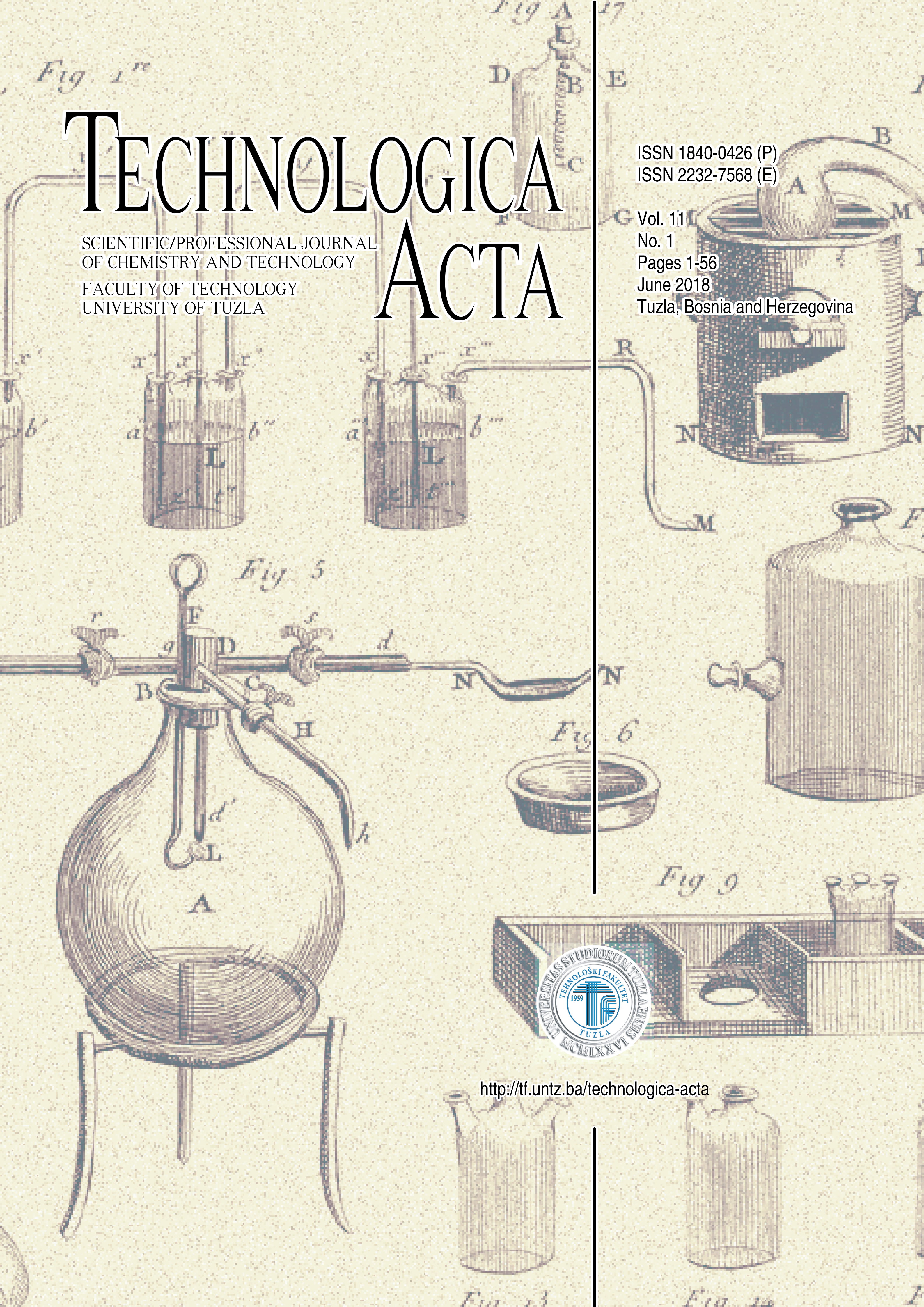


TECHNOLOGICA ACTA

SCIENTIFIC/PROFESSIONAL JOURNAL
OF CHEMISTRY AND TECHNOLOGY

FACULTY OF TECHNOLOGY
UNIVERSITY OF TUZLA

ISSN 1840-0426 (P)
ISSN 2232-7568 (E)

Vol. 11
No. 1
Pages 1-56
June 2018
Tuzla, Bosnia and Herzegovina

http://tf.untz.ba/technologica-acta

TECHNOLOGICA ACTA

SCIENTIFIC/PROFESSIONAL JOURNAL
OF CHEMISTRY AND TECHNOLOGY
FACULTY OF TECHNOLOGY
UNIVERSITY OF TUZLA

ISSN 1840-0426 (P)
ISSN 2232-7568 (E)

Vol. 11
No. 1
Pages 1-56
June 2018
Tuzla, Bosnia and Herzegovina

http://tf.untz.ba/technologica-acta

TECHNOLOGICA ACTA

SCIENTIFIC/PROFESIONAL JOURNAL OF CHEMISTRY AND TECHNOLOGY
FACULTY OF TECHNOLOGY UNIVERSITY OF TUZLA

Vol. 11, No. 1, June 2018 Pages 1-56

Publisher	Faculty of Technology University in Tuzla
Editor-in-Chief	Vahida Selimbašić
Deputy Editors	Dijana Miličević Zahida Ademović
Administrative Secretary	Vedran Stuhli
Scientific Board	Sadik Latifagić, Nihada Latifagić, Ranka Kubiček, Jozo Budimir, Muhamed Bijedić, Drago Šubarić (Croatia), Zoltan Zavargo (Serbia), Vahida Selimbašić
Editorial Board	Midhat Jašić (BiH), Sead Ćatić (BiH), Gordan Avdić (BiH), Elvis Ahmetović (BiH), Tatjana Krička (Croatia), Jovica Hardi (Croatia), Radoslav Grujić (BiH), Stanko Blatnik (Slovenia), Bruno Zelić (Croatia), Zorka Novak Pintarić (Slovenia), Branka Andričić (Croatia), Mitja Kolar (Slovenia), Vlado Guberac (Croatia), Jadranka Blaževska-Gilev (Macedonia), Matjaž Kunaver (Slovenia), Ljubica Dokić (Serbia)
Proofreader/English Language Adviser	Milja Jogunčić
Technical Editor & Layout	Franc Andrejaš
Cover Picture	Derived from the illustration made by Marie-Anne Paulze Lavoisier, published as a Planche IV in the Antoine Laurent Lavoisier's book "Traité élémentaire de chimie", Paris, France, 1789.
Editorial Office	Nermina Jahić (Technical Secretary) Faculty of Technology, University in Tuzla Univerzitetska 8, 75000 Tuzla, Bosnia and Herzegovina Phone/fax: +387 35 320 740 / +387 35 320 741
Printing	″HARFOGRAF″ Tuzla
Edition	100
Technologica Acta is indexed in	CAB Abstracts, COBISS, Index Copernicus Journal Master List, EBSCO, HRČAK, ROAD, Directory of Journal Quality Factor...

Technologica Acta is beeing published twice a year.

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

TECHNOLOGICA ACTA
SCIENTIFIC/PROFESIONAL JOURNAL OF CHEMISTRY AND TECHNOLOGY
FACULTY OF TECHNOLOGY UNIVERSITY OF TUZLA

Vol. 11, No. 1, June 2018 Pages 1-56

CONTENT


UNIVERSITAS STUDIORUM TUZLAENSIS
MCMLXXVI
TEHNOLOŠKI FAKULTET
1959
TUZLA

DETERMINATION OF THE BIOMETHANIC POTENTIAL OF CORN GRAIN

ORIGINAL SCIENTIFIC PAPER

Samra Halilović[1], Vedran Stuhli[2], Mirnesa Zohorović[2], Zoran Iličković[2]*

RECEIVED 2017-12-16
ACCEPTED 2018-05-18

[1]Institute for Chemical Engineering, Tuzla, Bosnia and Herzegovina
[2]Faculty of Technology, , Bosnia and Herzegovina
*✉ samra.halilovic95@gmail.com

ABSTRACT: Although it is primarily grown as food, corn also represents a very good raw material for the production of biogas due to the favourable starch and fibre ratio that is the basis of its structure. In addition, an important fact is that there are large arable or potentially arable areas in our country that are triturated with different pollutants, so that the production of plant crops for dietary purposes on these surfaces is not possible.

The aim of this paper was to determine the biometric potential of various varieties of corn grain in laboratory conditions through a series of experimental measurements. The obtained results are based on the assessment of the possibility of applying energy plantations of corn grown on contaminated arable or recultivated surfaces in the production of biogas.

KEYWORDS: corn, biomethane potential, sludge, anaerobic digestion, biogas

INTRODUCTION

Owing to increased energy consumption and reduction of fossil fuels in the world, systems have recently been developed in order to meet renewable energy needs, which is the cause of development of new technological processes of energy production. In addition, renewable energy sources form part of the European fight against climate change, while at the same time increasing the number of employees contributes to economic growth and development of the whole region [1], [2], [3].

At the global level of biomass energy production, very high potential has been estimated that can be unambiguously linked to the potential of biogas. Existing estimates are made on the basis of different assumptions, but all the results indicate the use of a very small part of that potential. The European Biomass Association (AEBIOM) estimates that biomass energy can increase from 72 Mtoe in 2004 to 220 Mtoe in 2020 (1 Mtoe = 11.63 TWh) [4].

Biogas plants using agricultural raw materials are one of the most important examples of anaerobic digestion in the world. Compared to other biofuels, anaerobic digestion biogas is an important priority in European transport and energy guidelines, and is of increasing importance because it offers many environmental benefits and an additional source of income for farmers [5].

Maize is the most dominant crop for biogas production. It is cultivated all over the world, and the breeding area is very broad, resulting from the variety of corn utilization and abilities that can thrive on various soils and in various climatic conditions. Corn crops are the third world crops, after wheat and rice. All parts of corn can be used either as food or for industrial processing [6].

MATERIAL AND METHODS

The basic substrate was chopped corn grain (diameter 0.5-1 mm) of two varieties, hybrid maize NS 444 *Ultra* and a homemade Bosanac variety. For the purpose of achieving the production of biogas as an inoculum, sludge from the treatment plant for communal waste water in Živinice was used.

For the purposes of this paper, a laboratory reactor system for anaerobic digestion of organic matter with glass eudiometric pipes (manufacturers Šurlan-Medulin), mounted on glass bottles of 500 ml were used. Provision of anaerobic conditions was performed by sparging of nitrogen in order to displace the air from the reactor, while providing the required constant temperature of the reactor system at 35°C ± 2°C was carried out by heating in a water bath with circulating water (Figure 1). Using the eudiometric pipes, the production of biogas is simple to read off, because produced gas pushes the liquid level down, while the fluid goes back into the storage bottle.

The pressure and temperature of the ambient air were measured on the set pressure hydrometers or eudiometric tubes, whose values were used to convert the volume of the resulting biogas to normal conditions. Mixing of the substrate was realized mechanically using a magnetic stirrer.

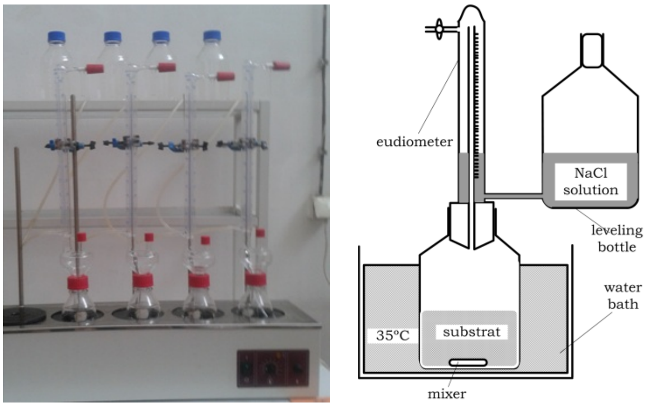


Figure 1. Laboratory reactor system: a. system layout; b. reactor block scheme

Measuring the volume of biogas produced in the reactor was performed in accordance with DIN 38 414 [7], and a gas composition analysis was performed on a gas chromatograph "PERKIN ELMER", equipped with the software package "Arnel". Helium was used as a gas carrier (flow rate 34 ml / min). A mixture of gases was used to calibrate the device which consists of CO, CO_2, CH_4 and O_2 (Messer, Germany). The gas chromatograph has two columns: 70 HayeSep N 60/80, 1/800 SF (maximum temperature 150°C) and 90 Molecular Sieve 1345-1360, 1/800 SF (maximum temperature 375°C). Conditions governing the furnace chromatograph are: initial temperature 60°C, total retention time 12 min, maximum temperature 150°C.

Because of the inhibition of anaerobic substrate degradation due to a sudden increase in the concentration of volatile fatty acids at the beginning of the process, which was reflected in a decrease of pH below 4, it was necessary to buffer the substrate by combining two bicarbonate buffers. By adding 150 mg of $NaHCO_3$ and 170 mg of $KHCO_3$ in relation to 1 g of total organic volatile matter [8] in the substrate, the optimum pH range was obtained.

To determine the necessary parameters in the substrates, the methods described below were used.

Determination of dry and volatile organic matter was performed according to Method 2540-B and 2540 Solid-Solid E. Standard Methods for the Examination of Water and Wastewater [9]. Electrometric measurement of pH was carried out by direct measurement, with the pH meter METTLER TOLEDO FE 20/EL 20. Prior to each measurement, an internal control was performed with certified reference materials of pH- value 4.01; 7.01; 10.01.

The nitrogen content by Kjeldahl was determined according to Method 4500-N_{org} B. Standard Methods for the Examination of Water and Wastewater 20nd edition. APHA, Washington, DC [10]. The method consists of three stages: digestion at a temperature of 340°C (boiling point of H_2SO_4) in the presence of concentrated sulfuric acid and selenium Kjeldahl catalyst; distillation in the presence of NaOH where distillate accepts in the solution of boric acid, and titration with 0.1 M HCl in the presence of indicator bromocresol green. Determination was made on Kjeldahl apparatus Gerhardt. To determine the chemical oxygen demand, the standard method was used according to the ISO 6060:2000 [11].

RESULTS AND DISCUSSION

The anaerobic digestion experiment of two grain varieties and waste sludge from municipal wastewater treatment plants was carried out at mesophilic conditions (36 ± 1°C). When experimenting with the mixture of corn grain substrate and waste sludge, experimental data [12] were used as a blank test waste sludge was used without additives (Table 1).

Table 1. Composition of the cosubstrate in the formed blends (2&3) and the blank test (1)

Test	sludge mas%	*Bosanac* mas %	*NS 444 Ultra* mas %	H_2O mas %
1	100	0	0	0
2	49.67	0.33	0	50
3	49.64	0	0.36	50

The values of the physico-chemical parameters of the treadmill are shown in the Table 2.

Table 2. Physico-chemical characteristics of the cosubstrates used at the beginning of the experiment

Parameter	Unit	1	2	3
TS	%	5.05	2.83	2.84
VS	%	3.36	1.96	1.99
VS/TS	-	0.66	0.69	0.70
pH	-	6.41	6.21	6.09
COD	g/kg	48.6	102.58	117.96
TKN	g/kg	2.80	2.20	2.00

TS – total solids; VS – volatile solids; COD - chemical oxygen demand; TKN - total Kjeldahl nitrogen

The ratio of VS / TS to the blank test sample (1) and the formed coagulates (2 and 3) approx. 0.7 the condition of the presence of sufficient quantity of organic matter in the reactors is satisfied. The content of dry matter in reactors 2 and 3 is lower than the content of dry matter in the control sample (1).

The optimum ratio of organic matter and nitrogen in the ratio of 30:1 in this case is expressed through HPK:TKN and ranges from 17:1 in the control sample up to 60:1 in reactor 3 (HPK reactor: TKN =

47:1). It is clear that substrates are not loaded with nutrients especially nitrogen.

The graphs show daily biogas production (Figure 2) and cumulative yield of biogas (Figure 3) in reactors 1, 2 and 3. On the biogas daily production diagram, discontinuous biogas production is observed.

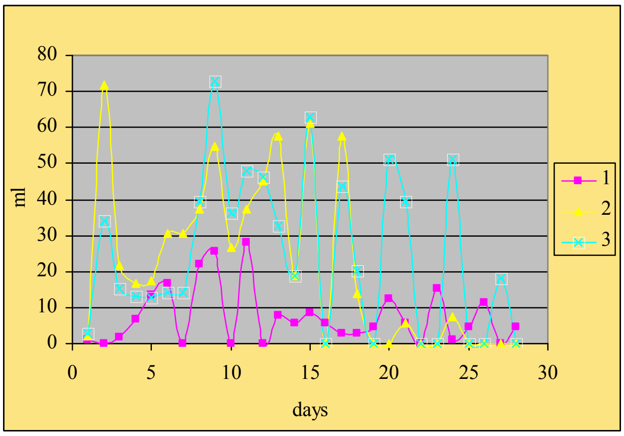


Figure 2. Daily biogas production diagram

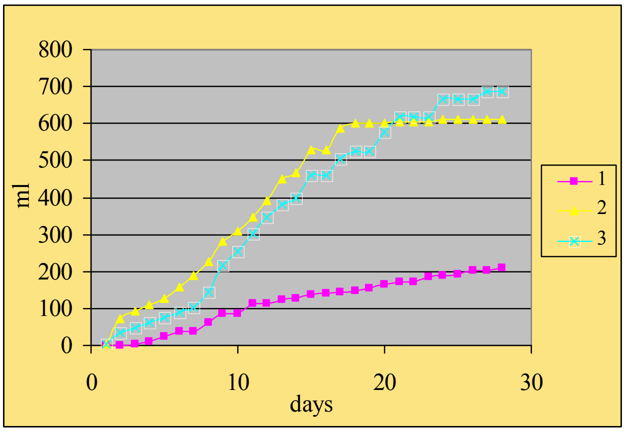


Figure 3. Cumulative biogas yield

The cumulative yield of the biogas (Figure 3) confirms the adaptation phases and the beginning of hydrolysis in the first days of the process. The diagram clearly shows a significantly larger biogas production in reactors 2 and 3 compared to the controlled sample 1.

Table 3 gives characteristics of the digested residue (digestate) which remains after the experiment has been realized. If we compare the content of dry and volatile organic matter before and after the process, it is easy to conclude that a certain part of the matter has been transformed into gas products. Also, the value of HPK for all three samples is significantly lower in the digestate (Table 2) compared to HPK values in substrates prior to the decomposition process (Table 1). In addition, the lower value of TKN content in digestates can be noted with respect to the TKN values in the initial coagulants. Based on the proportion of methane in biogas and biogas production, a calculation of methane production was performed for all three analyzed cases (Figure 4).

Table 3. Final results of batch anaerobic digestion after experiment

Parameter	Unit	1	2	3
TS	%	2.79	2.22	2.19
VS	%	1.36	1.26	1.09
pH	-	7.57	7.44	7.47
HPK	g/kg	14.43	15.12	10.47
TKN	g/kg	1.06	1.35	1.15
V biogas	ml	208.2	612.84	685.75
V methane	ml	64.77	366.05	411.38
W methane	%	31.11	59.73	59.99
spec. gas yield	ml CH_4/gVS	14.49	466.90	524.72

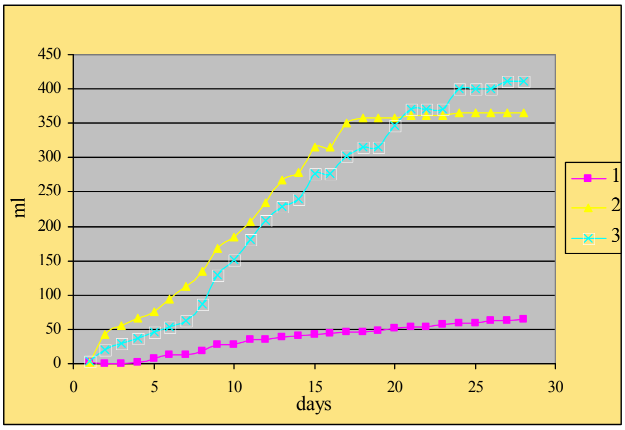


Figure 4 Cumulative yield of methane

Similarly to the case of cumulative production of biogas and methane production from cobblestone 2 and 3, it is considerably higher than methane production of controlled sample 1. In addition, the share of methane in biogas in samples 2 and 3 is almost identical and is close to 60 % while the share in the biogas of the sample is almost twice as small.

CONCLUSIONS

Based on the physical-chemical analysis of waste sludge from the municipal wastewater treatment plant and the maize hybrid cultivar “NS 444 Ultra”, as well as the preserved Bosanac variety, all three types of substrates represent potential biogas raw materials owing to very favourable VS content and HPK representing the amount of organic matter from which methane is obtained, and a favourable ratio of the macronutrient expressed through the ratio HPK: TKN.

By comparing the content of dry and volatile organic matter before and after the process, it is easy to conclude that a certain part of matter has been transformed into gas products. Also, the value of HPK and TKN for all three samples is significantly lower at the end of the process compared to the HPK and TKN values in the substrates prior to the decomposition process, which is particularly relevant for the 2 and 3 blends. Methane production from both substrates 2 and 3 is considerably larger in comparison to the methane production of the controlled sample 1. In addition, the proportion of methane in the biogas in samples 2 and 3 is almost identical and is close to 60 % while the biogas fraction of the blank test sample is almost twice as small.

The specific production of methane produced from the sands and grains of hybrid maize NS 444 Ultra is 524.72 ml of CH_4/gVS, while the sludge and the Bosanac grain is 466.90 ml of CH_4/gVS.

REFERENCES

[1] T. Amon, B. Amon, V. Kryvoruchko, W. Zollitsch, K. Mayer, L. Gruber, "Biogas production from maize and dairy cattle manure-Influence of biomass composition on the methane yield", *Agriculture, Ecosystems and Environment*, pp. 173-182, 2007.

[2] P. Balsari, P. Bonfanti, E. Bozza, F. Sangiorgi, "Evaluation of the influence of animal feeding on the performances of a biogas installation (mathematical model)", *Proceedings of the 3rd International Symposium "Anaerobic Digestion*, Boston, 1983.

[3] P. Vindiš, P. Muršec, C. Rozman, F. Cus, "A multi-criteria assessment of energy crops for biogas production", *Journal of Mechanical Engineering*, 2009.

[4] Z. Osman, "Projekt biolinskog postrojenja", Diplomski rad, 2015.

[5] T. Al Seadi, D. Rutz, H. Prassl, M. Kottner, T. Finsterwalder, S. Volk, R. Janssen, *Priručnik za bioplin*, Intelligent Energy Europe, 2008.

[6] D. Čakija, "Proizvodnja silažnog kukuruza na OPG-u Čakija za potrebe bio-plinskog postrojenja bioplinara Ogranica, Gregurovec" Diplomski rad, 2-5, 2016.

[7] DIN 38 414-S1-1986-11 (1986): *Analyses of Water, Drinking Water, Waste Water, Sludge, Sediments and Residues*.

[8] A. Flor, L. Arroja, I. Capela, "Anaerobic co-digestion of organic fraction of municipal solid waste and waste activated sludge at different ratios", *Proceedings Sardinia 2003, Ninth International Waste Management and Landfill Symposium S. Margherita di Pula*, Cagliari, Italy, 2003.

[9] Method 2540-B and 2540 Solid-Solid E. *Standard Methods for the Examination of Water and Wastewater*.

[10] Method 4500-N_{org} B. *Standard Methods for the Examination of Water and Wastewater 20nd edition*. APHA, Washington, DC, 1998.

[11] BAS ISO 6060 *Određivanje hemijske potrošnje kisika*, 2000.

IMPACT OF THE FERMENTATION PROCESS WITH IMMOBILIZED YEAST CELLS ON THE AROMA PROFILE AND SENSORY QUALITY OF DISTILLATES PRODUCED FROM CAROB PODS (*CERATONIA SILIQUA L.*)

ORIGINAL SCIENTIFIC PAPER

Borislav Miličević[1], Đurđica Ačkar[1], Jurislav Babić[1], Antun Jozinović[1], Radoslav Miličević[2], Emil Petošić[3], Toni Kujundžić[4], Drago Šubarić[1]*

RECEIVED 2017-12-04
ACCEPTED 2018-03-24

[1]Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, F. Kuhača 20, HR-31000 Osijek, Croatia
[2]Progresys d.o.o., Ind. Park bb, HR-35400 Nova Gradiška, Croatia
[3]Zvečevo d.d., Food Industry, Kralja Zvonimira 1, HR-34000 Požega, Croatia
[4]Josip Juraj Strossmayer University of Osijek, Faculty of Agriculture Osijek, V. Preloga 1, HR-31000 Osijek, Croatia
*✉ dackar@ptfos.hr

ABSTRACT: The aim of this research was to investigate the influence of immobilized cell fermentation on aroma and sensory characteristics of distillates produced from carob pods (*Ceratonia siliqua L.*) commonly grown in coastal Croatia. Distillate samples were produced both by classical and immobilized yeast technology. The aroma profile was determined using GC/FID and a sensory analysis was conducted according to the German DLG model. Results showed that a immobilized cell technique gave distillates with lower ester contents, nevertheless satisfying sensory quality.

KEYWORDS: immobilized yeast cells, carob distillate, aroma, sensory quality

INTRODUCTION

Carob (*Ceratonia siliqua L.* - derived from the Greek word *keras* - horn and Latin *siliqua* - hard) is a wild tree or a shrub of the legume family (*Leguminosae*). Tree has a broad canopy and up to 15 m high, and the fruit is 20 cm long pods, at first green and maturing exceeds that in dark brown. The pods ripen in late summer. A mature follicle is of a sweet taste [1].

Endosperm in the mature follicle is processed into farina [2]. Carob grows wild or is cultivated in the coastal regions of the Mediterranean region countries: Croatia, Israel, Turkey, Algeria, Morocco, Tunisia, Spain, Portugal, Greece and Italy, under various names: Hebrew *kharuv*, Arab *Kharrūbah*, in Spain it is called, *Garrofer*, *Carrubo* in Italy*, Caroubier* in France, *Karubenbaum* in Germany, *Alfarrobeira* in Portugal, *Charaoupi* in Greece and *charnup* in Turkey. Carob has been used in the human diet for more than 4,000 years, it has been mentioned as food from Biblical times, which fed John the Baptist, and hence derives an English synonym for carob - *St.John's bread* [3].

The husk of the carob contains app. 13% of simple sugars (fructose, glucose and maltose), 20% sucrose, 2-3% pectin, 4% protein and 35% starch. It is rich in minerals: 36% calcium, 24% potassium, 29% copper. Carob pulp has a high content of phenolic compounds of which the most important are tannins (16-20%), while the proportion of phenolic compounds in the husk of the carob varies and depends on the climate, carob varieties, husk maturity degree and manner of processing [4]-[6].

Carob is offered to the market in fresh and dried state. In the food industry carob is used as thickener, stabilizer, cocoa replacement and medical dietetics use it as low-energy food. Carob extract is used as a flavour in the production of wide range of products (alcoholic and non-alcoholic beverages, frozen dairy products, candies, baked goods, puddings, meat products, spice mixes, fruit products, etc. [2], [7].
For distillate and brandy production, a completely dry carob pod with the high sugar content is used, as well as various essential oils and resinous substances, which give the distillate and brandy with a characteristic smell and taste [3].

The aim of this study was to investigate the influence of fermentation with immobilized yeast cells on the flavour and sensory properties of distillate produced from farina of the dried carob pod.

MATERIAL AND METHODS

SHUCK

The samples of shuck produced from flour of the dried carob pod (harvest 2015) were taken from the technological process of carob distillate production at “Zvečevo” JSC.

SHUCK FERMENTATION

The samples in two parallels were produced using the classical technological procedure and immobilized yeast fermentation as described previously [8].

All samples were taken at the end of fermentation before sedimentation, therefore the samples were insufficiently clear and slightly dull, which is appropriate for the selected procedure for the distillate production.

DISTILLATION

The selected samples were distilled in a copper clip distillation device, according to the procedure described previously [8].

The samples containing approximately 70% vol. alcohol were taken from the middle fraction, or with recommended alcohol concentration in distillates, while the first (head) and the last (tail) fraction were not used.

All selected samples were distilled according to the same distillation protocol.

ANALYSIS OF DISTILLATES

Instrumental analytical techniques were applied on the basis of the European Community Reference Methods for the analysis of spirits using gas chromatography [9], [10], [11], using a Hewlett Packard 5890 gas chromatograph with a split /splitless injector and a FID detector, as described previously [8].

SENSORY ANALYSES

The sensory analysis of samples was performed according to the method of positive ranking according to the German DLG model [12]. This model was based on 4 sensorial experiences, which were marked with grades from 0 to 5, including 0, while the average grade was multiplied by the significance factor.

The sensory assessment was conducted in two repetitive cycles; each group had ten qualified professional testers, from the alcoholic beverages industry, selected by selection procedure [13], with extensive experience in the sensory assessment of distillates.

RESULTS AND DISCUSSION

The results of the chemical analyses of distillate samples are shown in Table 1. The results show that the fermentation process with immobilised yeast cells resulted in increased content of ethanol in the immobilized yeast sample (69.87% vol. compared to 69.22% vol). A similar trend was recorded in our previous research [14].

Table 1. Selected chemical characteristics of carob distillate samples (mean±standard error).

Assessment characteristics	Classical fermentation	Immobilized yeast-fermentation	p
Total extract (g/L)	0.048±0.021	0.014±0.004	0.16575
Total SO_2 (mg/L)	4.255±0.007	6.16±0.070	**0.00069**
Total acidity (mg/L)	470.85±18.73	344.60±1.97	**0.01095**
Benzaldehyde (mg/L)	2.02±0.08	2.38±0.14	**0.09086**
Ethanol (% vol.)	69.22±0.04	69.87±0.04	**0.00423**
Methanol (mg/L)	0.135±0.02	0.04±0.05	0.15610
Propan-1-ol (mg/L)	2.67±0.04	1.80±0.14	**0.01434**
Butan-1-ol (mg/L)	0.175±0.049	0.090±0.014	0.14460
Isobutyl alcohol (mg/L)	3.71±0.14	3.41±0.014	0.09628
Isoamyl alcohol (mg/L)	8.79±0.02	6.18±0.28	**0.00583**
2-phenyl ethanol (mg/L)	4.13±0.388	2.59±0.212	**0.03873**
Linalool (mg/L)	0.73±0.18	0.76±0.16	0.88094
α-Terpineol (mg/L)	2.01±0.21	2.64±0.28	0.12790
Benzoic acid(mg/L)	21.74±0.87	60.48±7.16	**0.01690**
Quercetin (mg/L)	5.74±0.87	25.48±0.09	**0.00099**
Gallic acid (mg/L)	31.27±0.12	35.68±0.37	**0.00010**
Ethylgallate (mg/L)	20.20±0.21	35.68±0.37	**0.00039**
Ellagic acid (mg/L)	31.11±0.07	65.98±0.79	**0.00026**
Furfural (mg/L)	0.06±0.007	tr.	**0.00586**
5-Methylfurfural (mg/L)	0.025±0.035	n. d.	0.42226
5-Methyl -2-furancarboxaldehyde (mg/L)	0.100±0.014	0.055±0.007	0.05650
Pyranone (mg/L)	3.49±0.22	1.12±0.04	**0.00468**
2-Acetylpyrrole (mg/L)	1.56±0.01	1.06±0.01	**0.00079**
5,6-Dihydro-2-pyranone	0.88±0.01	0.36±0.01	**0.00073**
Ethyl lactate (mg/L)	0.09±0.01	n. d.	**0.01212**
Ethyl octanoate (mg/L)	2.74±0.09	2.40±0.31	0.27150
Ethyl decanoate (mg/L)	0.92±0.15	n. d.	**0.01399**
Ethyl acetate (mg/L)	6.33±0,18	4.45±0.04	**0.00049**
Isoamyl acetate (mg/L)	8.50±0.14	8.03±0.01	**0.04593**
Acetaldehyde(mg/L)	4.44±0.45	3.81±0.94	0.48552
Ethyl hexanoate (mg/L)	2.48±0.52	2.33±0.45	0.79617
Methyl octanoate (mg/L)	0.40±0.06	0.39±0.09	0.91091

Assessment characteristics	**Classical fermentation**	**Immobilized yeast-fermentation**	**p**
2-Phenylethyl acetate (mg/L)	0.71±0.14	0.58±0.007	0.34171
Methyl decanoate (mg/L)	0.31±0.02	0.23±0.01	**0.04215**
Benzyl acetate (mg/L)	0.22±0.007	n. d.	**0.00049**
Ethyl benzoate (mg/L)	0.15±0.03	n. d.	**0.02504**
Ethyl decanoate (mg/L)	28.43±0.007	27.89±0.07	**0.00839**
Isoamyloctanoate (mg/L)	0.41±0.02	0.35±0.04	0.23799
Ethylundecanoate (mg/L)	2.11±0.02	1.49±0.48	0.21443
3-Methylbutyl dodecanoate (mg/L)	0.35±0.02	0.24±0.02	0.05219
Ethyl dodecanoate (mg/L)	1.74±0.16	1.22±0.04	0.05219
3-Methylphenyl butanoate (mg/L)	0.25±0.007	0.21±0.01	0.05654

n. d. – not detected; tr. – traces; results with bolded p values are statistically different

The content of methanol, an important safety factor when producing alcoholic drinks [15], was in acceptable range in both samples [16], [17], with markedly lower content in the immobilized-yeast sample (0.04 mg/mL compared to 0.135 mg/mL).

On the other hand, total SO_2 was significantly higher in the immobilized-yeast sample (6.16 mg/L compared to 4.255 mg/mL). This potentially increases the risk of bonding SO_2 on acetaldehyde and results in an intense heavy smell [18], [19].

The total extract and total acidity were reduced by the fermentation process with the immobilised yeast cells method, which shows the higher degree of fermentation [14].

The content of main components that affect the aroma profile (Table 1) was reduced when using the fermentation process with immobilised yeast cells, which can result in reduced distillate sensory characteristics [17], [20].

Furthermore, the total share of typical phenolic substances, described by Rakib *et al.* [21], dominated by gallic acid was significantly higher in distillates produced by immobilized yeast cells, the share of quercetin increased nearly by 5-fold (25.48 mg/L compared to 5.74 mg/L), ellagic acid nearly 2-fold and ethylgallate by 1.5-fold.

Also, volatile substances typical for roasted carob beans [22] were notably more predominant in the samples produced by a classic method of fermentation. Furfural was not detected in the immobilized-cell sample, while in the classically produced sample the amount of 0.06 mg/L was measured. The content of pyranone was 3 times higher in the classically produced sample, acetopyrole 1.5 times and 5,6-dihydro-2-pyranone 2.4 times higher than in the immobilized-yeast sample (Table 1). Although not statistically significant, the content of 5-methylfurfural and 5-methyl-2-furancarboxaldehyde was also slightly higher in the classically produced sample.

Overall, the level of all identified esters in this study was reduced in the samples produced by the fermentation process with immobilized yeast cells, indicating that distillates produced by the classic fermentation process have a deeper aroma [23], [24].

This was confirmed by the sensory analysis where the samples produced by the fermentation process with immobilised yeast cells were given a significantly lower score for taste and odour in comparison to samples that were produced by the classical fermentation process (Figure 1), and this is in accordance with the research of Vila *et al.* [25] and Yajima and Yokotsuka [26]. Although the overall score of the sensory analysis was higher for the classically produced sample, the sample produced with immobilized yeast cells had over 90 points, which places it in a category of highly acceptable to consumers.

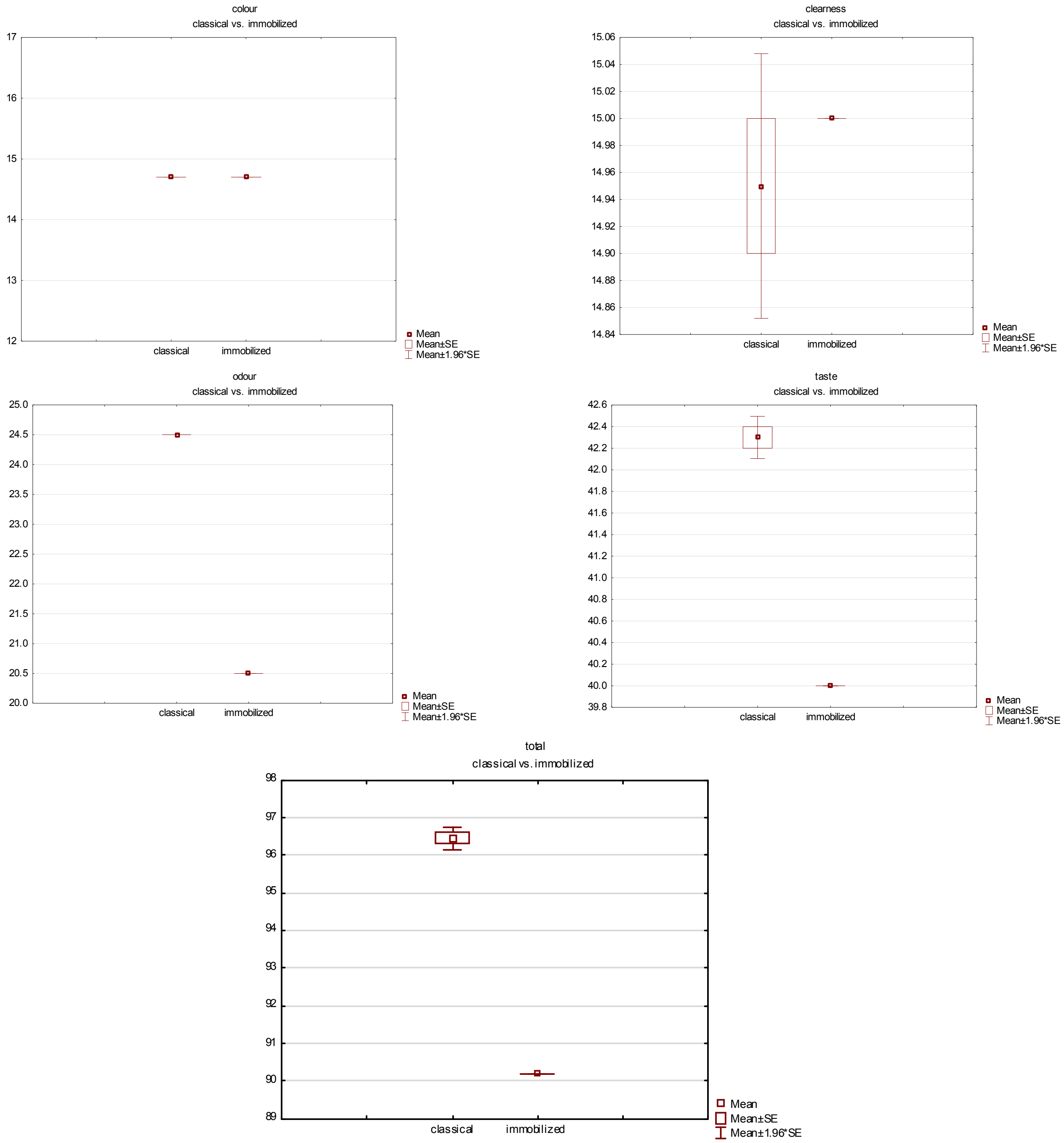


Figure 1. Results of sensory analysis of carob distillates produced by classical fermentation and fermentation with immobilized yeast cells - *German DLG model* (Koch, 1986.).

CONCLUSION

Although the present research shows that the original carob aroma was partly lost by immobilized yeast cell fermentation, as well as ester content, which gave less pronounced odour and taste of distillate, the overall sensory score shows that carob distillate acceptable to the consumers may be produced by immobilized yeast fermentation.

REFERENCES

[1] E. Karababa and Y. Coskunder, “Physical properties of carob bean (*Ceratonia siliqua L.): An industrial germ yielding crop,” Ind. Crops Prod., vol. ED-42, pp. 440–446, March 2013.*

[2] A. K. Yousif and H. M. Alghzawi, „Processing and characterization of carob powder,“ *Food Chem. vol. ED-69, pp. 283–287, May 2000.*

[3] R. Avallone, M. Plessi, M. Baraldi, and A. Monzan, "Determination of chemical composition of carob (*Ceratonia Siliqua*): protein, fat carbohydrates and tannins," J. *Food Compost. Anal., v*ol. ED-10, pp. 166 – 172, June 1997.

[4] C. Bengoechea, A. Romero, A. Villanueva, G. Moreno, M. Alaiz, F. Millan, A. Guerrero, and M. C. Puppo, "Composition and structure of carob (*Ceratonia Siliqua* L.) germ proteins," *Food Chem.,* vol. ED-107, pp. 675–683, March 2008.

[5] B. Matthaus and M. M. Ozcan, "Lipid evaluation of cultivated and wild carob (*Ceratonia Siliqua* L.) seed oil growing in Turkey," *Sci. Hortic*., vol. ED-130, pp. 181–184, August 2011.

[6] N. Bouzouita, A. Khaldi, S. Zgoulli, L. Cheli, R. Chekki, M. M. Chaabouni, and P. Thonart, "The analysis of crude and purified locust bean gum: A comparison of samples from different carob tree populations in Tunisia," *Food Chem*., vol. ED-101, pp. 1508–1515, February 2007.

[7] P. A. Dakia, B. Wathelet, and M. Paquot, "Isolation and chemical evaluation of carob (*Ceratonia Siliqua* L.)," *Food Chem*., vol. ED-102, pp. 1368 – 1374, May 2007.

[8] B. Miličević, Đ. Ačkar, J. Babić, A. Jozinović, R. Miličević, M. Oroz, and D. Šubarić, "Impact of the fermentation process with immobilized yeast cells on the aroma profile and sensory quality of distillates produced from two fig (*Ficus carica* L.) cultivars," *Poljoprivreda*, vol. ED- 23(1), pp. 49-55, June 2017.

[9] *AOAC, Official Methods of Analysis*. Association of Official Chemists, Arlington, VA, USA, 1995.

[10] *EEC Council Regulation 2870/00 laying down Community reference methods for the analysis of spirit drinks*, Off. J. Eur. Comm. L333, 2000.

[11] *EEC Council Regulation 110/2008 on the definition, description and presentation of spirit drinks*, Official Journal of European Communication L39, 2008.

[12] J. Koch, *Getränkebeurteilung*. Stuttgart: Eugen Ulmer GmbH Co., 1986, pp. 95-96.

[13] G. Jellinek, *Sensory Evaluation of Food, Theory and Practice*. Chichester: Ellis Horwod International Publishers in Science and Technology, 1985, pp. 252-255.

[14] B. Miličević, I. Lukić, J. Babić, D. Šubarić, R. Miličević, and Đ. Ačkar, "The influence of fermentation process with immobilized yeast cells on quality of tangarine distillates," *Glasnik zaštite bilja*, vol. ED-35, pp. 68-75, November 2012.

[15] M. Pohanka, "Toxicology and the biological role of methanol and ethanol: Current view," *Biomed. Pap. Med. Fac. Univ. Palacky. Olomouc. Czech Repub*., vol. ED-160, pp. 54-63, March 2016.

[16] A. J. Paine and A. D. Dayan, "Defining a tolerable concentration of methanol in alcoholic drinks," *Hum. Exp. Toxicol*., vol. ED- 20, pp. 563–568, November 2001.

[17] L. Nykanen and H. Suomalainen, *Aroma of Beer, Wine and Distilled Alcoholic Beverages*. Berlin: Akademie verlag, 1983.

[18] S. H. Guan and H. J. Pieper, "Examination of the distillation characteristics of the distillate from numerous fruit mashes using GC analysis," *Dtsch. Lebensmitt. Rundsch*., vol. ED-11, pp. 365-374, November 1998.

[19] S. C. Ferreira da Silva, J. Rodrigues, and K. Bertsch, "Ethyl esters of long chain fatty acids in Brandy: Rapid determination and stability tests," *Eux-de-vie 2007, 2nd International Symposium on Traditional Spirits of Viticultural Origin,* Bordeaux, France.

[20] C. Norbert, and C. C. Bauer, "Flavour of Spirit Drinks: Raw Materials, Fermentation, Distillation and Aging," In *Flavours and Fragrances Chemistry, Bioprocessing and Sustainability*, R. G. Berger (Ed). Berlin, Heidelberg: Springer-Verlag, 2007, pp. 219.

[21] M. el Rakib, H. Chicha, S. Abouricha, M. Alaoui, A. A. Bouli, M. Hansali, and R. W. Owen, „Determination of Phenolic Composition of Carob Pods Grown in Different Regions of Morocco," *J. Nat. Prod.,* vol. ED-3, pp. 134-140, 2010.

[22] M. A. Farag, and D. M. El-Kersh, „Volatiles profiling in *Ceratonia siliqua* (Carob bean) from Egypt and in response to roasting as analyzed via solid-phase microextraction coupled to chemometrics," *J. Adv. Res*., vol. ED-8, pp. 379–385, July, 2017.

[23] G. A. Burdock, (Ed), *Fenaroli's Handbook of Flavor Ingredients* 4th ed., Milton Park: Taylor & Francis, 2002, pp. 972.

[24] B. Plutowska, and W. Wardencki, "Headspace solid-phase microextraction and gas chromatography-olfactometry analysis of raw spirits of different organoleptic quality," *Flavour Fragr. J.*, vol. ED-24, pp. 177-185, July/August 2009.

[25] I. Vila, J. M. Sablayrolles, R. Baumes, C. Bayonove, and P. Barre, "Study of influence of yeast strain on fermentation aroma by sensory and chemical analyses," *J. Viticult. Enol.*, vol. ED-53, pp. 124-130, 1998.

M. Yajima, and K. Yokotsuka, "Volatile Compound Formation in White Wines Fermented Using Immobilized and Free Yeast," *Am. J. Enol. Viticult*., vol. ED-52, pp. 210-218, January 2001.

THE INFLUENCE OF RAW MATERIALS AND DIFFERENT FERMENTATION INTERVALS ON QUALITY PARAMETERS OF THE TRADITIONALLY PRODUCED BOSNIAN SUDŽUK

ORIGINAL SCIENTIFIC PAPER

Suzana Jahić, Nermin Pračić*

RECEIVED 2017-12-20
ACCEPTED 2018-05-18

Biotechnical faculty, University of Bihać, Luke Marjanovića bb, 77 000 Bihać, Bosnia and Herzegovina
*✉ suzanajahic2002@gmail.com

ABSTRACT: Bosanski sudžuk (The "Bosnian sudžuk" – The Bosnian Smoked Sausage) prepared in a traditional way is fermented and dried product made from high-quality ground beef and beef tallow with the addition of salt and spices. The production of Bosnian sudžuk in a traditional way comes down to filling the natural sausage casings with ground, salted beef and fat with the addition of salt and spices which are subsequently smoked, i.e. dried. This study was aimed to determine how the composition of raw materials and the extended fermentation interval, applied after stuffing natural casings, affect physicochemical and sensory properties of traditionally produced Bosnian sudžuk. The following samples of Bosnian sudžuk were prepared in the traditional way: Sample I – Bosnian sudžuk made from a combination of beef and beef fat tissue with the addition of spices and Sample II – Bosnian sudžuk made from a combination of beef, lamb and beef fat tissue with the addition of spices. After preparation of stuffing and filling into casings, the samples underwent prolonged liquid squeezing (fermentation) that lasted three days. During the aforementioned squeezing, the samples of the stuffing were analyzed on their chemical composition as well as pH changes of the stuffing. After the completion of the liquid squeezing process, the samples of Bosnian sudžuk were dried and smoked in the classical drier. The research results showed that the weight loss of dried Bosnian sudžuk was higher in the Sample II than in the Sample I. The course of changes of pH was balanced for both samples tested, and the final pH of the dried product was higher in the Sample II than in the Sample I. Regarding the chemical parameters of quality, the Sample II had a lower content of water and a higher content of fat and proteins compared to the Sample I. Sensory evaluation showed that the Sample I had better overall grade compared to Sample II.

KEYWORDS: Bosnian sudžuk, fermented sausage, physicochemical properties, sensory properties

INTRODUCTION

Bosnian sudžuk produced in a traditional way is a dried meat product, traditionally made of beef and beef fat, salt, garlic and pepper. It has been produced without strict technological norms and it has a well known horseshoe shape and a characteristic taste. A similar product can be found in Turkey and it is assumed that origin of Bosnian sudžuk is exactly from Turkey. Owing to production norms of sliced meat, semi-products and products of meat [1] sudžuk is a product made of sliced beef, beef fat, salt or substitute for salt, sugar, additives, spices or spice extracts and starter cultures. The mixture for sudžuk is stuffed into thin beef natural or synthetic casings. Ingredients of recepie and production conditions are parameters responsible for specifications andadvantages in sensory properties of fermented sausages compared to ones produced in controlled conditions [2]. Content of meat proteins is not allowed to be less than 16%, and relative protein content of connective tissue in proteins (content of collagen) is not allowed to be grater that 20% [1]. Since there are no strict regulations regarding production of Bosnian sudžuk, products of different quality are available on the market as a result of diverse technological approaches in manufacture, but also as a result of different climate factors [3].

In traditional production of dried sausages, natural lactic-acid bacteria, during fermentation, are transmuting, already existing or additional sugars, in lactic acid [4]. Sudžuk production starts at the end of autumn and the begining of winter, and has a natural process of ripening. During fermentation, lactic acid is made and pH is decreased. Acidification helps in creating colour and coagulation of proteins affects firmness and cohesiveness of a product, as well activation of protein muscle [5].

Important factor for a taste and smell of fermented sausages is a proteolysis process, caused by an activity of endogenous and microbial proteinases and peptidases, which results in increased content of polypeptids, peptids and free amino acids [6]. Fermented sausages are products with a high fat percentage responsible for many properties of sausages. During the oxidation and lipolytic changes in fat, important components of taste, smell and texture are created [7]. Spices used in production of fermented sausages, beside the taste, have the anti-oxidant impact and stimulatory impact on fermentation. Many spices have influence on microorganisms, and in specific conditions some spices can stimulate bacteria to create lactic acid [8].

MATERIAL AND METHODS

Experimental part of production was done in November 2015 by a local producer of dried meat products in Turija, a village in munipacility Bihać. Bosnian sudžuk has been mainly produced out of beef and sheep meat of I and II category.

Samples of sausages were produced in the following way: Sample I – a mixture for sudžuk production contained beef (92.59%) and beef tallow (7.41%); Sample II - a mixture for sudžuk production contained beef (74.07%), sheep meat (18.51%) and beef tallow (7.41%). All processes in production of Bosnian sudžuk were carried out in the same way for both mixtures. Meat and beef tallow were chopped manually, with a knife, in small pieces (4x4 cm); salt and black pepper were added, 3.1% and 0.07% respectively. After mixing meat and tallow together with spices, both samples (I and II) were left to ripen for 24 hours in a place at average temperature cca 10°C. Then garlic (1.9%) was added to the mixtures, mixed with meat and ground it in a meat grinder. The diameter of grid in the meat grinder was 4 mm. Sudžuk was stuffed in thin beef casings of 30-40 mm diameter. Before use, casings were salted and left in warm water to become elastic. A sausage stuffer was used to stuff the sausage mixture into casings. There is a hole with a thin tube on the bottom of the stuffer. A sausage casing was slid onto the funnel and pushed on the stuffer then, a casing was filled with the sausage mixture. Casings should be stuffed well, in order to become firm. After stuffing, the sudžuk was tied up in a shape of rings and settled on a stick to be equidistant; rings should not touch each other.

Prolonged liquid squeezing (fermentation) of the prepared samples lasted for 3 days. Room temperatures where samples were stored were in the range from 7°C up to 14°C. After prolonged liquid squeezing, the samples were settled on rounded sticks, separated from each other in order for every ring to be equally smoked and dried out. The samples were smoked and dried in a typical smokehouse with an open furnace, and it lasted for 12 days. Beech wood was used for fire. Distance between the sudžuk horseshoe and the furnace/fire bed was approximately three meters. Fire was made in the open furnace/fire bed without flame. Smoking and drying were done at room temperature between 0 °C and 10 °C. After the smoking process, the samples of sudžuk were left to ripen for 5 days in the same room at cca. 10 °C, while in the meantime sausages became of firmed consistency. During the whole process, mentioned above, the samples lost some of their initial weight which was determined by weighing the finished product and calculating lost weight (kalo). Afterwards, the samples of sausages were anylized in the Laboratory of Biotechnical Faculty, University of Bihać. Loss of weight during the liquid squeezing process (for 1st, 2nd and 3rd day) and at the end of process was determined using a scale (±0.01).

In the scope of physicochemical methods, the analyses of pH value were performed by pH metre according to the method ISO 2917:1999.

pH changes were recorded for samples I and II during the process of liquid squeezing (0, 1st, 2nd and 3rd day) and afterwards for the finished product. In the period of liquid squeezing process (for 0 and 3rd day) and for finished product, the following chemical analyses were performed: assessment of moisture content according to a method from drying to constant mass, BAS ISO 937; assessment of fat content according to a method by Soxhlet, BAS ISO 1443; assessment of protein content by Kjeldahl, determining nitrogen and multiplying by a factor (Nx6.25), BAS ISO 937; assessment of sodium chloride according to a volume method [9].

Sensory estimation of the finished product was done using quantitative-descriptive methods QDA [10]. The constant unstructured 10 cm long scale was used for the sensory estimation. Altogether 14 attributes of sudžuk were rated: view of cross section through four descriptors, connectivity of muscle and fat (adipose tissue), intensity in colour of muscle, intensity in colour of adipose tissue and presence of cortex; texture measuring in the mouth through four descriptors: softness, juiciness, toughness and fatness; taste through two descriptors: saltiness, sourness; aroma through four descriptors: flavour of garlic, flavour of black pepper, flavour of smoke and rustyness. A commission for evaluation of samples, consisted of 3 members, each of them having the following standards for tastes: saltiness (solution of 2% of salt) and sourness (solution of 0.1% of citric acid).

The obtained results were analyzed applying the statistical methods and significance of deviations was determined with an ANOVA test. When the main impact was significant, averages were split by Tukey's test of the smallest significant deviations at 5% level. Level of significance $p < 0.05$ was used for comparison and discussion of the obtained results.

The data were analyzed using statistical software SPSS (ver. 20).

RESULTS AND DISCUSSION

Table 1. Loss of weight (kalo) of Bosnian sudžuk after prolonged liquid squeezing (1st, 2nd and 3rd day) and of finished product

Liquid squeezing	Kalo %	
	Sample I	Sample II
1st day	5.50	6.25
2nd day	11.11	12.50
3rd day	18.88	18.75
Dried product	38.88	43.75

Loss of weight in the overall process (production) is due to process of drying, i.e. loss of moisture during the drying process. Loss of weight in fermented sausages is impacted by different factors: temperature, relative humidity, air circulation in a smokehouse, recipe of meat mixture, features of casings [11]. Operta et al. [10] stated that values for kalo ranged between 31.50% and 40.70% in Bosnian sudžuk production. Yildiz-Turp and Serdaroglu [12] determined kalo for Turkish fermented sausage of 35.10%. In Table 2, there are statistical parameters for pH of prolonged liquid squeezing (fermentation) (0 day, 1st day, 2nd day, 3rd day) and of dried product. On the 2nd day of liquid squeezing, Sample I had a quite low pH value compared to the 1st day. Sample II, which contained sheep meat, had a negligible increase in the pH value on the 2nd day, compared to the 1st day of liquid squeezing.

Table 2. Statistical-variation parameters for pH values of Bosnian sudžuk

Days	Sample I					Sample II				
	Parameters					Parameters				
	Σ	$\bar{x}$	SD	Stand. error	CV	Σ	$\bar{x}$	SD	Stand. error	CV
0 day	46.43	5.80^{A}	0.02	0.007	0.33	45.70	5.71^{B}	0.01	0.005	0.24
1st day	46.46	5.81^{A}	0.02	0.007	0.33	45.58	5.70^{B}	0.02	0.005	0.26
2nd day	45.76	5.72^{B}	0.02	0.008	0.41	45.91	5.74^{A}	0.01	0.004	0.21
3rd day	45.56	5.70^{B}	0.01	0.005	0.30	47.94	6.00^{A}	0.03	0.006	0.31
21st day –dried product	43.55	5.44^{B}	0.02	0.005	0.28	45.74	5.72^{A}	0.01	0.004	0.18

Σ – Summ, $\bar{x}$ – Average value of eight measurements, SD – Standard deviation, CV – Coefficient of variation; Average values in the same rows marked with supersricpts A,B are highly significantly different (p<0.01) (Tukey test)

Table 3. Statistical-variation parameters chemical analysis of samples during prolonged liquid squeezing (zero day and third day)

Chemical composition	Sample I –zero day					Sample II – zero day				
	Parameters					Parameters				
	Σ	$\bar{x}$	SD	Stand. error	CV	Σ	$\bar{x}$	SD	Stand. error	CV
Water %	513.12	64.14^{A}	0.80	0.281	1.45	490.84	61.35^{B}	1.08	0.383	1.77
Ash %	19.09	3.83^{B}	0.03	0.014	0.82	24.85	4.97^{A}	0.11	0.048	2.15
Fat %	52.04	10.41^{A}	0.08	0.035	0.75	42.49	8.50^{B}	0.05	0.022	0.58
Proteins %	97.43	19.49^{B}	0.03	0.014	0.16	114.02	22.80^{A}	0.08	0.038	0.37
NaCl %	15.00	3.00^{B}	0.05	0.023	1.72	19.69	3.94^{A}	0.03	0.016	0.89
	Sample I –third day					Sample II –third day				
	Parameters					Parameters				
	Σ	$\bar{x}$	SD	Stand. error	CV	Σ	$\bar{x}$	SD	Stand. error	CV
Water %	400.35	50.04^{b}	0.73	0.259	1.46	410.63	51.33^{a}	0.56	0.120	1.10
Ash %	27.81	5.56	0.06	0.026	1.06	27.62	5.52	0.25	0.115	4.66
Fat %	72.52	14.50^{A}	0.06	0.026	0.39	62.54	12.50^{B}	0.13	0.058	1.04
Proteins %	129.50	25.90^{b}	0.07	0.033	0.28	132.66	26.53^{a}	0.35	0.158	1.33
NaCl %	22.26	4.45^{b}	0.05	0.021	1.05	23.52	4.70^{a}	0.10	0.046	2.20

Σ – Summ, $\bar{x}$ – Average value of five measurements, SD – Standard deviation, CV – Coefficient of variation; Average values in the same rows marked with supersricpts A,B are highly significantly different (p<0.01); Average values in the same rows marked with supersricpts a,b are significantly different (p<0.05)

Table 4. Statistical-variation parameters chemical analysis of Bosnian sudžuk (dried product)

Chemical composition	**Sample I**					**Sample II**				
	Parameters					**Parameters**				
	Σ	$\bar{x}$	SD	Stand. error	CV	Σ	$\bar{x}$	SD	Stand. error	CV
Water %	348.64	43.58^A	0.03	0.012	0.08	330.08	41.26^B	0.03	0.010	0.07
Ash %	54.80	6.85	0.04	0.013	0.53	54.88	6.86	0.04	0.013	0.56
Fat %	85.49	17.10^B	0.05	0.021	0.28	90.00	18.00^A	0.04	0.017	0.21
Proteins %	157.34	31.47^B	0.03	0.012	0.08	164.39	32.88^A	0.03	0.012	0.08
NaCl %	31.20	6.24	0.03	0.013	0.47	31.04	6.21	0.03	0.015	0.54

Σ – Summ, $\bar{x}$ – Average value of five measurements, SD – Standard deviation, CV – Coefficient of variation; Average values in the same rows marked with supersricpts A,B are highly significantly different ($p<0.01$)

Table 5. Statistical-variation parameters sensory assesment of Bosnian sudžuk

Senzory properties	**Sample I**					**Sample II**				
	Parameters					**Parameters**				
	Σ	$\bar{x}$	SD	Stand. error	CV	Σ	$\bar{x}$	SD	Stand. error	CV
View of cross section										
Connectivity of muscle and fat	34.40	5.73	1.47	0.602	25.72	27.20	4.53	0.51	0.209	11.31
Colour of meat	28.60	4.77	1.14	0.466	23.94	32.50	5.42	2.50	1.020	46.12
Colour of fat	31.40	5.23^A	1.17	0.477	22.34	20.70	3.45^B	0.48	0.196	13.93
Presence of cortex	25.90	4.32	1.40	0.573	32.51	23.50	3.92	0.99	0.404	25.25
Texture in mouth										
Softness	23.40	3.90^B	0.45	0.183	11.47	34.00	5.67^A	1.13	0.461	19.92
Juiciness	29.10	4.85	0.97	0.395	19.94	23.90	3.98	0.65	0.266	16.38
Thougness	29.90	4.98	0.94	0.385	18.93	33.90	5.65	1.42	0.581	25.17
Fatness	29.00	4.83^b	0.75	0.307	15.57	38.40	6.40^a	1.04	0.426	16.30
Taste										
Saltiness	31.90	5.32	0.44	0.182	8.36	30.10	5.02	0.04	0.017	0.81
Sourness	25.80	4.30	0.55	0.225	12.82	28.90	4.82	1.36	0.554	28.17
Aroma										
Favour of garlic	33.10	5.52	1.23	0.504	22.36	20.60	3.43	2.29	0.937	66.82
Flavour of black pepper	23.30	3.88	1.85	0.756	47.72	16.00	2.67	1.96	0.802	73.62
Flavour of smoke	23.40	3.90	1.20	0.490	30.77	16.90	2.82	1.46	0.595	51.71
Rustyness	2.90	0.48	0.44	0.182	91.99	6.30	1.05	0.54	0.222	51.73
Overall grade	14.00	2.33	0.82	0.333	34.99	7.00	1.17	2.14	0.872	183.17

Σ – Summ, $\bar{x}$ – Average value of triplicate, SD – Standard deviation, CV – Coefficient of variation; Average values in the same rows marked with supersricpts A,B are highly significantly different ($p<0.01$); Average values in the same rows marked with supersricpts a,b are significantly different ($p<0.05$)

On the 3^{rd}day, a further decrease in pH value in Sample I was recorded, while in Sample II pH value was increasing. Salgado et al. [13] state that the increase in pH value in later phases affects the decrease in a content of lactic acid in a sausage mixture. Turkish Food Codex [14] concluded that pH value in fermented sausage should not be greater than 5.4. Lactic acid that occurred during the activity of bacteria of lactic acid on carbohydrates is responsible for the decrease in pH value [15].

Standards for production of traditional Turkish sudžuk [16] recommend a pH value of dried sausages between 4.7 and 5.4. Erkmen and Bozkurt [17] concluded that traditionally produced samples had pH value greater than 5.4. Siriken et al. [18] determined the average pH value for Turkish sudžuk 5.49, while Bozkurt and Bayram [19] determined pH for Turkish sudzuk 5.1. Table 3 shows the chemical results of the sample analysis for day 0 and 3^{rd} day. Sample I had a greater content of water compared to Sample II. Ercoskun et al. [20] found out that moisture content in Turkish sudžuk fluctuated between 57% and 58%. Sample II had a greater content of ash for day 0 compared to Sample I; 3^{rd} day of liquid squeezing ash content in the mixture was greater in Sample I than in Sample II. Ercoskun et al. [20] recorded ash content from 3.60% for day 0 to 5.11% for the fifth day. Sample I had a greater content of fat compared to Sample II. Ercoskun et al. [20] determined several values of fat content: from 25.66% of zero day to 36.57% of the fifth day. Sample II had a greater protein content compared to Sample I. Ercoskun at al. [20] determined lower values of proteins: from 13.26% for day zero 13.26% to 18.77% for the fifth day. Sodium chloride is responsible for the sensory properties and technological features in fermented sausage [21]. Sample II had a greater content of sodium chloride for day 0 compared to Sample I ($p<0.01$).

Third day Sample II had the increased content of sodium chloride compared to Sample I ($p<0,05$). Ercoskun et al. [20] determined a content of sodium chloride for day zero 2.56% to 3.66% for the fifth day. Table 4 shows the results of chemical analysis in the finished product. Sample I had greater water content compared to water content in Sample II ($p<0.01$). According to Operta et al. [22], a content of water in Bosnian sudžuk fluctuated between 28.65% and 33.45%. Siriken at al. [23] obtained variations in water content from 29.80% up to 47.60%. Sample II had greater fat content compared to fat content in Sample I ($p<0.01$). Operta et al. [10] determined high fat content for Bosnian sudžuk too, fluctuating from 27.33% up to 35.79%. Sample II had greater protein content compared to protein content in Sample I ($p<0.01$). Operta et al. [10] determined a content of proteins in samples of Bosnian sudžuk from 27.78% up to 33.40%.

Acording to Soyer et al. [24], a content of proteins in Turkish sudžuk fluctuated between 16.5% and 28.30%. NaCl (salt) is added to meat products because of its multiple impact on texture, aroma and sustainability, so that its decrease in meat products can have negative effects on water connectivity and emulsifying of fat, can damage the whole structure, increases losses in cooking, damages sensory quality, and especially affects taste [25]. In the survey by Operta et al. [26], a content of sodium chloride in Bosnian sudžuk was between 3.3% and 8.3%. Table 5 shows the results of sensory evaluation. Consistency of meat and fat in cross section was very good and uniform, also the colour of cross section was uniform. In both samples colour was dark red, except that colour tone in the sample with sheep meat was slightly darker. After ripening the cross section of product looked like mosaic, with small pieces of fat, which is characteristic for Bosnian sudžuk. There were no cracks inside the product, and the whole mixture had good consistency. Crust was standing out. According to the survey by Operta et al. [10], samples of sudžuk had good consistency in muscle and fat on cross section, while colour was dark red. The mark for softness (parameter of sensory feature texture in mouth) was greater for Sample II made with mixture of beef and sheep meat compared to Sample I, and Sample II had the greater mark for fatness than Sample I.

For sensory feature, saltiness in both of samples had a satisfactory salinity taste, without deviations in samples. Sourness was slightly stronger in Sample II compared to Sample I. Garlic aroma was stronger in Sample I compared to Sample II, which was the same for black pepper and smoke. The evaluators gave a greater grade for rustyness for Sample II. Toldra [27] suggested that rustyness and yellow colour of fat could be a consequence of oxidation unsaturated fat acids. The overall grade was better for Sample I compared to Sample II.

CONCLUSIONS

The results showed that loss of weight in Bosnian sudžuk (kalo) of a dried product was greater in Sample II in which recipe sheep meat was added. Fluctuation in changes of pH value in the stuffing mixture of sausage during the extended fermentation had a decreasing tendency in Sample I, while in Sample II the increase in pH was small. pH at the end of process (dried product) in both samples was lower than initial pH.

During the extended fermentation, the content of water decreased in both samples, and proportionally the content of other ingredients increased. After the sensory analysis, the overall result was better in case of Sample I compared to Sample II. Bosnian sudžuk produced from beef and from a mixture of beef and sheep meat in the prolonged liquid squeezing process (fermentation) is a high quality dried meat product.

Replacement in part of beef meat in the traditional recipe by sheep meat did not have a significant impact on quality and sensory acceptability, while the prolonged liquid squeezing process had a significant influence on the decrease in the pH value of mixture, that positively resulted in the quality of a finished product.

REFERENCES

[1] Pravilnik o usitnjenom mesu, poluproizvodima i proizvodima od mesa. Službeni Glasnik BiH 83/2013

[2] D. Karan, S. Vesković-Moračanin, N. Parunović, M. Rašeta, J. Babić , M. Đorđević, R. Tadić, Tehnologija mesa (50): 5-6 (2009) 335-341

[3] A. Smajić, Prerada mesa. Univerzitet u Sarajevu, Poljoprivredno – Prehrambeni fakultet Sarajevo (2014)

[4] B. Žlender, L. Gašperlin, Tehnologija mesa (45) 3-4 (2004) 81-88

[5] B.T. Cenci Goga, D. Ranucci, D. Miraglia, A. Cioffi, Meat Science, 78(2008)381-390

[6] P. Massimiliano, M. Bianchi, C. Cavani, Poultry Science 88 (2009) 1518-1523

[7] A. Olivares, J. Navarro, M. Flores, Food Cont. 115 (2010) 1464–1472

[8] I. Vuković. Osnove tehnologije mesa, četvrto izdanje. Veterinarska komora Srbije, (2012) Beograd

[9] Official Methods of Analysis of AOAC International, AOAC International, Gaithersburg, USA (2000)

[10] S. Operta, M. Dževdetbegović, S. Čorbo, J. Tahmaz, A. Šehović, Meso, Vol. XIV, 8 (2012b) 473-479

[11] J.G. Bloukas, E.D. Paneras, G.C. Fournitzis, Meat Science, 45 (1997) 133-144

[12] G. Yildiz-Turp, M. Serdaroglu, Meat Sciemce, 78 (2008) 447 – 454

[13] A. Salgado, M.C. García Fontán, I. Franco, M. López, J. Carballo, Food Chem. 92 (2005) 413-424

[14] Turkish Food Codex No 2000/4 (2000) Agriculture and Rural Affairs Minister, Ankara

[15] F.K.Lucke, Fermented meats. In B.M. Lund, A.C. (Tony) Baird-Parker,&G.W. Gould (Eds.), The microbiological safety and quality of food (3rd ed.) (2000) Maryland: Aspen Publishers.

[16] TSE (Turkish Standard Institution) (2002) Turkish sucuk. Turkish Standard, TS 1070, ICS 67.120.10, Ankara

[17] O. Erkmen, H. Bozkurt, Food Technol. Biotechnol 42 (2004) 63-69

[18] B. Siriken, H. Ozdemir, H. Yavuz, C. Pamuk,Food Control, 17 (2006) 923-928

[19] H. Bozkurt, M. Bayrym, Meat Science, 73 (2006) 344-350

[20] H. Ercoskun, S. Tagi, A. Hamdi Ertas, Meat Science 85 (2010) 174–181

[21] G. Comi, R. Urso, L. Iacumin, K. Rantsiou, P. Cattaneo, C. Cantoni, L. Cocolin, Meat Science 69 (2005) 381-392

[22] S. Operta, M. Dževdetbegović, S. Čorbo, J. Tahmaz, A. Šehović,Tehnologija mesa (53) 2 (2012a) 148–156

[23] B. Siriken, O. Cadirci, C. Yenisey, Journal of Animal and Veterinary Advences, (8) 10 (2009) 2027-2032

[24] A. Soyer, A.H. Ertas, U. Uzümcüoglu, Meat Science (69) 1 (2005) 135-141

[25] M. Rusunen, E. Puolanne, Meat Science 70 (2005) 531-541

[26] S. Operta, A. Smajić, A. Ganić, E. Karahmet, Vol LIII 59/1 (2008) 199-207

[27] F. Toldrá, Y. Sanz, M. Flores, Meat Fermentation Technology. In: *Meat Science and Application* (537–561), Hui Y. H., Wai-Kit Nip, Rogers R. W., Young O. A. (Eds.), (2001) New York: Marcel Dekker. Inc.

POSSIBLE APPLICATIONS OF INDUSTRY 4.0 BASED ON CUSTOMER RELATIONSHIP MANAGEMENT IN BAKERY

SCIENTIFIC REVIEW PAPER

Branimir Dukić[1*], *Robert Obraz*[2], *Stojanka Dukić*[3]

RECEIVED 2018-02-01
ACCEPTED 2018-05-18

[1]University of Josip Juraj Strossmayer in Osijek, Faculty of Economics in Osijek, Gajev trg 7, Osijek, Croatia
[2]Klimaoprema d.d. Samobor, Gradna 78A, Samobor, Croatia
[2]duxMission d.o.o. Osijek, Dalmatinska 21, Osijek, Croatia
*✉ bdukic@efos.hr

ABSTRACT: Production of bakery products went through a long and gradual transformation from individual, craft to modern industrial production. Today, the production of bakery products has been developed by mass high-automated industrial production. Although production plants that find their economic justification mostly on the expenditure side of reduction of production costs and production processes, the modern business requires a change of the income side, where development of relations with consumers has more important role. Production of bakery products had all the features of mass production until recently. Due to the importance of bakery products in the nutrition of people, particularly in developed parts of the world, demand for bakery products could be considered as non-elastic, since the price, quality and range of bakery products were basically irrelevant to the volume of demand for these products. However, at the end of the twentieth century the purchasing power of the population, as well as the level of information, knowledge and self-awareness, significantly influenced the consumption of bakery products. The modern consumer changes the structure of food in the nutrition and he is no longer satisfied with a mass-produced product, but requires a product that is “tailored” according to his needs. The manufacturing capabilities of modern, numerically controlled machines that make Industry 4.0, allow the production of “tailored” products according to the needs of individual consumers. Consequently, transformation and transition from mass to individualized bakery product production requires a scientific and systematic approach.

KEYWORDS: bakery, customer relationship marketing, industry 4.0, mass production.

INTRODUCTION

One of the challenges that human society has faced from its beginning to today is a problem of inertness, i.e. understanding and accepting changes which are an inevitable outcome of evolutionary social processes. The same problem is incurred in the process of preparing young people for their future life. When we talk about the education system, it is obvious that education processes are oriented toward the past, and learning principles and skills used in the past, or optionally those used–in modern conditions. However, the future is very rarely or never taken into consideration, or answers to the question what they would be like in the future are not offered either. Inertness of majority of human society was not an important problem in the past, because social changes happened very slowly. Things significantly changed during the 20th century, especially at the end of it, when society started galloping development under the influence of applying new scientific discoveries. Those changes were so rapid, that some fields of science were conservative and hardly accepted an idea that society would obtain totally different form in the near future, and that current principles of activities would almost not be worth the effort. Often, science in its study research perceives the current social situation without taking into consideration social changes and what will happen to society, and without considering how those changes will reflect on scientific research. One of the reflections of awareness of the pace of changes and importance of their acceptance is the “Bologna process”, which transforms education from partial to lifelong learning regardless to a fact of rapid obsolescence of acquired knowledge under dynamic developmental conditions, under which the modern world operates.

In science, inertness is generally a consequence of lack of interdisciplinary approach to research, which is necessary to accept new knowledge and understanding, especially those relating to social transformation. Technical sciences often ignore results of social and human science researches, focusing on scientific problems and considering them from the standpoint of today's developmental social scope, and not from the point of view of the major social changes, which are axiomatically held to be certain. A good example is that part of technological research sciences is oriented toward mass production processes, which were present during the 20th century, but which is to be ended. Often, the focus on investigating the process of mass production is justified by the thesis that in the future many products will remain products of mass production. However, it is a very questionable assumption, because changes in social behaviour influence social needs. It is evident that society enters the age of enormous awareness of individuals as well as of general rise in knowledge which strengthens self-awareness and complacency of each individual in that kind of society. That leads to rise of individual needs for a stronger identity, quality and safer life and increases the growth of individual needs. Banal examples of those changes are individualization and personalization of a large number of products and services. For instance, a modern tourist is increasingly oriented to individual travels than to mass travels. Mass and group fitness training sessions are more often replaced with individual training sessions with personal fitness coaches. An attitude toward clothing products and their substitution with those with brands has also evolved. Those social changes also produce the need to change the focus of technological research on the search for optimal production processes, which would ultimately result in lower production costs and more rational production. The focus would be on the low-level and individualized production of products with high added value, primarily in terms of services and knowledge, for the well-known consumer.

In accordance with the above stated, it is important to consider the question of possible changes in the sphere of production of bakery products. In order to give a relevant answer to this question, it is necessary to explore the future of bakery products and to answer the question whether bakery products are included in a group of products that are naturally products of mass production, or they are products that will be individualized products in the future, and then argue for taking a certain attitude. Results obtained from the research should be the starting point for further research related to the production processes in the bakery industry and cooperation of social sciences with technological science with the aim to optimally meet the needs of people in the new era.

RESEARCH METHODOLOGY

Modern human behaviour is largely determined by conditions in which the modern human exists. Unlike people in the last century, people today are much more informed, they possess a significantly higher level of knowledge, which results in a significantly higher level of self-awareness. The self-awareness of modern human is, among other things, reflected in his relation to both the environment and himself. Modern human is more environmentally friendly, more concerned about his health and appearance, but also about his social position. While the average reflected through the massive uniformity of society ruled during the mid of the last century, a modern man is an individual, “nobleman” of modern era who wants the best for himself. Therefore, a modern man is not satisfied with average uniform products, but seeks products tailored to his own needs and wishes. Modern industry can provide that, because modern manufacturing potential and capabilities have significantly changed owing to information and communication technology. It is this new industrial platform that can and should be studied by modern technological science to find solutions that satisfy individualized needs of modern consumers.

In accordance with the foregoing, it is possible to set the following hypothesis:

> It is possible to organize profitable individualized and personalized production of bakery products using a combination of Industry 4.0 concepts and consumer relationship management (CRM).

Research goals are:

1. Consider what Industry 4.0 is and prerequisites for using it in order to meet consumer needs for individualized and personalized products.
2. Consider what evolutionary processes are present in consumer’s behaviour, and how these changes have influenced the evolution of marketing and development of the concept of consumer relationship management.
3. Consider what the potential repercussions of evolutionary processes in industrial production and marketing are for production of bakery products, and determine whether bakery products will be more suitable for mass production or individualized production for the well-known consumer in the future.
4. Create a conceptual model of industry 4.0 concept in the bakery industry.

To achieve the goals of research the following scientific methods were used: deduction method, historical method, abstraction method, classification method, generalization method, aggregation method, combination method, causal conclusion method, descriptive and graphical modelling method methods.

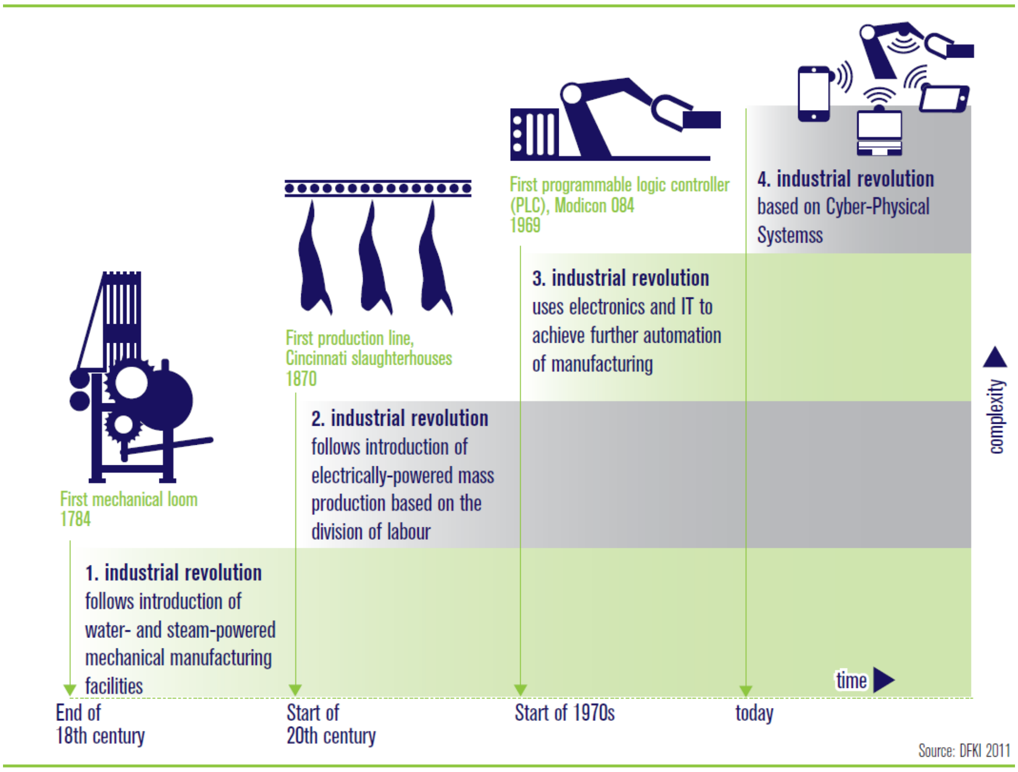
RESULTS OF RESEARCH

Until the beginning of the first industrial revolution at the end of the eighteenth century, the products were produced in a so called craftsmanship way which meant manufacturing without division of labour and use of manual tools, which made it slow and inefficient. Thus, until the onset of the industrial revolution, production was largely individualized or low-volume mainly for the known consumer. Given the level and scope of needs of the man at the time, most modern products did not exist or were produced in households that were self-sustainable or were the products of a craftsman [1]. The situation was the same with bakery products. It was significantly changed in the second industrial revolution, which took place from the end of 19th century to the second half of 20th century; yet its features are present in many industries even today.

The second industrial revolution was marked by creation of large industrial production capacities, especially in today’s developed western countries, which created conditions for mass industrial production [2]. The high division of labour and engagement of a large number of people was present in the production process.

The products were standardized and uniformed, and a business success was based on lowering fixed costs that drop with the increase of production volume. During the second industrial revolution, the focus of management was on production process optimization by increasing labour productivity and lowering production costs. Even today, a good part of the bakery industry has inherited the principles of behaviour that characterized the second industrial revolution. The characteristic of the third industrial revolution is high level automation following the use of electronics and robots in the production process [3]; while in the fourth industrial revolution the use of computerized production capacities, in particular the use of 3D production systems, enables personalized and individualized production of products tailored to the needs of modern human [4]. It can be concluded, therefore, that owing to the needs of modern consumers, who have become self-aware and seek products that are tailored to their needs, and owing to the potential that modern intelligent systems provide, the philosophy of production has made a full cycle, from production for own needs in households, individual and low volume production for well-known consumers, through mass, standardized and uniform production to modern low volume production, i.e. production tailored to the needs of small groups of consumers or even individualized consumers.

Figure 1 shows the evolutionary processes in industrial production.



Source: DFKI 2011

Figure 1. Evolution of industrial production [5]

The ratio of industrial production according to consumer needs is shown in Figure 2.

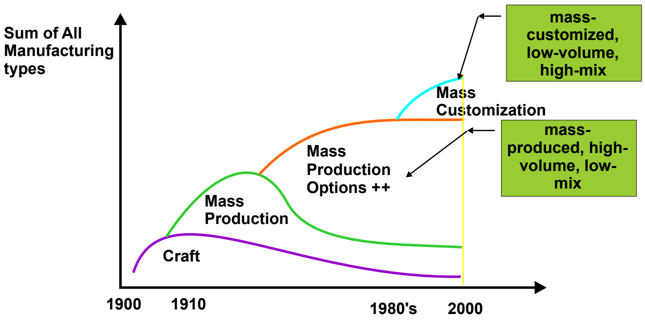


Figure 2. The ratio of industrial production to consumers [6]

In view of the evolutionary processes of pre-industrial age and industrial production, and the relation of production to the needs of consumers, the following can be differentiated:

- Domestic production for personal use
- Craft individual and low volume production
- Mass industrial production
- High volume industrial production with variations
- Low volume industrial production
- Individualized and personalized industrial production
- Domestic industrial production.

Figure 3 represents the circular process of industrial production from domestic, or craft individual production through mass production, again individualized and personalized production enabled by the production system within the Industry 4.0 concept.

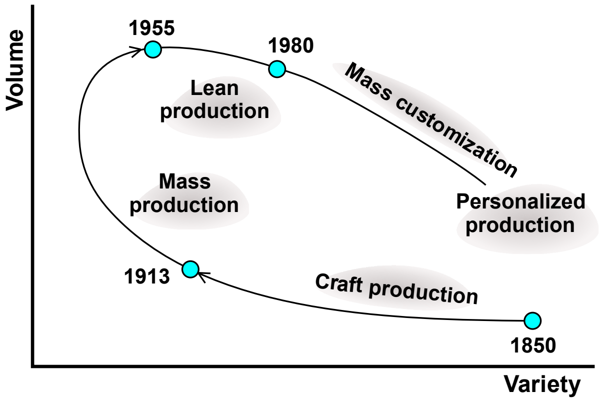


Figure 3. Circular process of industrial production [7]

Modern industrial production, which is defined by Industry 4.0 concept, is characterized by a high level of artificial intelligence, information and knowledge within the concept.

However, in order for Industry 4.0 to meet consumers’ needs, it is necessary to establish a stronger link between industrial production and consumers. If production concepts have no information on consumers’ needs, it is impossible to manufacture to satisfy those needs. Unlike mass production period when a manufacturer was not interested in customers’ needs due to the shortage of commodities in the market and high demand for everything that was produced; or unlike the period when care for consumers’ needs started and the needs were low and production of products that covered the modal consumer needs solved the problem of demand. Since today’s consumer is self-aware and wishes a product tailored to his own needs, such production is possible if a strong information and communication relationship between consumers and manufacturers is established.

When it comes to information about the needs of consumers, and the communication process with the consumer, then the focus of discussion should be on marketing, or relationship marketing as the last developmental version of the marketing philosophy. Marketing began in the middle of the last century in response to the problems of industrial production during the second half of the first half of the last century, when massive industrial production resulted in the emergence of surplus goods on the market and the problem of selling these goods. The solution to this problem was initially required within the framework of the so-called selling concepts by finding better ways to persuade the customer to buy the product. However, this concept soon showed its limitations, because people quickly learned to protect themselves from unscrupulous merchants and pushing goods. As an alternative to the sales concept, in the middle of the last century, marketing emerged. It focuses primarily on consumer’s needs and does not push the product and does not persuade the customer to buy the product, whether or not the customer needs the product. The primary idea is to investigate the needs of consumers, and based on those needs to produce goods. Logic implies that a consumer will buy a product if he needs it, or if a product meets his needs.

At its first stage, marketing was a mass concept that met average consumer’s needs, which was in line with development of consumers’ needs as well as with the power of industrial production. Therefore, information on consumers’ needs were gathered by market research prior to the beginning of production of a product, which was the basis for defining the product range selling price, as well as the basis for the choice of advertising the product and its distribution. As the needs of the population grew and people differentiated according to their needs, marketing also evolved into segmented marketing, then into marketing niche, only to evolve into a fine segmentation of the market, in the nineties of the last century, which transformed marketing into micro marketing, or 1:1 marketing relationship. Figure 4 shows evolutionary processes in marketing.

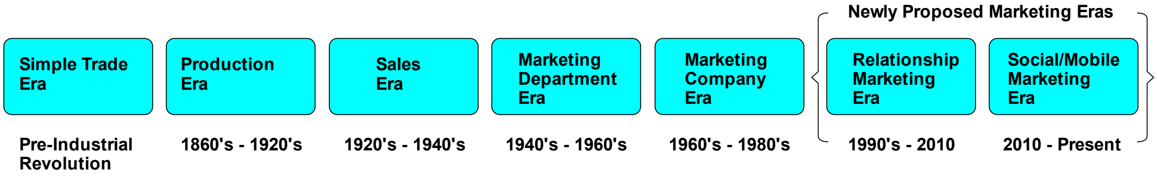


Figure 4. Evolutionary processes in marketing [8]

This last transformation of marketing should be related both to evolutionary processes in industrial production and to evolutionary processes in information and communication technology. Thereby, marketing relations are said to be an important component of digital marketing. Marketing relations is a consequence of evolution processes on the global market. In fact, in the era of global transparency and high competitiveness, it is very difficult to conquer new markets and find new free consumers. Recapturing a consumer from competition is an expensive and risky venture. Therefore, businesses try to keep their existing customers by building barriers around them, and take advantage of the repetitive and cross consumption in the best possible way. Accordingly, marketing from a former offensive business philosophy has transformed into a defensive business philosophy. In order to keep consumers and transform them into customers, it is necessary to optimally meet both their needs and their expectations. This can be done if you are well acquainted with consumers, or if you are familiar with their needs, but also if consumer dissatisfaction is quickly and efficiently eliminated. Therefore, it is necessary to continuously investigate consumer needs, i.e. consumer satisfaction with the products they consume. Permanent marketing research can be done only through permanent communication with the consumer in which a key role has a communication component of modern information and communication technology today. In order to realize in practice the theoretical framework set up by marketing relations, the application concept of consumer relationship management (CRM) has been built.

One of the key assumptions of the consumer relationship management concept is the systematic collection and recording of data on consumer characteristics and his behaviour in the database, for the acquisition of key knowledge of consumer needs and habits. Thus, any interaction with the consumer, whether in pre-sales, sales or post-sales activities, is recorded. The outcome of collecting data on the needs of the consumer and his behaviour results in a "genome" of consumers. Figure 5 represents the “genome” of a consumer.

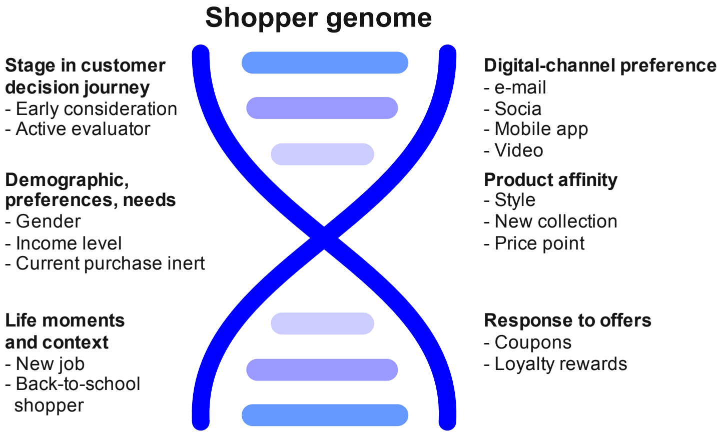


Figure 5. The customer or consumer genome [9]

When it comes to interaction between consumers and manufacturers in terms of designing and manufacturing products tailored to the needs and desires of an individual consumer, or a smaller group of consumers then, quality cooperation between Industry 4.0 concepts and consumer relationship management is necessary. The consumer relationship management concept should provide key information that is used to design and manufacture custom made products. Some important questions that arise are: for which products and to what extent it is important to customize the product, and whether the bakery products will remain mass production products or not, or if they are expected to be produced for small groups of consumers or individual consumers; furthermore, if it makes sense to produce bakery products for small groups, or individual consumers, then why and how to do it. In order to give answers to the above questions it is necessary to try to mentally model the future based on the trends that are present in human behaviour, and human society.

In the first half of the 20th century, due to the relatively low needs of people that were the result of low knowledge and awareness, the struggle for survival was the fundamental motive of work and action of most people. Ignorance of long-term results of the certain forms of behaviour was certainly one of the main reasons why a contemporary man accepted work conditions and activities that had an impact on his health and longevity. As science evolved particularly medical, chemical, biochemical, technology sciences and others, and also by spreading information through education and mass media, people's awareness of the need for curative and preventive action to restore and maintain long-term health, and to prolong life raised. An increase in the self-awareness of a modern man, particularly in the second half of the 20th century, resulted in an individual struggle of people to improve the quality of their own lives. Therefore, nutritionist science plays an increasingly important role in correlating the quality of nutrition with the health and quality of life. "Nutrition science began with modern chemistry and its founder Antoine Lavoisier infection, in the late 18th century. The basis for the establishment of new science, nutrition, has brought about the knowledge of general chemistry (identification of elements and compounds), development of chemical analysis methods, biochemistry and physiology, and the scientific and quantitative testing of old and new theories and ideas. Development of nutrition science was largely dependent on the development of analytical chemistry and general physiology."[10] Furthermore, the following can be stated: "Research is continued today. While in the middle of the last century classical nutrition was primarily concerned with hunger, rationalization of food and prevention of deficiency diseases, research today tends to determine the meaning of certain food ingredients (fibres, cholesterol, vitamins, minerals, phyto-substance) and eating habits on health and disease." [10].

If we take into account that food belongs to the basic human needs, and that there is a link between food, health and human illness, than modern manufacturing concepts supported by customer relationship management enable the production of individualized products, but also that further growth in the understanding of nutrition and individualization of the needs of people for the exact forms of nutrition, then it is expected that bakery products will be produced according to the individual needs. In addition, the growth of the needs and desires of people for individualized organoleptic properties [11] of bakery goods (appearance, colour, smell and taste), then it becomes certain that in the near future the bakery industry will be transformed into an individualized and personalized production based on the Industry 4.0 concept. The structural and process model of action of the production system is shown in Figure 6.

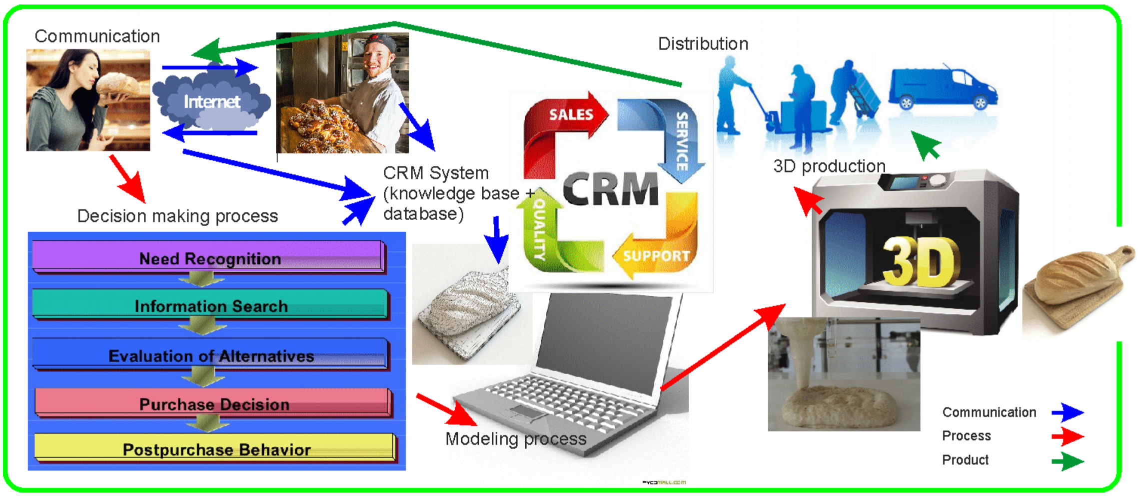


Figure 6. Structural and process model of bakery product production within the framework of Industry 4.0 [12] – [19]

CONCLUSION

Great part of modern production of bakery products is based on mass production or, to a lesser extent, on craft production. Mass production is a relic of the past that will probably become extinct due to the growth and changes in the needs of modern consumers. The modern consumer is no longer satisfied with the average product but wishes an individualized product that will meet his personal needs and preferences. However, bakery products as an individualized product of the future, besides having to satisfy the consumer with its organoleptic properties, it will have to respond nutritionally to consumer needs in terms of preserving his health and ensuring the quality of life. The reason for this lies in the fact that earlier demand for bakery products, as basic living food, was non-elastic, and that the price and quality of products did not affect a product demand. Therefore, mass production was a rational choice during that period. Today's survival of modern human does not depend on bakery products, so the demand for bakery products has become elastic, and bakery products have to adapt to new conditions, which implies transformation of bakery products from mass production to individualized production for the well-known consumer.

These expected changes also seek changes in the focus of science. At the time of mass production, the focus of technology but also economic sciences was on mass production in order to reduce fixed costs, as well as to optimize the product range and production processes. Nowadays, owing to the potentials of information and communication technology, the focus of bakery products production should be directed to optimally satisfy consumer needs to retain consumers. Thus, the new focus requires complete reorientation of technological, economic and information sciences which means leaving studies related to mass production and orienting to research which focuses on individualized and personalized production within Industry 4.0. A whole new series of problems arise, to which scientific answers should be found soon.

REFERENCES

[1] “History – Industrial Revolution”, *History.com.* [Online]. Available: http://www.history.com/topics/industrial-revolution [Accessed Dec 5, 2017]

[2] R. Engelman, “The Second Industrial Revolution, 1870-1914”, *U.S. History Scene*. [Online]. Available: http://ushistoryscene.com/article/second-industrial-revolution/ [Accessed Dec 5, 2017]

[3] J. Rifkin, “The Third Industrial Revolution: How the Internet, Green Electricity, and 3-D Printing are Ushering in a Sustainable Era of Distributed Capitalism”, *The World Financial Review*, Mar. 3, 2012. [Online]. Available: http://www.worldfinancialreview.com/?p=2271 [Accessed Dec 21, 2017]

[4] K. Schwab, “The Fourth Industrial Revolution: what it means, how to respond”, *World Economic Forum*, Jan. 14, 2016. [Online]. Available: https://www.weforum.org/agenda/2016/01/the-fourth-industrial-revolution-what-it-means-and-how-to-respond/ [Accessed May 29, 2017]

[5] T. Nguyen, “The 4th Industrial Revolution is Coming – But What Is It?”, *blog*, Mart 2015. [Online]. Available: http://www.apriso.com/blog/2015/03/the-4th-industrial-revolution-is-coming-but-what-is-it/ [Accessed Jun 17, 2017]

[6] S. Katoch, S. Singh, S. Piyush, D.N. Mohapatra, “Challenges & Opportunities for Operation Managers in case of Mass Customization in Supply Chain Management”, presentation, slideshare.net, 2015. [Online]. Available: https://www.slideshare.net/s2katoch/mass-customisation-in-scm-presentation [Accessed May 27, 2017]

[7] S. Bednar, V. Modrak, “Mass customization and its impact on Assembly process´ complexity”, *International Journal for Quality Research*, vol. 8(3), pp. 417-430, 2014.

[8] S. With, “The Evolution of Marketing”, *blog*, Jun 18. 2016. [Online]. Available: http://dstevenwhite.com/2010/06/18/the-evolution-of-marketing/ [Accessed Dec 6, 2017]

[9] G. BenMark, M, Masri, “Cracking the digital-shopper genome”, *ClikZ*, 2015. [Online]. Available: https://www.mckinsey.com/business-functions/marketing-and-sales/our-insights/cracking-the-digital-shopper-genome. [Accessed Dec 23, 2017]

[10] M. Šajin, “Povijest hrane, prehrane i nutricionizma”, *nutricionizam.com*, 2013. [Online]. Available: https://nutricionizam.com/ povijest-hrane-prehrane-i-nutricionizma/. [Accessed Jul 26, 2017]

[11] “Organoleptička svojstva”, Hrvatska enciklopedija, [Online]. Available: http://www.enciklopedija.hr/natuknica.aspx?id=45479 [Accessed Jul 26, 2017]

[12] “Small business 3d printers a beginners guide” [Online]. Available: http://www.smallbusinesscomputing.com/buyersguid/small-business-3d-printers-a-beginners-guide.html [Accessed Jan 2, 2017]

[13] K. Lee, “We Talk to Natural Machine’s Lynette Kucsma About the Foodini 3D Food Printer”, interview, inhabit.com. [Online]. Available: https://inhabitat.com/interview-we-talk-to-natural-machines-lynette-kucsma-about-the-foodini-3d-food-printer/ [Accessed Jan 2, 2017]

[14] “White Bread Loaf 3D model”. [Online]. Available: https://www.cgtrader.com/3d-models/food/miscellaneous/white-bread-loaf [Accessed Jan 2, 2017]

[15] “Gluten free bread aroma improved with precursors”, News & Analysis on the Bakery and Snacks Industries. [Online]. Available: https://www.bakeryandsnacks.com/ Article/2015/03/24/Gluten-free-bread-aroma-improved-with-precursors [Accessed Jan 2, 2017]

[16] “Baker Apprentice”, Sait. [Online]. Available: http://www.sait.ca/programs-and-courses/apprenticeships-and-trades/apprenticeship-programs/apprenticeship-baker [accessed Jan 2, 2017]

[17] “Internet cloud clipart”. [Online]. Available: http://www.okclipart.com/internet-cloud-clipart30jrfrsfge/. [Accessed Jan 2, 2017]

[18] “Consumer Behaviour”. [Online]. Available: https://www.slideshare.net/arjun.ramesh86/ consumer-behaviour-presentation-796587. [Accessed Jan 2, 2017]

[19] A. Nelly, “The Evolution of CRM”. [Online]. Available: http://www.dmnews.com/customer-experience/the-evolution-of-crm/article/570134/. [Accessed Jan 2, 20]

BIODEGRADABLE POLYMERS: PRODUCTION, PROPERTIES AND APPLICATION IN MEDICINE

SCIENTIFIC REVIEW PAPER

Amira Cipurković[1], Emir Horozić[2], Nusreta Đonlagić[1],
Snježana Marić[1], Mirzeta Saletović[1], Zahida Ademović[2]

RECEIVED 2018-01-22
ACCEPTED 2018-05-18

[1]Faculty of Natural Science and Mathematics, University of Tuzla, Bosnia and Herzegovina
[2]Faculty of Technology, University of Tuzla, Bosnia and Herzegovina
*✉ amira.cipurkovic@untz.ba

ABSTRACT: Biodegradable polymers (biopolymers) represent materials of new generation with application in different areas of human activity. Their production has recently reached a commercial level. They can be divided according to the origin (natural and synthetic), according to the chemical composition, methods of obtaining, application etc. The use of biopolymers in medicine depends on their biocompatibility, mechanical resistance, and sorptive characteristics. Today, they are the most commonly used as implants in vascular and orthopedic surgery, for the production of materials such as catheters, products for gynaecology and haemodialysis, tooth reconstruction, etc. In pharmacy, they are used as a medicine matrix-carrier to allow controlled release of drug within the body. Within this review paper, the properties and methods of production of certain biopolymers such as polyglycolic acid (PGA), polylactide acid (PLA), poly-ε-coprolactone (PCL) and polybutylene succinate (PBS) will be described in detail, as well as their application in medicine and pharmacy.

KEYWORDS: polyglycolic acid (PGA), polylactide acid (PLA), poly-ε-coprolactone (PCL), polybutylene succinate (PBS)

INTRODUCTION

A biomaterial is defined as any natural or synthetic substance engineered to interact with biological systems to direct medical treatment.

Biomaterials must be biocompatible meaning that they perform their function with an appropriate host response [1]. Biodegradable polymers comprise ester, amide, or ether chemical bonds. In general, biodegradable polymers can be grouped into two large groups on basis of their structure and synthesis. One of these groups is agro-polymers, i.e. those derived from biomass. The other consists of bio polyesters, which are those derived from microorganisms or synthetically made from either naturally or synthetic monomers [2].

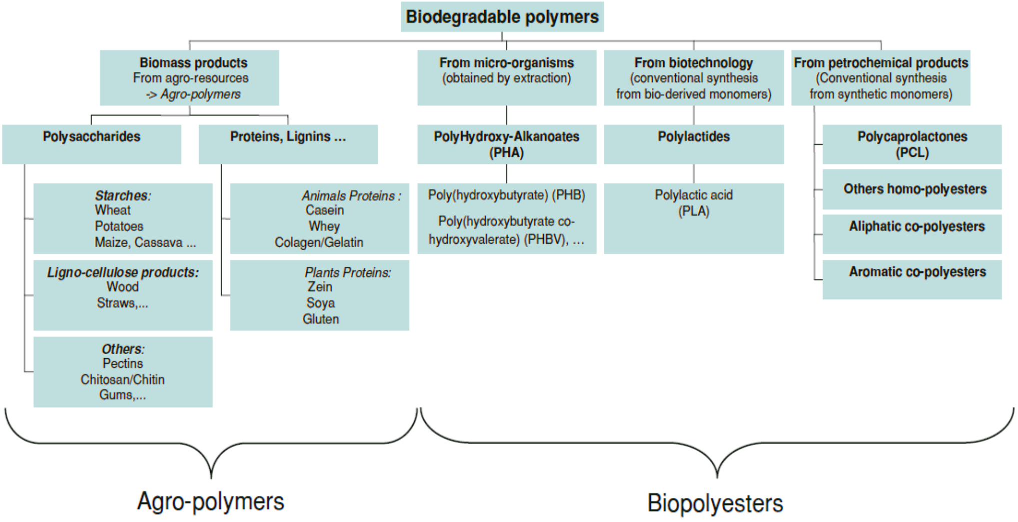


Figure 1. Classification of the main biodegradable polymers [3]

Biodegradable polymers represent a growing field. A vast number of biodegradable polymers (e.g. cellulose, chitin, starch, polyhydroxyalkanoates, polylactide, polycaprolactone, collagen and other polypeptides) have been synthesized or are formed in a natural environment during the growth cycles of organisms. Some microorganisms and enzymes capable of degrading such polymers have been identified. There are different classifications of biodegradable polymers, hereby is presented the classification according to the synthesis process, which is shown in Figure 1.

Polyesters are among the most used biodegradable plastics, given their hydrolysable ester bonds; therefore they represent a valid solution for biomedical applications. The properties of these materials strictly depend on the monomers used for their synthesis.

The aliphatic polyesters are almost the only high molecular weight biodegradable compounds and thus have been extensively investigated. Their hydrolysable ester bonds make them biodegradable. Aliphatic polyesters can be classified into two types according to the bonding of the constituent monomers. The first class consists of the polyhydroxyalkanoates. These are polymers synthesized from hydroxyacids, HO-R-COOH.

Examples are poly(glycolic acid) or poly(lactic acid). Poly(alkene dicarboxylate)s represent the second class. They are prepared by polycondensation of diols and dicarboxylic acids. Examples are poly(butylene succinate) and poly(ethylene succinate) [4]. Application of biomaterials in therapy are various and some of these applications are aimed at replacing a lost function or an organ and request a therapeutic device made of biomaterials (prosthesis) for the rest of the patient's lifetime. Many other biomedical applications require a therapeutic aid for a limited period of time. Accordingly, it is desirable that the temporary therapeutic aid disappear from the body after healing in order to avoid the storage of any foreign materials.

Whereas permanent aids require biostable biomaterials, temporary aids should be preferably made of degradable or biodegradable compounds that can be eliminated from the body or to be bioassimilated after use.

Historically, biopolymers, i.e. polymers of natural origin such as polysaccharides and proteins, were primarily used as sources of wound dressings and suture threads either under their natural forms or after some chemical treatments. Because macromolecular compounds are usually biodegradable, i.e. degraded via biological processes, biopolymers are often regarded as suitable compounds to make bioresorbable therapeutic devices [5]. Biodegradable polymers that are often used as biomaterials are shown in Figure 2.

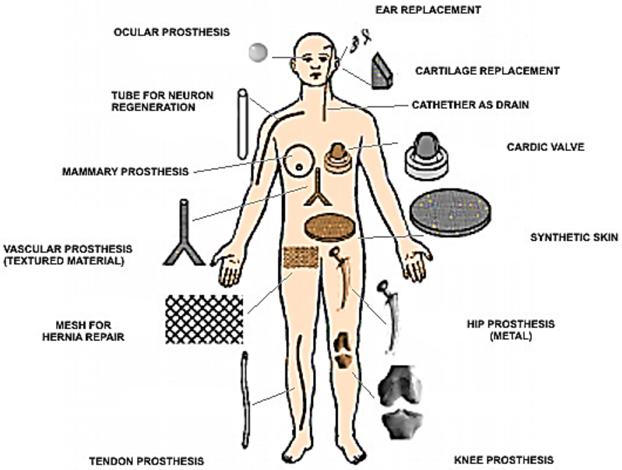


Figure 2. Biodegradable polymers used as biomaterials in human organism [7]

To be used as biomaterials, biodegradable polymers should have three important properties: biocompatibility, bioabsorbility and mechanical resistance. The use of enzymatically degradable natural polymers, as proteins or polysaccharides, in biomedical applications began thousands of years ago, whereas the application of synthetic biodegradable polymers dates back some fifty years. Current applications of biodegradable polymers include surgical implants in vascular or orthopaedic surgery and plain membranes. Biodegradable polyesters are widely employed as a porous structure in tissue engineering because they typically have good strength and an adjustable degradation speed [4]. Biodegradable polymers are also used as implantable matrices for the controlled release of drugs inside the body or as absorbable sutures [6].

SYNTHESIS, PROPERTIES AND APPLICATION OF SOME BIODEGRADABLE POLYMERS

Properties and methods of production of most common biopolymers: polyglycolic acid (PGA), polylactide acid (PLA), poly-ε-coprolactone (PCL) and polybutylene succinate (PBS) are described, as well as their application in medicine and pharmacy.

POLYGLYCOLIC ACID (PGA)

Polyglycolide or polyglycolic acid (PGA) is a biodegradable, thermoplastic polymer and the simplest linear, aliphatic polyester (Figure 3). PGA has been known since 1954 as a tough fiber-forming polymer [8]. Polyglycolide has a glass transition temperature between 35-40°C and its melting point is reported to be in the range of 225-230°C. PGA also exhibits an elevated degree of crystallinity, around 45-55%, thus resulting in insolubility in water. The solubility of this polyester is somewhat unique, in that its high molecular weight form is insoluble in almost all common organic solvents (acetone, dichloromethane, chloroform, ethyl acetate, tetrahydrofuran), while low molecular weight oligomers sufficiently differ in their physical properties to be more soluble. However, polyglycolide is soluble in highly fluorinated solvents like hexafluoroisopropanol (HFIP) and hexafluoroacetone sesquihydrate that can be used to prepare solutions of the high molecular weight polymer for melt spinning and film preparation. Fibers of PGA exhibit high strength and modulus (7 GPa) and are particularly stiff [9].

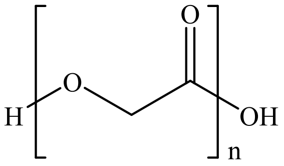


Figure 3. PGA structure

Synthesis. Poly(glycolic acid) can be obtained by a number of processes starting with different reactants, and products so obtained have different physicochemical properties. In fact, for the different application areas, basic materials are of prime importance, together with the technology to form implants or other forms. For instance, in the orthopaedic field, mechanical properties and design of the end product will be essential; to a lesser extent this will be the case in the pharmaceutical field, and so less attention needs to be paid to these aspects [10]. PGA can be obtained through several different processes starting with different materials: polycondensation of glycolic acid, ring-opening polymerization of glycolide and solid-state polycondensation of halogenoacetates. Polycondensation of glycolic acid is the simplest process available to prepare PGA, but it is not the most efficient because it yields a low molecular weight product. The most common synthesis used to produce a high molecular weight form of the polymer is ring-opening polymerization of "glycolide", the cyclic diester of glycolic acid (Figure 4).

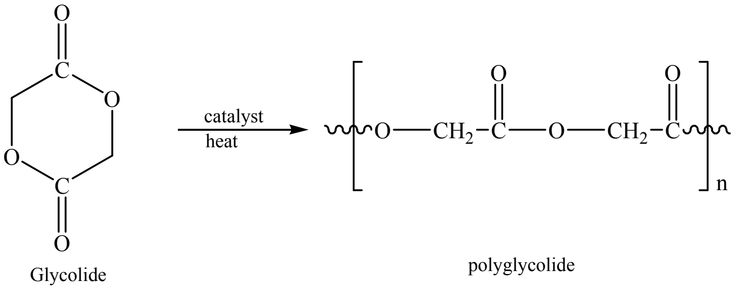


Figure 4. Ring-opening polymerization of glycolid In spite of its low solubility, this polymer has been fabricated into a variety of forms and structures. Extrusion, injection and compression molding as well as particulate leaching and solvent casting, are some of the techniques used to develop polyglycolide-based structures for biomedical applications. The high rate of degradation, acidic degradation products and low solubility however, limit the biomedical applications for PGA.

Therefore, several copolymers containing glycolide units are being developed to overcome the inherent disadvantages of PGA. Due to its hydrophilic nature, surgical sutures made of PGA tend to lose their mechanical strength rapidly, typically over a period of two to four weeks post-implantation. In order to adapt the materials properties of PGA to a wider range of possible applications, researchers undertook an intensive investi-gation of copolymers of PGA with the more hydrophobic PLA.

Polycondensation of glycolic acid is the simplest process available to prepare PGA, but it is not the most efficient one because it yields a low molecular weight product [11]. Briefly, the procedure is as follows: glycolic acid is heated at atmospheric pressure and a temperature of about 175–185°C is maintained until water ceases to distill. Subsequently, pressure is reduced to 150 mm Hg, still keeping the temperature unchanged for about two hours and the low molecular weight poly(glycolic acid) is obtained. The polymer obtained has a low molecular weight, because it is hard to remove water completely from the highly viscous reaction mixture; therefore a polymer of a molecular weight of a few ten thousands is obtained. In the polycondensation system of PGA, two principal equilibrium exist, one is dehydration equilibrium for esterification [12]. The cationic-ring opening polymerization reaction of lactones has been achieved using alkylating agents, acylating agents, Lewis acids, and protic acids.

However, the quality of end product varies with the agents used. There are reports that polymers prepared using protic acid such as sulphuric acid and phosphoric, yield brittle and highly coloured polymers in high yield. While the polymers prepared using Lewis acids such as zinc chloride, ferric chloride, aluminium chloride, titanium tetrachloride, boron triflouride etherate, and antimony triflouride yield high molecular weight and high tensile strength PGA, especially antimony triflouride gave a tough and colourless almost quantitatively, whose reduced viscosity was higher than 0.7 [13]. Boron triflouride was moderately active at low temperature of 110°C.

Chemical and physical properties. Polyglycolide has a glass transition temperature between 35-40°C and its melting point is reported to be in the range of 225-230°C. PGA also exhibits an elevated degree of crystallinity, around 45-55%, thus resulting in insolubility in water [11].

PGA can be easily crystallized as spherulites and hedrites in a hedritic rosette. Braided sutures from melt-extruded, stretched, and heat-set PGA fibres were chosen for their high strength, excellent handling properties, minimal tissue reactivity, and a similar but more reproducible absorption rate than catgut, as comparing to nylon-4, poly(β-hydroxybutyric acid), poly(ethylene oxide), oxidized regenerated cellulose, and poly(vinyl alcohol) as absorbable sutures [14].

However, one of the two PGA polymorphs from ring-opening polymerization is readily degradable in the presence of moisture. In 1973, using the thermogravimetric gas evolution analysis together with kinetic study, It was confirmed that degradation of PGA was a first-order reaction mainly via an intramolecular ester interchange mechanism, as shown in Figure 5 [14].

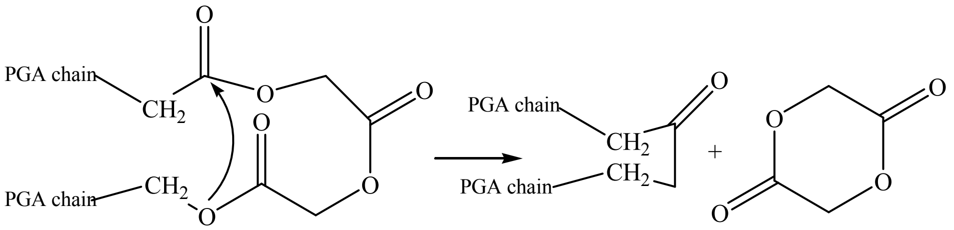


Figure 5. Intramolecular ester interchange mechanism of poly(glycolide) degradation (the intramolecular arrow indicates the direction in which the ester interchange occurs)

Application. Polyglycolide and its copolymers poly(lactic-co-glycolic acid) (PLA–PGA), poly(glycolide-*co*-caprolactone) and poly(glycolide-*co*-trimethylene carbonate) are widely used as materials for the synthesis of absorbable sutures. In most cases, PGA is copolymerized with other organic acids such as with PLA to make a PLA–PGA copolymer for improving its property. PGA–PLA copolymers have been known to be biodegradable and histocompatible for the past 40 years. Their physicochemical and biological properties have been found suitable, in many instances, for sustaining drug release *in vivo* for days or months. Microencapsulation technique is chosen frequently for its unique properties because microcapsules can be made using different traditional and nontraditional techniques containing core materials ranging from biological proteins to synthetic drugs. A biocompatible delivery system using poly(-DLlactide-*co*-glycolide) microspheres was developed as a controlled release antigen for parenteral administration. It offers several advantages in terms of immune adjuvanticity over other compounds. In contrast to other carriers, microspheres are more stable, thus permitting administration by the oral or parenteral route [14]. Because of PGA’s rapid degradation and insolubility in many common solvents, limited research has been conducted with PGA-based drug delivery devices. Instead, the most recent research has focused on short-term tissue engineering scaffolds and the utilization of PGA as a filler material coupled with other degradable polymer networks [1].

PGA is often fabricated into a mesh network and has been used as a scaffold for bone, cartilage, tendon, tooth, vaginal, intestinal, lymphatic, and spinal regeneration. Although there has been research conducted into a wide range of applications, there exist significant issues with PGA. Rapid degradation leads to the loss of mechanical strength and significant local production of glycolic acid. Although glycolic acid is bioresorbable by cells via the citric acid cycle, high level of glycolic acid have been linked to a strong, undesired inflammatory response. In addition, PGA has mechanically failed as a biomaterial when used to facilitate colonic anastomosis formation and prevent intrapericardial adhesions [1].

POLYLACTIC ACID (PLA)

Polylactic acid or polylactide (PLA) is a biodegradable thermoplastic aliphatic polyester derived from renewable resources (corn starch, cassiva roots, sugarcane etc.). PLA is the most consumed biopolymer in the world (Figure 6) [15]. Polylactic acid belongs to the family of aliphatic polyesters commonly made from α-hydroxy acids, which include polyglycolic acid or polymandelic acid, and are considered biodegradable and compostable. PLA was discovered in 1932 by *Carothers (at DuPont)*. He produced a low molecular weight PLA by heating lactic acid under vacuum while removing condensed water. By ring-opening polymerization of the lactide, high-molecular PLA was synthesized. PLA was first used in combination with polyglycolic acid (PGA) and sold under name *Vicryl* in the USA in 1974 [16].

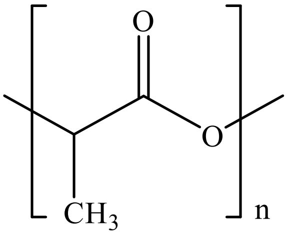


Figure 6. PLA structure

Synthesis. The basic building block for PLA is lactic acid, which was first isolated in 1780 from sour milk by the Swedish chemist *Scheele* and first commercially produced in 1881 [17]. The production of PLA is a multistep process which starts from the production of lactic acid, (2-hydroxy propionic acid), as single monomer of PLA, which is produced via fermentation or chemical synthesis and -ends with its polymerization. An intermediate step is often the formation of lactide. Polymerization of lactic acid can follow three main routes: direct condensation polymerization, direct polycondensation in an azeotropic solution and polymerization through lactide formation. Lactic acid can be manufactured either by carbohydrate fermentation or chemical synthesis, although fermentation predominates [18]. The majority of the world's commercially produced lactic acid is made by bacterial fermentation of carbohydrates, using homolactic organisms such as various optimized or modified strains of the genus Lactobacilli, which exclusively form lactic acid. The organisms that predominantly yield the L(+)-isomer are *Lactobacilli amylophilus*, *L. bavaricus*, *L. casei*, *L. maltaromicus* and *L. salivarius* [17].

These strains yield high carbon conversions from feed stocks at standard fermentation conditions, pH range of 5.4 to 6.4, a temperature range of 38 to 42 °C, and a low oxygen concentration. The various types of carbohydrates that can be utilized in the fermentation depend on the particular strain of *Lactobacillus*. Most of the simple sugars obtained from agricultural byproducts can be used. These sugars include: glucose, maltose and dextrose from corn or potato starch; sucrose from cane or beet sugar and lactose from cheese whey.

Commercial fermentation is usually conducted in a batch process. Sugar concentrations of 5-10% are used, with the production rates of 2 grams of acid per 1 litre of broth per hour. Various methods of extraction of the produced acid have been developed. The major method of separation is based on calcium hydroxide addition to neutralize the fermentation acid and give soluble calcium lactate solutions, which is recrystallized and purified to give crude lactic acid.

Direct condensation polymerisation of lactic acid yields low-molecular-weight glassy polymer which is unusable for any application unless external coupling agents are used to increase the molecular weight of the polymer. Producing high molecular weight PLA polymers by direct polycondensation in an azeotropic solution application of catalysts is more efficient. The choice of catalysts and solvent volume percentages enabled the higher molecular weights of PLA up to 6.6 x 10^4 [19]. The third mechanism of producing PLA is to purify and ring-open polymerize (ROP) lactide to yield high-weight-average molecular weight (M>100.000) PLA (Figure 7).

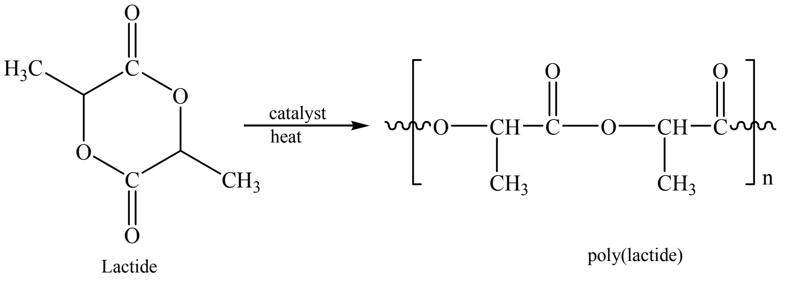


Figure 7. Synthesis of poly(lactide) (PLA)

The lactide method was the only method of producing pure, high-molecular-weight PLA until *Mitsui Toatsu Chemicals* commercialized the catalytic polymerization process in aprotic solvent, i.e., azeotropic dehydration condensation and obtained PLA with average molecular weights greater than 300.000 [20]. To increase the molecular weight, chain-coupling agents must be added, and it will preferentially react with either the hydroxyl or carboxyl groups. The condensed PLA can be modified to produce either all hydroxyl or all carboxyl groups. The PLA can also be treated with anhydrides such as maleic or succinic to convert the hydroxyl to a carboxylic end-group [21]. Various esterification–promoting adjuvants and chain-extending agents have been reported that can be used to increase the molecular weight of the PLA condensation products [22]. The use of chain-extending agents overcomes many of the disadvantages associated with esterification – promoting adjuvents. The azeotropic condensation polymerization is a method to obtain high-molecular-weight polymer without the use of chain extenders or adjuvents. The ring-opening polymerization of lactide was first demonstrated by *Carothers* in 1932, but high molecular weights were not obtained until improved lactide purification techniques were developed by *Du Pont* in 1954 [23]. Polymerization through lactide formation is being industrially accomplished for high molecular weight PLA production. Ring-opening polymerization of lactide can be carried out in melt or solution by cationic, anionic and coordination mechanisms, depending on the initiator utilized. The most considered active initiator for the L-lactide ring–opening polymerization is stannous octoate (bis-2-ethyl hexanoate, $SnOct_2$), which causes a low degree of racemization at high temperature. The choice of initiator system, co-initiator as chain control agent, catalyst concentration, monomer-to-initiator ratio, and polymerization temperature and time significantly affect the polymer properties. Properties such as molecular weight, degree of crystallinity, and residual monomer content, in turn affect the psychico-mechanical properties of polylactide and its copolymers [24].

Chemical and physical properties. Poly(lactic acid) exists as a polymeric helix, with an orthorombic unit cell. Due to chiral nature of lactic acid, several forms of polylactide exist. PLA is soluble in chlorinated solvents, such as benzene, tetrahydrofuran and dioxane [22]. The tensile properties of PLA can vary widely, depending on whether or not it is annealed or oriented or what its degree of crystallinity is. Polyactic acid can be processed into fibre and film on standard plastics equipment. PLA undergoes thermal degradation at temperatures above 200^{o}C by hydrolysis, lactide reformation, oxidative main chain scission, and inter- or intramolecular transesterification reactions. PLA degradation is dependent on time, temperature, low-molecular-weight impurities and catalyst concentration [25]. Catalysts and oligomers decrease the degradation temperature and increase the degradation rate of PLA. Poly(lactic) homopolymers have a glass-transition and a melt temperature of about 55°C and 175°C, respectively. PLA have a very narrow processing window. The most widely used method for improving PLA processability is based on melting point depression by the random incorporation of small amounts of lactide enantiomers of opposite configuration into the polymer (i.e., adding a small amount of D-lactide to the L-lactide) [26]. High-molecular-weight poly(lactic acid) is a colourless, glossy, stiff thermoplastic polymer with properties similar to polystyrene. The amorphous PLA is soluble in most organic solvents (tetrahydrofuran, THF), chlorinated solvents, benzene, acetonitrile and dioxane [17].

Application. PLA has potential for use in a wide range of applications. It is used as a buffering agent, acidic flavouring agent, acidulant and bacterial inhibitor in many processed foods. PLA is growing alternative as a „green“ food packaging polymer. Due to the larger thermal processing ability compared to other biomaterials like polyethylene-glycol, polyhydroxyalkanoates (PHA) and poly α-caprolactone; the processing of PLA can be achieved by film casting, extrusion, blow molding and fiber spinning [27]. The major PLA application today is packaging (70%); the estimation for 2020 shows the increase of other applications especially in biomedicine.

Commercialized PLA products demonstrate the fact that PLA is not being used solely because of its degradability, nor because it is made from renewable resources; it is being used because it functions very well and provides excellent properties at a competitive price [28]. Applications of PLA are limited by several factors such as low glass transition temperature, weak thermal stability and low toughness and ductility [29].

A large number of investigations have been performed on the blending of PLA with various polymers, such as: thermoplastic starch, poly(ethylene oxide), poly(ethylene glycol), poly(ε-caprolactone), poly(vinyl acetate), poly(butylene succinate) etc. Low molecular weight compounds have also been used as plasticizers for PLA, for example, oligomeric lactic acid, glycerol, low molecular citrates etc. [30].

The choice of polymers or plasticizers that are to be used as modifiers of PLA is limited by the requirements of application. The final properties of these blends depend on the chemical structure of the original components, the mixing ratio of the constituent polymers, the interaction between the components and the processing steps to which they are then subjected. PLA is also used in biomedical applications, with various uses as internal body components, interference screews in ankle, knee and hand; tacks and pins for ligament attachment; rods and pins in bone, plates and screews for craniomaxillofacial bone fixation [31] and also for surgical sutures, implants and drug delivery systems [32]. This is based on their advantages over nondegradable biomaterials with respect to long term biocompatibility. PLA offers biodegradability, biocompatibility and thermoplastic process ability, and it is used as surgical implant material and drug delivery systems, as well as porous scaffolds for the growth of neo-tissue [33]. Tissue engineering is a technique whose concept was introduced in 1988 by the reconstruction of the biological tissues using biomaterials. Three dimensional porous scaffolds of PLA have been created for culturing different cell types used in cell based gene therapy for cardiovascular diseases, muscle tissues, bone and cartilage regeneration [34].

Different surface modification strategies, such as physical, chemical, plasma and radiation induced methods for creating desirable surface properties of PLA biomaterials. PLA fibres are used for ligament and tendon reconstruction and stents for vascular and urological surgery. One application of PLA in the form of injectable microspheres is for temporary fillings in facial reconstructive surgery, as well as embolic material in trans catheter arterial embolization [35].

Microspheres and microcapsules have been applied in drug delivery systems (DDS). Release of drugs from these systems is based on several mechanisms that include diffusion and polymer degradation (hydrolysis or enzymatic degradation) [36]. Erosion, diffusion and swelling are one of the ways by which polymeric drug release occurs. In the encapsulation process of many drugs, PLA and their copolymers have been utilized in nanoparticle form [37]. Different methods were used to obtain nano-particles, such as solvent evaporation, solvent displacement, salting out and emulsion solvent diffusion [38]. PLA nano particles have been tested in human skin revealing that they can propose the active sites into hair follicles which makes them a stellar candidate as a drug delivery layout [39].

Due to high strength it is possible to create 3D structures for bone fixation in the forms of plates, pins, screws and wires. Three dimensional (3D) electrowave fibrous scaffolds is a possibility tissue engineering device for bone renewal. PLA and its copolymers are utilized in wound management, such as surgical sutures, healing dental extraction wounds [40].

PLA can be blended with other monomers, such as glycolic acid, and such copolymer is used in drug release. PLA is often mixed with starch to increase biodegradability and to reduce its price and to increase the water absorption.

POLY (E-CAPROLACTONE) (PCL)

Poly ε-caprolactone, PCL is one of biodegradable and biocompatible polymers, which have received significant attention because they are environmentally friendly and are extensively used in biomedical applications. PCL structure is shown in Figure 8.

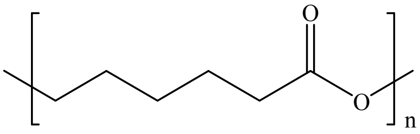


Figure 8. PCL structure

Synthesis. Poly (ε-caprolactone) PCL is synthesized by polymerization of hydroxy acids, HO-R-COOH, or by ring-opening-polymerization of cyclic monomers, -R-COO-. PCL is prepared by the ring opening polymerisation of the cyclic monomer (ROP) of ε-capro lactone (ε-CL) using ammonium heptamolybdate as a catalyst at 155 °C. It is composed of five methy lenes [$(CH_2)_5$] and an ester functional group as the repeating unit (Figure 9) and was studied as early as the 1930s.

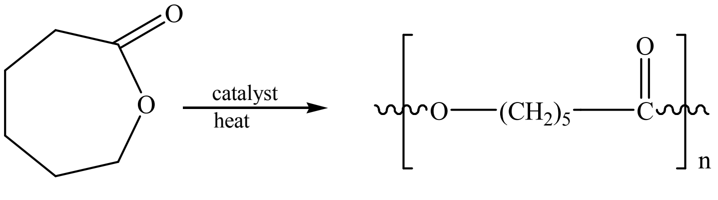


Figure 9. Synthesis of poly(ε-caprolactone) (PCL)

PCL and its derivatives are polymers that have been of great interest in the pharmaceutical industry as matrices for medical formulas. Recently a wide range of catalysts for the ring opening polymerization of caprolactone has been reviewed. Catalysts such as stannous octoate are used to catalyze the polymerization and low molecular weight alcohols can be used to control the molecular weight of the polymer. There are various mechanisms which affect the polymerization of PCL and these are anionic, cationic, coordination and radical. Each method affects the resulting molecular weight, molecular weight distribution, end group composition and chemical structure of the copolymers [41]. The obtained PCL was purified by dissolution into chloroform and precipitated ion with methanol, finally being isolated by vacuum filtration [42].

Chemical and physical properties. PCL is a semicrystalline aliphatic polymer that has a slow degradation rate 12-24 months. It has a low glass transition temperature at -60°C, a melting temperature at about 60°C, and a high thermal stability [43]. PCL has been shown to be degraded by the action of aerobic and anaerobic microorganisms that are widely distributed in various ecosystems [44]. The biodegradability of PCL can be increased by copolymerization with aliphatic polyesters [45]. PCL is soluble in tetrahydrofuran, chloroform, methylene chloride, carbon tetrachloride, benzene, toluene, cyclohexanone dihydropyran and 2-nitropropane and only partially soluble in acetone, 2-butanone, ethyl acetate, acetonitrile and dimethyl fumarate [46]. Recently, other studies have considered the fabrication of PCL nanofibres using less harmful solvents, like formic acid. In fact, formic acid is relevant for the reduction of the fibre diameter [47]. PCL is degradable in several biotic environments, including river and lake waters, sewage sludge, farm soil, paddy soil, creek sediment, roadside sediment, pond sediment, and compost [48]. The degradation times of PCL varies with molecular weight, crystallinity degree and morphology [49].

Application. Due to PCL's very low in vivo degradation rate and high drug permeability, it has found favour as a long-term implant delivery device. Current research is being conducted into the development of micro- and nano-sized drug delivery vehicles, but the degradation rate (2–3 years) is a significant issue for pure PCL products to be FDA approved for this use. PCL and PCL composites have been used as tissue engineering scaffolds for regeneration of bone, ligament, cartilage, skin, nerve, and vascular tissues [1]. A recent advancement using PCL hybrid scaffolds has been used in interfacial tissue engineering [1].

PCL nanofibres show viscoelastic properties. It could be that higher molecular weight PCL results in softer nanofibres. These different values suggest that different nanofibre properties can be achieved by varying the preparation methods. The mechanical properties of electrospun PCL nanofibres do not closely mimic any of the natural or electrospun protein fibres. However, they seem to come closer to fibrin fibres and electrospun fibrinogen fibres, rather than collagen fibres [50]. PCL fibres may have suitable mechanical properties for various applications in biomedical and tissue engineering including blood vessels, skin grafts, and tendons. Viscoelastic properties have been found to depend on the age of the fibres. Younger fibres could be pulled to a greater strain before permanent deformation than older fibres. This is an important property for determining how long a scaffold made from these single fibres can be stored before the mechanical properties are significantly altered. The relaxation times and total and elastic moduli also show age-relate dependencies. This dependence on age gives a better understanding of how PCL degrades over time, from a mechanical perspective. Combining these findings with PCL's bioresorbable properties will allow for better fabrication of specific bioengineered scaffolds and devices [50].

POLY(BUTYLENE SUCCINATE), PBS

Polycaprolactone (PCL), and poly(butylene succinate) (PBS) are petroleum based, but they can be degraded by microorganisms. PBS degrading microorganisms are widely distributed in the environment, but their ratio to the total microorganisms is lower than PCL-degraders. PBS structure is shown in Figure 10.

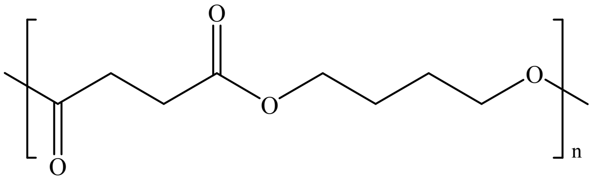


Figure 10. PBS structure

PBS is nowadays one of the most appealing biodegradable polymer because it is fully sustainable, commercially available and exhibits a good balance of thermal and mechanical properties [51]. PBS also referred to as polytetramethylene succinate, is a biodegradable thermoplastic polyester resin. PBS exhibits good thermal and mechanical properties that are comparable with those of widely used polymers such as polyethylene and polypropylene, with high melting points of 112-114 °C. The favourable properties coupled with ease of processing have resulted in emergence of a variety of application areas across diverse end use industries for PBS. Efforts are channelized towards developing bio-based PBS from bio based 1,4 butanediol and succinic acid. Succinic acid is one of the two primary intermediates used to manufacture PBS, and is a building block chemical in the production of 1,4-butanediol (BDO). Although diglycollic acid has a similar structure to succinic acid, it possesses ether-oxygen in molecules and thus, thermal, mechanical and biodegradable properties are different [52]. Biodegradable plastic like PBS has conventionally been made using petroleum-based feedstock but it moved the packaging industry a step in the right environmental direction. Mechanical properties are comparable to polypropylene and low-density polyethylene (LDPE) [44]. PBS is synthesized from dicarboxylic acids (e.g., succinic and adipic acid) and glycols (e.g., ethylene glycol and 1,4-butanediol) [53]. Synthesis of PBS is presented in Figure 11.

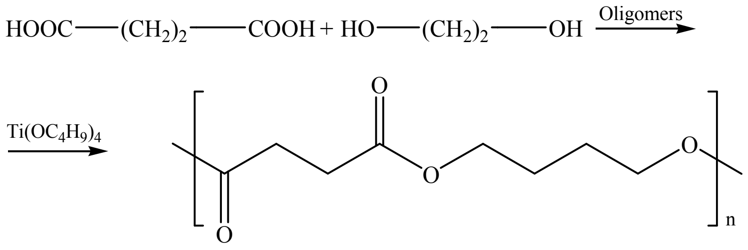


Figure 11. Synthesis of PBS

It has high flexibility, excellent impact strength, and thermal and chemical resistance. However, its high cost and other properties such as elastic modulus, tensile strength, gas barrier properties, and melt viscosity for further processing, restrict its extensive application [54].

CONCLUSIONS

Biodegradable polyesters have been intensively investigated in the last two decades because of their biodegradability and superb physical properties. The biodegradable polymers are indispensable in drug delivery due to their unrivalled physicochemical properties. These physicochemical properties have a significant effect on the drug delivery system, as well as on the pharmacological effect. Currently, a wide range of degradable polymers exist that hold potential as biomaterials. As a result, the market of these environmentally friendly materials is in rapid expansion. Utilization of polymers as biomaterials has greatly impacted the advancement of medicine and pharmacy. Biopolymers with desired physical, chemical, biological, biochemical and degradation properties can be designed, and a wide range of these novel materials have been investigated for biomedical applications. The field of degradable polymeric biomaterials will continue to progress since these new materials present the great challenge to sophisticated multidisciplinary research as well as to their economic technological production.

REFERENCES

[1] B. D. Ulery, L. S. Nair, C. T. Laurencin, „Biomedical Applications of Biodegradable Polymers“, *Journal of polymer science part B: Polymer Physics*, vol. 49, no. 12, pp. 832-864, 2011.

[2] R. D. Saini, „Biodegradable Polymers“, *International Journal of Applied Chemistry*. vol. 13, no. 2, pp. 179-196, 2017.

[3] L. Avérous and E. Pollet, „Biodegradable Polymers“ in Environmental Silicate Nano-Biocomposites, pp. 15-16, Springer, 2012.

[4] I. Vroman and L. Tighzert, „Biodegradable Polymers“, *Materials*, vol. 2, no. 2, pp. 307-344, 2009.

[5] M. Vert, „Biopolymers and Artificial Biopolymers in Biomedical Applications, an Overview“ in Biorelated Polymers, pp. 63-79, Springer, 2001.

[6] J. C. Middleton and A. J. Tipton, „Synthetic biodegradable polymers as medical devices“, *Medical Plastics and Biomaterials Magazine*, vol. 3-4, pp. 31-38, 1998.

[7] http://mitr.p.lodz.pl/biomat/old_site/raport/1_radiation_hydrogels.html

[8] J. M. Pachence, M. P. Bohrer and J. Kohn, Cahpter 23 „Biodegradable Polymers“ in: R. P. Lanza, R. Langer, J. P. Vacanti (Eds.) Principles of Tissue Engineering (3rd Edition), Academic Press, San Diego, 2007, pp. 323-339.

[9] L. S. Nair and C. T. Laurencin, „Biodegradable polymers as biomaterials“, *Progress in Polymer Science*, vol. 32, no. 8-9, pp. 762-798, 2007.

[10] V. Singh, M. Tiwari, „Structure-Processing-Property Relationship of Poly(Glycolic Acid) for Drug Delivery Systems 1: Synthesis and Catalysis“, International Journal of Polymer Science vol. 2010, pp. 1-23, 2010.

[11] B. Ghanbarzadeh and H. Almasi, „Biodegradable Polymers“ in Biodegradation - Life of Science, pp. 141-185, InTech, 2013.

[12] K. Takahashi, I. Taniguchi, M. Miyamoto and Y. Kimura, “Melt/solid polycondensation of glycolic acid to obtain highmolecular-weight poly(glycolic acid),” *Polymer*, vol. 41, no. 24, pp. 8725–8728, 2000.

[13] K. Chujo, H. Kobayashi, J. Suzuki, S. Tokuhara and M.Tanabe, “Ring-opening polymerization of glycolide,” *Die Makromolekulare Chemie*, vol. 100, no. 1, pp. 262–266, 1967.

[14] S. Fakirov, “Biodegradable Polyesters”, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany 2015.

[15] Market Study Bioplastics, Ceresana, Dec.2011, http://news.bio-based.eu/ceresana-market-study-bioplastics/

[16] R. Mehta, V. Kumar, S. N. Upadhyay, „Mathematical modelling of the poly(lactic acid) ring-opening polymerization using stannous octoate as a catalyst“, *Polymer-Plastics Technology and Engineering*, vol. 46, no. 10, pp. 933-937, 2007.

[17] M. H. Hartmann, „High Molecular Weight Polylactic Acid Polymers“ in Biopolymers from Renewable Resources, pp. 367-411, Springer, 1998.

[18] R. Datta, S. Tsai, P. Bonsignore, S. Moon and J. Franck, „Technological and economic potential of poly(lactic acid) and lactic acid derivatives“, *FEMS Microbiology Reviews*, vol. 16, no. 2-3, pp. 221-231, 1995.

[19] L. L. Tang, S. C. Wang, Q. H. Pan, J. K. Wang, „Preparation of poly(lactic acid) by direct polycondensation in azeotropic solution“, *J. East Chins. Univ. Sci. Technol* 32, pp. 672-675, 2006.

[20] M. Ohta, Y. Yoshida, S. Obuchi “Preparation process of polyhydroxy-carboxylic acid,”U.S. Patent 5,444,143 (1995)

[21] A. C. Ibay, L.P. Tenney, „Polymers from hydroxy acids and polycarboxylic acids“ U.S. patent 5,206,341 (1993)

[22] D. Garlotta, „A literature Review of Poly(lactic Acid)“, *Journal of Polymers and the Environment*, vol. 9, no. 2, pp. 63-84, 2001.

[23] C. E. Lowe, „Preparation of high molecular weight polyhydroxyacetic ester“ U.S.Patent 2,668,162 (1954)

[24] E. T. H. Vink, K. R. Rabago, D. A. Glassner, P. R. Gruber, Applications of life cycle assessment to NatureWorks polylactide (PLA) production, *Polymer Degradation and Stability*, vol. 80, pp. 403-419, 2003.

[25] K. Jamshidi, S. H. Hyon and Y. Ikada, „Thermal characterization of polylactides“, *Polymer* vol. 29, no. 12, pp. 2229-2234, 1988.

[26] M. Spinu, C. Jackson, M. Y. Keating and K. H. Gardner, „Material design in poly (lactic acid) systems: block copolymers, star homo-and copolymers, and stereocomplexes“, *Journal of Macromolecular Science, Pure and Applied Chemistry,* A33(10), pp. 1497-1530, 1996.

[27] K. Yuniarto, B. A. Welt, A. Purwanto, H. K. Purwadaria and A. Abdellatief, T. C. Sunarti and S. Purwanto, „Effect of Plasticizer on Oxygen Permeability of Cast Polylactic Acid (PLA) Films Determined Using Dynamic Accumulation Method“, *Journal of Applied Packaging Research*, vol. 6, no. 2, pp 51-57, 2014.

[28] M. Jamshidian, E. A. Tehrany, M. Imran, M. Jacquot and S. Desobry, „Poly-lactic acid: Production, Applications, Nanocomposites and Release Studies“, *Comprehensive Reviews in Food Science and Food Safety*, vol. 9, no. 5, pp. 552-571, 2010.

[29] M. Harada, T. Ohya, K. Iida, H. Hayashi, K. Hirano and H. Fukuda, „Increased impact strenght of biodegradable poly(lactic)/poly(butylene succinate) blend composites by using isocyanate as a reactive processing agent“, *Journal of Applied Polymer Science*, vol 106, no. 3, pp. 1813-1820, 2007.

[30] N. Ljungberg and B. Wesslén, „The effects of plasticizers on the dynamic mechanical and thermal properties of poly(lactic acid)“, *Journal of Applied Polymer Science*, vol 86, no. 5, pp. 1227-1234, 2002.

[31] J. Y. Lim, S. H. Kim, S. Lim and Y. H. Kim, „Improvement of flexural strengths of poly(lactic acid) by solid-state extrusion“, *Macromolecular Materials and Engineering*, vol. 288, no. 1, pp. 50-57, 2003.

[32] T. Furukawa, H. Sato, R. Murakami, J. Zhang, Y. Duan, I. Noda, S. Ochiai and Y. Ozaki, „Structure, Dispersibility, and Crystallinity of Poly(hydroxybutyrate)/Poly(l-lactic acid) Blends Studied by FT-IR Microspectroscopy and Differential Scanning Calorimetry“, *Macromolecules*, vol. 38, no. 15, pp. 6445–6454, 2005.

[33] B. N. Gupta, N. Revagade and J. Hilborn, „Poly(lactic acid) fiber: An overview“, *Progress in Polymer Science*, vol. 32, no. 4, pp. 455-482, 2007.

[34] B. J. Papenburg, J. Liu, G. A. Higuera, A. M. C. Barradas, J. Boer, C. A. Blitterswijk, M. Wessling and D. Stamatialis, „Development and analysis of multi-layer scaffolds for tissue engineering“, *Biomaterials*, vol. 30, no. 31, pp. 6228-6239, 2009.

[35] M. J. Imola and V. L. Schramm, „Resorbable Internal Fixation in Pediatric Cranial Base Surgery“, *The Laryngoscope*, vol. 112, no. 10, pp. 1897-1901, 2002.

[36] B. J. Papenburg, J. Liu, G. A. Higuera, A. M. C. Barradas, J. de Boer, C. A. van Blitterswijk, M.Wessling, D. Stamatialis, „Development and analysis of multi-layer scaffolds for tissue engineering”, *Biomaterials*, vol. 30, no. 31, pp. 6228-6239, 2009.

[37] K. Fukushima and Y. Kimura, „An efficient solid-state polycondensation method for synthesizing stereocomplexed poly(lactic acid)s with high molecular weight“, *Journal of Polymer Science part A: Polymer Chemistry*, vol. 46, no. 11, pp. 3714-3722, 2008.

[38] F. Esmaeili, M. H. Ghahremani, S. N. Ostad, F. Atyabi, M. Seyedabadi, M. R. Malekshahi, M. Amini and R. Dinarvand, „Folate-receptor-targeted delivery of docetaxel nanoparticles prepared by PLGA–PEG–folate conjugate“, *Journal of Drug Targeting*, vol. 16, no. 5, pp. 415-423, 2008.

[39] V. Lassalle and M. L. Ferreira, „PLA Nano- and Microparticles for Drug Delivery: An Overview of the Methods of Preparation“, *Macromolecular Bioscience*, vol. 7, no. 6, pp. 767-783, 2007.

[40] X. Q. Zhang, J. Intra and A. K. Salem, „Comparative study of poly (lactic-co-glycolic acid)-poly ethyleneimine-plasmid DNA microparticles prepared using double emulsion methods“, *Journal of Microencapsulation*, vol. 25, no. 1, pp. 1-12, 2008.

[41] B. Azimi, P. Nourpanah, M. Rabiee and S. Arbab, „Poly (ε-caprolactone) Fiber: An Overview“, *Journal of Engineered Fibers and Fabrics*, vol. 9, no. 3, pp. 74-90, 2014.

[42] A. R. Hernández, O. C. Contreras, J. C. Acevedo and L. G. N. Moreno, „Poly(ε-caprolactone) Degradation Under Acidic and Alkaline Conditions“, American Journal of Polymer Science, vol. 3, no. 4, pp. 70-75, 2013.

[43] G. B. C. Cardoso, S. L. F. Ramos, A. C. D. Rodas, C. A. C. Zavaglia and A. C. F. Arruda, “Scaffolds of Poly (E-Caprolactone) with Whiskers of Hydroxyapatite,” *Journal of Materials Science*, vol. 45, no. 18, pp. 4990-4993, 2010.

[44] Y. Tokiwa 1, B. P. Calabia, C. U. Ugwu and S. Aiba, „Biodegradability of Plastics“, *International Journal of Molecular Sciences*, vol. 10, no. 9, pp. 3722-3742, 2009.

[45] Y. Tokiwa, T. Suzuki and K. Takeda, „Two types of lipases in hydrolysis of polyester“, *Agricultural and Biological Chemistry*, vol. 52, no. 8, pp. 1937-1943, 1988.

[46] L. J. Suggs, S. A. Moore and A. G. Mikos, „Physical Properties of Polymers Handbook“ in Physical Properties of Polymers Handbook, pp. 942-943, Springer, 2007.

[47] L. V. Schueren, B. D. Schoenmaker, Ö. I. Kalaoglu and K. D. Clerck, „An alternative solvent system for the steady state electrospinning of polycaprolactone“, *European Polymer Journal*, vol. 47, no. 6, pp. 1256-1263, 2011.

[48] A. C. Albertsson, R. Renstad, B. Erlandsson, C. Eldsäter and S. Karlsson, „Effect of processing additives on (bio)degradability of film-blown poly(ε-caprolactone)“, *Journal of Applied Polymer Science*, vol. 70, no. 1, pp. 61-74, 1998.

[49] P. Jarrett, C. V. Benedict, J. P. Bell, J. A. Cameron, S. J. Huang, in Polymers as Biomaterials, S. W. Shalaby, A. S. Hoffman, B. D. Ratner and T. A. Horbett (eds.), Plenum Press, New York, p. 181, 1991.

[50] S. R. Baker, S. Banerjee, K. Bonin and M. Guthold, „Determining the mechanical properties of electrospun poly-ε-caprolactone (PCL) nanofibers using AFM and a novel fiber anchoring technique“, *Materials Science and Engineering: C*, vol. 59, pp. 203-212, 2016.

[51] M. Bautista, A. M. Ilarduya, A. Alla and S. Muñoz-Guerra, „Poly(butylene succinate) Ionomers with Enhanced Hydrodegradability“, *Polymers*, vol. 7, pp. 1232-1247, 2015.

[52] A. Oishi, M. Zhang, K. Nakayama, T. Masuda and Y. Taguchi, „Synthesis of Poly(butylene succinate) and Poly(ethylene succinate) Including Diglycollate Moiety“, *Polymer Journal*, vol. 38, no. 7, pp. 710-715, 2006.

[53] A. Díaz, R. Katsarava and J. Puiggalí, „Synthesis, Properties and Applications of Biodegradable Polymers Derived from Diols and Dicarboxylic Acids: From Polyesters to Poly(ester amide)s“, International Journal of Molecular Sciences, vol. 15, no. 5, pp. 7064-7123, 2014.

[54] Y. Tachibana, N. T. G. Giang, F. Ninomiya, M. Funabashi, M. Kunioka, „Cellulose acetate butyrate as multifunctional additive for poly(butylene succinate) by melt blending: Mechanical properties, biomass carbon ratio, and control of biodegradability“, Polymer Degradation and Stability, vol. 95, no. 8, pp. 1406-1413, 2010.

POSSIBILITY OF CROSSLINKING OF RAW XANTHAN WITH DIVALENT AND TRIVALENT METAL IONS

ORIGINAL SCIENTIFIC PAPER

Ida Zahović, Zorana Rončević, Siniša Dodić, Jovana Grahovac, Jelena Dodić*

Faculty of Technology Novi Sad, University of Novi Sad, Bulevar cara Lazara 1, Novi Sad 21000, Serbia
✉ idaidaza@gmail.com

RECEIVED 2018-03-09
ACCEPTED 2018-05-18

ABSTRACT: Biopolymers are biodegradable, biocompatible and non-toxic natural compounds with large molecular masses. Xanthan is a biopolymer produced by bacteria of genus Xanthomonas. Nowadays scientists are focused on examining the possibilities of crosslinking of xanthan using different agents. The aim of this paper was to investigate the possibility of crosslinking of xanthan with divalent and trivalent metal ions. During these studies, xanthan produced by reference strain Xanthomonas campestris ATCC 13951 on medium based on wastewater from white wine production was used. Xanthan solutions of different concentrations (1.0%, 2.0% and 2.5% (w/v)) were prepared in 0.1M sodium chloride. Salts of barium, calcium, magnesium, manganese, zinc, lead, as well as divalent and trivalent iron at different concentrations (0.025 M, 0.050 M and 0.075 M) and pH values (3, 5, 7 and 9) were used. The obtained results indicate that the success of crosslinking and the stability of the formed structures highly depend on the pH value of the salt solutions and the biopolymer concentration. The most stable, solid structures were formed by dropping 2.5% xanthan solution into a 0.075 M solution of FeCl3 at highly acidic pH value. The dry matter content of structures was 2.37%, and average dimensions were about 4.02 x 5.08 mm.

KEYWORDS: biopolymer, xanthan, crosslinking, metal ions

INTRODUCTION

The extensive industrialization results in a large resource exploitation and generation of significant quantities of waste effluents [1]. Taking into account the amount of waste streams generated by the food industry, as well as the content of organic and inorganic ingredients in them, it is clear that they cause high environmental problems. The emission of large volumes of effluents into natural recipient without adequate treatment is a big problem today, and with the evident trend of increasing the load in qualitative and quantitative terms, the inevitable issue will be active in the future also [2].

The pollution is mostly expanded by water flow, which is precisely the reason for the increase in the number of scientific research related to development of wastewater treatment [3]. In order to safely discharge wastewaters into the environment, it is necessary to purify those using appropriate mechanical, chemical and/or biological methods. Mechanical methods of purification of wastewater involve procedures for the removal of larger impurities and suspended particles, and are based on an action of physical forces such as gravity and pressure. Chemical methods are processes in which the removal of pollutants is carried out by performing certain chemical reactions or physicochemical phenomena. Biological processes of wastewater treatment are based on the application of microorganisms that adopt and metabolize numerous organic and inorganic substances from wastewater [4-6].

These methods have a number of advantages and disadvantages. Some of them require the use of toxic and expensive materials, while some are not completely effective and can lead to the accumulation of new waste [7]. Therefore, the attention of the scientific public is focused on testing the possibility of applying biocompatible sewage treatment materials, of which biopolymers have been most suitable [8].

Due to ability to absorb large amounts of water or biological fluids, with the formation of hydrogels [9], biopolymers have found a variety of applications, and among others they are used to remove individual metal ions from different wastewaters [10]. The most dangerous pollutants originating from industrial wastewater include toxic metals such as iron, lead, cobalt, copper, aluminum, sodium and others [3].

In a previous study, lead and iron ions have been successfully removed with xanthan, microbial biopolymer [3]. A large number of biopolymers of microbiological origin are known, but xanthan, produced by the *Xanthomonas* bacteria on semi-synthetic substrates, which are the basis of various industrial effluents [11], is the most important commercially. Xanthan has a unique structure and exceptional rheological characteristics [12] which are very appreciated in processes of purifying of wastewater. The aim of this paper was to examine the possibility of crosslinking of xanthan with divalent and trivalent metal ions. Based on the success of crosslinking of xanthan and the stability of formed structures, the potential for the use of this biopolymer for the removal of metal ions from wastewater was estimated.

EXPERIMENTAL

Preparation of xanthan solutions

1.0%, 2.0% and 2.5% (w/v) solutions of raw xanthan synthesized on wastewaters from white wine production using *Xanthomonas campestris* ATCC 13951, prepared in 0.1M sodium chloride, were used for a crosslinking procedure. Preparation of xanthan solution meant suspending the required amount of biopolymer in 0.1 M sodium chloride, and its long-term dissolution at constant mixing.

Preparation of metal ion solutions

Ions of divalent and trivalent metals were used in this experiment as crosslinking agents. Salts (chlorides, sulphates and nitrates) of barium, calcium, magnesium, manganese, zinc and lead were used as metal ion sources. For the purposes of this research, aqueous solutions of the mentioned metal ions of different concentrations (0.025 M, 0.050 M and 0.075 M) and pH values (3, 5, 7 and 9) were prepared.

Crosslinking procedure

The crosslinking of raw xanthan was carried out at ambient temperature by dropping xanthan solution into a solution of metal ions with a peristaltic pump at a rate of 1 drop/s (tube diameter: 0.3 mm) with a constant stirring of 150 rpm provided by placing a vessel for crosslinking on a magnetic stirrer. Figure 1 shows a schematic view of the used apparatus.

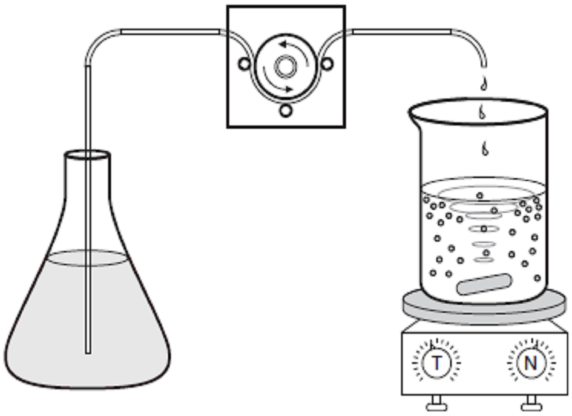


Figure 1. Schematic view of the apparatus used for the laboratory crosslinking of xanthan.

Hardening, i.e. the mechanical stability of the formed xanthan structures, was achieved by their aging in ionic metal solution for 10 minutes with constant mixing.

Characterization of formed structures

At the end of the hardening, characterization of the formed xanthan structures was carried out. For this purpose, dimensions of the cross-linked structures were measured, and the content of the dry matter was determined.

Dimensions of the cross-linked xanthan structures were determined by measuring using a nonius. A randomly selected sample of ten cross-linked structures was used for measurement.

The dry matter content of cross-linked xanthan structures was determined gravimetrically, by drying them at a temperature of 105ºC until a constant mass was achieved [13]. A randomly selected sample of twenty cross-linked structures was used for measurement.

RESULTS AND DISCUSSION

In accordance with the defined aim, during this research, the possibility of crosslinking of xanthan with divalent (Ba^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Pb^{2+}, Fe^{2+}) and trivalent (Fe^{3+}) metal ions was investigated. Crosslinking was performed by dropping the xanthan solution into the metal ion solutions as described above. In order to examine the influence of the pH value and the concentration of metal ions on the success of crosslinking, the characterization of formed structures was carried out, which, in addition to visual characterization, included measurement of the size, as well as determining the dry matter of the structures. The visual characterization was performed in order to estimate formation and stability of cross-linked structures.

Influence of pH of metal ion solution on crosslinking efficiency

In order to examine the influence of the pH value of the metal ion solution on the crosslinking efficiency, 1.0% (w/v) xanthan solution was added to 0.050 M metal ion solution whose pH value was adjusted to 3, 5, 7 or 9. The possibility of crosslinking, appearance and stability of formed structures for all investigated ions, other than lead and iron, is described and summarized in Table 1.

The results shown in Table 1 indicate that the formation of solid and stable structures could not be achieved in all the analyzed conditions. The influence of pH value of the lead and iron ion solution on the crosslinking of xanthan was not tested because it was impossible to correct this parameter in solutions of the mentioned ions. It was impossible to adjust pH value of the iron ion solution above 3, while in the solution of the lead ions a precipitate was formed when adding both acids and bases. Therefore, the solutions of the mentioned metal ions without correction of pH values were used in further experiments for the crosslinking of xanthan.

Table 1. Influence of the pH value of 0.050 M metal ion solution on the success of the crosslinking of 1.0% (w/v) raw xanthan solution

Metal ion source	Initial pH value	Characteristics of formed structures			
		pH=3	pH=5	pH=7	pH=9
		CHLORIDES			
$BaCl_2·2H_2O$	6.66	very unstable, disintegrated structures	unstable, disintegrated structures	unstable structures that lasted for a few seconds	very unstable, disintegrated structures
$CaCl_2$	6.94	unstable structures that in first few seconds tend to spherical form, but after a few seconds, they elongate and disintegrate	unstable spherical, long lasting structures	unstable structures, decay after a few seconds	completely unstable structures
$MgCl_2·6H_2O$	7.29	unstable spherical structures	in first few seconds structures tend to spherical form, but after a few seconds, they elongate and disintegrate	unstable spherical structures	completely unstable structures
$MnCl_2·4H_2O$	6.86	*	*	unstable structures that dissolve very quickly	*
$FeCl_3·6H_2O$	1.95	*	*	*	*
		SULPHATES			
MgSO4	7.05	unstable structures that disintegrate very quickly	unstable structures	unstable structures	unstable structures, last for a long period of time
$MnSO_4·H_2O$	6.84	elongated spherical structures, which decay after a long time	unstable elongated spherical structures, last for a short period of time	unstable elongated spherical structures, last for a short period of time	*
$ZnSO_4·7H_2O$	6.21	unstable ellipsoidal structures, last for a long period of time	smaller unstable ellipsoidal structures, last for a short period of time	*	*
$FeSO_4·7H_2O$	4.34	unstable structures of indefinite forms, eventually disintegrate and dissolve	*	*	*
		NITRATES			
$Pb(NO_3)_2$	4.62	spherical structures that are very unstable, disintegrate on touch	*	*	*

*- It was impossible to adjust pH value.

Based on the results in Table 1, it can be noticed that if chlorides of barium, magnesium and manganese ions are used as crosslinking agents, the most stable structures are formed at a pH value of 7. The most defined shaped structures are formed in the calcium ion solution at a pH value of 5.

The results of crosslinking of xanthan with sulphates solutions show the possibility of application of solution with different pH values. According to the obtained results, it can be found that if solutions of ions of zinc and iron are used as crosslinking agents, the most stable structures are formed at a pH value of 3.

Further, if manganese ion solutions are used as crosslinking agents, very durable, elongated spherical structures are formed at a pH value of 7. The most durable structures can be formed by crosslinking of xanthan with solution of magnesium ions at a pH value of 9. The most defined spherical structures in this experiment were formed by crosslinking of xanthan with nitrate solution of lead ions at a pH value of 3. Bearing in mind that xanthan crosslinking is possible with solutions of the mentioned metals ions, but it does not result in a stable and solid structure, it can be said that these metals do not crosslink xanthan well enough at a concentration of 0.05 M.

Table 2. Influence of the concentration of metal ions in solution on the success of the crosslinking of 2.0% (w/v) raw xanthan solution

Metal ion source	pH value of metal ion solution	Characteristics of formed structures		
		c=0.025 M	c=0.050 M	c=0.075 M
		CHLORIDES		
$BaCl_2 \cdot 2H_2O$	7.00	unspecified structures, disintegrate on the touch, very unstable, dissolves after standing in solution	rod shaped structures, unstable, disintegrate on the touch	very unstable ellipsoidal structures, disintegrate on the touch
$CaCl_2$	5.00	very unstable ellipsoidal structures, disintegrate on the touch	ellipsoidal structures, unstable on the touch, disintegrate after few seconds	ellipsoidal structures, very unstable, disintegrate fast on the touch
$MgCl_2 \cdot 6H_2O$	7.00	unspecified structures, disintegrate on the touch, very unstable	ellipsoidal structures, look stable but disintegrate fast on the touch	unstable structures that tend to spherical form, disintegrate on the touch
$MnCl_2 \cdot 4H_2O$	7.00	ellipsoidal structures, break down on the touch, very unstable, dissolve after standing in solution	ellipsoidal structures, look stable but disintegrate on the touch	ellipsoidal structures, unstable, disintegrate on the touch
$FeCl_3 \cdot 6H_2O$	1.95	very stable ellipsoidal structures, remain in solution for a long time, do not dissolve	stable solid structures that do not disintegrate on touch	ellipsoidal stable structures, do not disintegrate
		SULPHATES		
MgSO4	9.00	rod shaped structures, unstable, disintegrate on the touch	rod shaped structures, more stable and long-lasting, but disintegrate on the touch	ellipsoidal structures, unstable, disintegrate on the touch
$MnSO_4 \cdot H_2O$	7.00	ellipsoidal structures, very unstable, disintegrate on the touch	ellipsoidal structures, unstable, disintegrate on the touch	unspecified structures, disintegrate on the touch, unstable
$ZnSO_4 \cdot 7H_2O$	3.00	rod shaped structures, unstable, disintegrate on the touch	rod shaped structures, unstable, disintegrate on the touch	ellipsoidal structures, unstable, disintegrate fast on the touch
$FeSO_4 \cdot 7H_2O$	3.00	structures almost do not form, difficult to see due to the colour of the solution	ellipsoidal structures, more stable and long-lasting, but disintegrate on the touch	supersaturated solution
		NITRATES		
$Pb(NO_3)_2$	3.00	unspecified structures, disintegrate on the touch, very unstable	ellipsoidal stable structures, disintegrate on the touch	ellipsoidal, stable but very soft structures

Influence of metal ion concentration on crosslinking efficiency

In order to investigate the effect of concentration of metal ions on crosslinking process, a xanthan solution of different concentrations (1.0%, 2.0% and 2.5% (w/v)) was dropped into 0.025 M, 0.050 M and 0.075 M solutions of metal ions whose pH value was set to the value that was found to be the most suitable in the previous set of experiments except for the solutions of lead and iron ions whose pH value was not corrected. By crosslinking of 1.0% (w/v) xanthan solution, no stable structures were formed (Table 1), so no further analyses were performed. The possibility of crosslinking of 2.0% and 2.5% (w/v) xanthan solutions, appearance and stability of formed structures for all investigated ions, is described and summarized in Table 2 and Table 3, respectively.

The results represented in Table 2 show that the concentration of metal ions in the solution significantly affects the success of crosslinking, but also the stability of the formed structures. From the examined bivalent ions of metals (Ba^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, $Pb^{2\ +}$, Fe^{2}), the best crosslinking was accomplished with lead ions, while the solutions of other ions did not show positive crosslinking results in the applied experimental conditions. By using xanthan with a solution of lead ions at all investigated concentrations stable structures were formed.

Nevertheless, it was noticed that the stability of the formed structures increased with the increase of the concentration of lead ions in the solution. The most stable, ellipsoidal shaped structures were formed at concentration of lead ions of 0.075 M.

Table 3. Influence of the concentration of metal ions in solution on the success of the crosslinking of 2.5% (w/v) raw xanthan solution

Metal ion source	pH value of metal ion solution	Characteristics of formed structures		
		c=0.025 M	c=0.050 M	c=0.075 M
CHLORIDES				
$BaCl_2 \cdot 2H_2O$	7.00	unstable ellipsoidal structures, disintegrate on the touch	ellipsoidal structures, very unstable, disintegrate on the touch	unstable ellipsoidal structures, disintegrate on the touch
$CaCl_2$	5.00	unstable ellipsoidal structures, disintegrate on the touch	unstable ellipsoidal structures, disintegrate on the touch	ellipsoidal structures, very unstable, disintegrate on the touch
$MgCl_2 \cdot 6H_2O$	7.00	unstable unspecified structures, disintegrate on the touch	unspecified structures, look stable but disintegrate fast on the touch	unstable unspecified structures, disintegrate on the touch
$MnCl_2 \cdot 4H_2O$	7.00	unstable structures, disintegrate on the touch, dissolves after standing in solution	unstable structures, disintegrate on the touch	unstable unspecified structures, disintegrate fast on the touch
$FeCl_3 \cdot 6H_2O$	1.95	very stable ellipsoidal structures, remain in solution for a long time, do not dissolve	stable solid structures, do not disintegrate on the touch	stable structures that tend to spherical form, do not disintegrate
SULPHATES				
MgSO4	9.00	rod shaped structures, unstable, disintegrate on the touch	rod shaped structures, more stable and long-lasting, but disintegrate on the touch	unstable ellipsoidal structures, disintegrate on the touch
$MnSO_4 \cdot H_2O$	7.00	ellipsoidal structures, very unstable, disintegrate on the touch	unstable ellipsoidal structures, disintegrate on the touch	unstable ellipsoidal structures, disintegrate on the touch
$ZnSO_4 \cdot 7H_2O$	3.00	unstable ellipsoidal structures, disintegrate on the touch	unstable ellipsoidal structures, disintegrate on the touch	unstable ellipsoidal structures, disintegrate fast on the touch
$FeSO_4 \cdot 7H_2O$	3.00	very unstable structures, disintegrate after a few seconds	unstable structures, disintegrate on the touch	supersaturated solution
NITRATES				
$Pb(NO_3)_2$	3.00	ellipsoidal structures, disintegrate on the touch, very unstable	stable ellipsoidal structures, disintegrate on the touch	ellipsoidal, stable but very soft structures

Crosslinking of 2.0% (w/v) solution of xanthan with trivalent iron ions was very successful. Crosslinked structures were ellipsoidal and stable, and they did not break apart, unlike most structures crosslinked with divalent ions. The most stable structures were formed by using 0.075 M solution of trivalent iron ions.

Rod shaped structures of xanthan, cross-linked with barium and zinc ions were unstable. They disintegrated few seconds after staying in solution. Structures cross-linked with calcium, magnesium and manganese ions were ellipsoidal but also unstable. 0.075 M solution of iron ions did not crosslink xanthan, and at smaller concentrations, structures were very unstable and difficult to see due to the colour of the solution.

The results represented in Table 3 are very similar to the results in Table 2. It is confirmed that the concentration of metal ions in the solution affects the success of crosslinking, but also the stability of the formed structures.

From the examined bivalent metals ions (Ba^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Pb^{2+}, Fe^{2+}), the best crosslinking was accomplished again with lead ions, while the solutions of other ions did not show positive crosslinking results in the applied conditions.
Compared to crosslinking of 2.0% (w/v) xanthan, structures of 2.5% (w/v) xanthan cross-linked with the mentioned ions are a little bit harder but still unstable.

Both, structures of 2.5% (w/v) xanthan cross-linked with lead and iron ions showed similar characteristics like in previous research [3].

By using xanthan with a solution of lead ions at all investigated concentrations stable structures were formed. Nevertheless, it was noticed that the stability of the formed structures increased with the increase of the concentration of lead ions in the solution. The most stable, ellipsoidal shaped structures were formed at concentration of lead ions of 0.075 M. One fraction of stable structures cross-linked with 0.075 M solution of Pb^{2+} ions is shown in Figure 2.

Figure 2. Structures formed with 2.5% (w/v) xanthan and 0.075 M lead ions solution

Crosslinking of 2.5% (w/v) solution of xanthan with trivalent iron ions was very successful. Cross-linked structures were ellipsoidal and stable, and they did not break apart. Structures of 2.5 % (w/v) xanthan were a bit harder than structures formed with 2.0% (w/v) xanthan solution. The most stable structures were formed by using 0.075 M solution of trivalent iron ions. The interior of these structures was filled, and their long persistence in the solution was noted.

Dimensions of formed structures

Based on the results given in Tables 2 and 3, it is clear that by uniformly dropping xanthan solution in a solution of different metals ions, the structures with different characteristics and sizes are formed, due to the presence of metallic ions, its source and concentration in the solution, as well as the pH value of the solution. Considering that the most stable structures are formed in the presence of iron and lead ions, continuation of this research is devoted to the determination of their dimensions. Results shown in Table 4 represent dimensions of structures cross-linked with solution of lead and iron ions of different concentrations.

Table 4. The dimensions of structures cross-linked with lead and iron ions of different concentrations

Structures	Average size: width x length (mm)		
	0.025 M	0.050 M	0.075 M
2.0% (w/v) xanthan cross-linked with Pb^{2+}	structures were not formed	4.46 x 5.40	4.42 x 5.66
2.0% (w/v) xanthan cross-linked with Fe^{3+}	3.40 x 4.98	4.00 x 5.54	3.72 x 5.22
2.5% (w/v) xanthan cross-linked with Pb^{2+}	4.14 x 6.20	4.04 x 5.84	4.04 x 5.56
2.5% (w/v) xanthan cross-linked with Fe^{3+}	3.44 x 5.50	3.98 x 5.22	4.02 x 5.08

The results shown in Table 4 indicate that the dimensions of the structures formed in the presence of different metal ions depend mostly on concentration of solution of metal ions. There is a difference between structures formed with 2.0% and 2.5% (w/v) xanthan solution, but it is insignificant. The results shown in Table 4 indicate that the dimensions of the structures formed in the presence of different concentrations of lead ions are reduced with the increase of their content in the solution. It is noticed that addition of 2.0% (w/v) solution of xanthan to 0.025 M solution of lead ions does not result in the formation of any structures. The structures with the largest dimensions were formed in the 0.025M solution of lead ions, while the smallest structures were formed in 0.075 M solution of the lead ions.

Similar behaviour was observed with structures formed in the presence of iron ions. The dimensions of these structures also reduce with the increase in the concentration of metal ions in solution. Thus, the largest structures were formed by crosslinking with solution of iron 0.025 M solution of iron ions, while the smallest structures were formed in 0.075 M solution of the mentioned ions.

The largest structures were formed by crosslinking of 2.5% (w/v) solution of xanthan with 0.025 M solution of lead ions. This result is in accordance with the results from the previous research, where the structures with dimensions in the range from 3.4 to 6.8 mm were formed [3]. Reduction of dimensions of cross-linked xanthan structures with increase in concentration of metal ions in solution may be due to the fact that, in the presence of a higher concentration of ions, better crosslinking is performed, less porous structures are formed and the interior is filled better. Structures formed at lower concentration of ions are more porous, allowing diffusion of the solution into their interior, which results in the formation of larger-dimensional structures.

Dry matter content of formed structures

The characterization of the structures cross-linked with iron and lead ions of different concentrations also implied the determination of their dry matter. The results of the dry matter content of the structures cross-linked with lead and iron ions of different concentrations are shown in Table 5.

The results represented in Table 5 show the increase of dry matter content of structures formed with lead ions with the increase of concentration of mentioned ions and concentration of xanthan, both. Hence, if the lead ions are used as crosslinking agents, dry matter content of structures formed with 2.5% (w/v) xanthan solution is higher than for structures formed with 2.0% (w/v) xanthan solution. Structures cross-linked with 0.075 M solution of lead ions had the highest content of dry matter, while structures cross-linked with 0.025 M solution of lead ions had the lowest value of this parameter. The reason for the increase of dry matter content of structures by increasing of lead ions concentration may be a result of crosslinking of a large number of xanthan molecules with a large number of metal ions.

Table 5. Dry matter content of xanthan structures cross-linked with iron and lead ions of different concentrations

Structures	Dry matter content (%)		
	0.025 M	0.050 M	0.075 M
2.0% (w/v) xanthan cross-linked with Pb^{2+}	structures were not formed	2.2157	2.6809
2.0% (w/v) xanthan cross-linked with Fe^{3+}	1.4225	2.2466	3.8798
2.5% (w/v) xanthan cross-linked with Pb^{2+}	2.1175	2.9013	4.0461
2.5% (w/v) xanthan cross-linked with Fe^{3+}	1.5876	1.9249	2.3737

If the iron ions are used as crosslinking agents, dry matter content of structures formed with 2.5% (w/v) xanthan solution is a bit smaller than the same parameter for structures formed with 2.0% (w/v) xanthan solution. A reason for this kind of behaviour may be a result of different characteristics of this ion. Structures cross-linked with 0.075 M solution of iron ions had the highest content of dry matter, while structures cross-linked with 0.025 M solution had the lowest value of this parameter. The values of dry matter content of structures cross-linked with lead ions are insignificantly smaller than the values of dry matter content of structures cross-linked with iron ions.

CONCLUSION

In accordance with the defined aim, this paper examined the possibility of crosslinking of raw xanthan with divalent and trivalent metal ions in order to form stabile cross-linked structures. Based on the obtained results, it can be concluded that the success of the crosslinking of raw xanthan depends on the concentration of biopolymer solution, the concentration of metal ions solution, as well as on its pH value. It has been proved that the crosslinking of xanthan with Ba^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, $Zn^{2+}$and Fe^{2+} ions in the applied experimental conditions does not result in formation of stable cross-linked structures. Xanthan can be cross-linked with divalent lead and trivalent iron ions in concentrations of 0.025 M to 0.075 M and not adjusted pH value. The most stable ellipsoidal structures, with average size of 4.02x5.08 mm, are formed with 2.5% (w/v) xanthan solution and 0.075 M iron ions solution. In the same conditions, somewhat softer, ellipsoidal structures of average dimensions of 4.04x5.56 mm are formed with 0.075 M lead ions solution. Based on the successful crosslinking and stability of the formed structures, it can be concluded that xanthan has a high potential for the application in the removal of iron and lead ions from wastewater.

REFERENCES

[1] Y. Yavuz and Ü. B. Ögütveren, "Treatment of industrial estate wastewater by the application of electrocoagulation process using iron electrodes", *Journal of Environmental Management*, vol. 207, pp. 151-158, Feb. 2018.

[2] N. Veljković, D. Vidojević, M. Jovičić, "Uticaji zagađujućih materija iz urbanih otpadnih voda na životnu sredinu i zdravlje", in *Proceedings of the 40th International conference Waste Waters, Municipal Solid Wastes and Hazardous Wastes*, Subotica, Serbia, March 29- April 1, 2010, Association for water technology and sanitary engineering waterworks and sewerage association of Serbia, 2004. [Online]. Available: http://www.sepa.gov.rs/download/pollutantsWater.pdf. [Accessed June 11, 2018].

[3] I. Zahović, Z. Rončević, J. Dodić, J. Grahovac, S. Dodić, "The possibility of application of xanthan in removal of metal ions from wastewaters", *Journal of Engineering & Processing Management*, vol. 9, ED-1, pp. 86-93, Dec. 2017.

[4] R. Kitanović, V.Šušteršič, "Tretman otpadnih voda", *Military technical courier* vol. 61, ED-3, pp. 122-140, Oct. 2013.

[5] K. T. Ravndal, E. Opsahl, A. Bagi, R. Kommedal, "Wastewater characterisation by combining size fractionation, chemical composition and biodegradability", *Water Research*, vol. 131, pp. 151-160, Mar. 2018.

[6] T. Fazal et al., "Bioremediation of textile wastewater and successive biodiesel production using microalgae", *Renewable and Sustainable Energy Reviews*, vol. 82, ED-3, pp. 3107-3126, Feb. 2018.

[7] D. Povrenović and M. Knežević , *Osnove tehnologije prečišćavanja otpadnih voda*, Beograd, Srbija: Tehnološko-metalurški fakultet, 2013.

[8] C. S. Lee, J. Robinson, M. F. Chong, "A review on application of flocculants in wastewater treatment", *Process Safety and Environmental Protection*, vol. 92, ED-6, 489-508, Nov. 2014.

[9] V. Panić, “Sinteza i svojstva hidrogelova na bazi metakrilne kiseline modifikovanih zeolitima”, Ph.D. dissertation, Faculty of Technology and Metallurgy, Belgrade, Serbia, 2012.

[10] Q. Chen, L. Zhu, C.Zhao, J. Zheng, "Hydrogels for removal of heavy metals from aqueous Solution", *Environmental & analytical toxicology* S:2. ED-1, Jun. 2012.

[11] Z. Rončević, I. Zahović, S. Dodić, J. Grahovac, J. Dodić, "Xanthan biosynthesis by Xanthomonas campestris ATCC 13951 on wastewaters from white grape processing", *Field and Vegetable Crops Research*, vol. 54,ED-3, pp. 110-116, Dec. 2017.

[12] D. F. S. Petri, "Xanthan gum: A versatile biopolymer for biomedical and technological applications", *Journal of Applied Polymer Science* vol. 132, ED- 23, pp. 1-13, art. 42035, Feb. 2015.

[13] L. S. Clescerl, A. E. Greenberg, A. D. Eaton, *Standard Methods for the Examination of Water and Wastewater*, APHA American Public Health Association, 1998.

WATER DETERMINATION IN SAMPLES WITH HIGH SUGAR AND PROTEIN CONTENT

ORIGINAL SCIENTIFIC PAPER

Josip Jurković

RECEIVED 2018-03-27
ACCEPTED 2018-05-18

Faculty of Agriculture and Food Sciences, University of Sarajevo, Zmaja od Bosne 8, 71000 Sarajevo, Bosnia and Herzegovina
✉ josssjurkovic@yahoo.com

ABSTRACT: The aim of water determination should be detection of water and nothing but the water. Large number of methods use heating where the result shows loss of all volatile compounds and not only water. The result of these techniques is not water content, but the mass loss. One of the best techniques for determination of water content is Karl Fischer titration, based on a chemical reaction selective for water. Determination of water content by heating, in samples that are rich in proteins and sugars is especially hard, because of the Maillard reaction. During the Maillard reaction, water is produced, and it is hard to determine water which is originally from sample and water that is produced by Maillard reaction. In this investigation we used samples of different types of condensed milk - rich in proteins and sugars. Samples were measured in ten probes, and by four methods: Karl Fischer titration with different solvents, Classical Oven, IR drying and Oven Sample Processor. Classical Karl Fischer titration was used as a reference method. The best method was Classical Karl Fischer titration, because of precision, trueness and duration of measurement. Usage of boiling methanol for extraction is not recommended. Due to a small amount of sample, contribution in the amount of water from the Maillard reaction is not significant. The best method for measurement is KF titration.

KEYWORDS: Water; Maillard; Karl Fischer

INTRODUCTION

Water is one of the most important constituents of food. It is present in almost all foods, in a range from extremely low amounts in dried products to very high amounts in beverages. Water content is a parameter that affects many others, both of physical and chemical nature. Amount of water in food is a determinant for its nutritive value, taste, shelf life etc. The aim of water determination should be to detect all water, and nothing but the water. Water occurs in different bonding situations. This has an influence on the separability and the possibility to detect water. Water determination is, for practical reasons a challenge for the analysis as it is certainly the most frequent analysis preformed in foods [1]. It is also a challenge for a second reason. Because of many different methods for water determination, one must ask himself which one yields the correct value [1], [2], [3]. There are three main problems when we want to determine water content in foods:

Heating of a sample could cause loosing of all volatile compounds and not just water.

Classical methods for determination of water content use heating of a sample during a certain period of time, and the result is mass loss before and after heating. [4] It is basically a physical separation of water from a sample. The problem with these methods is that we measure mass loss of every volatile compound (we get a higher result than the correct one). The results of drying techniques should therefore not be termed as „water content“. One can use the term „moisture“, but the most suitable is „mass loss“. Another possibility is to determine water content by a selective chemical reaction. Also, we can use indirect methods which determine property of a sample which depends on water content, such as, density, sound velocity, electrical conductivity etc [5], [6].

Contamination of a sample.

It is very easy to contaminate a sample with water since water is all around us: on our hands, in the air, on the laboratory table, in our breath.

Production of water in samples during heating or storage (Maillard reaction).

The Maillard reaction is a very complex network of chemical reactions which happens in samples which contain reducing sugars and proteins (or amino acids). This type of reaction occurs during heating of samples or during storage. Volatile compounds of low molecular mass, non volatile coloured compounds of intermediate molecular mass and brown substances of high molecular mass can occur as products of these reactions [7, 8]. In some steps of the Maillard reaction water is produced (dehydration) [8].

So, there is a question: how to determine water content that is originally in samples, and not water that is originally in samples plus water which is formed in the Maillard reaction.

Determination of water content is often considered as an easy task, but if we want to determine only water task is not so easy.

Because it is very difficult to measure amount of water content in samples, the aim of this research is to recommend the best method for determination of water content in samples with high amounts of sugars and proteins. For this investigation we used samples of condensed milk which are very complex and Maillard reaction is easy to occur.

MATERIALS AND METHODS

In this investigation we used different kinds of condensed milk, which have quite high amounts of proteins and sugars. The first type of condensed milk (Milch Mdchen) had 7,6 grams of protein and 55,0 g of sugars per 100 g. The second type (Milblu) had the same amounts of proteins and sugars. The third type (Молоко) had 6,9 g of proteins and 56 g of sugars per 100 g. For determination of water content, we used several different methods of analysis which are used in today’s laboratories:

1. Classical Karl-Fischer titration
2. Oven drying
3. Automated Karl Fischer titration
4. IR drying

Karl Fischer titration

Measurements were carried out on Titrando 890 Metrohm (Metrohm, Herisau Schwitzerland). This method uses selective chemical reaction which is selective for water. It is a direct method for determination of water content [9] [10]. This method was reference method in this investigation, since it is known as one of the best methods for determination of water content. The instrument used volumetric Karl-Fischer titration cell with a thermostat. With this instrument we made measurements in two different solvents (Solvent-Riedel de Haën and boiling methanol - Riedel de Haën), and at two different temperatures: room temperature and 50°C. The duration of measurement was 250 s. The mass of sample was between 0.1300 and 0.2000 g. The sample was introduced into the titration cell with the usage of a syringe with a needle. The end point of titration was potentiometric. Measurements were made on every kind of sample in ten repetitions.

Automated Karl Fischer titration

774 Oven Sample Processor (Metrohm, Herisau Schwitzerland) was used for automated determination of water content. In this method there is a combination of vaporisation of water from a sample and Karl Fischer titration [11], [12]. A sample is heated in the oven, and water that is formed, as vapour is transformed by usage of the pump, into the Karl Fischer Cell. It is also a direct method- combined direct method. A device which we used is equipped with the coulometric Karl-Fischer titration cell. The measurement duration was between 65 and 100 min. Stop criteria was absolute drift of 20 μg/min. Sample mass was between 0.1500 and 0.2500 g. The sample was introduced into the vial with the usage of a syringe with a needle, after that the vial was closed. Before the measurement of water content in samples, the „temperature ramping“was made. It is the part of the program in which a sample is heated from 20 to 250°C (1°C/min), in which we can see the temperature at which it is the best to measure the water content. In the samples of condensed milk we got the temperature of 120°C. After determination of the measurement temperature we put three blanks (vials with only air) in the sample changer and samples in ten repetitions.

Oven drying

Oven drying measurements were made on Binder FDL 115 (Binder, Mount Holly, USA). It is a direct method. This method does not measure water as such. The result is a mass loss. The mass los is not only caused bay water, but also by all volatile compounds under the drying conditions. The analysed samples (2.000 – 4.000 g) were weighed into the glass weighing bottles, where were mixed with predried sand, and then dried at 105°C, until the constant mass was reached. These measurements were preformed in five repetitions.

IR dryer

IR drying is rapid method for water determination aimed to determine water content thermogravimetricaly [7]. IR drying measurements were made on Sartorius MA 40 (Sartorius, Göttingen, Germany). IR drying was made at 100°C, duration of the measurement was 60-70 min. Sample mass was between 1.000 and 2.000 g. The sample was introduced in the device on filter paper, with a syringe. Measurements by means of IR dryer were made in five replicates.

RESULTS AND DISCUSSION

In this research an investigation of water content in samples of condensed milk was done. For determination of moisture (water) content in condensed milk several different techniques are used, i.e. Volumetric Karl-Fischer titration with different solvents and at different temperatures, Automatic Karl-Fischer titration, Oven drying and IR Drying. All of these methods are used for moisture content determination in food samples. Results for different samples are very similar. The differences between methods are significant regarding the optimisation, time of measurement, mass of the sample and standard deviation. All methods are compared with classical Karl Fischer titration which was used as reference. First step before analysis was the optimisation of the method.

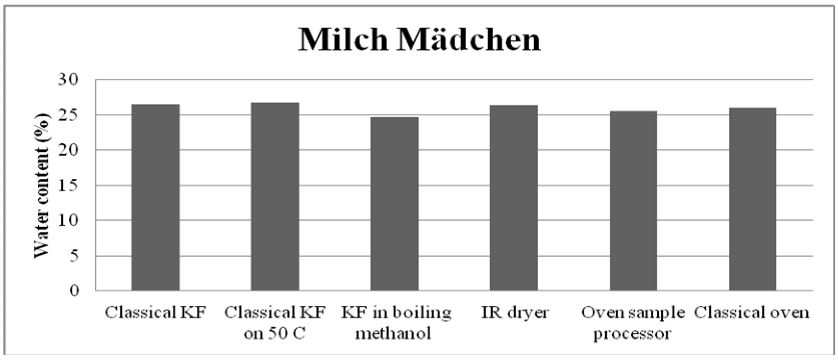


Diagram 1. Water content (mass loss) by different methods in Milch Mädchen

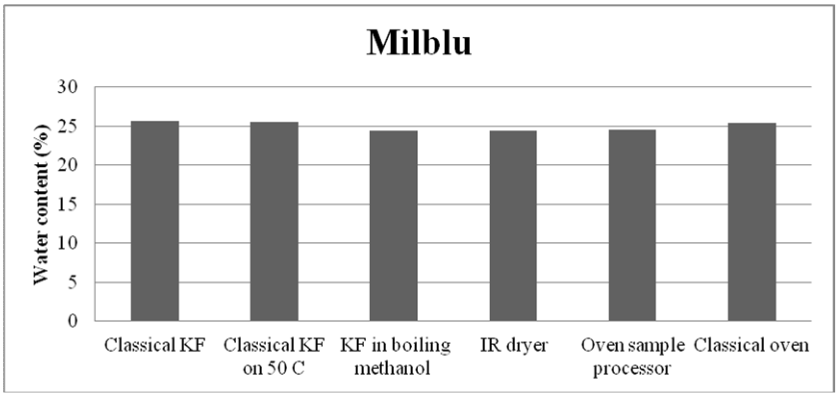


Diagram 2. Water content (mass loss) by different methods in Milblu

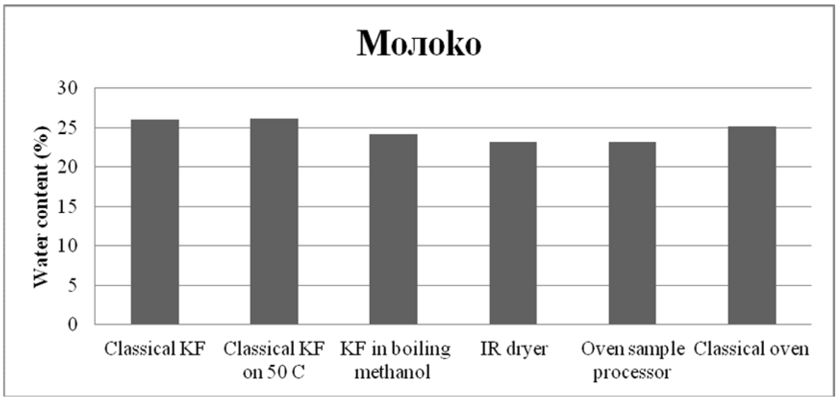


Diagram 3. Water content (mass loss) by different methods in Молоко

Optimisation and application of Karl Fischer titration

Before measurement and extraction of water it was necessary to choose the right solvent for extraction [5]. After several solvents used in testing [13], we chose „Solvent 1“and boiling methanol. Boiling methanol was chosen because of its vapours which can put water from hidden parts of a titration cell back into the titration cell. After solvent selection, we had to choose the extraction time (time at which all water from a sample is extracted). If we choose too short time for extraction, we could get too low results. For standardisation of measurement we used standards with the known amount of water content (10 mg/mL).

For determination of water content we applied two different temperatures: room temperature and 50°C. The second temperature was chosen do see possible water formation during measurement, due to Maillard reaction.

Optimisation and application of Automated Karl Fischer titration

For this method we need to find the best temperature for measurement. The adequate temperature was chosen with “temperature ramping” (1°C/min), with temperature ramping we see the best temperature for measuring. Also, because this method uses heating of a sample and bringing it to the titration cell, we must find the best gas flow rate. For gas carrier we used dried air. The first step in the measurement is to measure amount of water in blanks and then in the standards. As a standard we used lactose with the amount of water of 5.05%. As a blank we used only vial with air.

Optimisation and application of Classical Oven

Classical Oven is a well defined and easy to use method for determination of mass loss. The sand which was used for heating process is pre-dried. The heating was at the standard temperature of 105°C. As a reference material for determination of water content a lactose standard was used with 5.05% of water.

Optimisation and application of IR dryer

IR dryer uses Infra Red radiation for heating of a sample. The drying process is highly dependent on the radiation temperature and distribution of a sample in the sample holder. A sample must be distributed evenly. For distribution of a sample we used a plastic syringe.

On diagrams 1, 2 and 3 are shown the results of measurement of water content by different methods. The results and basic statistical parameters of water determination by different methods in samples of condensed milk are shown in Table 1.

Table 1. Results of water determination gained from different methods

Sample: **Milblu**						
	Classical KF	Classical KF (50 °C)	KF in boiling methanol	Oven sample processor	Classical Oven	IR dryer
Time	250 s	250 s	250 s	65-100 min	6 h	60-70 min
Sample mass	0.02-0.03	0.02-0.03	0.02-0.03	0.01-0.02	2-3	1-2
Maximum	26.33	26.30	25.44	25.11	25.57	24.90
Minimum	24.61	24.05	23.48	22.36	24.94	23.16
Median	25.62	25.75	24.22	24.87	25.41	24.07
Average	25.67	25.54	24.38	24.52	25.30	24.15
STDEV	0.48	0.68	0.71	0.85	0.25	0.67
Sample: **Milch Mädchen**						
	Classical KF	Classical KF (50 °C)	KF in boiling methanol	Oven sample processor	Classical Oven	IR dryer
Time (min.)	180 s	250 s	250 s	65-100 min	6 h	60-70 min
Sample mass (g)	0.02-0.03	0.02-0.03	0.02-0.03	0.01-0.02	2-3	1-2
Maximum	26.80	27.33	25.51	26.19	26.82	27.50
Minimum	26.05	26.33	23.96	24.70	24.20	22.90
Median	26.50	26.67	24.65	25.42	26.11	26.25
Average	26.47	26.73	24.62	25.49	25.69	25.67
STDEV	0.26	0.35	0.49	0.46	1.03	1.73
Sample: **Молоко**						
	Classical KF	Classical KF (50 °C)	KF in boiling methanol	Oven sample processor	Classical Oven	IR dryer
Time (min.)	250 s	250 s	250 s	65-100 min	6 h	60-70 min
Sample mass (g)	0.02-0.03	0.02-0.03	0.02-0.03	0.01-0.02	2-3	1-2
Maximum	26.72	26.76	25.14	23.70	25.74	23.96
Minimum	24.55	25.40	23.19	22.62	24.77	21.68
Median	26.13	26.26	24.39	23.08	25.51	22.72
Average	26.03	26.19	24.22	23.13	25.37	22.84
STDEV	0.64	0.47	0.66	0.36	0.37	0.83

Results gained with all of used methods are similar. The differences in methods are mainly in speed of measurement and precision. The shortest time in measurement was with Karl Fischer titration, and the longest with Classical Oven. Duration of measurement with Classical Karl Fischer titration is around four minutes. Even shorter time is possible if we use higher temperature for analysis, since extraction of water from samples depends on temperature.

Classical Oven has the longest time of measurement. That is the main disadvantage of this method because reaching the constant mass if very time consuming. On the other hand this method has quite high precision and it does not show significant difference from reference method (Classical Karl Fischer).

Karl Fischer in boiling methanol showed lower results from reference method. From this we can conclude that methanol as a solvent is not suitable for extraction of water from condensed milk samples. For purposes of determination of water in condensed milk with Karl Fischer titration it is better to use other solvents, for example: solvent 1, or other mixtures of solvents.

Oven sample processor has one advantage which does not have any of other used methods, and that is sample changer. Disadvantage of this method is many parameters that need to be controlled and time of measurement. It is not possible to have absolutely dried air as a carrier. It is hard to extract all of water from the samples of condensed milk, since during the measurement the crust is formed on the surface of samples.

The mass of the samples was quite small and instrumentation is not precise enough to record this small addition of water because of Maillard reaction. Higher temperature does not give significantly higher amount of water (Maillard reaction).

Precision of methods and comparison to reference method

The precision of Oven drying and Classical Karl-Fischer titration was the best of all tested methods (lowest STDEV).

The next comparison of all above mentioned methods was made by the ANOVA at $p<0.005$ in SPSS statistical program. It was found that differences between Classical KF (which was used as a reference method) and Classical KF in boiling methanol were not significant. Also differences between the reference method and Classical dryer were not significant. The largest difference between the reference method and the other method was found with IR dryer and Automated Karl Fischer titration.

CONCLUSIONS

Water content in samples that contain high amounts of proteins and sugars (condensed milk – Maillard reaction is possible to occur) was determined by several methods.

The results obtained by Classical Karl Fischer titration were used as referential.

The amount of water produced by Maillard reaction during the measurement was not significant (due to small mass of a sample).

For determination of water content in samples of condensed milk we suggest the usage of classical Karl-Fischer titration, because of high precision of the method, low amount of a sample needed and fast measurement.

Instead of classical Karl Fischer titration, one can use Karl Fischer at 50°C, or Classical Oven.

The usage of boiling methanol for determination of water content in samples of condensed milk is not recommended, because it cannot extract all of the water in reasonable time.

In the largest part of samples, Classical Karl Fischer gives lowest, and IR dryer gives the highest standard deviation (precision of method).

Determination of water content in samples that contain proteins and sugars should be made as soon as possible (Maillard reaction).

ACKNOWLEDGEMENTS

Author would like to thank University of Hohenheim (Germany), Department for Food Chemistry and Biotechnology.

REFERENCES

[1] H-D. Isengard, "Water content, one of the most important properties of food" Food Control, Elsevier, Vol. 12, pp. 395-400, 2001.

[2] H. Bünung – Pfaue, Analysis of water in Food by near infrared spectroscopy, Food Chemistry, vol. 82, pp. 107-115, 2003

[3] M. Matlouhi, Water content, water activity, water structure and the stability of foodstuffs, Food Control, vol. 12, pp. 409-417, 2001

[4] H-D. Isengard, "Water Determination in Food", in: Ensuring the Global Food Safety, Cambridge, Elsevier, 2010, pp. 109-123.

[5] H-D. Isengard, Water determination-Scientific and economic dimensions, Food Chemistry, vol. 106, pp. 1393-1398, 2008

[6] M. Adam, P. Dobiáš, P. Bajerová, K. Ventura, Comparison of various methods for determination of water in white yoghurts, Food Chemistry, vol. 115, pp. 1069-1073, 2009

[7] F.J. Morales and M.A.J.S. van Boekel "A study on Advanced Maillard Reaction in heated Casein/Sugar Solutions:

Color formation, Int. Diary Journal, Vol. 8, pp. 907-915, 1998.

[8] M. Namiki ”Chemistry of Maillard reaction: recent studies on the browning reaction mechanism and the development of antioxidants and mutagens”, Adv. Food Res. Vol. 32, pp. 115-184, 1988.

[9] H-D. Isengard, R. King, C.T. Reh, Proposal of a new reference method to determine the water content of dried dairy products, Food Chemistry, vol. 96, pp. 418-422, 2006.

[10] H-D. Isengard and H. Präger, Water determination in products with high sugar content by infrared drying, Food Chemistry, vol. 82, pp. 161-167,2003

[11] A. Felgner, R. Schlink, P. Kirschenbühler, B. Faas, H-D. Isengard, Automated KarlFischer titration for liquid samples-water determination in edible oils, Food Chemistry, vol. 106, pp. 1379-1384.

[12] V. Kestens, P. Connely, A. Bernreuther, Vaporisation coulometric Karl Fischer titration: A perfect tool for water content determination of difficult matrix reference materials, Food Chemistry, vol. 106, pp. 1454-1459, 2008.

[13] K. Schöffski, New Karl Fischer reagents for the water determination in Food, Food Control, vol. 12, pp. 427-429, 2001.

Josip Juraj Strossmayer University of Osijek – Faculty of Food Technology Osijek,
Croatian Society of Chemical Engineers,
The International Union of Food Science and Technology (IUFoST),
European Hygienic Engineering & Design Group – EHEDG
and The European Association for Chemical and Molecular Sciences (EuCheMS)

Proudly announce

INTERNATIONAL CONFERENCE 17TH RUŽIČKA DAYS „TODAY SCIENCE – TOMORROW INDUSTRY“

Which will be held in Vukovar, Croatia, September 19th – 21st 2018.

CONFERENCE TOPICS:

1. Chemical analysis and synthesis
2. Chemical and biochemical engineering
3. Food technology and biotechnology
4. Medical chemistry and pharmacy
5. Chemistry in agriculture and forestry
6. Environmental protection

Will be addressed through plenary and invited lectures, oral and poster presentations.

Until now, following *plenary lecturers* confirmed participation:

- Igor Štagljar, PhD, University of Toronto, Department of Biochemistry, Department of molecular genetics, Donelly Centre, Canada
- David Smith, PhD, Ruđer Bošković Institute, Zagreb, Croatia
- Nenad Bolf, PhD, full prof., University of Zagreb, Faculty of chemical engineering and technology, Zagreb, Croatia
- Sandra Kraljević Pavelić, PhD, assoc. prof., University of Rijeka, Department of Biotechnology, Rijeka, Croatia
- Mojca Škerget, PhD, University of Maribor, Fakulteta za kemijo in kemijsko tehnologijo, Maribor, Slovenia

MEETING OF YOUNG CHEMISTS

The 6th meeting of young chemists will be held under moto “In young people chemistry remains”, where students of secondary school, supervised by their mentors, present their research in the field of chemistry.

For the complete list of plenary and invited lecturers,
preliminary programme, instructions for abstract submission
and all other information regarding the Conference,
please, visit: **http://www.ptfos.unios.hr/ruzicka/**

INSTRUCTION FOR AUTHORS

GENERAL INFORMATION

Technologica Acta is an open access, double-blind peer-reviewed, semiannual international journal devoted to fundamental and applied chemistry, as well as materials chemistry and technology, chemical engineering and technology, environmental protection engineering and food technology research, published by the Faculty of Technology Tuzla, University in Tuzla (Bosnia and Herzegovina), since 2004.

Technologica Acta is an APC-free (there is not an article processing charge or some hidden costs), open access journal.

The Journal is indexed by CAB Abstracts, EBSCO, Index Copernicus Journal Master List, HRČAK, ROAD, COBISS, The Quality Factor and many others.

CATEGORIES OF CONTRIBUTIONS

Original scientific paper reports original research, previously unpublished – except in a preliminary form. It must contain significant and original observations to be critically evaluated. Experimental data should be presented in a way that enables reproduction and verification of analyses and deductions on which the conclusions are based.

Preliminary communication includes short information on the results of scientific research which require and merrit immediate publication.

Scientific note includes report on short but completed original research or describe original laboratory techniques (methods, apparatus etc.).

Scientific review paper is original, critical and up-to-date survey of an specific research area. Generally, these are prepared by the invitation of the Editor.

Professional paper reports on applications of an already described innovation, known methods and facts.

Conference paper presents lecture given (or poster presented) at a conference, workshop or similar event. It is not passing the peer review process.

Book and Web site reviews. It is not passing the peer review process.

REVIEWING THE MANUSCRIPT

All contributors are evaluated according to the criteria of originality and quality of their scientific content, and only those deemed worthy will be accepted for publication. To facilitate the reviewing process, authors are encouraged to suggest three persons competent to review their manuscript. Such suggestions will be taken into consideration but not always accepted.

The Editor-In-Chief and Editors have the right to decline formal review of a manuscript when it is deemed that the manuscript is:

- on a topic outside the scope of the Journal;
- lacking technical merit;
- of insufficient novelty for a wide international readership;
- fragmentary and providing marginally incremental results; or
- is poorly written.

PROOFS

When a manuscript is ready for printing, the corresponding author will receive a PDF-formatted manuscript for proof reading, which should be returned to the journal within one week. Failure to do so will be taken as the authors are in agreement with any alteration which may have occurred during the preparation of the manuscript.

COPYRIGHT

LICENSE AGREEMENT

“I, as a corresponding author, in submitting an article to Technologica Acta certify that:

- I am authorized by my co-authors to enter into these arrangements.
- I warrant, on behalf of myself and my co-authors, that:
  - the article is original, has not been formally published in any other peer-reviewed journal, is not under consideration by any other journal and does not infringe any existing copyright or any other third party rights;
  - I am/we are the sole author(s) of the article and have full authority to enter into this agreement and in granting rights to Technoploica Actar are not in breach of any other obligation;
  - the article contains nothing that is unlawful, libellous, or which would, if published, constitute a breach of contract or of confidence or of commitment given to secrecy;
  - I/we have taken due care to ensure the integrity of the article. To my/our – and currently accepted scientific – knowledge all statements contained in it purporting to be facts

> are true and any formula or instruction contained in the article will not, if followed accurately, cause any injury, illness or damage to the user.

I, and all co-authors, agree that the article, if editorially accepted for publication, shall be licensed under the Creative Commons Attribution License CC BY 4.0."

PROFESSIONAL ETHICS AND PUBLICATION POLICY

The journal expects the Editors, Referees and authors to adhere to the well-known standards of professional ethics. Authors are responsible for the factual accuracy of their contributions. Submission of the paper commits the author not to submit the same material elsewhere. Referees should act promptly. If certain circumstances preclude prompt attention to the manuscript at the time it is received, the non-received manuscript should be returned immediately to the Editor or the Referee should contact the Editor for possible delay of the report submission date. The Editor accepts full responsibility for his decisions on the manuscripts.

MANUSCRIPT PREPARATION

Manuscript 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.

Manuscripts with grammar or vocabulary deficiencies are disadvantaged during the scientific review process and, even if accepted, may be returned to the author to be rewritten in regular English, either standard British or American English, but consistent throughout. The authors are requested to seek the assistance of competent English language expert, if necessary, to ensure their English is of a reasonable standard. This journal maintains its policy and takes the liberty of correcting the English of manuscripts scientifically accepted for publication.

The submitted articles must be prepared solely with Microsoft Word; with single spacing (12 points Times New Roman; Greek letters in the character font Symbol) in A4 format leaving 2.5 cm for margins.

The size of the article (text, along with abstract, figures, tables and list of literature references should be limited to 7-10 pages. An exception can be negotiated with the editorial board, and to receive a larger volume of work if the content and quality justifies it.

IUPAC and International Union of Biochemistry and Molecular Biology recommendations for the naming of compounds should be followed.

Symbols of physical values should be in *cursive* (*italic*), and unit of measure in regular font, *eg*. V, m, p, t, T, but: m^3, kg, Pa, °C, K.

SI units, or other permissible units, should be employed. The designation of physical quantities should be in Times New Roman font. In text, graphs, and tables, brackets should be used to separate the designation of a physical quantity from the unit.

Please do not use the axes of graphs for additional explanations; these should be mentioned in the figure captions and/or the manuscript (example: "pressure at the inlet of the system, kPa" should be avoided).

Percents and per mills, although not being units in the same sense as the units of dimensioned quantities, can be treated as such. Unit symbols should never be modified (for instance: w/w%, vol.%, mol.%) but the quantity measured has to be named, e.g. mass fraction, w=95%; amount (mole) fraction, x=20%.

Latin words, as well as the names of species, should be in italic, as for example: *i.e., e.g., in vivo, ibid, Artemisia annua L.*, *etc*. The branching of organic compound should also be indicated in *italic*, for example, *n*-butanol, *tert*-butanol, *etc*.

Decimal numbers must have decimal points and not commas in the text, tables and axis labels in graphical presentations of results. Thousands are separated, if at all, by a comma and not a point.

Tables are part of the text together with their captions. They should be made so that they are understandable without reading the text, font Times New Roman 10 pt. in table. Table caption have to be positioned above the table. The tables should be numbered consequently in Latin numbers. Quantities should be separated from units by brackets. Footnotes to tables, in size 9 font, are to be indicated consequently (line-by-line) in superscript letters. Tables should be prepared solely using the Word table function, without vertical lines. Table columns must not be formatted using multiple spaces. Table rows must not be formatted using Carriage returns (enter key). Tables should not be incorporated as graphical objects.

Figures and diagrams are also part of the text together with their captions. They 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. Figure caption have to be positioned below the table. Every figure and/or diagram should be prepared according to the artwork instructions and, even embedded in text, submitted also as a separate file. All these files should be archived in the *.zip or *rar archive and named as follows: TA_*last name of first author_first word of title*_figures.extension. The extension must match the format of archive (zip or rar).

Mathematical and chemical equations should be numbered by Arabic numbers, consecutively in parenthesis at the end of the line. All equations should be embedded in the text except when they contain graphical elements (tables, figures, schemes and formulae). Complex equations (fractions, inegrals, matrix…) should be prepared using the Word MS Equation Editor or MathType.

The main file, containing the text of the manuscript with all elements embedded, should be named as follows: TA_*last name of first author_first word of title*.doc

Artwork Instructions. High resolution illustrations in TIF, JPG, PNG or GIF format are acceptable and must be uploaded as a separate archived (.zip or .rar) file. MS files (Word, PowerPoint, Excel, Visio) are NOT acceptable. Generally, scanned instrument data sheets should be avoided. Authors are responsible for the quality of their submitted artwork.

Image quality: keep figures as simple as possible for clarity - avoid unnecessary complexity, colouring and excessive detail. Images should be of sufficient quality for the printed version, i.e. 300 dpi minimum. Image size: illustrations should be submitted at its final size (8 cm for single column width or 17 cm for double column width) so that neither reduction nor enlargement is required. Please, keep in mind that colour photographs rarely reproduce satisfactorily in black and white.

STRUCTURE OF THE MANUSCRIPT

The manuscript must contain the title of the manuscript, full name(s) of the author(s) without abbreviation, abstract, the list of key words, the main text with all the tables and figures embedded and list of references. Can contain also the “Acknowledgement” section.

Title should be specific and informative, in order to exactly determine the content of the paper. It is desirable to be as short as possible

Authors are listed with full first name(s) and family name(s), without abbreviation. The corresponding author should be marked witf the asterix (*) at the end of his family name.

A one-paragraph A**bstract** written of 150–200 words in an impersonal form indicating the aims of the work, the main results and conclusions should be given and clearly set off from the text. It must finish with the list of keywords (up to 6, separated by “;”)

Main text should have the following form (though this proposed form is not fixed):

Introduction should include the aim of the research and a concise description of background information and related studies directly connected to the paper.

Experimental section should give the purity and source of all employed materials, as well as details of the instruments used. The employed methods should be described in sufficient detail to enable experienced persons to repeat them. Standard procedures should be referenced and only modifications described in detail.

Results and Discussion should include concisely presented results and their significance discussed and compared to relevant literature data. The results and discussion may be combined or kept separate.

The inclusion of a *Conclusion* section, which briefly summarizes the principal conclusions, is highly recommended.

Acknowledgement (optional).

Reference List should be selective rather than extensive. Generally, no more than 30 references should be cited in your manuscript. except when it comes to review article. Please ensure that every reference cited in the text is also present in the Reference List (and vice versa).

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.

Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text.

From this issue of Technologica Acta (vol. 10, no. 2) forward, the Journal will strictly follow the IEEE citation style. The brief explanation of IEEE citation style is given below.

IEEE citation style includes in-text citations, numbered in square brackets, which refer to the full citation listed in the reference list at the end of the paper. The reference list is organized numerically, not alphabetically.

THE BASICS OF IEEE CITATION STYLE:

IN-TEXT CITING

Refer to the source with a number in a square bracket, e.g. [1], that will then correspond to the full citation in your reference list.

- Place bracketed citations within the line of text, before any punctuation, with a space before the first bracket.
- Number your sources as you cite them in the paper. Once you have referred to a source and given it a number, continue to use that number as you cite that source throughout the paper.
- When citing multiple sources at once, the preferred method is to list each number separately, in its own brackets, using a comma or dash between numbers, as such: [1], [3], [5] or [1]-[5].

EXAMPLES OF IN-TEXT CITATIONS:

"...end of the line for my research [13]."
"This theory was first put forward in 1987 [1]."
"Scholtz [2] has argued that..."
"Several recent studies [3], [4], [15], [16] have suggested that...."
"For example, see [7]."

EXAMPLES OF CITATIONS FOR DIFFERENT MATERIALS:

Material Type	Works Cited
Book in print	[1] B. Klaus and P. Horn, *Robot Vision*. Cambridge, MA: MIT Press, 1986.
Chapter in book	[2] L. Stein, “Random patterns,” in *Computers and You*, J. S. Brake, Ed. New York: Wiley, 1994, pp. 55-70.
eBook	[3] L. Bass, P. Clements, and R. Kazman, *Software Architecture in Practice*, 2nd ed. Reading, MA: Addison Wesley, 2003. [E-book] Available: Safari e-book.
Journal article	[4] J. U. Duncombe, "Infrared navigation - Part I: An assessment of feasability," *IEEE Trans. Electron. Devices*, vol. ED-11, pp. 34-39, Jan. 1959.
eJournal (from database)	[5] H. K. Edwards and V. Sridhar, "Analysis of software requirements engineering exercises in a global virtual team setup," *Journal of Global Information Management*, vol. 13, no. 2, p. 21+, April-June 2005. [Online]. Available: Academic OneFile, http://find.galegroup.com. [Accessed May 31, 2005].
eJournal (from internet)	[6] A. Altun, "Understanding hypertext in the context of reading on the web: Language learners' experience," *Current Issues in Education*, vol. 6, no. 12, July 2003. [Online]. Available: http://cie.ed.asu.edu/volume6/number12/. [Accessed Dec. 2, 2004].
Conference paper	[7] L. Liu and H. Miao, "A specification based approach to testing polymorphic attributes," in *Formal Methods and Software Engineering: Proceedings of the 6th International Conference on Formal Engineering Methods, ICFEM 2004, Seattle, WA, USA, November 8-12, 2004*, J. Davies, W. Schulte, M. Barnett, Eds. Berlin: Springer, 2004. pp. 306-19.
Conference proceedings	[8] T. J. van Weert and R. K. Munro, Eds., *Informatics and the Digital Society: Social, ethical and cognitive issues*: IFIP TC3/WG3.1&3.2 Open Conference on Social, Ethical and Cognitive Issues of Informatics and ICT, July 22-26, 2002, Dortmund, Germany. Boston: Kluwer Academic, 2003.
Newspaper article (from database)	[9] J. Riley, "Call for new look at skilled migrants," *The Australian*, p. 35, May 31, 2005. [Online]. Available: Factiva, http://global.factiva.com. [Accessed May 31, 2005].
Technical report	[10] J. H. Davis and J. R. Cogdell, “Calibration program for the 16-foot antenna,” Elect. Eng. Res. Lab., Univ. Texas, Austin, Tech. Memo. NGL-006-69-3, Nov. 15, 1987.
Patent	[11] J. P. Wilkinson, “Nonlinear resonant circuit devices,” U.S. Patent 3 624 125, July 16, 1990.
Standard	[12] *IEEE Criteria for Class IE Electric Systems*, IEEE Standard 308, 1969.
Thesis/Dissertation	[1] J. O. Williams, “Narrow-band analyzer,” Ph.D. dissertation, Dept. Elect. Eng., Harvard Univ., Cambridge, MA, 1993.

SUBMISSION OF MANUSCRIPT

COVER LETTER

Manuscripts must be accompanied by a cover letter It should contain:

- title of the manuscript without any abbreviations,
- proposed type of contribution,
- full name(s) of the author(s) without abbreviations,
- full affiliation of all the authors (department, institution, city and country), along with the ORCID ID and ResearcherID (if they have them),
- mailing address (address, phone and fax numbers, e-mail) of the author to whom correspondence should be addressed.

The file with cover letter should be named as follows: TA_*last name of first author_first word of title*_cover.doc.

SUBMISSION

Submissions containing all needed files should be sent to e-mail address: dijana.milicevic@untz.ba with the subject “*Manuscript for Technologica Acta*”. All manuscripts will be acknowledged on receipt (by e-mail) and given a reference number, which should be quoted in all subsequent correspondence.