JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

SPECIES-DEPENDENT 5'-HYDROXYMETHYLFURFURAL FORMATION IN SLOWLY DRIED FRUITS

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doi: 10.15414/jmbfs.2021.10.4.586-591

ARTICLE INFO

Received 21. 2. 2019
Revised 2. 9. 2020
Accepted 5. 10. 2020
Published 1. 2. 2021

Regular article

OPEN ACCESS

ABSTRACT

The aim of the work was to determine 5-hydoxymethylfurfural (HMF) content in fruit samples dried by slow convective method. Additionally, the nutritional value, mineral composition and antioxidant activity were investigated as parameters confirming the quality of the product. Seven different kinds of dried fruits certified as ecological products (apricot, gooseberry, cherry, cornel, blackcurrant, plum and apple) were tested. Analysis covered: reducing sugars, protein content, moisture content, energy value, antioxidant activity, total phenolics content, mineral composition and HMF content (chromatographic and spectrophotometric method). Samples were abundant in reducing sugars (25-51% of dry weight), protein (1.0-5.4%), minerals (Ca, K, Fe, Mg) and were heavy metals-free. The strongest antioxidant activity for blackcurrant and the weakest for dried apple were observed. The highest average content of HMF was found in blackcurrant fruits (3102.2 mg/kg), while the lowest in apple (75.6 mg/kg). Results obtained by Winkler's and HPLC methods were strongly correlated (r = 0.976). It was found that applied slow convective drying method provide to obtain dried fruits rich in health-promoting properties and HMF quantified in examined fruits was found on the safety level for recommended daily consumption.

Keywords: 5-hydoxymethylfurfural, antioxidant activity, dried fruits, energy value, minerals, protein content

INTRODUCTION

Drying is one of the physical methods of food preservation based on water removal from dried products. Lower moisture content in dried food products causes inhibition of enzymatic processes and microorganisms development. The most often dried foods include mainly fruits and vegetables, allowing to ensure the availability of those important components of the human diet regardless from the season and prolonging their shelf life. Dried fruits are known as a source of antioxidants (e.g. flavonoids, anthocyanins, phenolic acids, carotenoids), micro- and macroelements, carbohydrates etc. Many literature reports about beneficial effects of dried fruits on human health. They may have antidiabetic (**Zhu *et al.*, 2011**), antibiotic (**Sengupta *et al.*, 2011**), cardioprotective (**Valentová *et al.*, 2007**), hepatoprotective (**Ahmed *et al.*, 2010; Yurt and Celik, 2011**) and even cancer chemopreventive effects (**Kundu and Chun, 2014**).
The crucial issue in drying processes is to preserve beneficial properties of dried fruits, such as nutritional value, polyphenols content and antioxidant activity. However, it is also important to maintain food safety standards. Due to water is a major component of most food products, dehydration causes many physicochemical changes in their structure. One of the important aspects of product quality after thermal processing is the presence of non-enzymatic browning reaction products, including Maillard products. Among them, toxic compounds should be distinguished, i.e. acrylamide, furane and its derivatives (furfural, 5-metyhylfurfural and 5-hydroxymethylfurfural (HMF) (**Michalska and Zieliński, 2007; Kowalski *et al.*, 2013**). HMF is a heterocyclic compound, a derivative of furan, containing both aldehyde and alcoholic functional groups. The main factor of its formation in foodstuff may be thermal processing, including drying. Because of high sugars content, especially fructose, and organic acids content, formation of HMF during thermal processing or long term storage is possible (**Murkovic and Pichler, 2006; Kowalski *et al.*, 2013; Akkaya and Karataş, 2016**). HMF as a main heat-induced food contaminant could be a risk factor for human, due to its potential toxic, effects. In living organism HMF could be transformed to 5-sulfooxymethyl-2-furaldehyde, exhibiting confirmed mutagenic action (**Lee *et al.* 1995**). To potentially harmful consequences of HMF presence in food products also cytotoxic, neurotoxic, genotoxic and even cancerogenic action may be included (**Teixidó *et al.*, 2011; Nikolov and Yaylan, 2011**). HMF level in food is often used as an indicator of product quality. There is a lack of results of HMF formation in dried fruits.

The main objective of our research was to determine HMF content in several fruit samples dried by slow convective method. The nutritional value, mineral composition and antioxidant activity was also investigated as indicators of the quality of fruits dried by slow convective method. In addition, HMF determination results obtained with two analytical methods were compared to assess their usefulness for estimating the content of this compound in the dried fruits.

MATERIAL AND METHODS

Samples

Dried fruits used for research were obtained directly from local manufacturer (AWB Alina Becla, Handzlówka, Poland) operating in Podkarpackie region in south-east Poland who has a certificate for ecological production and trade. The studies covered seven fruit species: apricot (*Prunus armeniaca* L.), gooseberry (*Ribes uva-crispa* L.), cherry (*Prunus cerasus* L.), cornel (*Cornus mas* L.), blackcurrant (*Ribes nigrum* L.), plum (*Prunus domestica* L.) and apple (*Malus domestica* L.), dried by slow convective method at the temperature 65-68°C and during 36-60 h (depending on the kind of fruit), without artificial additives and not candied. For drying of fruits, the trolley-chamber dryer of own design was used.

Extraction

Prior to extraction the fruit samples were grinded in MMK-060M grinder (MPM, Milanówek, Poland), then 2 g of each sample was weighted and 20 mL of distilled water was added. The extraction was carried out in ultrasonic bath (SONIC-10, Polsonic, Warszawa, Poland) for 60 min at 40°C. After sonication 20 mL of distilled water was added and the extract was filtered through medium hardness paper filter. For chromatographic analyses the extracts were additionally filtered through the 0,22 μm syringe filter. Every extract was prepared in two repetitions.

Reducing sugars

Reducing sugars content in dried fruits samples was determined using the dinitrosalicylic acid reagent (DNS) according to **Negrulescu *et al*. (2012)** with minor modification. DNS reagent contained a 1:1:1:1 volumetric mixture of 3,5-dinitrosalicylic acid (1%), Rochelle salt (potassium sodium tartrate tetrahydrate) (40%), phenol (0.2%) and potassium disulphide (0.5%), all dissolved in sodium hydroxide (1.5%). 1.5 mL of extract was mixed with 1.5 mL of such prepared DNS reagent and the mixture was heated at 90°C for 10 min and then cooled on an ice bath. The absorbance of samples was measured at 540 nm against blank (Biomate 3 spectrophotometer, ThermoFisher Scientific, Waltham, Massachusetts, USA) and the results were expressed as grams of glucose per kilogram of dried fruits based on calibration curve (Eq. 1) prepared in the range 0.20-1.85 mg/mL.

$$y = 0.4089x;\ R^2 = 0.9914 \qquad /1/$$

Protein content

Total protein was determined based on nitrogen content using carbon/hydrogen/oxygen analyzer TruSpec (LECO, Saint Joseph, Michigan, USA), which is based on the Dumas dry combustion technique. The grinded material was directly suspended to CHN analyzer according to ISO 16948:2015-07. Obtained nitrogen percentage (N) was calculated into protein content (P) by Eq. 2 and expressed as grams per kilogram.

$$P = N \times 6.25 \qquad /2/$$

Water content

The moisture analyzer MA50/1.R (Radwag, Radom, Poland), equipped with infrared heater was used for water content determination. Samples were heated at 105°C until their weight (in milligrams) was not changed within 60 s. Results were expressed as percent of water.

Energy value

The energy value of tested dried fruits was identified by AC 500 calorimeter (LECO) (oxygen bomb system) in accordance with ISO 18125:2017-07.

Mineral contents

The content of 18 elements (Al, As, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Sr and Zn) were determined by optical emission spectrometry with inductively-induced plasma (ICP-OES) using a ThermoiCAP 6500 spectrophotometer (ThermoFisher Scientific). Before the determination of elements, wet mineralization was carried out. The samples of dried fruits were weighed (about 1 g) into Teflon vessels and added to 8 mL of concentrated HNO3 (65%). The mineralization of the samples was performed using a microwave mineralizer Milestone Ethos Ultrawave-One (Milestone, Sorisole, Italy). A microwave-assisted heating program was implemented using 15 min for ramp time and 15 min for hold time at applied adjustable power (up to 1800 W). Temperatures were set at 150°C and 200°C. Maximum pressure was set at 1.2·107 Pa. After cooling, the samples were transferred quantitatively to flasks with a capacity of 50 mL and supplemented with redistilled water to the mark. Such prepared samples were analyzed by ICP-OES. The detection threshold obtained for each element was not lower than 0.01 mg/kg (with an assumed detection capacity of the measuring apparatus at a level exceeding 0,001 mg/kg. A curve fit factor for the studied elements was above 0.99. All the analyses were done in three independent repetitions.

Total phenolics content

Total phenolics content was determined using Folin-Ciocalteu reagent, according to **Singleton and Rossi (1965)** with minor modifications. 0.2 mL of each extract was added to 1 mL of 10% Folin-Ciocalteu reagent followed by 0.8 mL of Na_2CO_3 solution (75 g/L). After mixing the samples were kept in the dark for 120 min and then the absorbance was read at 760 nm against blank (Biomate 3 spectrophotometer). Results are expressed as milligrams of gallic acid equivalents (GAE) per kilogram of dry weight based on calibration curve (Eq. 3). The samples were prepared in triplicate and the mean value was calculated.

$$y = 0.0555x;\ R^2 = 0.9976 \qquad /3/$$

Antioxidant activity

DPPH assay

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical inhibition was measured according to assay described by **Blois (1958)** with minor modifications. The 0.2 mL of each extract was mixed with 1.8 mL of DPPH radical methanolic solution (0.1 mmol/L) and kept in the dark for 60 min. After incubation the absorbance of samples was measured at 517 nm against blank. The reduction of DPPH radical (R) was calculated using Eq. 4 and expressed in percent:

$$R = \frac{(A_0 - A_s)}{A_0} \times 100 \qquad /4/$$

where A0 is the absorbance of control, and As is the absorbance of samples with extract.
The analyses were performed in triplicates and the mean value was calculated.

FRAP assay

The ferric reducing antioxidant power (FRAP) assay was carried out as previously described by **Benzie and Strain (1996)** with some minor modifications introduced by **Bertoncelj *et al*. (2007)**. The FRAP reagent contained 2.5 mL of a 10 mmol/L 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) solution in 40 mmol/L HCl, 2.5 mL of 20 mmol/L $FeCl_3$ and 25 mL of 0.3 mol/L acetate buffer (pH 3.6). 1.8 mL of such prepared reagent was added to 0.2 mL of examined extract and the absorbance of the reaction mixture were measured spectrophotometrically (Biomate 3 spectrophotometer) at 593 nm after incubation at 37°C for 10 min against blank. The results were expressed as micromoles of Trolox equivalents (TE) per kilogram of fruits dry weight based on calibration curve (Eq. 5), prepared with 0.1 mmol/L Trolox in range (15 – 200 nmol). The analyses were performed in triplicates and the mean value was calculated.

$$y = 0.026x;\ R^2 = 0.998 \qquad /5/$$

5-hydroxymethylfurfural determination

Spectrophotometric method

Determination of HMF was made by Winkler's method according to the procedure modified by **Zappala *et al*. (2005)**. To 1 mL of filtered extract 2.5 mL of p-toluidine solution (10 g dissolved in 100 mL of isopropanol with 10% of acetic acid) was added, then 0.5 mL of barbituric acid solution (2.5 g/L) or water in case of blank sample was introduced. The samples were thoroughly mixed and after 3 min the absorbance was read ad 550 nm against blank Biomate 3 spectrophotometer (ThermoFisher Scientific). The samples were prepared in duplicates and the mean value was calculated. HMF content was calculated using prepared standard curve (Eq. 6).

$$y = 0.0364x,\ R^2 = 0.9968 \qquad /6/$$

HPLC method

The HPLC analyses were carried out in Laboratory of Plant Biotechnology Aeropolis (Podkarpackie Science and Technology Park, Rzeszów, Poland) using Gilson chromatographic set (Gilson, Middleton, Wisconsin, USA), equipped with binary pump (Gilson 322), diode-array detector (DAD) detector (Gilson 172), column thermostat (Knauer, Berlin, Germany) and autosampler with fraction collector (GX-271 Liquid Handler) according to **Polak-Śliwińska *et al*. (2013)**. The separation was performed using Eurosphere II RP-18H 100-5 column (250 mm × 4.6 mm, Knauer, Berlin, Germany) with precolumn (Gilson), operated at 35°C. The elution was performed isocratically with 10% methanol (in water) mobile phase, flow rate 1 mL/min, time of analysis 15 min. The injection volume of samples was 20 µl. Analyte was detected at wavelength 285 nm. For quantitative analysis of HMF the standard curve (Eq. 7) was prepared using standard solution of HMF within the concentration range 0.25–6.00 µg. The analyses were performed in duplicates and the mean value was calculated.

$$y = 5123.8x,\ R^2 = 0.9989 \qquad /7/$$

Statistical analysis

All assays were done in three repetitions. The results were expressed as mean values with standard deviations. The significant differences in the level of tested parameters depending on the kind of fruit were calculated by one-way analysis of variance followed by Tukey's honest significant difference test ($P < 0.05$). Correlations between tested parameters were established using Spearman's rank correlation coefficient (r). All calculations were done using StatSoft Statistica 13.1 software (StatSoft, Tulsa, Oklahoma, USA).

RESULTS AND DISCUSSION

Nutritional value

In the tested samples reducing sugars, protein content, water content and energy value were measured. The results are summarized in Tab 1.

The content of reducing sugars was ranging from 253.5 mg/kg to 510.3 mg/kg of dry weight, the highest value was tested for dried plum and the lowest for gooseberry. The protein content varied from 9.9 mg/kg to 54.0 mg/kg of dry weight. The obtained results are comparable to other authors findings. **Hussain *et al*. (2010)** tested the protein and reducing sugars content in several varieties of apricot from Pakistan, and found lower level of those parameters (10 g of protein and between 30 – 60 g/kg of sugars) as compared to our study. What is important, the nutrient composition varies depending on the fruit variety, it can be also influenced by the heat treatment method. Reducing sugars content at the level from 97.2 g/kg for rose hips to 612.1 g/kg for cranberry was acquired by **Cvetković *et al*. (2009)**. They obtained similar result for dried apricot (232.3 g·kg-1), for wild apple the sugar content was half as high in our sample, wherein the apple variety may play a big role.

Table 1 Nutrient composition of tested dried fruits.

Dried fruit	Reducing sugars [g/kg]	Proteins [g/kg]	Water [%]	Energy value [kJ/kg]
Apricot	318.7±8.6^{a}	32.5±2.8^{a}	10.0±0.7^{a}	14013±105^{a}
Gooseberry	253.5±32.9^{b}	52.1±7.7^{b}	6.0±0.2^{b}	15974±318^{b}
Cherry	454.9±22.2^{c}	38.2±5.0^{a}	11.9±2.8^{a}	14403±77^{ac}
Cornel	411.7±24.5^{c}	10.6±3.3^{c}	5.5±0.4^{bc}	14562±276^{c}
Blackcurrant	329.3±12.2^{a}	54.0±4.7^{b}	3.4±0.8^{c}	15622±85^{b}
Plum	510.3±5.6^{d}	9.9±6.8^{c}	8.2±0.1^{ab}	14454±110^{c}
Apple	500.5±13.2^{d}	22.0±6.8^{d}	4.2±1.4^{bc}	12151±153^{d}

Data are presented as mean value ± standard deviation (n = 3) per kilogram of dry weight. Means sharing the same superscript in a column are not significantly different from each other (Tukey's HSD test, P < 0.05).

The percentage of water content in fruit samples is an indicator of drying efficiency. Among tested samples, the most dried ones were blackcurrant and apple, while the most water remained in cherry and apricot. Moisture content in several dried fruits and vegetables was determined by **Lutz *et al*. (2015)**, the lowest moisture content was observed in eggplant sample (0.2%), the highest for red delicious apple cultivar (5.8%) and vegetables, such as pepper or tomatoes (up to 7.7%). The water content within 10% was also marked by **Hussain *et al*. (2010)**. Fruits generally contained a large amount of water, so the results we obtained (moisture level not exceeded 10% of dried weight) indicate the effectiveness of the drying process.

Energetic value of all tested products was in the range from 12151 kJ/kg of dried apple to 15974 kJ/kg of dried gooseberry. Similar energy values are given by United States Department of Agriculture (USDA) (27): the most energetic are cranberries (12890 kJ/kg of dried weight), the least – apples (10170 kJ/kg of dried weight). This data are in this area consistent with ours, the fruits can be ranked by terms of energetic value from apples to berry fruits (cranberry or gooseberry). The database also contains data about among other parameters protein, sugars and water content.

Protein content ranged in this case from 7 g/kg for cranberries to 40.8 g/kg for blackcurrants, just like in our case the sample of blackcurrant was characterized by the highest protein content. The order of magnitude also agrees for sugars content (381.3 g/kg for prunes to 725.6 g/kg for cranberries). The fruits included in this data were characterized by a much higher water content, which probably results from differences in drying technology (**USDA, 2015**).

Mineral composition

Fruits and vegetables are rich source of minerals, they can provide the human organism with both macro- and microelements. The results of mineral composition determined for tested fruit samples are shown in Tab 2.

Table 2 Mineral composition of dried fruits.

Element content [mg/kg]	Apricot	Gooseberry	Cherry	Cornel	Blackcurrant	Plum	Apple
Al	27.3±0.9^{a}	31.0±2.0^{a}	5.2±1.8^{bc}	8.7±2.2^{bc}	1.9±0.6^{b}	10.9±2.5^{c}	7.8±1.8^{c}
Ca	660±25^{a}	1670±306^{b}	1289±39^{c}	4511±12^{d}	989±20^{e}	1620±22^{b}	360±13^{f}
Cr	12.8±0.4^{a}	0.6±0.2^{b}	nd	nd	0.8±0.1^{b}	0.9±0.2^{b}	0.1±0.0^{b}
Cu	9.4±0.5^{a}	2.5±0.3^{b}	24.4±0.4^{c}	5.1±0.5^{d}	2.6±0.5^{b}	7.1±0.6^{e}	2.0±0.5^{b}
Fe	432.7±9.7^{a}	172.6±20.0^{b}	91.1±1.1^{c}	67.5±7.6^{c}	78.1±2.9^{c}	81.8±1.8^{c}	37.4±2.0^{d}
K	27880±134^{a}	15000±310^{b}	15689±692^{b}	7311±100^{c}	14731±670^{b}	9578±150^{d}	6642±110^{c}
Mg	670±26^{a}	780±35^{b}	1100±28^{c}	1112±300^{d}	778±29^{b}	440±42^{e}	451±32^{e}
Mn	6.4±0.4^{abc}	20.0±1.4^{d}	15.6±0.6^{e}	7.8±1.3^{a}	25.6±1.5^{d}	6.8±0.6^{be}	3.9±1.0^{c}
Mo	0.1±0.0^{a}	0.1±0.0^{a}	nd	0.1±0.0^{a}	nd	nd	0.1±0.0^{a}
Na	10.2±0.6^{a}	16.4±0.5^{b}	nd	17.1±0.6^{b}	9.8±0.9^{a}	4.8±0.8^{c}	33.2±1.1^{d}
Ni	1150±22.2^{a}	1200±51.4^{a}	370±23^{bcd}	263±54^{e}	300±18^{bf}	290±25^{def}	453±22^{c}
P	1970±67^{a}	3410±49^{b}	1952±5^{a}	1730±32^{c}	2940±96^{d}	1171±67^{e}	711±39^{f}
S	540±22^{ab}	841±42^{c}	503±30^{a}	592±44^{b}	871±29^{c}	130±31^{d}	220±20^{d}
Sr	4.5±0.5^{ab}	5.5±1.0^{a}	3.6±0.3^{b}	17.9±0.8^{c}	5.6±0.4^{a}	4.1±0.1^{ab}	1.9±0. 2^{d}
Zn	8.7±0.7^{a}	9.8±0.9^{a}	32.6±0.7^{b}	6.3±0.4^{c}	nd	39.6±1.2^{d}	1.1±0.2^{e}
As	nd	nd	nd	nd	nd	nd	nd
Cd	0.1±0.0^{a}	0.1±0.0^{a}	nd	0.1±0.0^{a}	nd	nd	nd
Pb	nd	nd	nd	nd	nd	nd	nd

Data presented as mean value ± standard deviation (n = 3) per kilogram of dry weight. Means sharing the same superscript in a row are not significantly different from each other (Tukey's HSD test, P<0.05). nd – not detected.

The most abundant element in all the fruits studied was potassium – the highest level of such mineral was found in apricot (28 g/kg of dry weight). Calcium was the second element found in big quantities in tested fruits - the most in cornel. Apricots stood out also with high content of iron, more than twice as many as in the second in order gooseberry. Relatively poor in microelements were cherries, sodium, molybdenum and chromium were not detected in them. Importantly, toxic elements (arsenic, mercury, lead and cadmium) were not detected in any of the fruits. Obtained results can be compared with data about content of some elements has been provided by the **USDA (2015)**. The highest potassium content is also given for apricots, which confirms that these fruits are a particularly rich source of this element. Other determined elements (Ca, Fe, Mg, Na, Cu) are generally in a similar order of magnitude. Apricots also showed high content of potassium in the study of Serbian authors (**Cvetković *et al*., 2009**). In turn, particularly rich in calcium and iron were wild apples and rose hips.

Total phenolics content and antioxidant capacity

The results of total phenolics content determination and antioxidant capacity measured by two methods DPPH and FRAP are summarized in Tab 3.

Table 3 Total phenolics content and antioxidant activity of dried fruits

Dried fruit	Total phenolics content [mg/kg]	Antioxidant activity	
		FRAP [µmol/kg]	DPPH [%]
Apricot	1456.8±22.7^{a}	3850.0±104.4^{a}	72.6±0.5^{a}
Gooseberry	583.3±30.9^{b}	2283.7±75.7^{b}	67.9±0.7^{b}
Cherry	6465.8±135.0^{c}	9488.5±63.3^{c}	89.5±0.3^{c}
Cornel	931.5±156.5^{d}	6009.6±869.5^{d}	89.9±0.2^{c}
Blackcurrant	1652.7±62.7^{e}	7184.6±299.5^{e}	83.3±1.6^{d}
Plum	888.3±25.0^{d}	2190.4±104.4^{b}	68.4±0.5^{b}
Apple	400.0±6.2^{b}	1265.4±66.6^{f}	66.3±0.1^{b}

Data presented as mean value ± standard deviation (n = 3); Means sharing the same superscript in a column are not significantly different from each other (Tukey's HSD test, P<0.05).

Total phenolics content is expressed as milligrams of gallic acid equivalents per kilogram of dry weight. FRAP – ferric reducing antioxidant power (expressed as micromoles of Trolox equivalents per kilogram), DPPH – 2,2-Diphenyl-1-picrylhydrazyl (expressed as percentage reduction of DPPH radical)

Among examined fruit extracts cherry showed the highest phenolics content, above 6 g/kg of dry weight (expressed as GAE) and the significant amount of polyphenols has also been detected in apricot and blackcurrant. It is probably related with high flavonoids content in these fruits, including anthocyanins, responsible for dark color of fruits. The content of polyphenols in apricot extract (1456.8 mg/kg of dry weight) is comparable to the result obtained by **Erdoğan**

and Erdemoğlu (2011) in the case of using water as a solvent. The lowest phenolics content were found in apple and gooseberry extracts. However, it should be remembered, that the Folin-Ciocalteu assay used for phenolics determination is a non-specific method, a positive result may also give other compounds, such as amino acids, sugars and metal complexes (**Everette *et al.*, 2010**).

Antioxidant capacity was tested with use of DPPH radical reduction method and FRAP method. In DPPH assay the highest antiradical effect showed cherry, cornel and blackcurrant extracts (above 80% of DPPH radical inhibition), similarly FRAP antioxidant power is the largest for the same samples. Total phenolic content is a parameter highly related to antioxidant capacity of tested extracts, this is confirmed by a significant correlation coefficients for both used methods (r = 0.832 for FRAP and r = 0.682 for DPPH method). Also between both antioxidant assays we observed high correlation (r = 0.947). The highest total phenolics content and also the strongest antiradical properties were usually obtained for berry fruits, like blackberry, blueberry (**Lutz *et al.*, 2015**), chokeberry, billberry (**Miletić *et al.*, 2014**) or raisins (**Oukhemoukh *et al.*, 2012**). The high content of antioxidant compounds has been observed for apricot samples, it was also confirmed, that processing parameters and sample extraction method significantly affect the total polyphenols content, also the phenolic profile and individual content of flavonoids and phenolic acids (**Madrau *et al.*, 2008; Čanadanović-Brunet *et al.*, 2013**). Relatively often studied dried fruit species are apples (**Joshi *et al.*, 2011; Schulze *et al.*, 2014**). In the case of these fruits it was confirmed that additionally the polyphenols content and profile and also antioxidant effect is influenced by the apple cultivar. Because the tests are done by different methods, the way of sample preparation (antioxidants extraction) is also different, the results cannot be directly compared.

5-hydroxymethylfurfural content

HMF was quantitated in fruit extracts by Winkler's spectrophotometric method and chromatographic method (HPLC-DAD). Identification of HMF peak on chromatograms was performed on the basis of retention time and UV-Vis spectrum comparison (Fig 1).

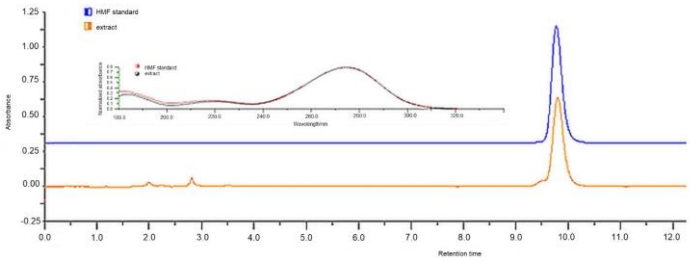

Figure 1 Chromatographic analyses results for example fruit extract – overlayed chromatograms of extract and 5-hydroxymethylfurfural standard, (inset – UV-Vis spectra comparison)

The highest HMF content, measured with the use of both analytical methods, was determined in blackcurrant and cherry samples, above 2500 mg/kg and 2000 mg/kg of dry weight, respectively (Fig 2). Both analytical protocols used by us to quantify HMF in fruit samples yielded comparable results, the correlation coefficient of both methods was 0.976 (Fig 3.), which showed very good compatibility. A chromatographic method could be a good alternative to spectrophotometric Winkler's assay in which p-toluidine, a known carcinogen is used.

HMF has been also determined in preserves of these fruits (sherbets) by Polish scientists, but their results indicate much lower level of this compound – order of micrograms per 100 grams of product (**Rój and Przybyłowski, 2014**). In our case blackcurrant and cherry were in a dried form, the drying process probably strongly influenced the formation of HMF. The lowest level of HMF we observed in apple, only 63.4 mg/kg determined with Winkler method and 87.8 mg/kg determined with HPLC. There is no data about HMF quantitation in dried apple, available data about HMF content in apple juice indicate content of HMF at the level of a few milligrams per liter (**Akkaya and Karataş, 2016**), but the data are not directly comparable. Meanwhile, data about HMF content in dried plum and apricot are available in literature. In plum samples the variable content: from 220–290 mg/kg (**Donovan *et al.*, 1998; Michalska and Łysiak, 2014**) up to 1600–2200 mg/kg (**Murkovic and Pichler, 2006**) was registered. In the case of dried apricot the content was specified from 7–22 mg/kg (**Kowalski and Łukasiewicz, 2012**) even to 780 mg/kg (**Murkovic and Pichler, 2006**). The differences arise most likely from drying technology and conditions or also analytical method used to determine the quantitative level of HMF.

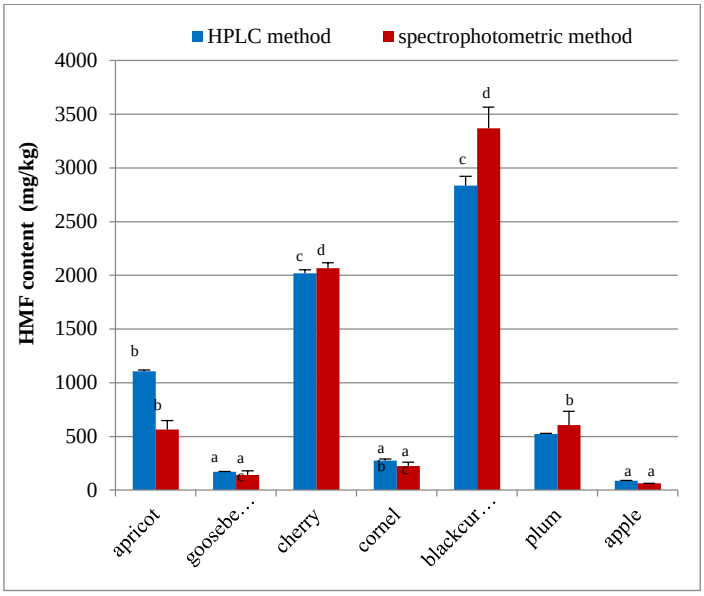

Figure 2 5-hydroxymethylfurfural content in dried fruits samples quantified by two methods. Letters (a, b c, d) above bars means data that differs significantly according to Tukey's test ($P \leq 0.05$)

The indicators of acute toxicity for HMF are relatively high which indicates that its toxicity is rather low. An acute oral lethal dose, 50% (LD_{50}) value for rats amounts 3100 mg/kg body weight, for mice 1910 mg/kg body weight (**Ulbricht *et al.*, 1984**). It can be inferred from this data that also for humans values of several dozens or even several hundreds of HMF in 100 g of dried fruits should not cause toxic effects. Assuming 20 grams as a daily portion of dried fruits, the amount of HMF consumed in such a portion according to obtained data is from 1.3 mg in apples to 18.2 mg in blackcurrant. After conversion into kilograms of body weight for an average person with a weight 60 kg it gives the result 22–303 µg/kg, which is a safe value, regarding the data available for animals. Because of limited data and deficiencies in toxicological studies, currently the tolerable daily intake for humans cannot be determined. The suggested NOAEL (No-Observed Adverse Effect Level) for HMF in animal experiments is ca. 80-100 mg/kg body weight per day (**Abraham *et al.*, 2011**). The dose calculated above falls within this range. However, it must be remembered about the other sources of HMF in food.

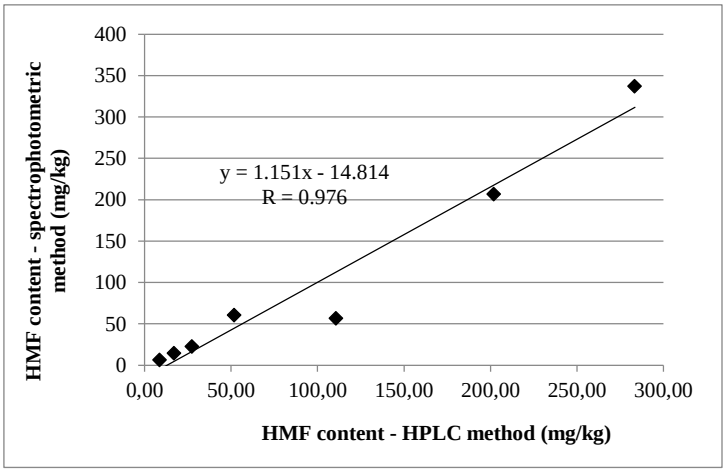

Figure 3 Correlation between data of 5-hydroxymethylfurfural content obtained by spectrophotometric and chromatographic method

HMF formation during drying process as well as long-term storage of food products is a well-known fact, although the content of this compound was determined by several authors only in dried fruits. **Del Caro *et al.* (2004)** described effect of drying conditions and storage time on fruit properties, including HMF level. They observed an initial decrease of HMF content during storage of prunes (after 4 months) and the maximal level of this molecule after 12 months in the case of plums dried at temperature 85°C and continuous increase in this value in the case of fruits dried at 65°C. Moreover, the fruits dried at a higher temperature contained more HMF. Along with the increase in HMF content, higher antioxidant capacity was also observed. A positive correlation between these values suggest a connection of Maillard reaction products formation and antiradical activity (**Del Caro *et al.*, 2004**). Also in present study we tested the connection between HMF content and antioxidant capacity (r = 0.787 for HMF

content and FRAP capacity and r = 0.580 for HMF content and DPPH inhibition). Moreover, a very good correlation was also observed for HMF and total phenolics content values (r = 0.924). A group of scientists from Iran studied content and bioaccessibility of HMF in dried fruits samples commercially available in their country (**Rahimzadeh *et al*., 2014**). They found the highest content in blackcurrant and plum and proved that HMF is biologically available after digestion (in vitro gastrointestinal digestion model) up to about 95% in case of berries. High content of HMF in dried blackcurrant, plum and apricot was also confirmed by other authors (**Mousavi *et al*., 2016**). Moreover, it was observed that chlorogenic acid presence in systems containing glucose and some amino acids significantly increases formation of HMF at elevated temperature (**Jiang *et al*., 2013**). As chlorogenic acid is one of the most common polyphenols in different species of fruits, so it may contribute to increase in HMF level during drying.

CONCLUSION

All tested dried fruits samples, originating from the ecological manufacture, were characterized by high nutritional value, content of essential micro- and macroelements, polyphenols and antioxidant capacity as compared to available literature data. The results proves the high quality of fruits dried by slow convective method. Applied method provide to obtain dried fruits rich in health-promoting properties and the HMF quantified in examined fruits was found on the safety level for recommended daily consumption. Moreover, for the first time good correlation between assessment of HMF in dried fruit by HPLC and spectrophotometric methods was establish.

REFERENCES

ABRAHAM, K., GÜRTLER, R., BERG, K., HEINEMEYER, G., LAMPEN, A., APPEL, K. E. 2011. Toxicology and risk assessment of 5-Hydroxymethylfurfural in food. *Molecular Nutrition & Food Research*, 55(5), 667-678. http://dx.doi.org/10.1002/mnfr.201000564

AHMED, T., SADIA, H., KHALID, A., BATOOL, S., JANJUA, A. 2010. Report: Prunes and liver function: A clinical trial. *Pakistan Journal of Pharmaceutical Sciences*, 23, 463-436.

AKKAYA, D. E., KARATAŞ, Ş. 2016. Determination of hydroxymethylfurfural contents of some apple juices on the Market by HPLC method. *International Journal of Food Engineering Research*, 2, 19-27.

BENZIE, I. F. F., STRAIN, J. J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant Power": The FRAP assay. *Analytical Biochemistry*, 239(1), 70-76. http://dx.doi.org/10.1006/abio.1996.0292

BERTONCELJ, J. J., DOBERŠEK, U., JAMNIK, M., GOLOB, T. 2007. Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. *Food Chemistry* 105(2), 822-828. http://dx.doi.org/10.1016/j.foodchem.2007.01.060

BLOIS, M. 1958. Antioxidant determinations by the use of a stable free radical. *Nature*, 181, 1199-1200. http://dx.doi.org/10.1038/1811199a0.

ČANADANOVIĆ-BRUNET, J. M., VULIĆ, J., ĆETKOVIĆ, G. S., DJILAS, S. M., ŠAPONJAC, V. T. 2013. Bioactive compounds and antioxidant properties of dried apricot. *Acta Periodica Technologica*, 44, 193-205. http://dx.doi.org/10.2298/APT1344193C

CVETKOVIĆ, B. R., FILIPČEV, B. V., BODROŽA-SOLAROV, M. I., BARDIĆ, Ž. M., SAKAČ, M.B. 2009. Chemical composition of dried fruits as a value added ingredient in bakery product. *Food Processing, Quality and Safety*, 1-2, 15-19.

DEL CARO, A., PIGA, A., PINNA, I., FENU, P.M., AGABBIO, M. 2004. Effect of drying conditions and storage period on polyphenolic content, antioxidant capacity, and ascorbic acid of prunes. *Journal of Agricultural and Food Chemistry*, 52(15), 4780-4784. http://dx.doi.org/10.1021/jf049889j

DONOVAN, J. L., MEYER, A. S., WATERHOUSE, A. L. 1998. Phenolic composition and antioxidant activity of prunes and prune juice (*Prunus domestica*). *Journal of Agricultural and Food Chemistry*, 46(4), 1247-1252. http://dx.doi.org/10.1021/jf970831x

ERDOĞAN, S., ERDEMOĞLU, S. 2011 Evaluation of polyphenol contents in differently processed apricots using accelerated solvent extraction followed by high-performance liquid chromatography–diode array detector. *International Journal of Food Sciences and Nutrition*, 62(7), 729-739. http://dx.doi.org/10.3109/09637486.2011.573469

EVERETTE, J. D., BRYANT, Q. M., GREEN A. M., ABBEY, Y. A., WANGILA, G. W., WALKER, R. B. 2010. Thorough study of reactivity of various compounds classes toward the Folin-Ciocalteu reagent. *Journal of Agricultural and Food Chemistry*, 58(14), 289-295. http://dx.doi.org/10.1021/jf1005935

HUSSAIN, A., YASMIN, A., ALI, J. 2010. Comparative study of chemical composition of some dried apricot varieties grown in northern areas of Pakistan. *Pakistan Journal of Botany*, 42, 2497-2502.

JIANG, S. S., OU, S. Y., LIANG, E., YU, M., HUANG, C. H., ZHANG, G. W. 2013. Effect of chlorogenic acid on hydroxymethylfurfural in different Maillard reaction systems. *International Food Research Journal*, 20,1239-1242.

JOSHI, A. P. K., RUPASINGHE, H. P. V., KHANIZADEH, S. 2011. Impact of drying processes on bioactive phenolics, vitamin c and antioxidant capacity of red-fleshed apple slices. *Journal of Food Processing and Preservation*, 35(4), 453-437. http://dx.doi.org/10.1111/j.1745-4549.2010.00487.x

KOWALSKI, S., ŁUKASIEWICZ, M. 2012. Occurrence of 5-hydroxymfthyl-2-furfural (HMF) in selected food products. *Bromatologia i Chemia Toksykologiczna* XLV, 1239-2415 (in Polish).

KOWALSKI, S., ŁUKASIEWICZ, M., DUDA-CHODAK, A., ZIĘĆ, G. 2013. 5-Hydroxymethyl-2-Furfural (HMF) – heat-induced formation, occurrence in food and biotransformation – a review. *Polish Journal of Food and Nutrition Sciences*, 63(4), 207-225. http://dx.doi.org/10.2478/v10222-012-0082-4

KUNDU, J. K., CHUN, K. S. 2014. The promise of dried fruits in cancer chemoprevention. *Asian Pacific Journal of Cancer Prevention*, 15(8), 3343-3352. http://dx.doi.org/10.7314/APJCP.2014.15.8.3343

LEE, Y. C., SHLYANKEVICH, M., JEONG, H. K., DOUGLAS, J. S., SURH, Y. J. 1995. Bioactivation of 5-hydroxymethyl-2-furaldehyde to an electrophilic and mutagenic allylic sulfuric acid ester. *Biochemical and Biophysical Research Communications*, 209(3), 996-1002. http://dx.doi.org/10.1006/bbrc.1995.1596

LUTZ, M., HERNÁNDEZ, J., HENRÍQUEZ, C. 2015 Phenolic content and antioxidant capacity in fresh and dry fruits and vegetables grown in Chile. *CyTA Journal of Food*, 13(4), 541-547. http://dx.doi.org/10.1080/19476337.2015.1012743

MADRAU, M. A., PISCOPO, A., SANGUINETTI, A. M., DEL CARO, A., POIANA, M., ROMEO, F. V., PIGA, A. 2008. Effect of drying temperature on polyphenolic content and antioxidant activity of apricots. *European Food Research and Technology*, 228, 441-448. http://dx.doi.org/10.1007/s00217-008-0951-6

MICHALSKA, A., ŁYSIAK, G. 2014. Applicability of plums grown in Poland to drying process in terms of changes in bioactive compounds and of Maillard reaction products being formed. *Żywność: nauka - technologia – jakość*, 21(6), 29-38 (in Polish).

MICHALSKA, A., ZIELIŃSKI, H. 2007. Maillard reaction products in food. *Żywność: nauka - technologia – jakość*, 2(51), 5-16 (in Polish).

MILETIĆ, N., POPOVIĆ, B., MITROVIĆ, O., KANDIĆ, M., LEPOSAVIĆ, A. 2014. Phenolic compounds and antioxidant capacity of dried and candied fruits commonly consumed in Serbia. *Czech Journal of Food Sciences*, 32, 360-368.

MOUSAVI, R., ALIZADEH, M., SALEH-GHADIMI, S. 2016. Consumption of 5-hydroxymethylfurfural-rich dried fruits is associated with reduction in urinary excretion of 8-hydroxy-2'-deoxyguanosine: a randomized clinical trial. *European Food Research and Technology*, 242(5), 677-684.http://dx.doi.org/10.1007/s00217-015-2575-y

MURKOVIC, M., PICHLER, N. 2006. Analysis of 5-hydroxymethylfurfual in coffee, dried fruits and urine. *Molecular Nutrition and Food Research*, 50(9),842-846. http://dx.doi.org/10.1002/mnfr.200500262

NEGRULESCU, A., PATRULEA, V., MINCEA, M. M., IONASCU, C., VLAD-OROS, B. A., OSTAFE, V. 2012. Adapting the reducing sugars method with dinitrosalicylic acid to microtiter plates and microwave heating. *Journal of the Brazilian Chemical Society*, 23(12), 2176-2178. http://dx.doi.org/10.1590/S0103-50532013005000003

NIKOLOV, P. Y., YAYLAN, V. A. 2011. Reversible and covalent binding of 5-(hydroxymethyl)-2-furaldehyde (HMF) with lysine and selected amino acids. *Journal of Agricultural and Food Chemistry*, 59(11), 6099-6107. http://dx.doi.org/10.1021/jf200735c

OUCHEMOUKH, S., HACHOUD, S., BOUDRAHAM, H., MOKRANI, A., LOUAILECHE, H. 2012. Antioxidant activities of some dried fruits consumed in Algeria. *LWT-Food Science and Technology*, 49(2), 329-332. http://dx.doi.org/10.1016/j.lwt.2012.07.022

POLAK-ŚLIWIŃSKA, M., ŁAMEJKO, Ł., KUBIAK, M. S. 2013. Patulin and 5-HMF content in fruit and vegetable juices from ecological and commercial production. *Bromatologia i Chemia Toksykologiczna*, XLVI, 80-88 (in Polish).

RAHIMZADEH, N., ALIZADEH, M., HEZAVEH, S. J. G. 2014. Estimated bioaccessibility to 5-hydroxymethylfurfural from frequently consumed dried fruits in Iran. *Journal of Chemical Health Risks*, 4, 15-23.

RÓJ, A., PRZYBYŁOWSKI, P. 2014. Estimate the content of HMF in fruit sorbets. *Przemysł Spożywczy,* 68, 27-28 (in Polish).

SCHULZE, B., HUBBERMANN, E. M., SCHWARZ, K. 2014. Stability of quercetin derivatives in vacuum impregnated apple slices after drying (microwave vacuum drying, air drying, freeze drying) and storage. *LWT-Food Science and Technology*, 57(1), 426-433. http://dx.doi.org/10.1016/j.lwt.2013.11.021

SENGUPTA, K., ALLURI, K. V., GOLAKOTI, T., GOTTUMUKKALA, G. V., RAAVI, J., KOTCHRLAKOTA, L., SIGALAN, S. C., DEY, D., GHOSH, S., CHATTERJEE, A. 2011. A randomized, double blind, controlled, dose dependent clinical trial to evaluate the efficacy of a proanthocyanidin standardized whole cranberry (*Vaccinium macrocarpon*) powder on infections of the urinary tract. *Current Bioactive Compounds*, 7(1), 39-46. http://dx.doi.org/10.2174/157340711795163820

SINGLETON, V. L., ROSSI, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*,16, 144-158.

TEIXIDÓ, E., NÚÑEZ, O., SANTOS, F. J., GALCERAN, M. T. 2011. 5-Hydroxymethylfurfural content in foodstuffs determined by micellar electrokinetic chromatography. *Food Chemistry*, 126(4), 1902-1908. http://dx.doi.org/10.1016/j.foodchem.2010.12.016

ULBRICHT, R. J., NORTHUP, S. J.,THOMAS, J. A. 1984. A review of 5-hydroxymethylfurfural HMF in parental solutions, *Fundamental and Applied Toxicology*, 4(5), 843-853. http://dx.doi.org/10.1016/0272-0590(84)90106-4

USDA. 2015. National Nutrient Database for Standard Reference, Release 28 (revised), Beltsville, Maryland (USA). Beltsville Human Nutrition Research Center, Available form: http://www.ars.usda.gov/ba/bhnrc/ndl

VALENTOVÁ, K., STEJSKAL, D., BEDNÁŘ, P., VOSTÁLOVÁ, J., ČÍHALÍK, Č., VEČEŘOVÁ, R., KOUKALOVÁ, D., KOLÁŘ, M. REICHENBACH, R., ŠKŇOUŘIL, L., ULRICHOVÁ, J., ŠIMÁNEK, V. 2007. Biosafety, antioxidant status, and metabolites in urine after consumption of dried cranberry juice in healthy women: A Pilot double-blind placebo-controlled trial. *Journal of Agricultural and Food Chemistry*, 55(8), 3217-3224. http://dx.doi.org/10.1021/jf0636014

YURT, B., CELIK, I. 2011. Hepatoprotective effect and antioxidant role of sun, sulphited-dried apricot (*Prunus armeniaca* L.), and its kernel against ethanol-induced oxidative stress in rats. *Food and Chemical Toxicology*, 49(2), 508-513. http://dx.doi.org/10.1016/j.fct.2010.11.035

ZAPPALA, M., FALLICO, B., ARENA, E., VERZERA, A. 2005. Methods for the determination of HMF in honey: a comparison. *Food Control*, 16(3), 273-277. http://dx.doi.org/10.1016/j.foodcont.2004.03.006

ZHU, M., HI, J., PEREZ, E., PHILLIPS, D., KIM, W., GHAEDIAN, R., NAPORA, J. K., ZOU, S. 2011. Effects of long-term cranberry supplementation on endocrine pancreas in aging rats. *The Journals of Gerontology: Series A*, 66A(11), 1139-1151. http://dx.doi.org/10.1093/gerona/glr105