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CONTENT


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MICROENCAPSULATION OF DRONEDARONE HYDROCHLORIDE BY SPRAY DRYING

ORIGINAL SCIENTIFIC PAPER

Matija Gretić✉, Gordana Matijašić, Juraj Petanjek

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Faculty of Chemical Engineering and Technology, University of Zagreb, Marulićev trg 19, 10000 Zagreb, Croatia

✉ mgretic@fkit.hr

ABSTRACT:

Microencapsulation is a method often used in various industries and one of the many advantages is that controlled release of the active ingredient can be achieved. The aim of this study was to prepare microcapsules by spray drying by changing the process conditions and ratios of the used components, polymer excipient poly(vinyl alcohol) and active ingredient dronedarone hydrochloride. Dosage forms of poly(vinyl alcohol) and dronedarone hydrochloride were prepared in 1:1, 2:1 and 3:1 weight ratios and the solutions were dried in a laboratory spray dryer at four different atomization flowrates. The morphology of the obtained microcapsules was examined using a scanning electron microscope (SEM). The release kinetics of the active ingredient from the microcapsules were examined by in vitro laboratory testing and the resulting release profiles were described using the Weibull model. The results showed that the change of weight ratio has influence on the morphology of microcapsules, the efficiency of microencapsulation and drug release.

KEYWORDS: microencapsulation, spray drying, dronedarone hydrochloride, poly(vinyl alcohol), *in vitro* drug release

INTRODUCTION

In the pharmaceutical industry, coating processes of active ingredients are increasingly used to obtain controlled and prolonged drug release. A well-designed, controlled release drug delivery system can overcome some of the problems of conventional delivery of the active ingredient and improve the therapeutic efficacy of a given drug [1]. One of the methods that enables the delivery of the active ingredient to the targeted location is microencapsulation [2]. Microencapsulation is a process where very tiny droplets or particles of liquid or solid material are surrounded or coated with a continuous film of polymeric material [3]. The primary reasons for microencapsulation are controlled drug release, improvement of the physical and chemical properties of the dosage form, protection of the active ingredient, masking of bad taste masking and easier handling of the dosage form [4]. An important step in microencapsulation process is selection of the polymer material for coating of the active ingredient. When choosing a polymer, it is important to pay attention to the following characteristics: polymer biodegradability, physical, chemical and thermal properties of polymer, compatibility between the polymer and active ingredient, etc. [5]. There are many different methods of microencapsulation and the most commonly used method is spray drying. Spray drying is a method of drying liquid and semi-liquid solutions, suspensions, emulsions and droplets, in just one production step. The principle of spray drying is to disperse the drug solution into very small droplets in the atomizer. Solvent rapidly evaporates in hot dry media to obtain a dry product in powder or granular form. In this process, a product with a high degree of purity and a narrow particle size distribution is produced [6]. The aim of this work was to prepare prolonged release dosage form. Dronedarone hydrochloride (DNR) was used as the active ingredient and poly(vinyl alcohol) (PVA) was used as a polymer for the coating of the active ingredient. Microencapsulation was performed in a spray dryer and the release kinetic of dronedarone hydrochloride was studied by *in vitro* laboratory testing.

MATERIALS AND METHODS

MATERIALS

Antiarrhythmic drug, dronedarone hydrochloride, used in the experiments is received from TAPI Pilot, PLIVA Croatia Ltd. Dronedarone hydrochloride (DNR) is fine white crystalline powder, with melting point in range of 149 – 153°C [7]. Particle size of used DNR, expressed with as $x_{90,}$ is 39.92 μm [8]. Poly(vinyl alcohol) (Acros Organic) with average molecule weight of 50000 – 85000 g mol^{-1} was used for microencapsulation.

PREPARATION OF MICROCAPSULES

Solutions with a different weight ratio of DNR and PVA (Table 1) were prepared for the spray drying process. DNR was dissolved in 100 mL of the ethanol under stirring conditions at room temperature. PVA solution was prepared in 100 mL of the distilled water preheated at 80°C. Prepared solutions were combined together and then used as a feed solution for the spray drying process in Büchi Mini Spray Dryer B-290 (Büchi Labortechnik AG, Switzerland) with 1.4 mm nozzle. The process parameters were set as follows: inlet temperature 75°C, atomization air flowrates 1.22 - 4.84 x 10^{-4} m^2 s^{-1}, feed flowrate 2.5 x 10^{-8} m^3 s^{-1}, and drying air flowrate 1.06 x 10^{-2} m^3 s^{-1}.

Table 1. PVA:DNR weight ratios, atomization air flowrates and viscosity of used in experiments

Sample	Weight ratio PVA:DNR	Atomization air flowrate, x 10^{-4} (m^3 s^{-1})	Viscosity (mPa s)
PS 1	1:1	4.84	3.7
PS 2	1:1	2.92	
PS 3	1:1	1.85	
PS 4	1:1	1.22	
PS 5	2:1	4.84	4.8
PS 6	2:1	2.92	
PS 7	2:1	1.85	
PS 8	2:1	1.22	
PS 9	3:1	4.84	6.4
PS 10	3:1	2.92	
PS 11	3:1	1.85	
PS 12	3:1	1.22	

SCANNING ELECTRON MICROSCOPY

The morphology of the microcapsules was examined by scanning electron microscopy (SEM; VEGA3, TESCAN, Czech Republic). The samples were fixed to the holder, placed in the Quorum SC7620 sputter coater and coated with a thin layer of gold and palladium in an inert argon atmosphere and a 10^{-2} mbar vacuum. The SEM was operated at an acceleration voltage of 5-10 kV.

EQUILIBRIUM MOISTURE CONTENT

The equilibrium moisture content was determined using infrared moisture analyzer Kern MLS 50-3C (KERN, Balingen, Germany). Drying was carried out at 75 °C and the equilibrium moisture content was calculated from Equation 1.

$$X_{\text{eq}} = \frac{m(\text{microcapsules}) - m(\text{dry microcapsules})}{m(\text{dry microcapsules})} \quad (1)$$

IN VITRO DRUG RELEASE

The microcapsules were filled into standard gelatin capsules, size 00, before *in vitro* studies. The weight of microcapsules was calculated according to the ration of DNR and PVA so that each gelatin capsule would contain approximately the same amount of DNR (42.75 mg). The release profiles of DNR were determined using the European Pharmacopeia method-II dissolution apparatus [9] (RC-6D, Zhengzhou Nanbei Instrument, China). Food & Drug Administration (FDA) method defined for referent drug MULTAQ® (dronedarone hydrochloride tablets) [10] was used for *in vitro* tests of prepared microcapsules. Each experiment was carried out in triplicate, using 1000 mL of a phosphate buffer with pH 4.5, at a temperature of 37 ± 0.5 °C. The paddle rotational speed was 75 rpm. The gelatin capsules were placed into Japanese sinkers, which were placed into the beakers. Sampling was performed over a 24h period. The samples were filtered using Chromafil Xtra H-PTFE-20/25 with a pore size of 0.45 μm and subsequently analyzed using a UV/Vis spectrophotometer (UV-1280, Shimadzu, Japan) to determine the concentration by monitoring the absorbance of the DNR at 289 nm.

DISSOLUTION KINETICS

The DDSolver was used for the fitting of the dissolution profile. Various mathematical models of the drug dissolution are already integrated into the DDSolver [11]. In this study, the applicability of different models was tested and the value of R^2 was used as model selection criteria. The release profiles are described by the Weibull model (Eq 2). Where, M_t is the amount of drug dissolved as a function of time and M_0 is total amount of drug being released. Parameters *a* and *b* are kinetic parameters of Weibull model.

$$\frac{M_t}{M_0} = 1 - e^{\frac{-(t)^b}{a}} \quad (2)$$

RESULTS AND DISCUSSION

The objective of this research was to obtained microcapsules of dronedarone hydrochloride to investigate the possibility of drug release control by using PVA as coating polymer. Microcapsules of different DNR:PVA ratios were successfully pre-

pared. Microencapsulation was performed by spray drying at four different atomization air flowrates. In addition, the PVA and DNR mass ratios (1:1, 2:1 and 3:1) were changed which resulted in a total of 12 experiments. Samples are labeled from PS1 to PS12, and the corresponding mass ratios and flowrates are given in Table 1. Samples PS4, PS8 and PS12 were dried at the lowest atomization flowrate 1.22 $x10^{-4}$ m^3 s^{-1}. The efficacy in experiments PS4, PS8 and PS12 was extremely low due to the huge remaining of the samples at the cyclone walls. This could be explained by low atomization flowrates that produce large droplets which, due to their size, do not completely dry at 75°C. Those samples were not used in *in vitro* study due to the low yield.

The efficiency of microencapsulation, the morphology of the obtained microcapsules, as well as the equilibrium moisture content of the samples were examined. Table 2 shows the efficiency of microencapsulation expressed by the ratio of DNR weight in samples before and after drying. In addition, in Table 2 the values of equilibrium moisture content in obtained samples are presented.

Table 2. Microencapsulation efficiency and equilibrium moisture content

Sample	Mass ratio PVA:DNR	Atomization flowrate, $x10^{-4}$ (m^3 s^{-1})	Initial mass of components (g)	Mass of DNR in spray dried samples (g)	Efficiency (%)	Equilibrium moisture content (%)
PS1	1:1	4.84	2.0	0.773	77.3	6.4
PS2	1:1	2.92	2.0	0.647	64.7	7.5
PS3	1:1	1.85	2.0	0.295	29.5	6.4
PS5	2:1	4.84	3.0	0.773	77.3	7.5
PS6	2:1	2.92	3.0	0.677	67.7	8.7
PS7	2:1	1.85	3.0	0.614	61.4	8.7
PS9	3:1	4.84	4.0	0.542	54.2	8.7
PS10	3:1	2.92	4.0	0.524	52.4	9.9
PS11	3:1	1.85	4.0	0.556	55.6	8.7

Process efficiency increases with the increase of the atomization flowrate for samples with the same ratio of polymer and the active ingredient (1:1) (PS1, PS2, PS3; Table 2). On the other hand, smaller differences in the dependence of efficiency on the atomization flowrate were observed for the ratio 2:1 (samples PS5, PS6 and PS7). The effect of the atomization flowrate is insignificant for microcapsules with the highest polymer content (3:1) (samples PS9, PS10 and PS11) where the values of efficiency are similar in all three experiments. Increase of the microencapsulation efficiency can be explained through the atomization step and droplet size. Higher atomization flowrate results in droplets of smaller sizes [12] resulting in faster drying at low temperatures. Smaller particles will have enough time to complete the drying process before reaching the cyclone chamber. An increase in the polymer content leads to an increase in the viscosity of the solution, thereby reducing the microencapsulation efficiency for the samples PS9, PS10 and PS11. The formation of droplets is more influenced by viscosity than the atomization air flowrate [13]. Solution with higher viscosity will give larger droplets, which is directly related to the drying process and microencapsulation efficiency [14]. Dried microcapsules were tested for equilibrium moisture content at 75°C and the values are present in Table 2. Increased equilibrium moisture content was observed for samples with higher polymer content, which is consistent with previously described droplet size and drying conditions. The equilibrium moisture content of all samples is less than 10 %.

The samples were characterized by a scanning electron microscope (SEM) to determine their morphology. The micrographs are shown in Figures 1-4.

Figure 1 shows micrographs of the sample PS1 corresponding to an equal ratio of PVA and DNR and maximum atomization flowrate. The microcapsules are very small and due to their size agglomeration problem occurred.

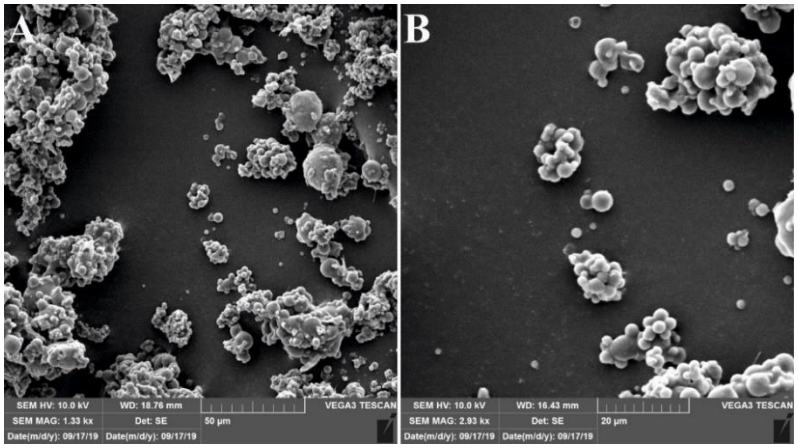

Figure 1. SEM micrographs of the sample PS1; a) 1000x, b) 2000x

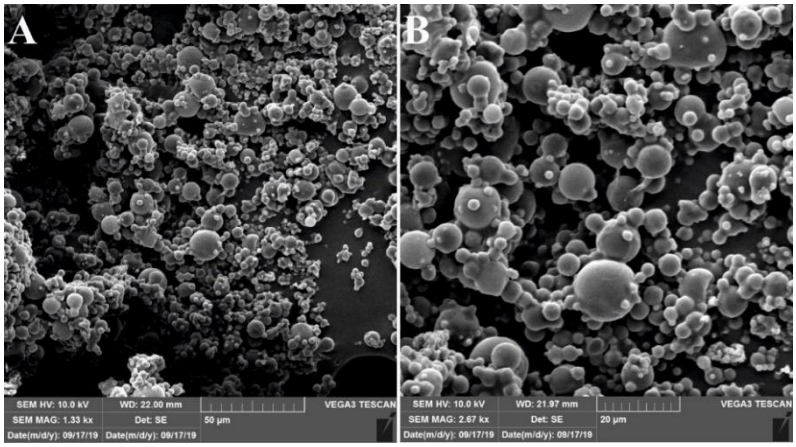

Figure 2. SEM micrographs of the sample PS5; a) 1000x, b) 2000x

Figure 2 shows micrographs of a PS5 sample containing polymer and an active ingredient in the ratio of 2:1. Both solutions (PS1 and PS5) where dried at maximal atomization flowrate resulting in small microcapsules. Same results were obtained for sample PS9 (Fig. 3).

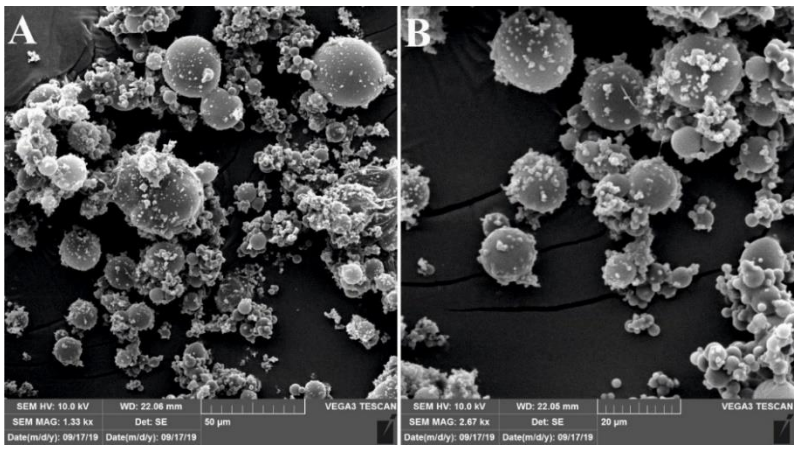

Figure 3. SEM micographs of the sample PS9; a) 1000x b) 2000x

On the other hand, if one compares polymer content in samples PS1 (1:1), PS5 (2:1) and PS9 (3:1) it can be seen from SEM micrographs that particle size increases, which is in accordance with the efficiency and equilibrium moisture content. Increasing the viscosity of the solution causes the formation of larger particles at same atomization flowrate.

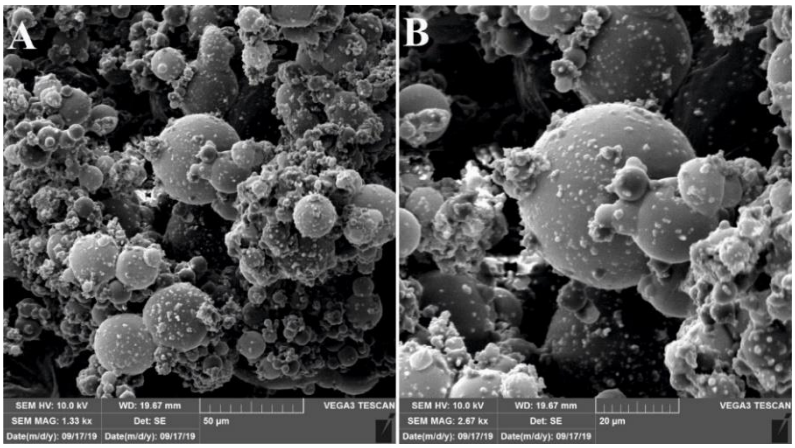

Figure 4. SEM micrographs of the sample PS10; a) 1000x b) 2000x

Figures 3 and 4 show micrographs of obtained microcapsules in which the PVA:DNR ratio is 3:1. The influence of different atomization air flowrate is considered. By reducing the flowrate of atomization air, a significant increase in particle size was seen. These samples clearly show spherical shape of microcapsules.

In vitro drug release profiles of DNR are presented in Figures 5-7.

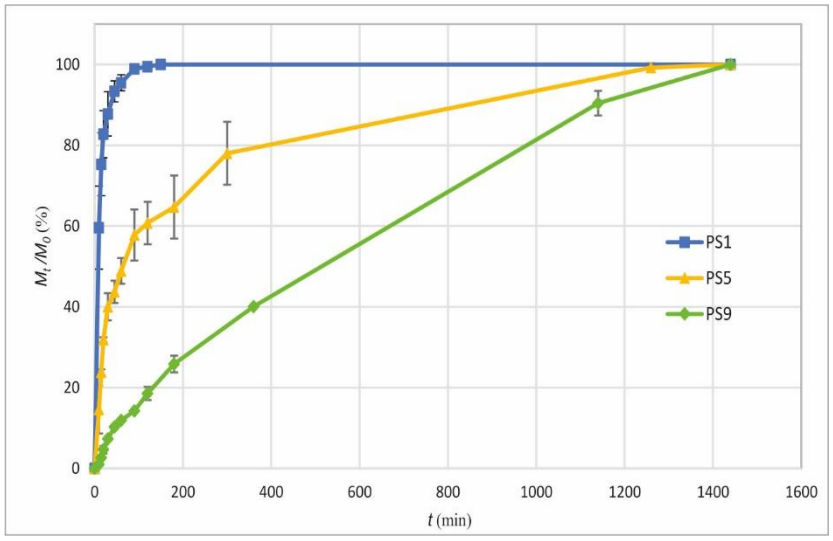

Figure 5. Dissolution profiles for different polymer content at atomization air flowrate 4.84 x 10^{-4} m^3 s^{-1}

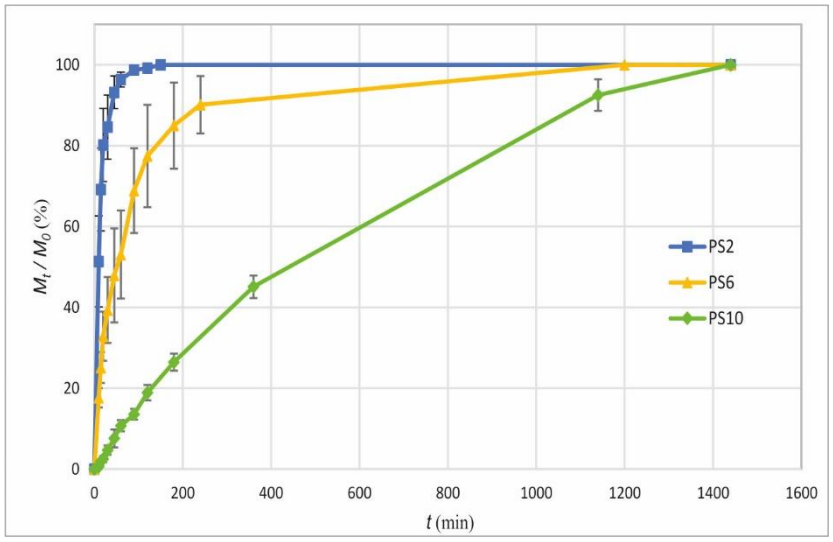

Figure 6. Dissolution profiles for different polymer content at atomization air flowrate 2.92 x 10^{-4} m^3 s^{-1}

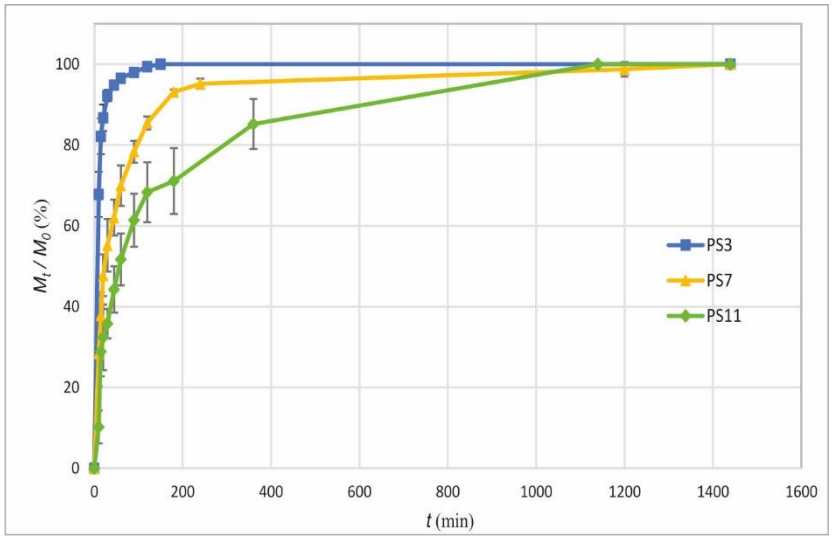

Figure 7. Dissolution profiles for different polymer content at atomization air flowrate 1.85 x 10^{-4} m^3 s^{-1}

The release profiles for the same atomization flowrate with different PVA and DNR ratios were compared. Increase in polymer content results in a prolonged release of DNR over 24h period.

Figures 8-10 provide comparisons of the *in vitro* release profiles for the samples obtained with spray drying at a different atomization air flowrate and equal PVA and DNR ratios.

Samples PS1, PS2 and PS3 have almost the same release profiles. In samples with equal PVA and DNR ratio, a large amount of DNR is released very quickly and its maximum is reached after 90 minutes. Independent of the atomization air flowrate, all samples with equal PVA and DNR ratios have the same release profile. This is explained by insufficient amount of polymer to form a shell around the DNR. The differences in the release profiles are more pronounced for different atomization flowrates by increasing the polymer content (Fig. 10 and 11).

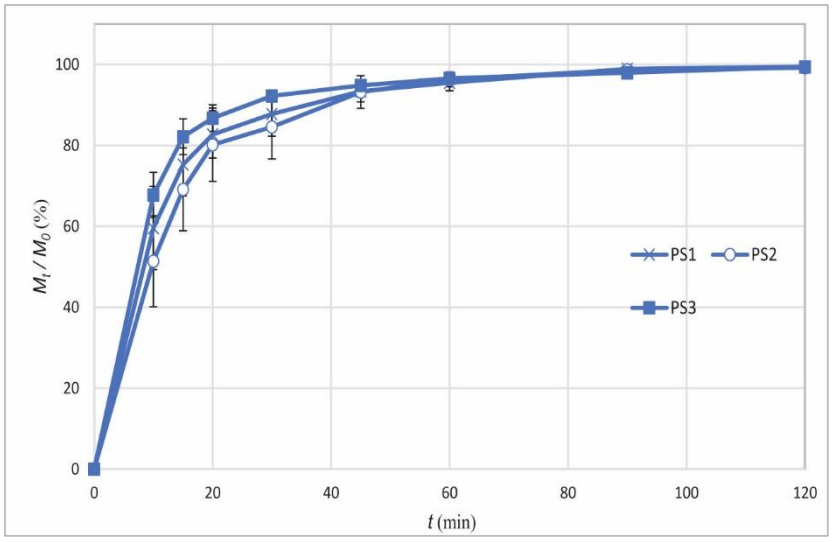

Figure 8. Dissolution profiles for different atomization air flowrate at same polymer content

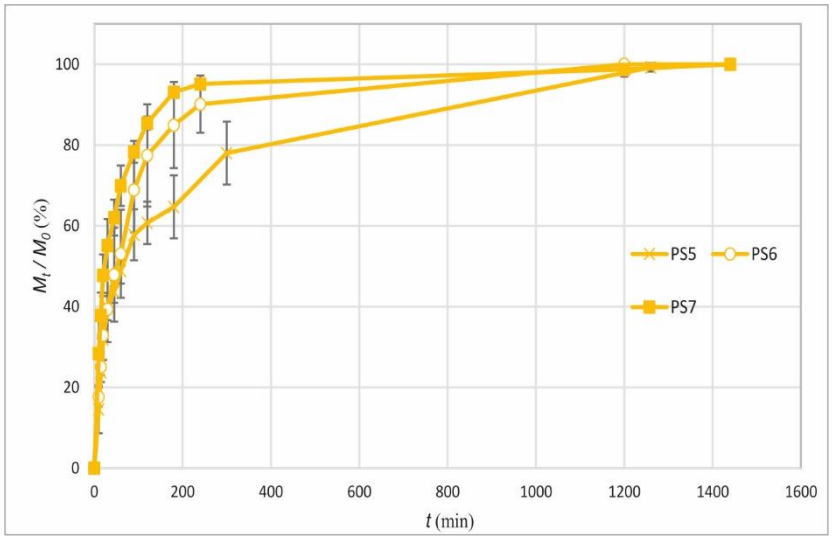

Figure 9. Dissolution profiles for different atomization air flowrate at same polymer content

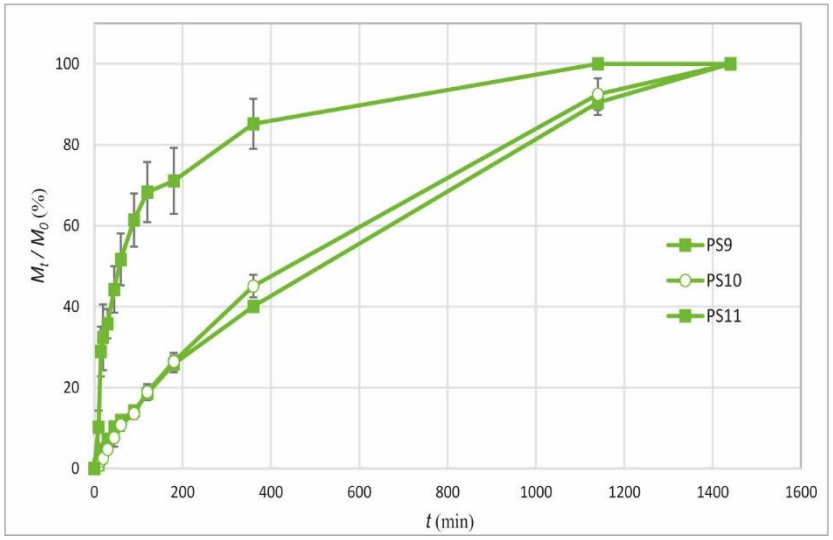

Figure 10. Dissolution profiles for different atomization air flowrate at same polymer content

The release kinetics of the active ingredient was described by the Weibull model (Eq. 2), which was selected based on the high R^2 values. The Weibull model is often used for comparison of the release profiles of an active ingredient from matrix dosage form [15]. Table 3 shows the parameter values of the selected kinetic model.

Table 3. Weibull model parameters

Sample	a, min^b	b	R^2
PS1	5.218	0.666	0.994
PS2	9.572	0.754	0.989
PS3	2.880	0.536	0.994
PS5	21.279	0.622	0.972
PS6	32.009	0.805	0.994
PS7	11.756	0.650	0.988
PS9	656.62	1.021	0.986
PS10	1277.7	1.144	0.994
PS11	29.185	0.706	0.971

Parameter *b* is related to the curve type. When the value of parameter *b* is less than 1, curve is parabolic with high initial slope and a consistent exponential character. That corresponds to the rapid release of a large amount of the active ingredient from the dosage form. When *b* is greater than 1, curve is sigmoidal with inflection point. Such curves indicate slower drug release [16]. Samples with the highest polymer content (3:1) (PS9, PS10 and PS11) show the highest values of parameter *b*, as expected, since the increase of polymer content will prolong drug release. Parameter *a* defines the release process timescale [16]. Higher values of this parameter indicate longer process time, which is, in this case, prolonged drug release. The highest values were observed for the samples with PVA and DNR ratio of 3:1, which was confirmed by parameter *b*. The only discrepancy was observed for sample PS11. We can attribute this to the lowest R^2 value and inadequate model fit for obtained experimental data.

CONCLUSION

Microcapsules of dronedarone hydrochloride coated with poly(vinyl alcohol), with different polymer content and different process conditions of spray drying, were prepared. Selected process conditions (polymer content and atomization air flowrate) affect the rate of the drug release. Atomization air flowrate has direct influence on the microcapsules size, where smaller microcapsules were obtained by increasing the atomization air flowrate. The microencapsulation efficiency is greater than 50% for all samples. SEM micrographs confirmed that the finer particles were obtained at a higher atomization air flowrate. In addition, by increasing the polymer content, the larger particles were obtained. Microcapsules with the highest polymer content showed the prolonged release of the dronedarone hydrochloride over 24h period.

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QUALITY EVALUATION OF BREAD WITH ADDITION OF SUNFLOWER SEEDS, OLIVES AND TURMERIC

ORIGINAL SCIENTIFIC PAPER

Aldin Karić✉, Amela Kusur, Martina Andrejaš, Hurija Alibašić

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Faculty of Technology, University of Tuzla, Urfeta Vejzagića 8, 75000 Tuzla, Bosnia and Herzegovina

✉ aldinkaric96@hotmail.com

ABSTRACT:
This paper will show that adding some spices and other raw materials can increase the nutritional value of bread and provide a product that will be acceptable for consumption. Sunflower seeds, olives or turmeric are added in amounts up to 10 %, relative to the total amount of flour. Nutritional value of bread was determined, the sensory analysis was performed and the durability or preservation of freshness was monitored.

KEYWORDS: bread with addition of sunflower seeds, olives or turmeric; quality; enriching white bread

INTRODUCTION

White wheat flour bread was considered as the highest quality bread product for a long time due to its large volume, shape and color. This is all due to the quantity and properties of gluten. However, this bread is poor in vitamins and minerals because during milling in the production of white flour, peel wheat parts and germs are removed. Those parts contain high value ingredients. For this reason, the use of black, integral, whole wheat flour and other cereal grains, which were mainly mixed with wheat flour has begun. Another way of enriching white wheat flour is to add the variety of raw materials that will increase its nutritional value [4].

The bakery industry is one of the branches of the food industry. The first bread was baked back in the Neolithic, almost 12,000 years ago, most likely from coarse-grained grains mixed with water. By the time of ancient Rome, milling and baking had already developed to an almost industrial level [1]. Bakery products mean a very wide range of bread and pastries. Most of the pastries differ from bread mainly by weight and very little by raw material composition. Flour products form the basis of nutrition, especially in lower-standard countries. In some countries, over 60% of the total energy is consumed with these products. With the rise in living standards, consumption of cereal products is declining, and they are being replaced by fat, sugar and animal products, which are much more expensive [2].

In many high-standard countries, cereal consumption has fallen below the limit, which is a physiological minimum, which has had a serious impact on the health of the population [2]. Cereal replacement products are generally poor in ballast [3]. A large number of medical tests indicate the benefits of darker types of bread [2]. This bread provides significant amounts of vitamins and minerals as well as ballast that are essential in the diet. The consumption of grain is influenced not only by living standards but also by other factors, most notably the habits of the local population. In most countries, the production of bread and pastries as essential foodstuffs is regulated by law and quality regulations [4].

A large number of raw materials are used in the bakery. These are raw materials of plant and animal origin and some of the raw materials are from other sources.

Raw materials in bakery are divided into:

1. Basic raw materials - flour and water.
2. Additional raw materials - yeast and salt.
3. Auxiliary raw materials - sugar, sweeteners, milk and milk products, fats, margarine, chocolate, cocoa powder, fruits and fruit products, spices, additives, emulsifiers, nutritional or technological quality enhancers, etc [5].

Nutritionally valuable foods were used as additional raw materials for the production of bread with additives: sunflower seeds, turmeric and olives. In laboratory conditions it is possible to define precisely the best recipe to be used in production. Trial baking

is the most relevant criterion for evaluating the baking properties of flour and cannot be completely replaced by any instrumental method [3].

Sunflower seeds are rich in vitamins E, B1, B3, B6, folic acid and minerals such as copper, manganese, selenium, phosphorus and magnesium. At the same time, they have a high calorie value (584 kcal in 100 g).

Olives are distinguished by their high content of single unsaturated fatty acids (as high as 75%), with a beneficial effect on health. They are also a good source of vitamin E, a powerful antioxidant essential in the daily fight against harmful free radicals. In addition, olives are a good source of iron and copper minerals and dietary fiber. In addition to vitamin E, the antioxidant properties of olives are also enhanced by active phytochemicals such as polyphenols and flavonoids.

Turmeric is medicinal herb and has been in use for over 5000 years. It is rich in vitamins B complex, contains vitamin C as well as minerals. The active ingredient in turmeric is curcumin, which, even at very low concentrations, has a curative effect. First of all it is known for its very powerful antioxidant, anti-inflammatory and antibacterial properties [4].

EXPERIMENTAL

Bread without additives control samples is made according to the following recipe: 300 g flour, humidity of 14%,

Sugar solution: 5 g of sugar dissolved in 95 g of water, 5.4 g of dry yeast suspended in 21.6 g of sugar solution, 4.5 g of table salt, 5.58 g of sugar, 210 ml of water.

The process of making the other three breads differs only in the addition of additional raw materials in quantities of 10%: turmeric, sunflower seeds, olives. The process of making all the samples is exactly the same.

MATERIALS

The following raw materials were used to make the bread without additives:

1. flour: ("Ljubače type 500")
2. baker's yeast: ("Di-Go")
3. salt: ("Tuzlanska so")
4. sugar: ("Bingo Šećer")
5. water: ("Voda Kristal)
6. vegetable oil: ("Bimal")

For the preparation of bread samples fortified with the additives, in addition to the basic raw materials, in each bread sample were added one of the following additives (in amount of 10%):

- turmeric powder,
- olives,
- sunflower seeds.

For the bread making followed materials were used:

- plastic containers,
- fermentation chamber (30 ° C and 85% humidity, water bath),
- kneading board,
- dough roller,
- baking mold,
- oven with temperature control.

METHODS

Bread samples were obtained after a trial baking according to a following recipe:

300 g of flour is poured into a pan, added the prepared yeast solution, salt, sugar, and stirred. After the dough of the desired structure is obtained, it is transferred to a suitable container and placed under fermentation for 30 minutes, at a temperature of 30 ± 1 ° C and a relative humidity of 85%. When fermentation is complete, stirring takes 2-3 minutes to achieve better structure and quality of the finished product. Then a second fermentation was carried out, 50 minutes at 30 ± 1 ° C. The dough was placed in an oven heated to 230 ± 10 ° C. After 5 minutes the temperature was reduced to 200 ° C and after 15 minutes (from the beginning of baking) to 175 ° C. Total baking time is 30 ± 3 minutes. After this time, bread is left for 1 h to cool. To obtain the data used to calculate the losses, the bread must be weighed after 1 h of cooling and after 24 h.

Sensory evaluation:

After the trial baking, an organoleptic or sensory evaluation of the finished products was performed. Organoleptic evaluation involves monitoring and evaluating the following factors: volume, outer appearance, apperance of the bread crumb, flavour of bread crumb and crust, taste of bread crumb and crust. For each bread sample, 10 evaluators participated in the evaluation. Each evaluator received a certain amount of bread sample that he consumed and then entered his grades into a rating sheet.

Determination of water content (dry matter) in bread was made by drying method:

The proportion of water (dry matter) in bread was determined by drying the weighed sample (3 g) at 130 ° C (± 1 ° C) to constant weight. The water or

dry matter content was calculated as the difference in weight of the sample before and after drying.

$$\%dry\ matter = \frac{M-m}{M}\cdot 100 \quad \text{.....(1)}$$

$$\%water = 100 - \%\ dry\ matter \quad \text{.....(2)}$$

where is:
M- mass of bread sample before drying,
m- mass of bread sample after drying.

The acidity of the bread crumb was determined by the titration method:

6 g of the bread crumb of the bread sample is measured, moistened it in a porcelain dish with 5 ml of neutral acetone and then homogenized it with 100 ml of freshly boiled and cooled water. To the mixture was added 1 ml ethanol solution of phenolphthalein and immediately titrated with 0.1 mol (NaOH) / l to a reddish color, which should last at least 15 seconds.

The acidity was expressed as an acidic degree, denoting the number of milliliters of a 1- molar alkali solution required to neutralize the total acids in 100 g of the bread crumb, and was calculated by the following formula:

$$acid\ \deg ree = \frac{a\cdot 10}{b} \quad \text{.....(3)}$$

where is:
a - spent milliliters of 0.1 mol (NaOH) / l to neutralize total acids,
b - sample weight.

The determination of the amount of ash (mineral matter) in bread is determined by burning and annealing:

2 g of the sample is weighed and dried according to the procedure for determining the water in bakery products. From the homogenized sample, the required amount of sample is measured and combusted it on the grid until complete carbonation. As soon as the contents of the vessel are charcoal-coated, the vessel is carefully inserted into the muffle furnace. The annealing was carried out at 800 ºC. When the combustion was completed, the pan was removed from the oven and cooled for 1 min. Then the contents of the pan are weighed.

The amount of ash is expressed as a percentage by mass relative to the dry matter and is calculated by the following formula:

$$Ash\ amount(\%) = m_1 \cdot \frac{100}{m_0}\cdot\frac{100}{100-V} \quad \text{.....(4)}$$

where is:
m_0 - mass of test sample, in grams,
m_1 - mass of the remainder, in grams,
V - the amount of water, expressed as a percentage, in the sample to be tested.

Determination of fat content in bread (Soxlet method):

5 g (m_0) of the sample is weighed and transferred to a paper sleeve. The sleeve is inserted into the Soxhlet extractor so that the height of the sleeve is less than the edge of the solvent siphon tube. The extractor is then combined with a flask of known mass. Petroleum ether was poured over the bushing so that its volume is at least 1.5 volumes of the extractor and at the same time not more than ¾ the volume of the flask. The extractor is then combined with the reflux through which the water flows. A heater is placed under the flask. The extraction takes 4 to 6 hours, depending on the type of sample. After extraction was complete, the distillation was stopped as soon as the solvent was poured into the flask. The appliance was removed from the heating body, removed the sleeve, then reassembled the appliance, and the solvent was pre-distilled into the extractor. The fat flask was separated from the extractor and dried in an oven at 105 ° C for about 1 hour or until constant mass. The flask is cooled and weighed (m_1). The difference in the mass of the flask after drying and the empty flask represents the amount of extracted fat in the sample. The calculation of the amount of crude fat is expressed as a percentage and is calculated by the following formula:

$$Fat\ content(\%) = \frac{m_1}{m_0} - \frac{m_2}{m_0}\cdot 100 \quad \text{.....(5)}$$

where is:
m_0 - measured quantity of sample, in grams,
m_1 - mass of the flask with extract (fat), in grams,
m_2 - mass of empty flask, in grams,
V - moisture content of the sample (%).

Determination of protein in bread (Kjeldahl method):

The method is based on the destruction of organic matter by sulfuric acid, the nitrogen compounds are converted into ammonia, which forms ammonium sulfate with acid. From such a solution, ammonia is

displaced by NaOH and its amount after distillation is determined by titration with standard acid. On the basis of the amount of acid consumed, the nitrogen content of the sample is calculated and converted to proteins by multiplying by the appropriate factor. This factor for flour is 6.25.

RESULTS AND DISCUSSION

Based on the analysis we have obtained the following results.

Figures 1 and 2 show the appearance of the bread samples after the trial baking.

Figure 1. Bread without additives (right), bread with sunflower seeds (left).

Figure 2. Bread with olives (right), bread with turmeric (left).

Table 1. Results of trial baking.

Sample	Bread without additives	Bread with sunflower seeds	Bread with turmeric	Bread with olives
Mass of dough (g)	531.45	540.81	543.34	539.87
Mass of bread after 1h (g)	454.55	470.74	473.41	465.19
Mass of bread after 24 h (g)	476.86	489.65	490.75	486.42
Yield of dough	1.77	1.80	1.81	1.79
Yield of bread (1 h)	1.51	1.57	1.58	1.55
Yield of bread (24 h)	1.59	1.63	1.64	1.62
Total losses %	24.73	22.41	22.55	23.73
Losses by roasting %	14.46	12.95	12.87	13.83
Losses by drying %	10.27	9.46	9.68	9.9

Table 2. Results of sensory evaluation of bread.

	Bread without additives	Bread with sunflower seeds	Bread with turmeric	Bread with olives
Volume	20	20	20	20
Outer appearance	15	15	15	15
Apperance of the bread crumb	25	24.375	25	25
Flavour of bread crumb and crust	14.625	14.625	14.667	15
Taste of bread crumb and crust	24.375	24.375	23.889	25
TOTAL (max 100)	99	98.375	98.556	100

Table 3. Results of nutritional composition of bread.

Sample	Water content (%)	Dry matter content (%)	Ash content (%)	Fat content (%)	Protein content (%)	Acid degree
Bread without additives	40.6	59.4	0.64	1.12	5.3	2.61
Bread with sunflower seeds	38.7	61.2	0.96	2.22	8.2	2.61
Bread with turmeric	38.91	61.08	0.80	0.66	5.4	1.72
Bread with olives	41.87	58.13	1.08	1.72	7.1	2.42

Table 4. Nutrients composition of bread samples.

Sample	Fat content (%)	Protein content (%)	Carbohydrate content (%)	Energy (kcal)
Bread without additives	1.12	5.3	52.34	240.64
Bread with sunflower seeds	2.22	8.2	49.82	252.06
Bread with turmeric	0.66	5.4	54.22	244.42
Bread with olives	1.72	7.1	48.23	236.80

From figure 1. it can be concluded that the dough of bread without additives has a lighter color. The volume achieved is of satisfactory quality. The bark looks uniform and crack-free with a characteristic brown color. The bread crumb is soft with pores that are evenly spaced. For the bread with addition of sunflower seeds we can conclude that the color is much darker due to the presence of sunflower seeds. Sunflower seeds give this sample its characteristic aroma and taste. The volume is satisfactory and the bread crumb looks porous. Most pores contain fragments of sunflower seeds.

From figure 2. it can be concluded that the bread with addition of turmeric have a characteristic yellow - orange hue that comes from turmeric. The aroma and taste are characteristic of turmeric but not too pronounced. The volume is of adequate quality and the medium is porous. The color is uniform throughout the cross-section. The bread with addition of olives has a characteristic green hue due to the presence of olives. The crust of the bread has the same color as the sample without additives, but the bread crumb has a pale green hue. The volume achieved is appropriate and the bread crumb contains fragments of olives that give this bread its characteristic aroma and taste.

From table 1. it can be concluded that the dough weight of bread with additive of sunflower seeds, olives or turmeric is higher compared to the dough of bread without additives. From the above it can be concluded that doughs of bread with additives have stronger ability to bind water. Also the total losses by roasting and drying are less value for bread with the addition of sunflower seeds, olives or turmeric.

From table 2. it can be concluded that all breads received very high marks for all five sensor categories that were evaluated.

From table 3. it can be concluded that the bread with additives contain more minerals (ash) and protein, except for bread with turmeric containing almost the same protein content as bread without additives. Bread with sunflower seeds and olives contain more fat than bread without additives, while turmeric bread contains less fat than bread without additives. The acidic degree of bread with additives is very similar to that of bread without additives, except for bread with the addition of turmeric, whose acidic level is lower.

From table 4. it can be concluded that bread with addition of sunflower seeds, olives or turmeric have characteristic energy values and carbohydrate content for bread samples.

CONCLUSION

Based on the analyzes performed and the experimental part, the following can be concluded:

Breads with addition of sunflower seeds, olives or turmeric have proven to be a nutritionally very affordable alternative to white bread and are more economically viable for production due to lower production losses. The addition of sunflower seeds, turmeric or olives to bread improves its organoleptic properties. The additives give the bread a fuller flavor, aroma and taste. Trial baking has proven to be a

very good criterion for evaluating the baking properties of flour, and for reconciling the recipe and production parameters. By using these breads in our diet we increase the possibility to provide our body with vitamins, antioxidants and ballast substances, which are very important substances for maintaining health.

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EFFECT OF COPPER ON THERMAL STABILITY OF NANOCRYSTALLINE CERIA

ORIGINAL SCIENTIFIC PAPER

Katarina Mužina,[1]✉ Aleksandar Bajrović,[1] Martina Župančić,[2] Stanislav Kurajica[1]

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[1] University of Zagreb, Faculty of Chemical Engineering and Technology, Marulićev trg 19, 10000 Zagreb, Croatia
[2] Leibniz-Institut für Kristallzüchtung, Max-Born-Straße 2, 12489 Berlin, Germany

✉ kmuzina@fkit.hr

ABSTRACT:
Cerium (IV) oxide (CeO_2) is extensively used as a catalyst because it has numerous advantages over conventional catalysts, like low cost, better poisoning resistance, high catalytic activity due to the facile Ce^{4+} / Ce^{3+} redox reaction and high oxygen storage capacity. CeO_2 nanoparticles have higher specific surface area and better redox properties, and thus an increased catalytic activity in comparison to bulk materials. However, at elevated temperatures ceria nanoparticles are very prone to coarsening. In order to increase the thermal stability of CeO_2 nanoparticles, but also improve its catalytic properties, metal ions are incorporated into the CeO_2 crystal structure. The aim of this study was to compare coarsening kinetics of nanocrystalline CeO_2 and nanocrystalline CeO_2 doped with 10 mol. % of copper in order to determine the effect of doping on thermal stability. Samples were prepared by hydrothermal synthesis and thermally treated at different temperatures and processing times. The samples were analyzed by X-ray diffraction analysis and the crystallite sizes were calculated using the Scherrer's equation. Based on the obtained crystallite sizes, kinetic parameters were determined and it was found that copper addition has a positive effect on the thermal stability of CeO_2.

KEYWORDS: cerium (IV) oxide; coarsening kinetics; hydrothermal synthesis; doping

INTRODUCTION

Cerium (IV) oxide (CeO_2), also called ceria, is one of the most important and generally applied lanthanide oxides. It possesses a fluorite crystal structure with space group Fm3m, which is stable from room temperature till the melting point (~2 400 °C). Cerium has two stable oxidation states, Ce^{3+} and Ce^{4+}, and the special feature of cerium (IV) oxide is that the fluorite lattice can withstand significant reduction of cerium without alteration or collapse of the structure, especially at elevated temperatures [1]. During the partial reduction of Ce^{4+} to Ce^{3+}, oxygen molecules are released in order to maintain the electroneutrality of the crystal lattice, which causes the formation of oxygen vacancies. These vacancies can freely move within the crystal lattice and can also readsorb oxygen molecules from the air. The good catalytic properties of cerium (IV) oxide are attributed to this easiness of transition between oxidation states, which allows the formation of oxygen vacancies and the good mobility and high oxygen storage capacity within the crystal lattice [2]. Ceria has many advantages over traditional catalysts, like low price and availability, lower degree of thermal sensitivity, higher resistance to poisoning, while also allowing high conversions to be achieved for certain types of chemical processes. Cerium (IV) oxide is therefore widely used as a heterogeneous catalyst in three-way catalytic converters in car exhaust systems, for the conversion of water gas to produce hydrogen for various industrial processes and for the removal of atmospheric pollutants such as volatile organic compounds [3-5]. Modern catalyst design is aimed at preparation of nanoscale catalysts because they possess superior properties in comparison to bulk materials due to greater surface to bulk atoms ratio. The atoms on the surface have unsatisfied chemical bonds and therefore possess greater energy than bulk atoms. Increased energy influences the band gap, morphology, reactivity, and catalytic potential of a heterogeneous nanocatalyst [6]. The problem with nanoparticles occurs at elevated temperatures since they are prone to aggregation and coarsening which cause the increase of crystallite sizes and the reduction of specific surface area. To solve this problem and to further improve the catalytic properties of ceria, transition metals having smaller radius and lower valence than cerium can be introduced into the ceria crystal lattice [7]. In this work, pure and Cu-doped ceria samples were prepared using a simple, affordable and eco-friendly hydrothermal method. The samples were characterized and then thermally treated at various temperatures and processing times. Coarsening kinetics analysis was performed in order to evaluate the

effect of the dopant on the thermal stability of cerium (IV) oxide.

EXPERIMENTAL

Samples of pure and copper doped ceria were prepared by hydrothermal method using the following procedure: The appropriate amounts of precursors, namely 0.8 mmol of $Ce(SO_4)_2{\times}4H_2O$ for pure sample as well as 0.72 mmol of $Ce(SO_4)_2{\times}4H_2O$ and 0.08 mmol of $CuSO_4x5H_2O$ for Cu doped sample, were put in a Teflon-lined stainless-steel autoclave with a capacity of 70 cm^3, which was then filled with 56 cm^3 of 8 mol dm^{-3} NaOH solution, sealed and placed in a temperature controlled oven at 120 °C for 16 hours. After the end of the reaction time, the autoclave was cooled down, the NaOH solution was decanted and the precipitate washed three times with demineralized water with help of sonification and centrifugation. The precipitate was then dried at 60 °C for 24 hours.

The pure and the doped sample were thermally treated at different temperatures and processing times (Table 1), after which X-ray powder diffraction (XRD) analysis was performed. The instrument used was Shimadzu XRD 6000 diffractometer with CuKα radiation operating at 40 kV and 30 mA in a step scan mode ranging from 5 to 105 °2θ, with steps of 0.02 °2θ and counting time of 0.6 s.

Table 1. Temperatures and processing times for the pure and Cu doped sample.

300 °C	350 °C	400 °C	450 °C	500 °C
15 min	15 min	15 min	15 min	-
30 min	30 min	30 min	30 min	-
1h	1h	1h	1h	-
2h	2h	2h	2h	2h
4h	4h	4h	4h	-

The as-prepared pure and Cu doped sample were additionally analyzed using high resolution transmission electron microscopy (HRTEM) on transmission microscope JEOL ARM 200 CF. The samples were applied directly to a nickel grid and analyzed at accelerating voltage of 80 kV. ImageJ program was used for the analysis of the obtained micrographs [8]. In order to analyze the chemical composition of the samples, energy dispersive X-ray spectroscopy (EDS) analysis was performed on Tescan Vega Easy Probe 3 scanning electron microscope operating at 10 kV. Samples were fixed on a sample holder using double-sided carbon conductive tape.

RESULTS AND DISCUSSION

The obtained pure and Cu doped sample were first analyzed by XRD in order to gain insight into their phase composition and purity. The X-ray patterns are shown in figure 1. As can be seen, both the pure and the doped sample only contain peaks characteristic for cerium (IV) oxide (ICDD PDF No. 34-0394), which indicates that copper is incorporated in the ceria crystal lattice. Diffraction patterns can be used for the determination of crystallite sizes using the Scherrer's method (Eq. 1).

$$d = \frac{K\lambda}{B\cos\theta} \tag{1}$$

where d is the crystallite size, K is the shape factor which is 0.94 for spherical particles of cubic symmetry, λ is the CuKα radiation wavelength, θ is the Bragg angle and B is the peak full width at half maximum corrected for instrumental broadening.

Broad peaks usually point out to small crystallite sizes, which were proven by calculation, as seen in Table 2. The doped sample has a slightly lower crystallite size, but taking into account the limitations of the method, the difference between the pure and the doped sample is not significant.

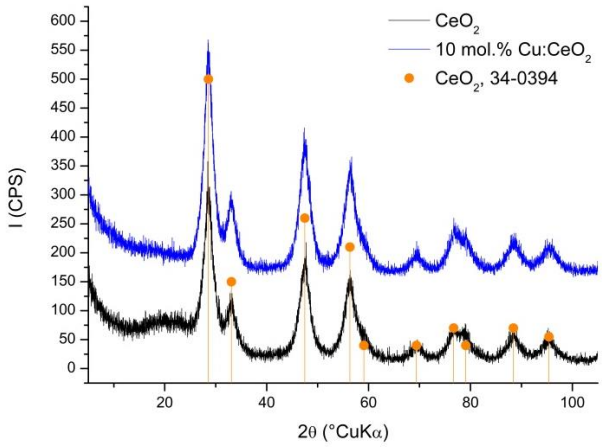

Figure 1. Diffraction patterns of the pure and the Cu doped sample.

HRTEM analysis (Figure 2) shows aggregates of fine nanocrystallites with sizes corresponding to the sizes obtained by the Scherrer's method (Table 3). The obtained nanoparticles have spherical morphology and there is no evident difference in morphology between the pure and the doped sample.The fringes distance of 2.7 Å for both samples matches the (200) crystal plane of cerium (IV) oxide (ICDD PDF No.

34-0394). Since neither XRD nor HRTEM analysis show the presence of copper in the doped sample, it can be indicated that a solid solution of copper in the ceria crystal lattice is formed.

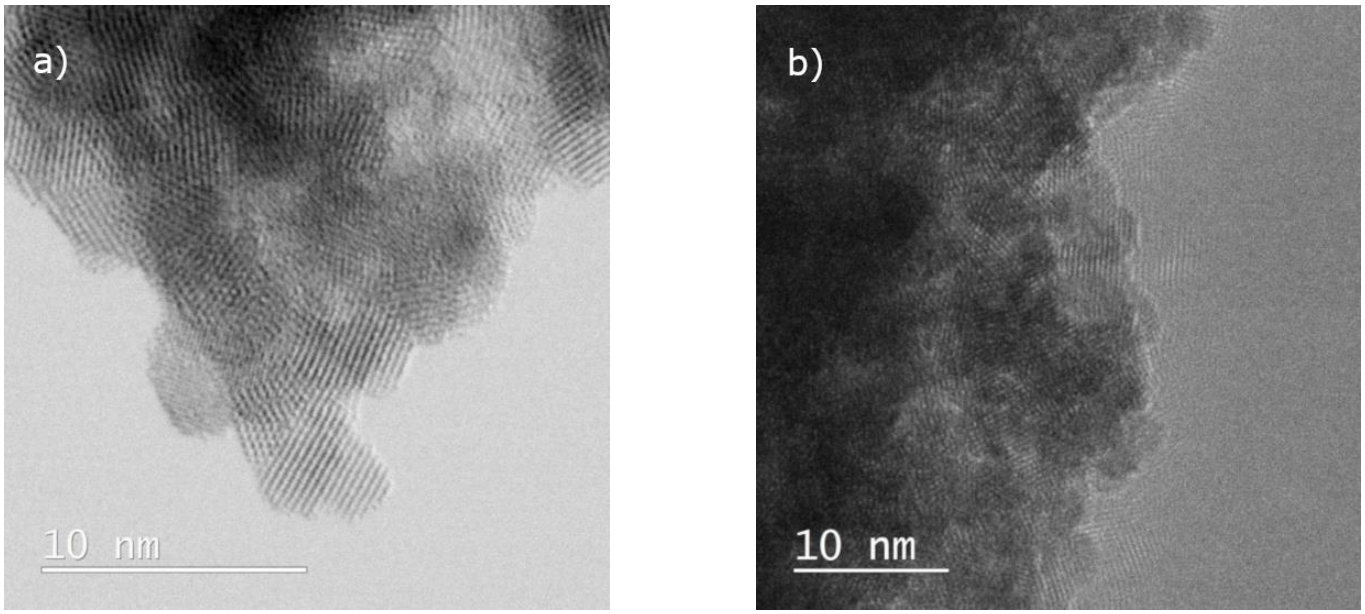

Figure 2. HRTEM micrograph of the pure (a) and Cu doped (b) sample.

Table 2. Crystallite sizes obtained from diffraction patterns by Scherrer's method and particle sizes and fringes distance obtained from micrographs using ImageJ program.

	CeO_2	10 mol. % Cu:CeO_2
Crystallite size (XRD)	4.2 nm	4.0 nm
Particle size (HRTEM)	~ 4 nm	~ 4 nm
Fringes distance	2.703 Å	2.730 Å

To confirm the presence of copper in the doped sample, EDS analysis was performed. Results are shown in figure 3. As can be seen, both the pure and the doped sample show the presence of cerium and oxygen, as well as carbon because of the carbon tape used for the fixation of the sample onto the sample holder. Peaks characteristic for copper are found in the doped sample proving the doping successful.

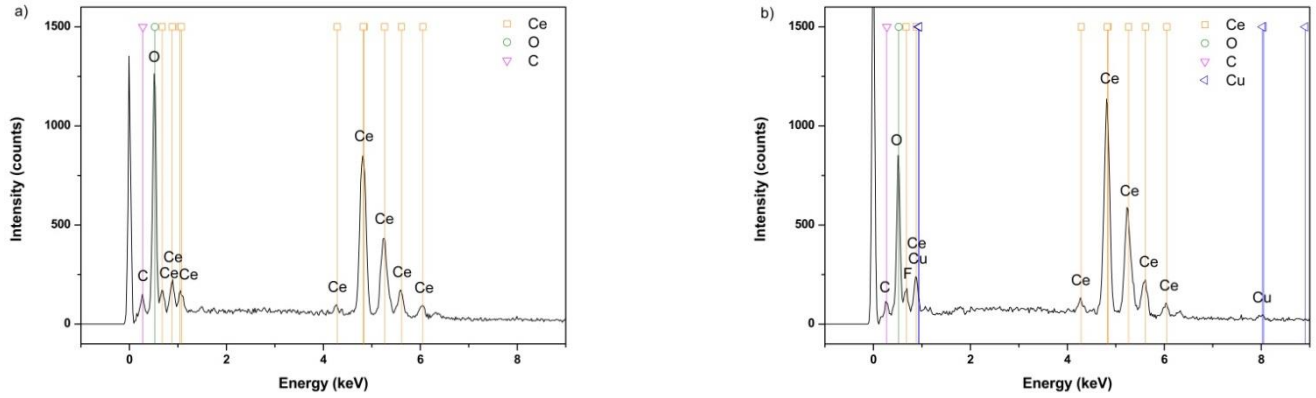

Figure 3. EDS spectra of the pure (a) and Cu doped (b) sample.

Both the pure and the doped sample were thermally treated at different temperatures and processing times, as shown in Table 2, analyzed by XRD, and the crystallite size values were obtained using the

Scherrer's equation. The crystallite sizes were then used in the coarsening kinetics analysis based on the equations from the work of Lopez et al. [9], which are as follows:

$$d^n - d_0^n = Kt \tag{2}$$

Where d is the average particle size, d_0 is the initial particle size, n is the particle growth exponent, K is a constant dependent on temperature ($K = Ko\ \exp(-Q/RT)$) and t is the time of thermal treatment.

This equation can be simplified to:

$$d = K't^m \tag{3}$$

Where m is equal to $1/n$, and it can be derived from the logarithmic plot of d versus t as the value of the slope:

$$lnd = lnK' + mlnt \tag{4}$$

While the intercept lnK' is further used in the Arrhenius plot to obtain the activation energy, Ea:

$$lnK' = lnA - \frac{Ea}{RT} \tag{5}$$

Where A is the pre-exponential factor, Ea activation energy, R universal gas constant and T is the temperature expressed in Kelvins [9].

Our chosen temperatures of thermal treatment were lower than the ones in the work of Lopez et al., because the targeted application of our ceria nanoparticles is in catalytic oxidation of volatile organic compounds and exhaust gases from automobiles which need to be accomplished at lower temperatures (less than 500 °C). Figure 4 shows the dependence of crystallite size on temperature for 2 hours duration of thermal treatment, including the values of initial crystallite size for both the pure and the doped sample. It can be seen that for both samples the crystal growth depends exponentially on the temperature. However, crystallites in the Cu doped sample grow slower at lower temperatures in comparison to the pure sample, although the difference is not drastic. Crystal growth accelerates significantly at 500 °C.

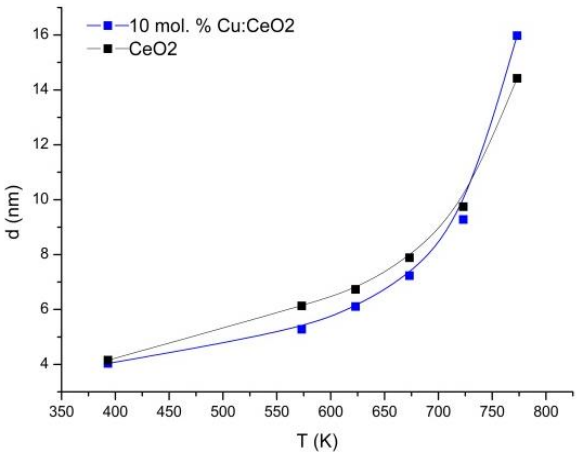

Figure 4. Initial size and crystallite sizes after thermal treatment for 2 hours for the pure and the doped sample.

The particle growth exponents (n) for the pure and the doped sample at various temperatures were determined from the logarithmic plots of d versus t (figure 5) and are listed in table 3.

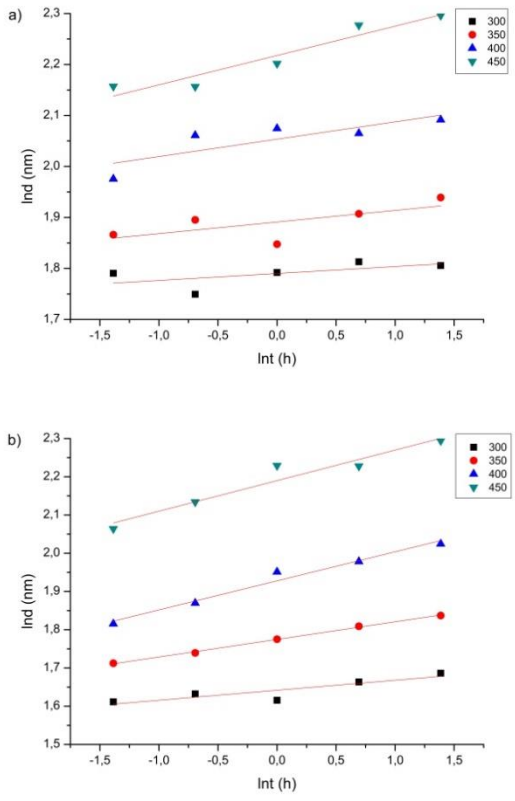

Figure 5. Plots of *lnd* vs *lnt* for the pure (a) and the doped (b) sample.

Equation 2 implies that high values of n point out to sluggish crystallite growth due to restricted boundary mobility [9], which is confirmed in this experiment since the n value is indeed higher at lower tem-

peratures. The values for the doped sample are lower which would indicate faster grain growth, but the calculated crystallite sizes are lower at lower temperatures than that of the pure sample, which would mean higher thermal stability.

Table 3. Particle growth exponent values for the pure and doped sample at various temperatures.

T (°C)	n (CeO_2)	n (Cu:CeO_2)
300	73	38
350	43	21
400	29	13
450	17	12

Activation energy values for the pure and the doped samples were obtained from the Arrhenius plots and are shown in figure 6.

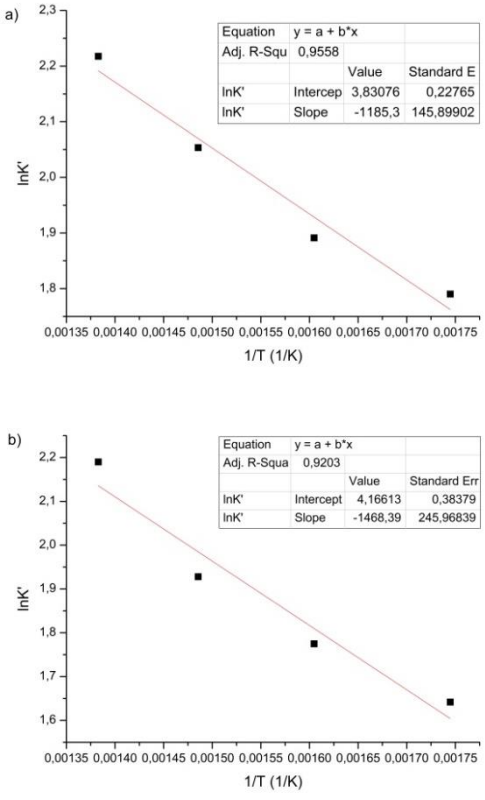

Figure 6. Arrhenius plot for the pure (a) and the doped (b) sample.

The obtained values are 9854 J/mol for the pure sample and 12208 J/mol for the Cu doped sample. The obtained values are much lower than in the literature [9, 10] because our research was done only for lower coarsening temperatures for the beforehand mentioned reasons. At lower temperatures no significant changes in ceria occur, except coarsening, which is not an extremely energy consuming process. The activation energy is higher for the doped sample, which means that it is necessary to bring more energy to the system to induce nanoparticles coarsening and that the doped sample is indeed more thermally stable than the pure one.

CONCLUSION

Nanoparticles of pure and Cu doped ceria were prepared by hydrothermal method. Samples were thermally treated at different temperatures and different processing times, and a coarsening kinetics analysis was conducted. Crystallites in both samples grow with increasing temperature and time. The addition of copper increases the thermal stability of ceria indicated by the higher activation energy of the doped sample.

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DEEP EUTECTIC SOLVENTS FOR PURIFICATION OF WASTE COOKING OIL AND CRUDE BIODIESEL

ORIGINAL SCIENTIFIC PAPER

Ana Petračić,✉ Matea Gavran, Ana Škunca, Lucija Štajduhar, Aleksandra Sander

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Department of Mechanical and Thermal Process Engineering, Faculty of Chemical Engineering and Technology, University of Zagreb, Marulićev trg 19, 10000 Zagreb, Croatia

✉ apetracic@fkit.hr

ABSTRACT:
The goal of this work was to explore the applicability of deep eutectic solvents in biodiesel production process. Biodiesel was produced from waste cooking oil via base-catalysed transesterification. The efficacy of two base catalysts KOH and NaOH on the conversion of triglycerides into biodiesel was tested. Eutectic mixtures DES 1 (K_2CO_3 : $C_2H_6O_2$ = 1 : 10) and DES 2 ($C_5H_{14}ClNO$: $C_2H_6O_2$ = 1 : 2) were prepared. DES 1 was used for feedstock deacidification and DES 2 for biodiesel purification via extraction. Effects of DES 2 to biodiesel ratio and the extraction duration on free glycerol removal were tested. Samples were analysed using FTIR and ^{1}H NMR spectroscopy. Synthesis of biodiesel was carried out for 3 h at 60 ° C with mass ratio KOH : methanol : oil = 1 : 40 : 100. DES 2 was effective for purification of biodiesel. Mass ratios 0.5:1 and 1:1 proved best and 45 minutes of extraction was enough to reduce free glycerol and increase the content of fatty acid methyl esters.

KEYWORDS: biodiesel, glycerol, extraction, deep eutectic solvent, waste edible oil

INTRODUCTION

The last decade has seen great interest in development of alternative fuels that could potentially replace existing fossil fuels [1]. Studies have shown that biodiesel has a great potential as an alternative fuel because its combustion reduces emissions compared to fossil fuels and it relieves dependence of the transport sector on fossil fuel prices and availability. It can also encourage economic development due to its available, renewable and environmentally friendly raw materials [2], [3].

Biodiesel is a mixture of fatty acid alkyl esters. It is produced from triglycerides via transesterification. Potential feedstocks for biodiesel production include not only high quality vegetable oils but also used animal fat, waste cooking oil, algae and even waste coffee ground oil [1], [4]. The reaction can be catalysed homogeneously and heterogeneously. Homogenous catalysis can be acidic or basic and heterogeneous catalysts can be solid catalysts or enzymes [5]. Most commonly, biodiesel is produced from vegetable oils and methanol in the presence of a basic catalyst such as sodium or potassium hydroxide. The reaction is fast and results in high content of fatty acid methyl esters (FAME) but it is very sensitive to impurities in the feedstock, most notably free fatty acids and water [6]. Potassium carbonate based deep eutectic solvent was previously used for deacidification of different feedstocks and the same method was applied to waste cooking oil in this work [7].

A by-product of transesterification reaction is glycerol. After the reaction, FAME and glycerol are separated under the force of gravity since the density of glycerol is higher than of FAME, but a small amount of glycerol is soluble in biodiesel and therefore has to be extracted from it [8]. Additionally, crude biodiesel may also contain unreacted methanol and mono-, di- and triglycerides, leftover catalyst and potentially formed soap [9]. All those impurities can negatively impact biodiesel storage properties and engine performance, making crude biodiesel purification a necessary step in the biodiesel production process.

Deep eutectic solvents are liquid mixtures consisting of two or three components, usually environmentally acceptable, cheap and non-toxic. Components bind together with hydrogen bonds and the resulting mixture has a lower melting point than the starting components [10].

A potential method for biodiesel purification is liquid-liquid extraction with deep eutectic solvents. Studies have shown that extraction with deep eutectic solvents reduces the content of all present impurities in biodiesel [11]-[13]. In this work, choline chloride based deep eutectic solvent was used to purify crude biodiesel.

MATERIALS AND METHODS

The goal of this work was to examine the applicability of deep eutectic solvents for purification of waste cooking oil and crude biodiesel produced via base catalysed transesterification. Experimental part includes characterisation and purification of the feedstock, preparation of deep eutectic solvents and lastly synthesis, characterisation and purification of crude biodiesel with deep eutectic solvents.

Table 1. Chemicals used in this work

Chemical	Manufacturer
Dietyl ether, $(C_2H_5)_2O$	Honeywell
Ethanol, C_2H_5OH	KEFO
Ethylene glycol, $C_2H_6O_2$	T.T.T.
Choline chloride, $C_5H_{14}ClNO$	Acros Organics
Potassium carbonate, K_2CO_3	Lach-Ner
Potassium hydroxide, KOH	Kemika
Methanol, CH_3OH	J.T. Baker
Sodium hydroxide, NaOH	T.T.T.

PREPARATION OF DEEP EUTECTIC SOLVENTS

Choline chloride, potassium carbonate and ethylene glycol were dried in a vacuum drier for 8 hours at 60 °C and pressure less than 10 mbar. After drying, components were weighed in a round bottom flask and mixed on a rotary evaporator until a clear homogenous liquid was formed. DES 1 was prepared for purification of the feedstock and it consists of potassium carbonate and ethylene glycol in molar ratio 1:10. DES 2 was prepared for purification of crude biodiesel and it consists of choline chloride and ethylene glycol in molar ratio 1:2.5.

PURIFICATION OF WASTE COOKING OIL

Waste cooking oil, obtained from a local company Agroproteinka, is a thick, viscous liquid containing different impurities such as metals and free fatty acids. In order to make it suitable for base catalysed transesterification, the feedstock was purified via extraction with DES 1. Waste cooking oil was first heated to 60 °C for 30 minutes and filtered to remove solid particles and a sample was taken to determine its acid number. After filtration it was mixed with DES 1 (mass ratio of DES 1 to fat was 1:10) for 30 minutes and then the mixture was separated and a sample of feedstock was taken to determine its acid number. Total acid number was determined titrimetrically according to the method ISO 660:2009.

BIODIESEL SYNTHESIS

Biodiesel was synthesized via base catalysed transesterification with potassium and sodium hydroxide dissolved in methanol. Hydroxide was dissolved in methanol and then added to preheated waste cooking oil and mixed for 3 hours at 60 °C. After synthesis, biodiesel was separated from glycerol using a centrifuge. Samples of biodiesel were taken for further analysis. Mass ratio of catalyst:MetOH:feedstock was 1:40:100. Efficacy of both catalysts was determined and potassium hydroxide was chosen for further experiments.

In order to determine the optimal amount of catalyst and methanol needed, experiments were carried out with different KOH:MetOH:feedstock mass ratios. First the optimal amount of catalyst was determined and then, with the chosen ratio of KOH to feedstock, amount of methanol was varied.

Table 2. Biodiesel synthesis was carried out for 3 hours at 60 °C and following mass ratios:

mass ratio – catalyst:methanol:feedstock		
0.1:40:100	0.5:40:100	1:40:100
1:30:100	1:40:100	1:50:100

PURIFICATION OF CRUDE BIODIESEL

Synthesized biodiesel contains a small amount of free glycerol and unreacted mono-, di- and triglycerides. Purification was conducted via extraction with DES 2. It was conducted in a glass reactor on a magnetic stirrer and afterwards the mixture was separated using a centrifuge. Four different DES 2 to biodiesel mass ratios were tested and after determining the optimal ratio, influence of extraction duration was investigated.

Table 3. Purification of crude biodiesel was conducted at room temperature under the following conditions:

	DES 2:biodiesel mass ratio			
Duration: 60 min	0.1:1	0.25:1	0.5:1	1:1
	Extraction duration [min]			
DES 2:biodiesel= 1:1	45	60	75	90

All biodiesel samples were analysed using FTIR and ^{1}HNMR spectroscopy.

RESULTS AND DISCUSSION

FEEDSTOCK PURIFICATION

Total acid value of the feedstock was 2.362 mg KOH/g feedstock. It was purified in order to avoid saponification during biodiesel synthesis. After 30

minutes of extraction with DES 1, total acid value of the feedstock was reduced to 0.574 mg KOH/g feedstock.

BIODIESEL SYNTHESIS

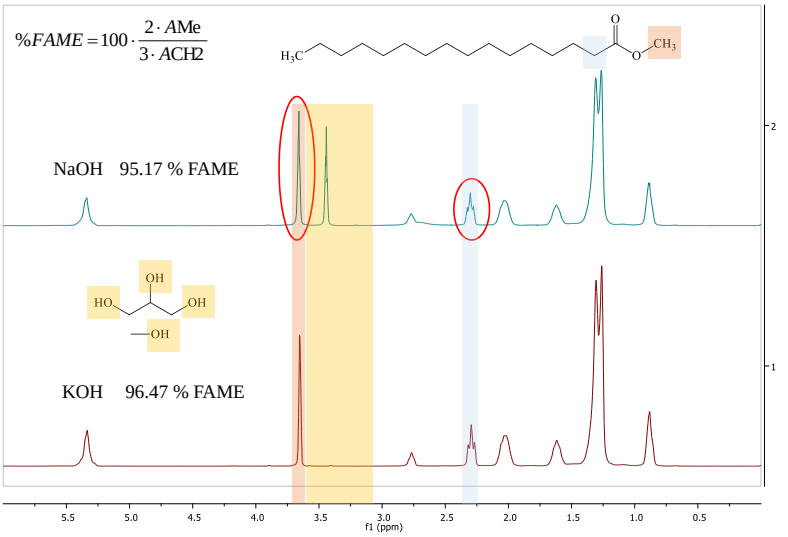

Figure 1. ^{1}HNMR spectra of biodiesel samples synthesized with NaOH and KOH catalysts

In order to choose a catalyst for further investigation, two experiments were conducted – biodiesel synthesis was done using potassium and sodium hydroxide as catalysts. Mass ratio of catalyst:methanol:feedstock was 1:40:100. ^{1}HNMR spectra of resulting biodiesel samples are shown in Figure 1. Several peaks can be observed here: the peak of ester groups at 3.64 ppm, which is characteristic for biodiesel and the peak of –OH groups at 3.45 ppm – corresponding to glycerol and unreacted methanol. The peak at 2.35 ppm corresponds to CH_2 group and the area under that peak was used, together with the area of the ester peak, to calculate the share of FAME in the sample. Biodiesel standards require a share of FAME higher than 96.5 %. Biodiesel produced with NaOH had 95.17 % of FAME and the one produced using KOH had 96.47%. Potassium hydroxide was chosen as a catalyst for further experiments, not only because it yielded a higher content of FAME but also because it dissolved in methanol much faster during preparation, saving valuable time.

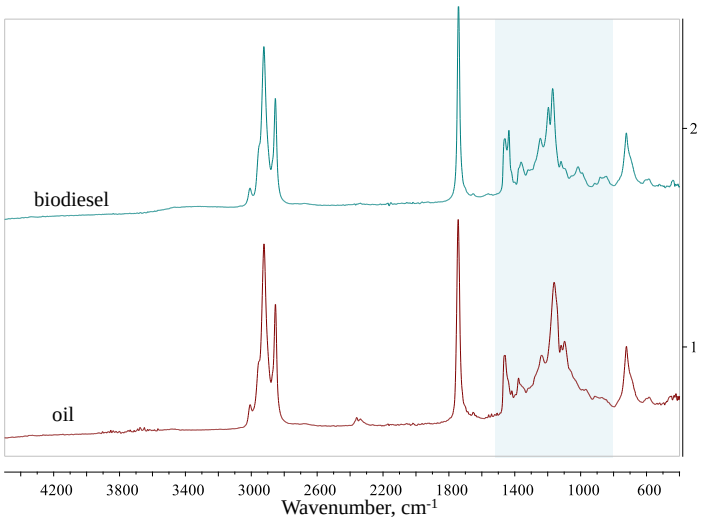

Figure 2. FTIR spectra of the feedstock and biodiesel (KOH:MetOH:feedstock mass ratio = 1:40:100)

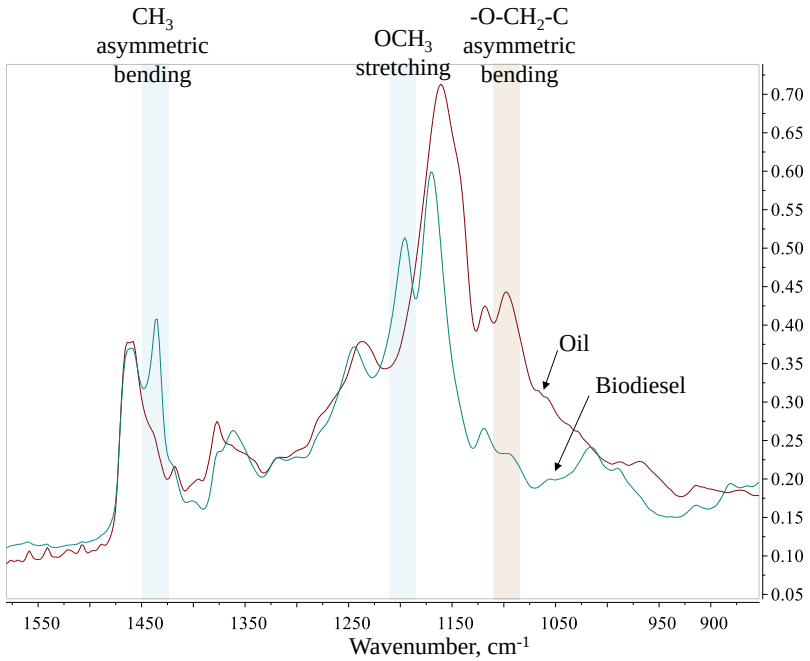

Figure 3. Enlarged part of the overlapped spectra of oil and biodiesel

FTIR spectra of the feedstock and biodiesel (KOH:MetOH:feedstock mass ratio = 1:40:100) are shown in Figure 2. An enlarged part of the overlapped spectra of oil and biodiesel is shown in Figure 3. Most notable differences can be observed at 1436 cm^{-1}, 1196 cm^{-1} and 1099 cm^{-1}. Peaks characteristic for oil disappear (asymmetric bending of –O-CH2-C group at 1099 cm^{-1}) and peaks characteristic for biodiesel appear (asymmetric bending of CH_3 group at 1436 cm^{-1} and stretching of $–OCH_3$ group at 1196 cm^{-1}).

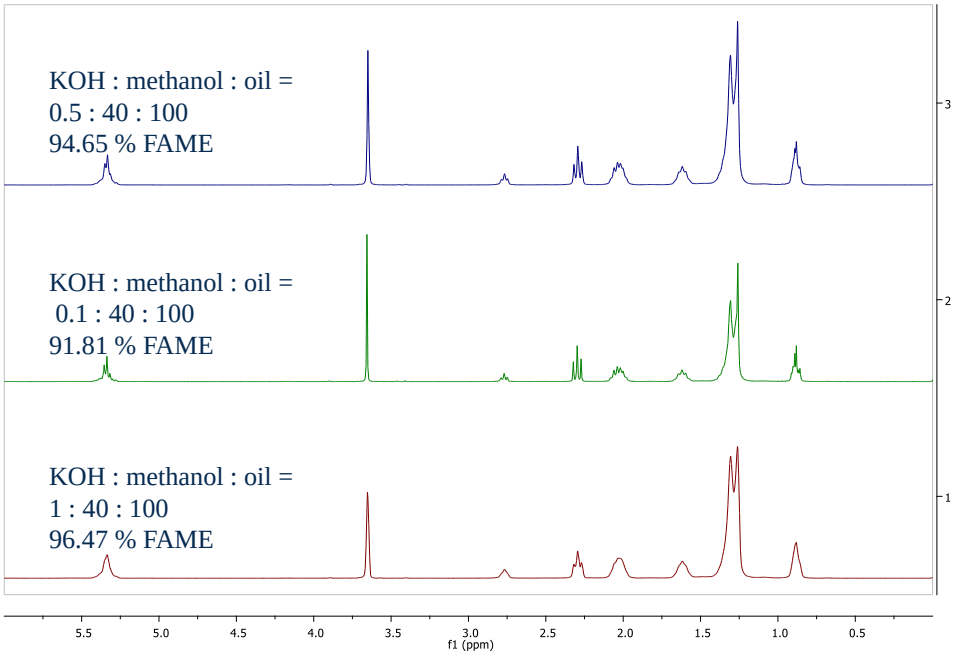

Figure 4. [1]HNMR spectra of biodiesel samples synthesized at 60 °C for 3 hours – influence of catalyst ratio on the amount of FAME in biodiesel

Next part of the investigation included finding the optimal amount of catalyst and methanol needed for transesterification. First set of experiments was conducted with three different amounts of KOH and fixed ratio of methanol to feedstock (as explained in Table 2).

^{1}HNMR spectra of the resulting biodiesel samples are shown in Figure 4. It can be observed that the share of FAME in biodiesel depends on the amount of catalyst used and the highest FAME content was obtained with the highest catalyst ratio. The best result was 96.47 % of FAME, which is very close to the 96.5 % limit required for biodiesel. Since crude biodiesel contains impurities such as free glycerol, mono-, di- and triglycerides and possibly unreacted methanol, it was expected that the share of FAME in biodiesel will increase after purification.

After determining the optimal amount of catalyst, mass ratio of catalyst to feedstock was fixed to 1:100 and the amount of methanol was varied (mass ratios for the experiments KOH:MetOH:feedstock were 1:x:100, x=30, 40, 50). The resulting biodiesel samples had a content of 96.12, 96.47 and 97.25 % FAME, respectively.

PURIFICATION OF CRUDE BIODIESEL

The last part of this work was focused on biodiesel purification in order to remove impurities and increase its FAME content. A larger amount of biodiesel was synthesized for next experiments (mass ratio KOH:MetOH:feedstock was 1:40:100). Influence of the amount of solvent used and extraction duration was investigated. First set of experiments was conducted for 60 minutes with varying mass ratios of DES 2 to biodiesel (0.1, 0.5, 0.75 and 1:1). Resulting FAME contents calculated from ^{1}HNMR spectra of the samples are given in TABLE. All obtained biodiesel samples had well above 96.5 % of FAME. Second set of experiments was conducted with mass ratio of DES to biodiesel 1:1 for 45, 60, 75 and 90 minutes. Results are given in Table 4. It was observed that 45 minutes was the optimal time of the extraction. Longer extraction duration resulted in the decrease of FAME content.

Table 4. Purification of biodiesel via extraction. Initial FAME content was 98.51 %

DES 2:biodiesel mass ratio	**0.1:1**	**0.25:1**	**0.5:1**	**1:1**
% FAME	97.98	98.04	99.02	98.55
Extraction duration [min]	**45**	**60**	**75**	**90**
% FAME	98.99	98.55	97.57	96.71

CONCLUSION

Applicability of two different deep eutectic solvents for purification of waste cooking oil and crude biodiesel was investigated. Potassium carbonate based DES proved successful for deacidification of the feedstock and choline chloride based DES was effective for purification of biodiesel.

For base-catalysed transesterification, KOH proved to be a better choice than NaOH, yielding a higher FAME content while also dissolving much faster in methanol during preparation.

The results show that FAME content increased with increasing catalyst and methanol to feedstock ratio. Varying catalyst ratio had a greater effect on the total FAME content than that of methanol.

Purification of biodiesel with DES 2 was conducted with different DES to biodiesel mass ratios. The ratios 0.5:1 and 1:1 were chosen as the best and further experiments were carried out with the ratio 1:1. Extraction duration of 45 minutes was found to be enough to extract free glycerol from crude biodiesel. Longer extraction times resulted in a small decrease of FAME content indicating possible occurrence of a partial extraction of FAME.

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CORROSION COUPON TESTING OF COMMERCIAL INHIBITOR IN SIMULATED COOLING WATER

ORIGINAL SCIENTIFIC PAPER

Borislav N. Malinovic[1], Dusko Zoric[2], Tijana Djuricic[1]✉

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[1] Faculty of Technology, University of Banja Luka, Stepe Stepanovica, 78000 Banja Luka, Bosnia and Herzegovina
[2] EFT Mine and Thermal Power Plant Stanari Ltd., Stanari, Bosnia and Herzegovina

✉ tijana.malinovic@tf.unibl.org

ABSTRACT:
In maintaining cooling systems, one of the biggest challenges is to control the corrosion process. Various corrosion inhibitors are often used for this purpose. Which type of corrosion inhibitor will be chosen depends on the material from which the plant has made. The main causes of corrosion in these systems are: pH, dissolved gas, ammonia, temperature and microbiology. In this paper it was studied the efficiency of two multicomponent commercial corrosion inhibitors based on phosphates and one of which containing zinc chloride. For the purposes of research, the pilot plant of open recirculation cooling system is constructed and made of stainless steel (EN 1.4301) and copper (EN 13601). Experiments were performed in a simulated cooling water witch recirculated for 3.5h. For the purpose of accelerating corrosion processes, it was added a corrosion activator (5% NaCl). It was monitored the corrosion rate of the mentioned materials in the cooling water with the corrosion activator, with and without inhibitor. Corrosion rate is determined by using corrosion coupons according standard ASTM D2688 and by analyzing physical-chemical parameters of cooling water. The results showed it was achieved higher protection efficiency for copper and stainless steel by using an inhibitor containing zinc chloride in addition to phosphate.

KEYWORDS: pilot plant, corrosion rate, stainless steel, copper.

INTRODUCTION

The corrosion in a cooling water system is defined as the electrochemical degradation of a metal that is in contact with cooling water. This process degrades the metal, reduces its strength, thickness, and in some extreme cases, creates pits and then holes in the material. At some point in the corrosion process, the metal can no longer do its job as a system component [1].

Circulating cooling water used to supply the cooling systems usually contains calcium and magnesium ions at concentrations of 10 to 600 ppm, in addition to these, there are bicarbonate ions. Because of continuous recycling, the water quality of cooling water becomes more concentrated leading to corrosion, fouling, scale forming and microbial breeding, which results in lower heat transfer efficiency of heat exchangers, corrosion and perforation of metal pipelines, and safety issues. In order to avoid this, cooling water is usually chemically treated with inhibitors and biocides to avoid corrosion, formation of scaling and fouling. It is common to use scaling inhibitor like phosphates, polyphosphates, and organophosphonates, and corrosion inhibitors such as zinc sulphate and azoles. Generally, chlorine and or sodium hypochlorite are the most widely used as antifouling biocide in cooling systems [2, 3, 4].

Different types of commercial multicomponent corrosion inhibitors are most commonly used to protect industrial cooling systems. Which inhibitor will be used to protect the system from corrosion depends on the plants construction materials, the quality of used supply water, the cost of the inhibitor and its environmental toxicity.

It has been experimentally confirmed that many organic and inorganic compounds containing heteroatoms of nitrogen, phosphorus, sulfur and oxygen with double or triple bonds in their structures are effective in protecting metals from corrosion. Phosphonates were developed and extensively used as scale and corrosion inhibitors in a variety of fields including circulating cooling systems in power plants, industrial equipment cleaning, industrial water treatment, corrosion inhibitors in concrete, coating, rubber blends, acids cleaners and anti-freeze coolants. Most of the inhibitors used in cooling water nowadays are based on phosphonates, either alone or in combination with one or more other corrosion inhibitors. Phosphonates give better inhibition efficiencies when used in combination with zinc salts [5-9].

A numerous of research has focused on the problem of corrosion in cooling systems, as well as the application of phosphonate-based inhibitors, alone or in combination with metal cations. Two commercial corrosion inhibitors were used in this paper, and both of them have 2-phosphonobutane 1,2,4-tricarboxylic acid (PBTC) in their composition.

Nessren i Wisam examined the efficiency of PBTCA as a carbon steel corrosion inhibitor in cooling water and found that the efficiency of a corrosion inhibitor increased with increasing its concentration [8]. A series of PBTCA modified hydroxyl terminated hyper-branched structure (HBPs) with hydroxyl and carboxyl were successfully prepared and used for scale and corrosion inhibition in the research of Wang et al. They concluded that increasing the concentration of the scale inhibitor will improve the effect of scale inhibition [10]. A similar research was conducted by Huang *et al.* New type of the scale and corrosion inhibitors with HBPs has been developed in this study to improve the scale and corrosion inhibition efficiency of existing scale inhibitors for industrial water. The experimental results show that the HBPs provides high scale inhibiting efficiency for $CaCO_3$ and $CaSO_4$ (96.7% and 93.9% at 20mg/L, respectively). HBPs inhibition effect on carbon steel corrosion in 3wt% NaCl medium was investigated. By the weight loss test it was obtained that corrosion inhibition efficiency depends on its concentration and reaches 91.3% [11].

The combination of zinc salts with carboxylic acid derivatives has proven to be effective in protecting the heat exchanger of industrial cooling systems [12]. By adding smaller amounts of zinc compounds to phosphate-based corrosion inhibitors, good corrosion protection is achieved, which is environmentally acceptable. In research of Jin *et al.* environmentally friendly inhibitor was designed (12.5% PBTCA, 21.8% ZnSO4, 18.7% PASP, 9.4% HPAA, 18.8% AA-ATMP-HPA polymer, and 18.8% HPMA) and it was examined its application in a pilot-scale cooling system with reclaimed wastewater. A protective film formed by the adsorption of inhibitor onto the surface of the carbon steel prevented corrosion and the inhibition efficiencies of carbonate scale and corrosion were 93.3 and 94.4%, respectively. [13].

EXPERIMENTAL

For the purposes of research, recirculation cooling pilot plant was constructed, designed as a real plant. During construction of the plant, attention was paid that a suitable heater (heat exchanger simulation) would be provided for the defined water flow, which would heat the water to the desired temperature, as well as the cooling tower height and fan strength, which would cool the water. The materials from which the construction of the plant is made are stainless steel EN 1.4301 and copper EN 13601. Schematic wiew of the pilot plant is given in Figure 1.

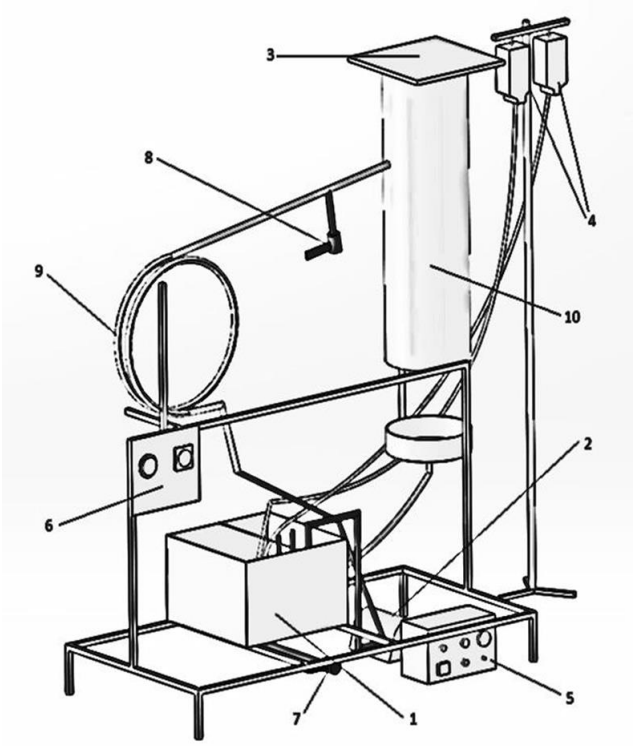

Figure 1. 3D model of open recirculation cooling system 1. Trunk with thermostat and heater, 2. Peristaltic pump, 3. Fan, 4. Dosing system for ammonia and sodium hypochlorite, 5. Control console for the pump, 6. Fan and heater control console, 7. Valve drain 8. Sampling valve, 9. Copper tube heater representing heat exchanger simulation and 10. Cooling tower

The parameter values of some parts of the plant are given in the Table 1.

Table 1. Parameters of a pilot plant

Parameter of Pilot plant	Value
Trunk	18 L
Peristaltic pump	1.1 L/min
Fan	410 m^3/h
Heater	0.25 kW
Height of the cooling tower	0.8 m
Height of cooling pad	0.15 m

The standard physical-chemical methods [14], as well as some specific methods [15], used to determine the physical-chemical quality of cooling

and raw water. In addition to the physical-chemical analysis of water, a corrosion coupon analysis was performed, (gravimetric mass loss of corrosion coupons) before and after immersion in cooling water, in accordance with standard method [16]. Raw water was used to supply this pilot plant, which is a mixture of tap water (90%) and distilled water (10%). Used corrosion coupons used are shown in Figure 2.

Figure 2. Corrosion coupons made of stailess steel and copper

Three commercially available inhibitors KURITA S-6300 and HEMOD MS 1E were used. The amount of corrosion inhibitor was evaluated based on the total phosphate content. Based on real industrial systems, the total phosphate content was from 3 to 4 mg/L. This scope was the manufacturer's recommendation for all three of the mentioned corrosion inhibitors, so in this paper no corrosion inhibitory efficiency at various concentrations of the corrosion inhibitor was examined. Following table (Table 2) shows amount of inhibitors that should be dosed to achieve the required values.

Table 2. The amount of dosed corrosion inhibitors depending on the total phosphate content

Corrosion inhibitor	Amount of dosed inhibitor per liter of cooling water (mL/L)	Total phosphate content (mg/L)
KURITA S-6300	0.20	3.27
BWT CS-1006	0.08	3.97
HEMOD MS 1E	0.15	3.17

Following tables (3, 4) shows the physical-chemical composition of the corrosion inhibitors given in their accompanying MSDS.

Table 3. Chemical composition of corrosion inhibitor KURITA S-6300 [17]

Name of the compound	%
zinc chloride	5-10
sulfuric acid (96-98%)	5-10
2-phosphonobutane-1,2,4-tricarboxylic acid	1-5
1-hydroxyethylidene 1,1-diphosphoric acid	1-5

Table 4. Chemical composition of corrosion inhibitor HEMOD MS 1E [18]

Name of the compound	%
Sodium hydroxide	10-15
2-phosphonobutane-1,2,4-tricarboxylic acid	20-25

In order to avoid the growth of microbiological processes in the cooling water, ω= 3% sodium hypochlorite solution was dosed, maintaining the residual chlorine content within the range of 0.1 - 0.2 mg L^{-1}. Since the corrosion processes are very slow, in addition to the corrosion inhibitor, corrosion activator is also dosed into cooling water to accelerate the corrosion process. As a corrosion activator 5% aqueous solution of NaCl was used. The pH was maintained in the range of 8.8 – 9.2 by dosing a ω= 5% NH_4OH aqueous solution. All used chemicals are p.a. The cooling water recirculation time in the pilot plant was 3.5 h, during which time all water was circulated through the system for a ten times.

RESULTS AND DISCUSSION

The experimental results can be divided into two parts. First, the impact of inhibitor addition on cooling water quality was examined, and then the effect of chloride addition (as a corrosion activator) and water recirculation in the system on cooling water quality was examined.

Physical-chemical analysis was performed on five different samples:

- *Raw water* (without the addition of inhibitors and corrosion activators),
- *Raw water + KURITA S-6300* (without corrosion activator, and this water recirculated for 3.5 hours on a pilot plant),
- *Raw water + KURITA S-6300* + NaCl (water with the corrosion inhibitor and the activator, and which recirculated for 3.5 hours on a pilot plant),

- *Raw water + HEMOD MS 1E* (without corrosion activator, and this water recirculated for 3.5 hours on a pilot plant) and
- *Raw water + HEMOD MS 1E* + NaCl (water with the corrosion inhibitor and the activator, and which recirculated for 3.5 hours on a pilot plant).

Physical-chemical analysis of raw water and cooling water with the addition of activator and corrosion inhibitors is given in Table 5.

Table 5. Physical-chemical composition of raw water and cooling water after 3.5 h recirculation using a corrosion inhibitor in 5% chloride solution

Parameter	Raw water	Raw water + KURITA S-6300	Raw water + KURITA S-6300 + NaCl	Raw water + HEMOD MS 1E	Raw water + HEMOD MS 1E + NaCl
pH	7.61	8.88	8.89	8.92	8.96
TDS	208	211	42800	295	42700
conductivity (μS/cm)	325	457	66900	461	66767
SiO_2 (mg/L)	2.83	2.89	2.81	2.96	2.91
total hardness (TH) ($mgCaCO_3$/L)	176.8	181	276.8	190.3	266.6
calcium hardness (CaH) (mg/L)	132.2	132.1	155.7	160.3	127
p- alkalinity ($mgCaCO_3$/L)	0	45	82	30	159
m- alkalinity ($mgCaCO_3$/L)	155	181	276	190.3	266.6
Cl^- (mg/L)	3.5	23.1	>500	8.6	>500
SO_4^{2-} (mg/L)	22.3	50.6	53.9	22.8	21.6
$PO_{4,\ ortho}^{3-}$ (mg/L)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
$PO_{4,\ total}^{3-}$ (mg/L)	< 0.5	3.1	3.1	3.17	3.1
Cu (μg/L)	4	395	1081	25	1150
Fe (μg/L)	13	91.8	4150	46.7	4238
Zn (mg/L)	< 0.1	2.61	0.16	< 0.1	< 0.1
$Cl_{rezidual}$ (mg/L)	0.3	0.2	0.2	0.2	0.1
carbonate hardness (CT) ($mgCaCO_3$/L)	155	181	276	190.3	266.6
NH_3 (mg/L)	< 0.02	108	111	70.8	69.9
O_2 (mg/L)	5.89	4.69	4.56	4.55	4.59

Addition of the *KURITA S-6300* in raw water results increase in the sulfate content, because sulfuric acid in the composition of this inhibitor. Also there is observed an increase in the zinc content of cooling water. Zinc content is increased because inhibitor contains zinc chloride. By adding corrosion activator and after recirculating cooling water, zinc-ammonia complex is formed, separated in the form of a precipitate, which result by reducing the zinc content in cooling water.

It can also be seen that both samples with *HEMOD MS 1E* have a slight increase in ammonia content, compared with samples with inhibitor *KURITA S-6300*. A slight increase in ammonia content occurs because sodium hydroxide is in composition of the *HEMOD MS 1E*, and therefore an aqueous solution of ammonia is required in less dosage to achieve a pH of 8.8 – 9.2.

The addition of corrosion activator accelerates corrosion processes in open recirculation cooling systems. This can be seen based on chemical composition, where is considerable increase in amount of Fe and Cu when the corrosion activator is added. The smaller separation of copper ions occurs because only one smaller part of the plant is made of copper (the pipe section around which the heater is located, which is a simulation of a heat exchanger), while the rest of plant is made of stainless steel.

The addition of the KURITA S6300 increases the zinc content, because the inhibitor contains zinc

chloride in its composition. It is also seen that the addition of corrosion activators leads to a decrease in the zinc content. Zn^{2+} is the most investigated cation for synergistic effect with metal corrosion inhibitors probably due to the ease of being replace by cation high up in the electrochemical series. It was supposed that in aqueous solution particularly alkaline and neutral solutions, Zn^{2+} –inhibitor complex forms in the solution. On the surface of the metal, the Zn^{2+} – inhibitor complex is converted into the metal–inhibitor complex, so the protective film consists principally of M^{2+} –inhibitor complex and Zn(OH)2, the reason for the remarkable improvement in inhibition efficiency [9].

The efficiency of corrosion inhibitors on steel and copper was determined by placing corrosion coupons in a pilot plant during recirculation of 3.5 h. First, corrosion rate of steel and copper was determined in a 5% NaCl solution without the presence of corrosion inhibitors, and after that with the application of corrosion inhibitors (Figures 2 and 3).

Based on mass loss method, a *negative mass corrosion index* can be calculated according to the following equation:

$$K_m^- = \frac{m_0 - m_1}{S_0 * \tau} \text{ (g/m}^2\text{h)} \qquad (1)$$

where:
m_0 – mass of corrosion coupon before corrosion process (g),
m_1 – mass of coupon after corrosion process, after removing of corrosion products (g),
S_0 – surface of corrosion coupon (m^2) i
τ – time of corrosion process.

Based on *negative mass corrosion index* it can bee calculated corrosion rate by following equation:

$$\pi = \frac{K_m^- * 8{,}76}{d_{Me}} \text{ (mm/year)} \qquad (2)$$

where:
π – thickness (length) of duct resulting from corrosion (mm/year),
K_m^- - negative mass corrosion index (g/m^2h) and
d_{Me} – density of metal.

Based on the obtained results, it can be seen both inhibitors achieved the same inhibitory efficiency on stainless steel (Figure 3), while higher inhibitory efficiency on copper was achieved with KURITA S-6300 inhibitor (Figure 4). However, based on the physicochemical composition of the cooling water (iron and copper content), we can see that better results are achieved on both materials by using the KURITA S-6300 inhibitor (Table 3).

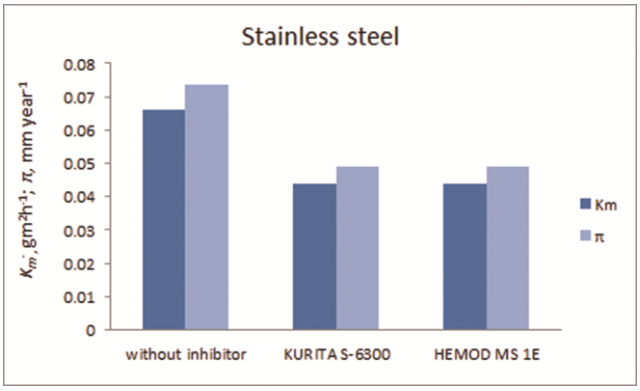

Figure 3. Corrosion inhibitor efficiency on stainless steel (EN 1.4301)

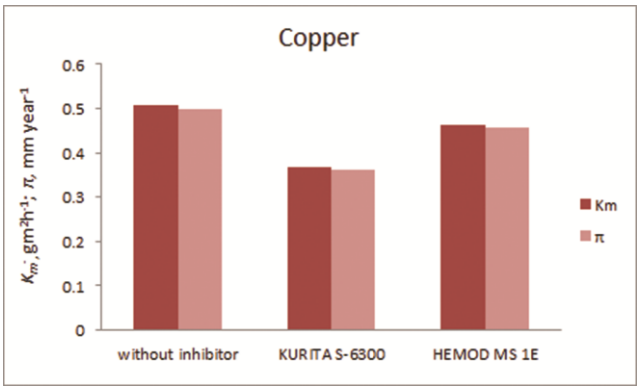

Figure 4. Corrosion inhibitor efficiency on copper (EN 13601)

CONCLUSION

The safe operation of the cooling system depends on the physical-chemical composition of the cooling water, as well as its quality monitoring. The use of proper quality cooling water slows down corrosion processes and prevents the separation of mineral and microbial deposits in the cooling systems, which results in an extended life of the plant.
The obtained results, based on the physicochemical analyze of the cooling water and weight loss method, shows that by use of KURITA S-6300 corrosion inhibitor in simulated cooling water better results are achieved in protection of stainless steel (EN 1.4301) and copper (EN 13601).

ACKNOWLEDGEMENTS

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QUALITY ASSESSMENT AND HEALTH SAFETY OF NATURAL SPRING WATER

ORIGINAL SCIENTIFIC PAPER

Amra Bratovcic[1]✉, Irena Petrinic[2]

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[1] Department of Physical Chemistry and Electrochemistry, Faculty of Technology, University of Tuzla, Univerzitetska 8, 75000 Tuzla, Bosnia and Herzegovina
[2] University of Maribor, Faculty of Chemistry and Chemical Engineering, Smetanova ulica 17, 2000 Maribor, Slovenia

✉ amra.bratovcic@untz.ba

ABSTRACT:
In this paper the natural spring water was investigated. The sample of spring water was taken at the beginning of July 2019 in Tuzla, Bosnia and Hercegovina (BIH), which is consumed by the local population. The source of investigated spring water is located above salt mine. All parameters of health safety were analysed by the methods of laboratory tests regulated by the State regulation on natural mineral and natural spring waters "Official Gazette BiH" No. 26/10 and 32/12, i.e. physical-chemical and microbiological analysis was performed. The results of the study showed that water is not for drinking because it contains higher concentrations of ammonium ion (NH_4^+) which is 0.89 mg/L, nitrite (NO_2^-) 2.20 mg/L and sulphate (SO_4^{2-}) 398.34 mg/L, and coliform bacteria are isolated. The pH value of 9.07 indicate that this spring water is alkaline. The concentrations of iron (Fe^{2+}) 0.007 mg/L and manganese (Mn^{2+}) 0.0144 mg/L are within the allowed limit values while the concentration of lead (Pb^{2+}) 0.0088 is at the upper maximum allowable value. The results of this research indicate that water in this untreated state is not for drinking.

KEYWORDS: spring water, ammonium, nitrites, sulphates, coliform bacteria.

INTRODUCTION

In recent years with the rapidly growing industries and population, water pollution has emerged as major challenge for scientific community that demands an intense and real-world solution. The problem with plastics contaminats present in water, as well as other organic compounds such as polycyclic aromatic hydrocarbons (PAHs), endocrine disrupting chemicals (EDCs), pesticides and herbicides, dyes and its photocatalytic degradation has been studied recently by Bratovcic [1-3]. According to the United Nation World Water Development report, around 748 million people around the world do not have provision for pure drinking water [4].

The civilization has managed to pollute the water supplies to the point where water for drinking has to be purified before using. The shortage of affordable pure water forces an estimated 1.2 billion people to drink unclean water. As a result, water related diseases kill 5 million people a year, mostly children, around the world. The UN estimates that 2.7 billion people will face water shortages by 2025. Only 0.06% is easily accessible from 3% of total freshwater available to us. This is reflected by the fact that over 80 countries now have water deficits. It is clear that water is a scarce and valuable commodity and we need to sustain its quality and use it judiciously, i.e. assure water sustainability. To achieve sustainability, we must ensure that as we meet our needs, we do not compromise the requirements of future generations [5].

Drinking water comes largely from rivers, lakes, wells, and natural springs. These sources are exposed to a variety of conditions that can contaminate water. The failure of safety measures relating to the production, utilization, and disposal of thousands of inorganic and organic compounds causes pollution of our water supplies [6].

Water is essential for life. Potable water is any water suitable for human consumption. It is an indispensable resource for the economy and also plays a fundamental role in the climate regulation cycle. Among the EU countries, Croatia, recorded the highest freshwater resources (with a long-term average of 27330 m^3 per inhabitant) followed by Finland and Sweden with around 20000 m^3 each [7]. Although there is an abundance of water resources, the water quality in Bosnia and Herzegovina is lacking. In most countries, the principal risks to human health associated with the consumption of polluted water are microbiological in nature, although the importance of chemical contamination should not be underestimated. Contaminated water and poor sanitation are linked to transmission of diseases such as cholera, diarrhoea, dysentery, hepatitis A, typhoid, and polio. Inadequate management of urban, industrial, and ag-

ricultural wastewater means the drinking-water of hundreds of millions of people is dangerously contaminated or chemically polluted. Some 829000 people are estimated to die each year from diarrhoea as a result of unsafe drinking-water, sanitation, and hand hygiene. Yet diarrhoea is largely preventable, and the deaths of 297000 children aged under 5 years could be avoided each year if these risk factors were addressed [8].

In Bosnia and Herzegovina only about 65 percent of the country's population has a connection to municipal or public water utilities – the average of European Union countries is 90 percent. Only large urban centres have a satisfactory supply of water, both in terms of quality and quantity. Unfortunately, the poorest and most vulnerable of Bosnia and Herzegovina's population live in rural areas [9]. There are several types of mineral, thermal and thermomineral waters known in the territory of Tuzla Canton (TC). From mineral waters in the area of TC, salt and carbonated waters are registered. Drinking groundwater on the territory of the TC is located in the coastal areas of the river and in the areas of the northern and southern parts of the Canton. In the watercourses on the river Spreca exists sources of Banovici, Tuzla, Zivinice and Kalesija. The total amount of water used for the water supply of Tuzla Canton is 3683032 m^3/year, and the total number of inhabitants serving water is 243900. From the total amount of the taken water from the sources, households are supplied with about 24% of the volume, the industry is 31%, and even 45% of the water makes up the losses.

The water supply of the Tuzla and surrounding places are operated through the inter-municipal water supply system Tuzla-Zivinice-Lukavac and make it the following sources:

groundwater source Sprecko polje, 120-200 l/sec, source Stupari, 220-300 l/sec, source Toplice, 140-230 l/sec.

Tuzla is the third-largest city of Bosnia and Herzegovina, located in the northeastern part of Bosnia and very well known by deposit of the rock salt (halite) and abundant coal deposits in the region around Tuzla. The salt deposits around Tuzla are younger geological age, compared to well-known deposits in Europe and the world, formed in the Miocene. The rock salt deposit "Tetima" is located about 8 km in the northeast of Tuzla. The construction of the salt mine was started in 1986, and it was a substitute capacity for the production of the saturated salty water (brine), from the Hukalo and Trnovac, whose uncontrolled exploitation provoked the ground surface settlement of the urban area, thus endangering the undisturbed development of life and existence of people in these city quarters. It is estimated that the balance reserves on this area is up to 342 million tons, out balance 33 million tons, while 54.7 million tons for exploitation. The life span of the mine with an annual production of 2.6 milion m^3 per annum is 62 years. The exploitation area is 406.38 hectares [10].

Water losses are estimated at about 37% and water supply reconstruction is expected in the forthcoming period. Safe and readily available water is important for public health, whether it is used for drinking, domestic use, food production or recreational purposes. Improved water supply and sanitation, and better management of water resources, can boost countries' economic growth and can contribute greatly to poverty reduction.

Groundwater is the water that lies beneath the surface of the earth and is formed by rainfall, from watercourses and condensation of water vapour in the earth [11]. Groundwater dissolves various minerals from the rocks through which they pass. In the groundwater solution there is CO_2 which is partly derived from the atmosphere and the second part is formed by the decomposition of plant material. Carbon dioxide affects the mild acidity of the water, and if such water comes into contact with limestone, carbonates dissolves and bicarbonates are formed and then a mild alkaline environment occurs. Ground waters can be soft or hard, depending on soil composition and dissolution of magnesium and calcium carbonates. In addition, this water dissolves all the minerals and organic materials present in the aqueous layer. Water depths up to 80 m are softer, with higher concentrations of ammonia, organic materials, iron and manganese, have a low sodium concentration and a slightly increased content of arsenic and chloride. The waters over 80 m deep have been trapped in underground tanks for tens of thousands of years are exceptionally soft, they have increased sodium content, organic matter, ammonia, arsenic and orthophosphate ion (HPO_4^{2-}), and the dominant anion in these waters is bicarbonate [12].

In this paper the quality and health safety of the spring water was studied. Extended physical-chemical analysis will be performed and it will include beside the determination of temperature, pH, electrical conductivity, alkalinity, organic matter, chlorine, hardness, total hardness, sulfate, sulfide and minerals such as iron, manganese and lead. The microbiological analysis that would be performed will covered the determination of the *Escherichia coli*, *Coliform bacteria*, *Enterococci*, the total number of microorganisms at 22°C and 37 °C and *Clostridium perfringens*.

MATERIAL AND METHODS

In order to determine health safety of the spring water, the sample was analyzed on organoleptic, microbiological and physical-chemical properties. The source of spring water is located in Tuzla in Bosnia and Herzegovina and more precisely in Tusanj near salt mine. A sample of water for physical-chemical analysis was taken in a clean plastic flask of 1.5 liter previously washed three times with water to be analyzed. A sample of water for microbiological analysis was taken in a sterile glass flask of 1 liter. The temperature of spring water at the place of sampling was 15 °C. The rate of water flow and the capacity of this source of spring water is 52704 m^3/day. This information is about the natural wealth that this source of natural water abounds in. It would be a great pity that due to the health deficiency, this water source remains unused, given the fact that there is a downward trend in clean spring water, as well as the final price of water. The sample was taken at the beginning of July 2019. The analysis of organoleptic properties, physical-chemical and microbiological analysis have been done.

PHYSICAL-CHEMICAL ANALYSIS

Physical – chemical analysis of the water has been done by Regulations on Natural mineral and natural spring waters [13]. Organoleptic properties were determined by sensory analysis and they included the determination of color, odor and taste. The turbidity of the tested water has been determined by standard method BAS EN ISO 7027-1:2017.

The extended physical-chemical analysis involves the determination of temperature, pH, electrical conductivity, alkalinity, organic matter, chlorine, hardness, total hardness, iron, manganese, lead, sulfate, and sulfide.

The spring water temperature is measured at the sampling site, by mercury thermometer. The pH value has been determined by BAS EN ISO 10523:2013. The electrical conductivity implies measuring electrical conductivity of water by conductometer following the standard method BAS EN 27888:2002 at 20 and 25 °C.

Organic matter analysis by using potassium permanganate ($KMnO_4$) can be used as an indicator of the content of organic matter in water. The potassium permanganate solution is heated in an acidic medium, oxidation of the organic substance occur and a certain amount of $KMnO_4$ is consumed, depending on the amount and the chemical structure of the organic substance present in the water. This indicator can only be conditional use as a measure of the content of organic substances because potassium permanganate is also reduced in the presence of some other substances in the sample of water [14].

Dry residue by gravimetric method was determined.

The concentration of ammonium ion (NH_4^+), nitrites (NO_2^-) and sulphates by spectrophotometry were determined. The instrument used was UV-VIS spectrophotometer Lambda 25.

Water hardness is the result of the presence of ions such as calcium, iron, manganese and magnesium dissolved in water. In natural waters, magnesium and calcium ions are more common. Thus, the sum of calcium and magnesium ions is expressed as hardness of water. The total hardness of water, alkalinity, calcium and magnesium were determined by a volumetric method.

Residual chlorine was determined by colorimetry.

Nitrates (NO_3^-) by Standard methods for examination of water and wastewater, 4500 NO_3^-B 2005 were determined. Sulfides were determined by standard iodometric method Standard methods 45000 S^{2-} F (Iodometric method, APHA-AWWA-WEF 2012).

Iron and Manganese were determined spectrophotometrically by Atomic spectroscopy Standard Methods 3111 (B), APHA-AWWA-WEF 2012.

Lead has been determined by BAS ISO 8288:2002 – Method of flame atomic absorption spectroscopy.

Microbial analysis implies determination of pathogenic and other microorganisms.

In this paper the Escherichia coli and Coliform bacteria were determined by standard method BAS ISO 9308-1:2015; BAS EN ISO 9308-2:2015. Enterococci were determined by BAS ISO 7899-2:2003 standard method, while Pseudomonas aeruginosa by BAS EN ISO 16266:2009 method. The total number of microorganisms at 22 °C and 37 °C by standard method BAS EN ISO 6222:2003 were determined, and Clostridium perfringens by BAS EN 26461-1:2003; BAS EN ISO 26461-2:2003.

MICROBIOLOGICAL ANALYSIS

To determine the total number of coliform bacteria such as *Escherichia coli* and *Enterobacter*, the membrane filtration firstly has to be done. Under sterile conditions, using a vacuum pump on the membrane filtration device, 100 ml of the sample is passed through a filter paper having a pore size of 0.45 μm. Thereafter, the filter paper is transferred to a sterile Petri dish with agar medium for the total number of microorganisms, and after 24 hours at 37 °C and after 72 hours at 22 °C, the total number of

microorganisms were determined. The presence of coliform bacteria after filtration, were determined by growing them on Endo agar in the thermostated incubator for 24 h-48 hours at 37 °C.

RESULTS AND DISCUSSION

The results include determination of organoleptic properties, extended physical – chemical properties and microbacterial analysis. Physical characteristics of water include temperature, colour, taste, odour, turbidity and total dissolved solids. Temperature is the single most important physical characteristic affecting the pH of water.

Table 1. Results of physical-chemical analysis

Parameter	Unit	Determined value	Allowed values
Colour	-	without	acceptable for the consumer
Odour	-	without	acceptable for the consumer
Taste	-	-	acceptable for the consumer
Turbidity	NTU	0.61	acceptable for the consumer
pH value (25°C)	pH	9.07	6.5-9.5
Consumption $KMnO_4$ (oxidation)	mg/l O_2	1.36	up to 5
Dry residue	mg/l	942	-
Electrical conductivity (25°C)	µS/cm	1537	-
Electrical conductivity (20°C)	µS/cm	1377	2500
Ammonium (NH_4^+)	mg/l	0.89	0.50
Residual chlorine	mg/l	0.00	0.50
Chloride (Cl^-)	mg/l	11.00	250
Nitrites (NO_2^-)	mg/l	2.20	0.50
Nitrates (NO_3^-)	mg/l	3.94	50.0
Iron	mg/l	0.007	0.2
Manganese	mg/l	0.0144	0.05
Total hardness	°nj	0.56	-
Alkalinity	°nj	27.44	-
Sulphates	mg/l	398.34	250
Sulphides	mg/l	0.0	0.1
Calcium	mg/l	12.01	-
Magnesium	mg/l	1.70	-
Lead (Pb)	mg/l	0.0088	0.01

In table 1 there are results of organoleptic properties which include the determination of colour, odour and taste by sensory analysis and extended physical – chemical parameters such as pH, turbidity, organic matter, dry residue, electrical conductivity, residual chlorine, the most relevant anions and cations, total hardness.

The results of the study showed that water is not for drinking because it contains higher concentrations of ammonium (NH_4^+) which is 0.89 mg/L, nitrite (NO_2^-) 2.20 mg/L and sulphate (SO_4^{2-}) 398.34 mg/L, and coliform bacteria are isolated. The pH of water as a measure of the acid and base equilibrium, and in most natural waters, is controlled by the carbon dioxide, bicarbonate and carbonate equilibrium system [15]. The pH value of 9.07 indicates that this spring water is alkaline and according to previous discussion suggests the lower concentration of carbon dioxide concentration and presence of carbonate or hydroxide ions. The pH level of water can affect, and in some cases be affected by, the physical, chemical and microbiological characteristics of the water.

The concentrations of iron (Fe^{2+}) 0.007 mg/L and manganese (Mn^{2+}) are 0.0144 mg/L a within the allowed limit values while the concentration of lead (Pb) 0.0088 is at the upper maximum allowable value. The maximum allowed concentrations (MAC) regulated by the State regulation on natural mineral and natural spring waters for (NH_4^+) is 0.50, for NO_2^- is 0.50 mg/L, (SO_4^{2-}) is 250 mg/L, (Fe^{2+}) is 0.2 mg/L, (Mn^{2+}) 0.05 mg/L and (Pb) 0.01.

In Figure 1 the concentrations of ammonia and nitrite are presented.

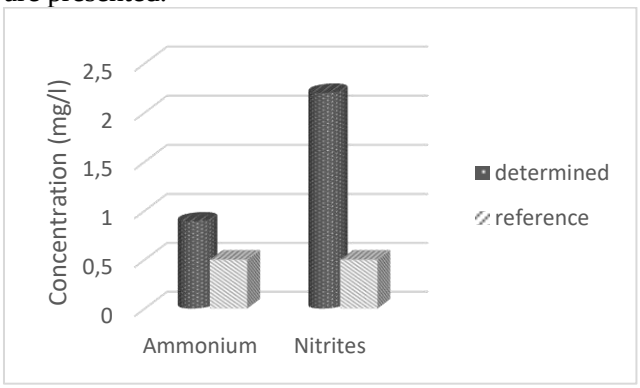

Figure 1. The concentrations of ammonia and nitrite in spring water

From Figure 1 is noticeable that the concentrations of ammonium and nitrites exscide the MAC. The concentration of ammonim is 1.8 times higher (0.89 mg/l) than MAC (0.5 mg/l), while the concentration of nitrites (2.2 mg/l) is 4.4 times higher than it is maximum allowed (0.5 mg/l).

The environmental cycling of nitrogen relies mainly on nitrate, followed by ammonia and the ammonium cation, which predominates. The ammonium cation is less mobile in soil and water than ammonia and is involved in the biological processes of nitrogen fixation, mineralization, and nitrification [16]. Since that ammonia is used in fertilizer and animal feed production we assume that this high concentration of ammonium ion may

originate from these sources. The local population where the water source is located is engaged in agriculture and livestock breeding. Nitrate can reach both surface water and groundwater as a consequence of agricultural activity (including excess application of inorganic nitrogenous fertilizers and manures), from wastewater treatment and from oxidation of nitrogenous waste products in human and animal excreta, including septic tanks [17].

Although the concentration of nitrate in the tested sample of water is within the permitted limits (3.94 mg/l) and maximum allowed is 50 mg/l, it is assumed that due to the presence of microorganisms nitrates are reduced to nitrite.

The nitrate concentration in surface water is normally low (0–18 mg/l) but can reach high levels as a result of agricultural runoff, refuse dump runoff or contamination with human or animal wastes. Concentrations of nitrate in rainwater of up to 5 mg/l have been observed in industrial areas [18]. In rural areas, concentrations are somewhat lower. Since that Tuzla is an industrial city and in our sample the concentration of nitrate is 3.94 mg/l which is in line with a previous results related to rainwater.

In Figure 2 the concentrations of sulphate is presented.

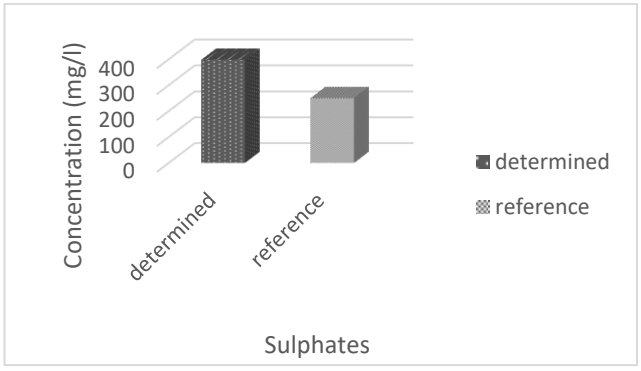

Figure 2. The concentrations of sulphate in spring water.

Sulphates occur naturally in numerous minerals which dissolved contribute to the mineral content of many drinking-waters. Sodium, potassium and magnesium sulphates are all highly soluble in water, whereas calcium and barium sulphates and many heavy metal sulfates are less soluble. Levels of sulphate in rainwater and surface water correlate with emissions of sulphur dioxide from anthropogenic sources [19].

Since the concentration of H_2S was not detected in the analyzed water sample, the relation between sulphate and sulphide concentrations is absent. The concentration of sulphate in analyzed sample was 398.34 mg/l and MAC is 250 mg/l. This indicates 1.6 times higher concentration upper maximum allowed limit which may originate from the sulphur containing detergents used for personal care.

The results of the physical-chemical analysis of the examined spring water indicate that the water does not meet the conditions of the *Regulation on natural mineral and natural spring waters" Official Gazette BiH" No. 26/10 and 32/12* because the tested water contains a higher content of ammonia, nitrite and sulfate.

Bacterial analysis included:

- *Escherichia coli* in 250 ml of water
- *Coliform bacteria* in 250 ml of water
- *Enterococci (Enterococcus faecalis)* in 250 ml of water
- *Pseudomonas aeruginosa* in 250 ml of water
- *Clostridium perfringens* in 100 ml of water
- Total number of microorganisms at 37 °C in 1 ml of water
- Total number of microorganisms at 22 °C in 1 ml of water

Table 2. Results of microbiological analysis

Parameter	Maximum allowed concentration (MAC) according to the Regulations (cfu)**	Results
Escherichia coli	0/250 ml	not isolated
Coliform bacteria	0/250 ml	are isolated
Enterococci	0/250 ml	3
Pseudomonas aeruginosa	0/250 ml	not isolated
Total number of microorganisms at 22 °C	100/1ml	50
Total number of microorganisms at 37 °C	20/1ml	20
Clostridium perfringens	0/100 ml	not isolated

** cfu – colony forming units

In Figure 3 the microbial analysis results are shown.

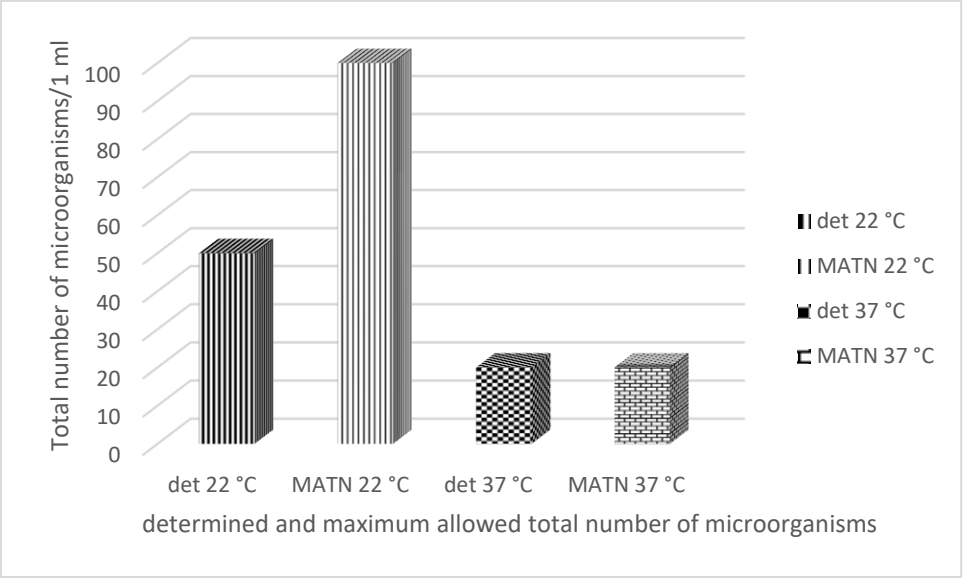

Figure 3. The microbial analysis of tested spring water

According to the Regulations, the total number of bacteria at the spring must not exceed 100 in 1 ml at 22 °C for 72 hours of incubation on nutritious agar and 20 in 1 ml at 37 °C for 24 hours of incubation on nutritious agar. In our sample were found 50 bacteria in 1 ml at 22 °C i.e. 50 % from maximum allowed value and 20 in 1 ml at 37 °C i.e. 100% of maximum allowed value.

The results of the microbiological analysis of the examined spring water indicate that water does not meet the conditions of the *Regulations on natural mineral and natural spring waters "Official Gazette BiH" No. 26/10 and 32/12*.

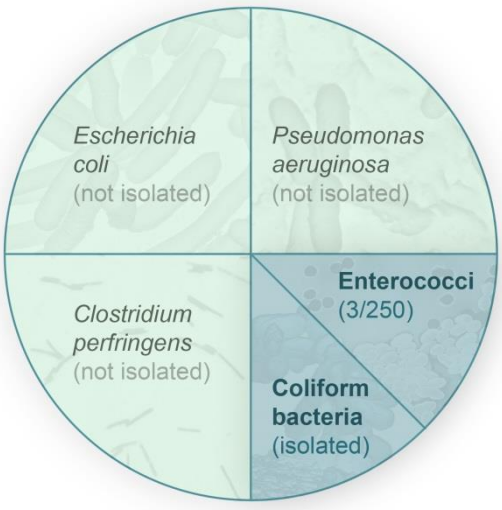

Figure 4. Results of microbial analysis of tested spring water.

Enterococci have been adopted as indicators of human fecal pollution in water. In drinking water, coliforms, including total and fecal coliforms (and *Escherichia coli* in particular) are the primary method of assessing contamination. In the European Union (EU), enterococci are used as indicators of drinking water contamination. In the EU, enterococci are not permitted in a 100 mL sample of tested drinking water that flows from a tap, and they are not permitted in a 250 mL sample of bottled water [20]. In our sample there are 3 of them in 250 ml of water. In Figure 4. The schematic presentation of the results of microbial analysis are presented.

Over the last ten years, about 10 residential houses have been built near the spring source water, and as a consequence it is reasonable to assume that the source of pollution is coming from the waste water used by these households. Namely, high concentrations of nitrite and ammonia as well as isolated coliform bacteria allude to fecal matter contamination, while high concentration of sulfate can be originated from the use of detergents to maintain personal hygiene.

One of the most important steps to make sure drinking water is safe is regular testing for coliform bacteria. Their presence in drinking water indicates that disease-causing organisms (pathogens) could be in the water system. Most pathogens that can contaminate water supplies come from the faeces of humans or animals. Total coliform bacteria are common in the environment (soil or vegetation) and are generally harmless.

Considering that total coliform bacteria in examined spring water were detected, it is important to

find and resolve the source of the contamination. It is assumed that the source is probably by faecal contaminated due to a large number of houses built in the surrounding area and inadequate sewage disposal. *Faecal coliform* bacteria are a subgroup of total coliform bacteria. They exist in the intestines and faeces of people and animals. *E. coli* is a subgroup of the faecal coliform group. Most *E. coli* bacteria are harmless and exist in the intestines of people and warm-blooded animals. However, some strains can cause illness. The presence of *E. coli* in a drinking water sample usually indicates recent faecal contamination. That means there is a greater risk that pathogens are present. In this study the *E. coli* were not detected.

The next step should be in finding out how the contamination got into the water. If the assessment identifies the cause of the contamination, the water system can usually correct the problem with repairs, treatment, or improved operation and maintenance practices. Since that this study has confirmed the presence of total coliform bacteria in spring drinking water, it is necessary to inform users as soon as possible. It is important to explain what is needed to do to correct the problem, when the problem will likely be resolved, and what users may need to do until then [21].

Ideally, drinking-water should not contain any microorganisms known to be pathogenic—capable of causing disease—or any bacteria indicative of faecal pollution. The detection of *Escherichia coli* provides definite evidence of faecal pollution; in practice, the detection of thermotolerant (faecal) coliform bacteria is an acceptable alternative.

When a guideline value is exceeded, the cause should be investigated and corrective action taken. Terminal disinfection is essential for surface waters after treatment and for protected groundwater sources when *E. coli* or thermotolerant (faecal) coliforms are detected. Chlorine in one form or another is the most commonly used disinfectant worldwide.

For terminal chlorination, there should be a free chlorine residual of at least 0.5 mg/litre after a minimum contact time of 30 minutes at a pH of less than 8.0, as for inactivation of enteric viruses. When chlorine is used as a disinfectant in a piped distribution system, it is desirable to maintain a free chlorine residual of 0.2–0.5 mg/litre throughout, to reduce the risk of microbial regrowth and the health risk of recontamination. In emergencies, e.g. in refugee camps, during outbreaks of potentially waterborne disease, or when faecal contamination of a water supply is detected, the concentration of free chlorine should be increased to greater than 0.5 mg/litre throughout the system [22].

CONCLUSION

The physical-chemical and microbiological analyzes of the investigated spring water clearly indicate that water in this untreated state is not for drinking. Due to the existence of a grounded suspicion that water could be contaminated due to inadequate disposal of municipal wastewater from the surrounding housing, it is urgent to inform the local population not to use water without prior cleaning or boiling water for health reasons. The results of this research clearly show the necessity of continuous monitoring of the quality of spring water consumed by the local population.

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THE EFFECT OF DIFFERENT METHODS FOR PRODUCTION OF CRACKERS ON THEIR PHYSICAL AND SENSORY CHARACTERISTICS

ORIGINAL SCIENTIFIC PAPER

Gjore Nakov✉, *Nastia Ivanova*

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Department of Chemical, Food and Biotechnology, University of Ruse "Angel Kanchev" - Branch Razgrad, Bulgaria

✉ gnakov@uni-ruse.bg

ABSTRACT:

The aim of this study was to compare the physical and sensory characteristics of crackers obtained by three different methods. To obtain the crackers by the first method, the dough was allowed to ferment for 16 h at 26 ^{0}C. In this method, fat (shortening) was added in the form of an oil-based semi-finished product. The second formulation differs from the first formulation by the time of fermentation (2 h) and the way in which the oil was added. Using this method of cracker production, fat was added in the form of lumps in the dough itself. The difference between the third and the first two methods was the raising agents used (the third method uses $NaHCO_3$ and the first two pressed yeast) and the lack of fermentation. The following were examined: temperature and pH before and after fermentation, fermentation loss, thickness, diameter, volume, color and sensory characteristics of the crackers obtained. The results showed that the temperature during fermentation increases and the pH decreases. The crackers obtained from the dough, which fermented for 2 hours, had the greatest thickness, diameter and volume. From the sensory analysis, it became clear that the crackers obtained by first method was the most appreciated.

KEYWORDS: crackers, physical characteristics, biscuits.

INTRODUCTION

Cereal products, especially those used for baking and pastry, are the most widely used in people's daily lives. Their use helps to improve the quality of bakery and pastry. In addition, they increase the functionality of the products and ensure the health of the body [1]. Crackers are dry bread products and they are consumed as breakfast or instead of bread. They can be consumed with butter, cheese and more.

Various types of crackers are known, which are mainly divided into unsweetened (salted) and slightly sweetened [2]. The main raw materials for crackers are wheat flour, water, fat and yeast. The most commonly used flour in the bakery and confectionery industry is white wheat flour T-500. This type of flour contains a large amount of starch (about 70% of the flour composition) [3]. The T-500 flour gives better gluten than the higher type flours. Due to the good properties of gluten and low enzyme activity, obtained dough is elastic, stable and easily stretchable [4]. On the other hand, gluten is a key ingredient that influences the texture of crackers [5]. There are different methods for making crackers. The first crackers that were produced were laminated [2]. Chonova and Karadjov [6] presented a method for obtaining the same crackers. Pressed yeast is used in it as a swell. Many authors also use the soda crackers method [7], [8], [9]. This method is also known as *"all in method"*, which means that all raw materials are mixed at the beginning of the process. With this technology, since soda bicarbonate ($NaHCO_3$) is used, the dough does not ferment, and the dough is immediately baked. The purpose of this article is to determine the physical characteristics of crackers obtained by three different methods. Additionally, by means of sensory analysis, the best method for cracker production was selected.

EXPERIMENTAL

MATERIAL

The following raw materials were used to make the crackers: flour type 500 (Sofia Mel), Salt (Familex), Pressed yeast (Yuva), Soda - $NaHCO_3$ (Radicot), Margarine (Kaliakra) and water from the water supply network of Razgrad.

METHODS

Production of crackers

To obtain the sample 1 crackers, flour (100 g), yeast (2.5 g), salt (1.2 g), margarine (15 g) and water (32.5 mL) were mixed to form dough at 27 °C. The dough was then left to ferment for 16 h at 27 °C.

Flour (25 g) and margarine (12.5g) were mixed separately to obtain an oiled semi-finished product which was stored at 4 °C until used. After the fermentation time has elapsed, the dough was laminated in order to obtain the same thickness over its entire length and an oiled semi-finished product was placed on it. The dough was laminated twice. This was followed by the formation of crackers using a circular shape form (d=44mm). The formed crackers was baked at 180 °C for 12 minutes.

To obtain sample 2 crackers, flour (100 g), yeast (3 g), salt (1.5 g), margarine (20 g) and water (38.00 mL) were mixed to form dough at 27 °C. The dough was then allowed to ferment for 2 hours at 27 °C. The other operations were the same as for sample 1 crackers.

The third type of crackers (sample 3) were the so-called soda crackers. For their preparation flour (100 g), $NaHCO_3$ (0.6 g), salt (1 g), margarine (10 g), water (45 mL) and sugar (2 g) were mixed by the "all in" method. After the dough was created, it was laminated to obtain the same thickness. Round-shaped crackers were formed and baked at 180 °C for 30 minutes.

Analysis of the parameters during the technological process

The temperature of the dough before and after fermentation was determined using an automatic thermometer. The pH values before and after fermentation was determined using a LovibondMicrodirect manual pH meter. The losses obtained during fermentation were calculated using equation (1):

$$Fermentation\ losses = \frac{m_b - m_a}{m_b} \times 100\ [\%] \quad (1),$$

where:
m_b- mass of dough before fermentation (g) and
m_a- mass of dough after fermentation (g).

Physical characteristics

The thickness and diameter of the crackers obtained were determined using an automatic caliper (Power Fixprofi, electronic digital calliper). The volume was determined by the AACC 10-05 method [10]. The color of the crackers was determined using a Minolta Chroma Meter CR-400 (Konica Minolta, Tokyo, Japan) via the CIE *Lab* system. The change between the color of the dough and the baked crackers (ΔE) was determined by equation (2):

$$\Delta E = \sqrt{(L^* - L_0)^2 + (b^* - b_0)^2 + (a^* - a_0)^2} \quad (2),$$

where:
L_0, b_0, a_0 – raw dough color data;
L^*, b^*, a^* – baked crackers color data.

Sensory analysis

Sensory analysis was performed by 26 untrained assessors. Each sample was evaluated by parameters: color, texture, odor, taste and overall acceptance. Each parameter was rated with points from 1 to 6, where 1 - I do not like it at all and 6 - I like it a lot.

Statistical analysis

Analysis of variance (ANOVA) and Fisher`s Least Significant Difference test (LSD) at $p<0.05$ were performed with the software's XLSTAT 2017 (Addinsoft Inc., Long Island City, NY, USA) and Office Excel 2013 (Microsoft, Redmond, WA, USA).

RESULTS AND DISCUSSION

The dough for crackers should be at an average temperature of 25-26 °C and should be subjected to continuous fermentation at almost the same temperature [6]. Figure 1 shows the initial and post-fermentation test data from which the three types of crackers were obtained.

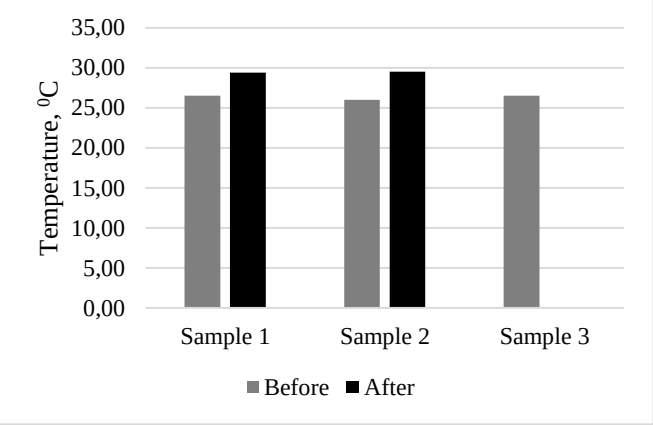

Figure 1. Dough temperature before and after fermentation

From the results presented in Figure 1, it can be seen that the dough temperature before fermentation is lower than that determined after fermentation. Because the crackers of sample 3 were not fermented, only the initial temperature was determined. The low temperature of the dough slows down the processes that take place during the fermentation of the dough, which leads to the prolongation of the final fermentation [11]. The ANOVA (not presented) highlighted

significant differences ($p < 0.05$) for pre- and post-fermentation temperatures.

The increase in temperature during fermentation depends primarily on the raising agent used. Most commonly, fermentation yeast *Saccharomyces cerevisiae* is used to prepare fermented dough. Bread yeast in the production of bread and bakery products has a dual role: it is responsible for increasing the volume of dough and helps to create the specific taste and aroma of the products. The taste and aroma depend mainly on the ingredients that are included in the recipe composition of the products (flour, yeast, sugar and salt) [12]. Baking soda crackers use baking soda bicarbonate ($NaHCO_3$). It is activated by heat when the crackers are baked.

During the fermentation of the dough there is an increase in its acidity (lower pH values), which contributes to the improvement of the shelf life, aroma, color and rheological properties of the products [13]. Figure 2 shows the pH values of the dough for crackers - before and after fermentation.

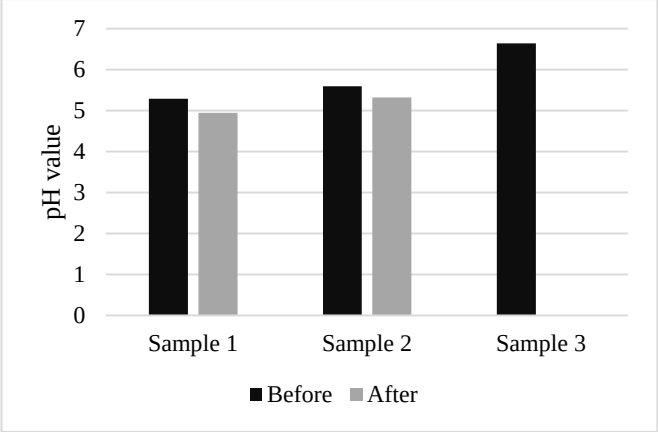

Figure 2. pH values of the dough before and after fermentation

The ANOVA (not presented) highlighted significant differences ($p < 0.05$) for pH value before and after fermentation of dough. The figure also shows that in the dough from which the crackers from sample 1 were obtained, the pH values decreased by 0.35, where as in the dough from which the crackers from sample 2 were obtained, the pH values decreased by 0.27. Of great importance is the duration of the fermentation of the dough. The sample cracker dough 1 fermented for 16 hours, while the sample cracker dough 2 fermented for 2 hours. In the first dough it is assumed that the alcoholic fermentation and the biochemical processes associated with the protein substances has proceeded completely, and in the second dough - partially.

As a result of the alcoholic fermentation, the carbon dioxide formed increases the proportion of the gas phase in the dough (the bubbles increase). As a result, the protein zippers tighten and the dough volume begins to increase. As a result of proteolysis, protein substances partially disaggregate and their solubility increases [11]. Enzymes break down some of the present starch and sugars. The acidity increases and affects the aroma of the baked crackers. The dough for the preparation of the classic oil crackers has an acidity of pH 6.0 [2].

During fermentation, the dough mass is reduced. The reason for this is that during fermentation in the dough, the gas phase increases and a porous structure is formed. Figure 3 shows the fermentation losses of the cracker dough.

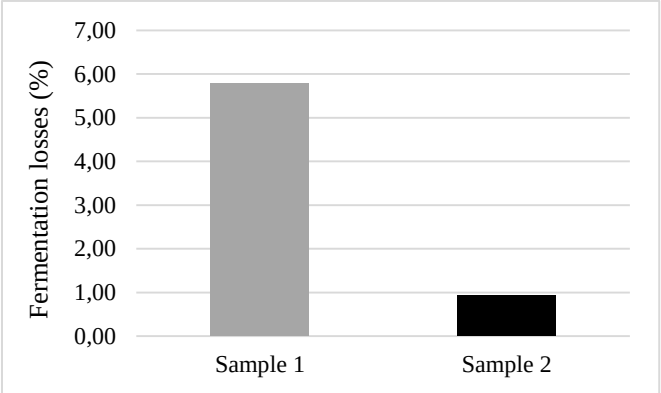

Figure 3. Fermentation losses

The ANOVA (not presented) highlighted significant differences ($p < 0.05$) in dough fermentation losses. The highest losses during fermentation were obtained with the sample 1 dough, which was allowed to ferment for 16 hours (5.79 %). In the test for the preparation of the sample 2 crackers, the fermentation losses were 0.92 %. During the process of maturing, the dough changes its structure, its physical properties and the content of aromatic substances. The dough is then suitable for forming and further processing. The matured dough should have: high intensity of gas formation; optimum physical formation properties; a sufficient quantity of finished and aromatic products, which determine the characteristic taste and aromatic properties of the crackers. These properties are acquired from the dough as a result of the simultaneous flow of various interrelated biochemical, colloidal and physical processes.

The biochemical processes occurring in the dough due to the activity of its microflora are fermentation and aerobic digestion of sugars. Alcohol fermentation accounts for the largest proportion of all fermentations in the dough. It is caused by the main microflora of the dough (yeast *Saccharomyces cerevisiae* of baker's yeast) [6].

Table 1. Physical properties of dough and crackers

	Thickness (mm)		Width (mm)		Volume (cm^3)
	Dough	Crackers	Dough	Crackers	Crackers
Sample 1	1.99 ± 0.04^b	1.40 ± 0.29^b	45.19 ± 0.08^a	40.53 ± 2.09^b	6.00 ± 0.70^a
Sample 2	4.78 ± 0.26^a	3.12 ± 0.24^a	42.35 ± 0.37^b	50.99 ± 3.42^a	7.00 ± 0.12^a
Sample 3	0.68 ± 0.20^c	3.69 ± 0.05^a	45.13 ± 0.01^a	37.81 ± 1.00^b	6.00 ± 0.02^a

Values are means SD ($n \geq 5$); values in the same row with different exponents have statistically significant differences ($p < 0.05$) following Fisher's LSD test.

The ANOVA (not presented) highlighted significant differences ($p < 0.05$) in thickness and width of dough. A decrease in the thickness of the crackers after baking is observed when using a biochemical method (pressed yeast) to swell the dough. Sample 1 crackers reduce their thickness by 0.59 mm and sample 2 crackers by 1.66 mm. On the other hand, crackers that use a chemical raising agent increase their thickness (become thicker) by 3.01 mm. One of the reasons for the increase in thickness could be the raising agent used. The chemical swelling mode is based on the ability of some compounds to decompose or interact with each other under the influence of heat, producing a large amount of gas [11]. During the heat treatment (baking), the diameter of the finished products was reduced. It can be seen from Table 1 that the diameter of the crackers of sample 1 decreases by 3.58 mm. Diameter reduction was also noticed for sample 3 crackers (7.3 mm). On the other hand, the diameter of the crackers of sample 2 increased by 10.64 mm. A round shape was used during the formation of the crackers. Due to the elasticity of the dough (sample 2), when forming the raw crackers, the latter lost their round shape. Data from the volume of crackers (Table 1) indicates that the crackers of sample 1 and 3 had a volume of 6.00 cm^3, while those of sample 2 had a volume of 7.00 cm^3. It is clear that the largest diameter crackers had the largest volume.

Table 2. Color determination

Dough	L^*	a^*	b^*	
Sample 1	75.48 ± 0.2^b	-6.92 ± 0.3^a	2.49 ± 0.1^b	
Sample 2	78.19 ± 0.4^a	-8.12 ± 0.5^a	4.52 ± 0.9^a	
Sample 3	71.66 ± 1.4^c	-8.46 ± 0.2^a	-4.93 ± 0.3^c	
Crackers	L^*	a^*	b^*	ΔE
Sample 1	71.90 ± 1.0^b	1.63 ± 0.2^a	24.58 ± 1.2^a	23.96
Sample 2	77.22 ± 0.1^a	3.51 ± 0.1^b	17.17 ± 1.3^b	17.21
Sample 3	69.31 ± 1.3^c	4.93 ± 0.6^b	15.64 ± 0.1^c	18.18

Values are means SD ($n \geq 5$); values in the same column with different superscripts are significantly different ($P < 0.05$) separately.

Table 2 presents the color data of the crackers through the CIE Lab model. The data presented in Table 2 shows that the values of parameter L^* decreased during baking and crackers became darker. The ANOVA (not presented) highlighted significant differences ($p < 0.05$) for L^* parameters of dough and crackers. Parameter values a^* decreased during baking for all samples and crackers turned red. An increase in values is also noticed for the parameter b^* after the heat treatment of the crackers. This parameter gives the yellow color of the crackers.

Due to browning caused by Maillard reaction, the dextrinisation of starch and caramelisation of sugars, significantly smaller colour differences have been noticed between dough and crackers, when compared with ΔE. These values between dough and crackers were considerably higher than 5, which is a colour difference value that observers could easily notice [14], [15].

The results of the sensory analysis performed are presented in Figure 4. Crackers from sample 1 had the best evaluation for all parameters tested.

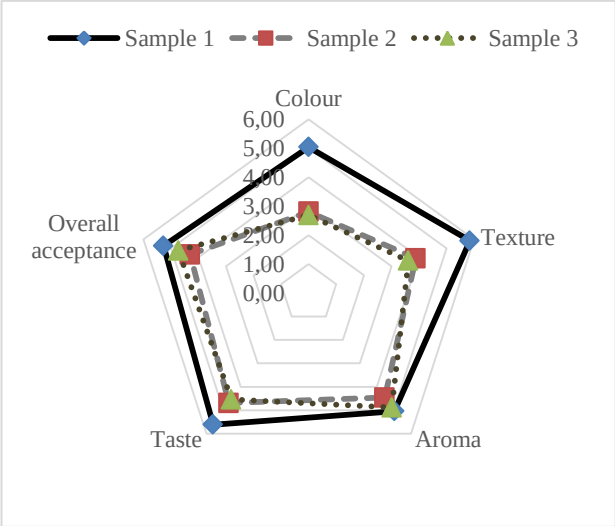

Figure 4. Sensory evaluation

At the end of the sensory analysis, we asked the cracker evaluators to choose one of the three types. 20 of them preferred sample 1 crackers, only 2 chose sample 2 crackers, and 4 preferred sample 3 crackers.

CONCLUSION

In the development of three different methods for making crackers, it has been shown that: the temperature during the fermentation of the dough increases and at the same time the pH value of the dough decreases (becomes more acidic); fermentation losses are greater with prolongation of fermentation time. From the crackers obtained, those of sample 2 have the largest thickness, diameter and volume. According to the color of the crackers it was found that there was a significant difference between the color of the dough and the crackers themselves. Sensory analysis revealed that sample 1 crackers were the most highly rated and preferred by the assessors.

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PRODUCTION OF THE ENRICHED MUESLI BARS UNDER MINIMAL PROCESSING TREATMENT

ORIGINAL SCIENTIFIC PAPER

Martina Andrejaš, Dijana Miličević✉, Gordan Avdić, Hurija Alibašić

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Faculty of Technology, University of Tuzla, Urfeta Vejzagića 8, 75000 Tuzla, Bosnia and Herzegovina

✉ dijana.milicevic@untz.ba

ABSTRACT:

Muesli bars is a generic term that refers to baked or cold-formed cereal-based snack bars, and may contain other ingredients such as fruit, nuts, seeds, chocolate, yoghurt, and a variety of other fillings and/or toppings. By changing the ingredients nutritional value of muesli bar can be modified. In this research, protein-rich and energy-high muesli bars have been produced, because they contain a higher amount of fat, proteins and sugar. However, fats and sugars were not added but come from specific ingredients (hazelnuts, walnuts, chia seeds, sesame, sunflower and pumpkin seeds, oat flakes, etc.). Four muesli bar formulations were developed, two with hazelnuts and two with walnuts, added in the amount of 25% and 50%, respectively. The other ingredients were added in the same amount. The product was dried at 80°C for several hours. The composition, polyphenolic compounds and antioxidant capacity were determined in the products before and after drying. Also, muesli bars were sensory evaluated.

KEYWORDS: muesli bar, hazelnut, walnut, oat flakes, drying.

INTRODUCTION

Snack consumption has increased significantly in recent years [1]. Chocolate bars, crisps, cakes and pastries are unhealthy snacks that have a high content of saturated fat, salt and refined sugar which can cause health problems [2]. Due to that, many people intend to change their unhealthy behaviors, such as their bad habit of consuming high-caloric foods [3]. Nowadays, the consumer demand of healthy snacks and minimally processed foods are increasingly popular because consumers want fresh like foods with their natural nutritive values and sensory attributes, such as flavor, odor, texture and taste [4]. This growing consumer's demand of minimally processed foods with no or lesser synthetic additives poses challenges to food technologists [5].

Minimally processed products have been defined as "any fruit or vegetable, or any combination thereof, which has been physically altered from its original form, but has remained in its fresh condition" [6]. The aims of minimal processing are: to make the food safe chemically and microbiologically; to retain the desired flavor, color and texture of the food products and to provide convenience to the consumers [7]. Minimal processing of foods with thermal methods is extensively used for the preservation and preparation of foods. HTST (high temperature short time) and LTLT (low temperature long time) concept are used. High temperatures will give the rapid inactivation of microorganisms and enzymes required in pasteurization or sterilization, and short times will give fewer undesired quality changes. If effective control is not possible, thermal processing is better done using the LTLT concept, using low temperatures over long times. This process improves shelf-life and sensory and nutritional quality by controlling causes of negative changes in quality such as exposure to oxygen and extreme temperatures [8].

In this research, muesli bars were produced under minimal processing treatment. The product was dried in a food dehydrator at 80°C for several hours. A mixture of different seeds with the addition of hazelnuts and walnuts has been used. Four muesli bar formulations were developed. Two formulations were with hazelnuts and two with walnuts, added in the amount of 25% and 50%, respectively. The other ingredients (sesame seeds, flaxseed, chia, sunflower seeds, pumpkin and oat flakes) were added in the same amount. Honey has been used as a sweetener.
Hazelnuts are good source of fats. The lipid fraction of hazelnuts is composed of non-polar and polar constituents. Major nonpolar lipids are the triacylglycerols. It is good source of monounsaturated fatty acid and polyunsaturated fatty acid. It contains predominantly palmitic acid, stearic acid, linoleic acid and linolenic acid. The omega-3 fatty acids are not synthesized by the human body. α-linolenic acid is precursor for omega-3 fatty acids [9].

Walnut is highly rich in terms of monounsaturated fatty acids. It is a perfect source of Omega 3 and arachidonic acid [10].Walnut contains much more antioxidants than the other hard shell nuts. Walnuts reduce: cholesterol, oxidative stresses caused by free radicals and the inflammations that damage the health [11].

Sesame seed contains 57–63% oil, 23–25% protein, 13.5% carbohydrate and 5% ash [12], [13]. Sesame is a rich source of iron, copper, manganese, magnesium and calcium [14]. The presence of sesamin (0.4-1.1) and sesamolin (0.3-0.6)% lignans contributes to oxidative stability and antioxidative activity. Sesamol is found in traces [15], [16].

Chemical composition of flaxseed shows that it contains 41% fat, 20% protein and 28% total dietary fibre. Flaxseed represents a good source of alpha-linoleic acid and phenolic compounds [17].

Chia seeds contain approximately 30–34 g dietary fiber. The insoluble fraction accounts for approximately 85–93%, while soluble dietary fiber is approximately 7–15% [18]. Chia seeds supply many minerals, such as: phosphorus (860–919 mg/100 g), calcium (456–631 mg/100 g), potassium (407–726 mg/100g) and magnesium (335–449 mg/100 g) [19].

100 g of sunflower seed contain 20.78 g protein, 51.46 g total lipid, 3.02 g ash, 20 g carbohydrate and 8.6 g fiber, with total energy of 2445 kJ. Sunflower seed is an excellent source of betaine and choline [20].

The pumpkin seed represents a good source of phosphorus, potassium and magnesium. It contains high amounts of other trace minerals, such as: calcium, sodium, manganese, iron, zinc, and copper. Elements like calcium, sodium, manganese, iron, zinc and copper make pumpkin seed valuable for food supplements [21].

Oat is a cereal that offers extra-functional properties as probiotics carriers over other cereals. Oats are rich source of β-glucan, antioxidant phenolic compounds, dietary lipids and soluble fibers [22].Compared with other cereals (wheat, rice, barley, buckwheat, and rice), oats contain higher content of protein. Oat lipids are rich in polyunsaturated fatty acids, Vitamin E, and plan sterols [23].

Honey can be used as a substitute for sugars, because it has a high content of fructose and a higher sweetness than refined sugars. Most sugars in honey are reducing sugars which are easily browned during baking and give a naturally darker color to the product. The flavor given by the honey to the product has proved to be desirable by consumers [24].

MATERIAL AND METHODS

Materials used for muesli bars preparation are: sesame seeds, flaxseed, chia, sunflower seeds, pumpkin seeds, oat flakes, hazelnuts, walnuts and honey.

PREPARATION OF MUESLI BARS

Muesli bars were made using a formulation described in Table 1. All ingredients were mixed and shaped in a shaper machine (Tupperware, USA). Hazelnuts, walnuts, pumpkin seeds and oat flakes were grinded in a grinder (Gorenje, Slovenia) before mixing. Other ingredients were added in a native form. The product was dried in a food dehydrator (Gorenje, Slovenia) at 80°C for six hours. During the preparation of this paper, the results of sensory analysis have shown that crispier products were more desirable, so the products were dried up to a moisture content of about 3%. Four muesli bar formulations were developed, two with hazelnuts and two with walnuts, added in the amount of 25% and 50%, respectively.

Table 1. Formulation for preparation of muesli bars

Materials	25% of hazelnut/walnut (g)	50% of hazelnut/walnut (g)
Hazelnuts/walnuts	90	260
Flaxseed	40	40
Sunflower seeds	20	20
Pumpkin seeds	20	20
Sesame	30	30
Chia	30	30
Oat flakes	60	60
Honey	60	60

DETERMINATION OF THE DRY MATTER CONTENT IN MUESLI BAR

Drying on 105°C in dryer until the constant mass in the muesli bar sample was used [25].

DETERMINATION OF THE TOTAL ASH IN MUESLI BAR

Burning on 600°C in laboratory furnace (Instrumentaria, Zagreb) was used. The total ash includes an inorganic leftover that remains after the burning and represents the total mineral content of the sample. During the burning, all the cations, beside ammonia cations, turn into carbonates or into others anhydrous inorganic salts. The total ash content was determined by standard gravimetric method [25].

DETERMINATION OF THE RAW FIBER CONTENT IN MUESLI BAR

The content of the raw fiber was determined by method upon Kurschner-Hanack. This method is based on insolubility of cellulose in water and its resistance to action of dilute acids and bases. The sample was degraded with a mixture of nitric acid and acetic acid and boiled in apparatus equipped with a Liebig's condenser. The solution was then filtered through a Büchner funnel. Then the filter paper containing an insoluble residue was dried in oven and measured [26].

DETERMINATION OF THE TOTAL AND REDUCED SUGARS IN MUESLI BAR

Reduction of Fehling's solution by a solution of reduced sugars in the muesli bar, using methylene blue as an indicator were used. The content of reducing sugars is determined by volumetric method upon Luff-Schoorl [25].

DETERMINATION OF THE SUCROSE CONTENT IN MUESLI BAR

The sucrose content is obtained from the difference between total and reduced sugars [25].

DETERMINATION OF THE FAT CONTENT IN MUESLI BAR

Soxhlet extraction method on the Soxhlet extractor (Velp Scientifica, Italy) was used. Petroleum-ether was used as organic solvent. The fat was collected by evaporating the solvent.

DETERMINATION OF THE PROTEIN CONTENT IN MUESLI BAR

Kjeldahl method on the Kjeltec TM 2300 device (Foss Tecator, Sweden) was used. Determination of the concentration of total nitrogen was conducted in three phases: wet burning of the sample, distillation and titration. In a cuvette the muesli bar sample was weighed and two catalyst tablets were placed and the sample was burned on the unit for burning. After the total burning, cuvette was transferred to the unit for a distillation. The strong base was added which caused a release of NH_3. NH_3 was transferred through the cooling system and treated with 1% boric acid which caused an increase of pH value in a solution. This solution was titrated with 0.1 N HCl. Bromine cresol and methil red were used as indicators. The concentration of ammonium ion was determined based on a volume of HCl that was needed for a titration.

DETERMINATION OF THE TOTAL POLYPHENOLS IN DOUGH AND MUESLI BAR

Folin-Ciocolteu method was used for total phenols determination using spectrophotometer (ThermoFisher Scientific, USA) at wavelength of 756 nm. Gallic acid was used to prepare the standard curve. 0.1 mL of prepared methanol extract was pipetted to the test tubes after which 1.9 mL of a distilled water, 10 mL of Follin-Ciocolteu reagent and 8 mL of sodium carbonate solution were added. Absorbance was measured after 2 hours. The blank was prepared with distilled water instead of the sample.

DETERMINATION OF THE ANTIOXIDANT CAPACITY IN DOUGH AND MUESLI BAR

FRAP method was used for antioxidant capacity determination using spectrophotometer (ThermoFisher Scientific, USA) at wavelength of 593 nm. $FeSO_4$ X $7H_2O$ was used to prepare the standard curve. 0.1 ml of prepared methanol extract was pipetted to the test tubes after which 3 mL of FRAP reagent was added. Absorbance was measured after 30 minutes. The blank was prepared with distilled water instead of the sample.

SENSORY ANALYSIS

For sensory analysis DLG method was used [27], [28]. Graded properties were: shape, color, surface; hardness, structure; chewiness; odour and taste.

RESULTS AND DISCUSSION

As shown in Table 2, dry matter content was similar in all samples, and it was from 96.784% to 97.644%. The addition of hazelnuts and walnuts did not cause major changes in the content of dry matter. The 50% addition of hazelnuts/walnuts caused a slight increase of dry matter content compared to the 25% addition of hazelnuts/walnuts. However, addition in higher amount caused decrease of the ash and raw fiber content, which can be seen in Table 1. The ash content was similar in all samples, and it was between 2.381% to 2.490%. It is interesting that the muesli bars with the addition of hazelnuts had much higher raw fiber content compared to the muesli bars with the addition of walnuts. That happened because 100 g of hazelnuts contains 10 g of raw fibers compared to the 100 g of walnuts, which contains 5 g of raw fiber [29].

Table 2. Dry matter, ash and raw fiber content in muesli bars

Sample	Dry matter content (%)	Ash content (%)	Raw fiber content (%)
25% of hazelnuts	96.784	2.468	7.067
50% of hazelnuts	97.688	2.381	6.966
25% of walnuts	97.360	2.490	2.690
50% of walnuts	97.644	2.407	2.390

Addition of hazelnuts/walnuts in higher amount caused a decrease of total and reduced sugars content compared to the 25% addition. The reduction was approximately 3%. The sucrose content was about the same in all the samples because all the sucrose came from honey which was used in the same amount for all formulations, which can be seen in Table 3.

Table 3. Total sugars, reduced sugars and sucrose content in muesli bars

Sample	Total sugars content (%)	Reduced sugars content (%)	Sucrose content (%)
25% of hazelnuts	14.000	11.600	2.280
50% of hazelnuts	11.000	7.600	3.230
25% of walnuts	13.400	10.000	3.230
50% of walnuts	10.000	6.200	3.610

No fat was added during the preparation of the muesli bars. All of the fat came from the used seeds and hazelnuts/walnuts. Addition of hazelnuts/walnuts in higher amount caused an increase of fat content and a decrease of protein content compared to the 25% addition, which can be seen in Table 4.

Table 4. Fat and protein content in muesli bars

Sample	Fat content (%)	Protein content (%)
25% of hazelnuts	24.888	25.112
50% of hazelnuts	36.432	24.340
25% of walnuts	33.419	27.706
50% of walnuts	39.695	27.580

As was expected drying in food dehydrator caused a slight decrease of total polyphenols and antioxidant capacity. Table 4 shows changes in the amount of total polyphenols and antioxidant capacity before and after drying. Addition of hazelnuts/walnuts in higher amount caused an increase of total polyphenols and antioxidant capacity compared to the 25% addition. The highest total polyphenols and antioxidant capacity was in the dough with 50% of walnuts. As for the product itself, the highest polyphenols content and antioxidant capacity was in the muesli bar with 50% of walnuts, which can be seen in Table 5.

Table 5. Total polyphenols content and antioxidant capacity in dough and muesli bars

Sample	Total polyphenols content (mg/L)	Antioxidant capacity (mmol/L)
Dough - 25% of hazelnuts	205.481	3428.333
Muesli bar - 25% of hazelnuts	107.333	3169.167
Dough - 50% of hazelnuts	331.901	4761.667
Muesli bar - 50% of hazelnuts	115.728	3425.000
Dough - 25% of walnuts	188.198	5263.333
Muesli bar - 25% of walnuts	173.753	4644.167
Dough - 50% of walnuts	877.086	6501.667
Muesli bar - 50% of walnuts	486.716	5084.167

As shown in Table 6, total sensory score was similar in all samples, and it was from 43.666 to 47.223. The muesli bar with the 25% of walnuts has had the highest sensory score, and the lowest goes to the muesli bar with the 50% of walnuts. All of the muesli bars were good graded. In general, the addition of 50% of hazelnuts or walnuts caused a decrease in sensory properties compared to the addition of 25%.

Table 6. Results obtained by sensory analysis of muesli bars samples (DLG method)

Graded property	25% of hazelnuts	50% of hazelnuts	25% of walnuts	50% of walnuts
shape, color, surface	4.778	4.000	4.889	3.667
hardness, structure	14.000	12.667	14.000	12.333
chewiness	15.000	14.333	14.667	14.333
odour	6.000	6.1667	6.500	6.500
taste	7.167	6.500	7.167	6.8333
Total score	**46.945**	**43.667**	**47.223**	**43.666**

CONCLUSION

Muesli bars can be made under minimal thermal treatment. Different seeds in combination with walnuts and hazelnuts represent a very good base for production of the muesli bars. All of these ingredients improve nutritive and sensory properties. The dry matter content was similar for all samples, within the expected range and appropriate values. The ash content was similar for all samples. The raw fiber content for the muesli bars made with hazelnuts was significantly higher than for the crackers with walnuts. Addition of hazelnuts/walnuts in higher amount caused a decrease of total and reduced sugars content compared to the 25% addition. The sucrose content was about the same in all the samples. Addition of hazelnuts/walnuts in higher amount caused an increase of fat content and a decrease of protein content compared to the 25% addition. Dough and muesli bar with 50% of walnuts had the highest total polyphenols content and antioxidant capacity, and the lowest results were obtained for dough and cracker with 25% of hazelnuts. The highest total sensory score was in muesli bar with 25% of walnuts. It can be concluded that the addition of 50% of hazelnuts or walnuts caused a decrease in sensory properties compared to the addition of 25%. However, all four muesli bars were good graded.

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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

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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.

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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 ";")

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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.

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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.

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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.
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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.

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