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THE INFLUENCE OF THE FOOD SURFACE AND MATRIX ON NOROVIRUS DETECTION BY RT-qPCR

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ABSTRACT

Human noroviruses are predominant cause of epidemic and sporadic food-borne gastroenteritis in industrialized countries. Priority food categories, vehicles of viruses in many reported outbreaks, beside shellfish are food of plant origin including berries, lettuce, tomatoes and green onions. These items, as well as deli ham are often components of many ready-to-eat foods.
The aim of this study was to assess the effect of different food matrices on norovirus detection. Stool sample with previously determined concentration of norovirus GII particles, was used to inoculate surface of lettuce, tomatoes, green onions and deli ham slices. For virus RNA extraction the direct method was used followed by RT-qPCR assay with SYBR green one-step kit and degenerate MON primers for the detection of GII norovirus. Obtained mean Ct values and Ct alterations calculated for each level of inoculation revealed that tomato had no inhibitory effect on NoV GII detection, followed by one log decrease of RT-PCRU due to properties of green onions, and two log decrease for lettuce and deli ham slices.
The adsorption of viruses to different solid surfaces depends on their complexity and food surface factors such as presence of hydrophobic domains and acid/base groups, virus specific ligands, etc. This is important since many fruits and vegetables have complex surfaces that prevent the removal of viruses by simple washing and may decrease desiccation effects that could lead to virus inactivation.

***Keywords**: noroviruses, food, detection, RNA extraction, RT-qPCR*

INTRODUCTION

Human enteric viruses, e.g. noroviruses, hepatitis A virus, rotaviruses, hepatitis E virus, are progressively identified as the leading cause of foodborne diseases worldwide. According to the latest report of EFSA and ECDC (2015), the most foodborne outbreaks, in 32 European countries in 2014, were caused by viruses. In the United States, estimation is that 31 pathogens cause 9.4 million foodborne illnesses, 128,000 hospitalizations, and 3,000 deaths annually (CDC, 2014). Viruses are at the leading position causing an estimated 58% (5.5 million) of those foodborne illnesses (Scallan *et al*., 2011). From all foodborne viruses, norovirus is recognized as a main cause of epidemic and sporadic foodborne gastroenteritis, therefore representing substantial public health burden (Head and Lopman, 2016). Generally, acute gastroenteritis (AGE) causes a significant global disease burden estimated at 89.5 million disability-adjusted life-years (DALYs) annually (Murray *et al*., 2012), and norovirus is associated with around one fifth of AGE episodes worldwide (Ahmed *et al*., 2014). The introduction of rotavirus vaccines has led to tremendous reductions in the AGE burden in some European states (Karafillakis *et al*., 2015), as well as in countries with national programs, which has, in turn, made norovirus the primary cause of AGE across all age groups. One of the most sensitive population group are young children, since the incidence among children aged <5 years is about 4 times higher than for those aged ≥5 years (O'Brien *et al*., 2016). Moreover, among young children and the elderly person severe and prolonged illness which lead to hospitalization is more frequently reported, as well as an important cause of chronic gastroenteritis for immunocompromised patients (Bok and Green, 2012).

Noroviruses belong to the Caliciviridae family, which is composed of five genera, of which *Norovirus* and *Sapovirus* genera contain primarily human viruses, while the other genera contain animal viruses (Clark *et al*., 2012). Human noroviruses are nonenveloped RNA viruses, approximately 27 to 38 nm in diameter, icosahedral in shape and contain single-stranded positive-sense RNA genomes ranging in size from 7.4 to 8.3 kb and, except for murine norovirus, contains 3 open reading frames (ORF1, ORF2, and ORF3). ORF1 encodes a polyprotein that is cleaved into seven nonstructural mature proteins (NS1 to NS7) that are involved in viral replication. ORF2 encodes the major structural protein (VP1) of approximately 60 kDa, and ORF3 encodes a minor structural protein (VP2). Noroviruses are genetically classified into 6 established genogroups (GI to GVI) (Green, 2013), while tentative genogroup VII is proposed by Vinjé (2015). GI and GII viruses are responsible for the majority of disease in humans.

Viruses are transmitted by contaminated food or water, through person-to-person contact, and by cross-contamination from surfaces (Teunis *et al*., 2008). Foods at risk for the presence of enteric viruses comprise those which are exposed to extensive handling, such as leafy vegetables, deli items, and other ready-to-eat foods that do not undergo further processing, and those subject to environmental contamination, such as seafood and fresh produce (Radin, 2013). Canada reports 20.5 million cases of enteric illness annually, and estimation of the relative role of various subcategories of food, water, and animal contact transmission of 28 enteric diseases (Butler *et al*., 2016) revealed that raw produce was most frequently identified as the dominant source for parasitic and viral infections. The number of reported foodborne outbreaks associated with fresh fruits and vegetables consumption in the United States and European Union during the period 2004–2012, in a total was 377 and 198, respectively. A wide spectrum of food vehicles have been involved in produce-associated outbreaks while among all microorganisms, norovirus with 59% in the United States and 53% in the European Union associated outbreaks, was the main pathogen responsible (Callejon *et al*., 2015).

Even though human noroviruses accounts for more than half of fresh produce-associated outbreaks, their detection is troublesome and the mechanisms of interaction with fresh produce are not quite well understood. The aim of this study was to assess the effect of different food surfaces and matrices on norovirus detection.

MATERIAL AND METHODS

For virus inoculation onto food items, stool sample containing norovirus genogroup GII, diluted in phosphate-buffer saline was used. Titers of these virus stocks were determined by PCR unit end point dilution using real-time RT-PCR assay for noroviruses. All commodities (tomato, lettuce, green onion and sliced deli ham) obtained from local retail store, were rinsed with sterile DDI H2O and 5% tri-sodium phosphate (TSP), kept under UV light for 3 min, and 25 g samples of produce were artificially contaminated with 100 µL of tenfold of serial dilutions of the stool samples containing norovirus GII. Experiment included the samples that were spiked with phosphate-buffer saline to serve as a negative control.

For virus RNA extraction the direct method with TRIzol reagent (INVITROGEN) was used. After rinsing the produce with 1 mL of reagent, the viral RNA-containing aqueous layer was extracted after the addition of 0.2 mL of chloroform, and precipitation in 0.5 mL of isopropanol. The resulting RNA pellet was washed with 70% ethanol, air dried, and suspended in 40 µL of RNase-free water. Additional purification of isolated virus RNA was carried out with QIAShredder Mini Spin Column (QIAGEN). Extracted RNA was stored at -80 °C.

Isolated RNA was analyzed by real-time RT-PCR using norovirus highly specific degenerate primers GII MON 431: 5' TGG ACI AGR GGI CCY AAY CA 3' and MON 433: 5' GGA YCT

CAT CCA YCT GAA CAT 3' (Richards *et al*., 2004). Primers were obtained from Sigma-GenoSys (St. Louis, MO).
Real-time RT-PCR was performed using the Invitrogen SuperScript™ III Platinum® SYBR® Green One-Step qRT-PCR Kit. Preparation of reaction mixes and assays was carried out ac cording to manufacturer protocol, reverse transcription at 50°C/40 min, denaturation 94° C/12 min, followed by 45 cycles at 94 °C/1 min, annealing at 50°C/1 min and extension at 61 °C/1 min and final extension at 77°C. RNA internal amplification control (IAC) at optimized concentration was added in order to eliminate false negative results due to inhibitors (Radin *et al*., 2012).

RESULTS AND DISCUSSION

For direct extraction of norovirus RNA from all tested food items (tomato, lettuce, green onion and sliced deli ham) the TRIzol method had been used. Based on end-point detection the standard curve of original stool samples was generated and used in the following experiments (Figure 1). Stool samples at decimal dilutions of 10,000, 1,000 and 100 RT-PCR units were used for spiking of food samples. Obtained threshold cycle (C_t) values in RT-qPCR assay and C_t alterations calculated for each level of inoculation revealed that surface of tomato had no inhibitory effect on norovirus GII detection, followed by approximately one log decrease of RT-PCR viral genome copies due to properties of green onions, and two log decrease for lettuce and deli ham slices (Table 1). The presence of IAC was confirmed by melting temperature (T_m) analysis displaying peaks at 84.5 °C, as well as agarose gel electrophoresis yielded amplification products of 213 bp for norovirus and 150 bp for the RNA IAC, as expected (results not showed).

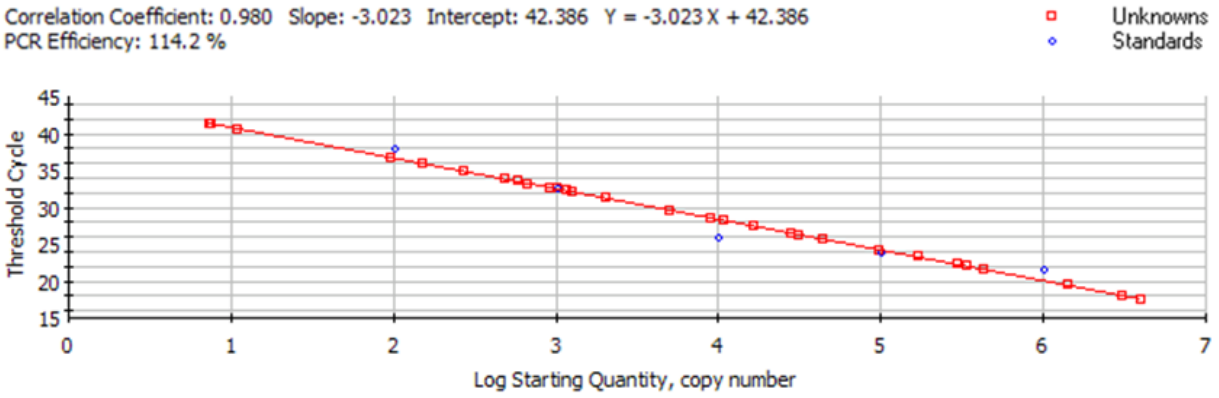

Figure 1. Standard curve for stool samples used for spiking coupled with data from RT-qPCR assay for norovirus detection from food products

Table 1. Obtained threshold cycle (C_t) values in RT-qPCR assay for detection from tomato, onion, lettuce and deli ham and stool samples used for inoculation

Commodity	**Norovirus GII RT-PCRU used for inoculation**		
	10 000	**1 000**	**100**
Stool	15.8±0.2	19.8±0.3	22.2±0.1
Tomato	16.0±0.5	19.6±0.4	23.0±0.2
Onion	18.9±0.5	21.6±0.1	25.5±0.5
Lettuce	22.2±0.6	24.7±0.5	27.6±0.6
Deli ham	22.7±0.6	24.2±0.5	26.8±0.6

In RT-qPCR assay the increase in threshold cycle (Ct) reflects decrease of viral genome copies (Bustin *et al*., 2005). It could be assumed that more virus particles have been attached on the surface of the food items that could not be eluted during the norovirus RNA isolation step. If they remain attached to the food matrix, it could be of significant health risk, because outside the human host, the norovirus is environmentally stable and has an estimated 50% human infectious dose (HID_{50}) ranging from 18 to 1,015 genome equivalents, although a recent study estimated that the HID_{50} is more similar to those of other RNA viruses (1,320 to 2,800 particles) (Atmar et al., 2014).

Virus binding to foods depends, among others on different physicochemical factors. The size of viruses is similar to that of colloids. The Derjagnin-Landau-Verwey-Overbeek (DLVO) theory of colloidal stability states that adherence of a colloid to a surface result from the interaction of two opposing forces: attractive Van der Waals forces and repulsive electrostatic forces (Le Guyader and Atmar, 2008). Factors that affect the electrostatic forces include: pH, ionic strength of the solution, the presence of compounds competing for sorption sites, properties of the virus (isoelectronic point, pI), and properties of the sorbent. For example, if pH values decrease the pI-net charge of the virus particle is positive, and vice versa.

For lettuce, as one of the most often implicated fresh vegetable in human norovirus foodborne outbreaks, it has been discussed that electrostatic forces play a major role in controlling virus adsorption (Lamhoujeb *et al*. 2009) and that lettuce seems to have the highest adsorption capacity and the most favorable conditions for viral persistence because of the size and the wrinkled texture of its leaves (Croci *et al*. 2002). On the other hand, human NoV virus-like particles associate with the surface of Romaine lettuce aggregating in and around the stomata, while in green onions between the cells of the epidermis and cell walls of both the shoots and roots, what suggests that viruses differ in their localization patterns to varieties of fresh produce (DiCaprio *et al.*, 2015). Additionally, the performance of elution buffers is not exactly the same even on two types of berries (raspberries and strawberries) suggesting that these two sorbents have different binding characteristics (Butot *et al*., 2007).

Different circumstances could be pointed out regarding tomato, as the capacity for human norovirus to persist in an infectious state on its surface is not known precisely (EFSA BIOHAZ Panel, 2014). Results showed in this study, could lead to the conclusion that its surface is not approachable for virus attachment. Tomato is very rarely identified in norovirus associated outbreaks and best to our knowledge (Radin, 2012) only one outbreak was reported in the EU between 2007 and 2012, due to a vomiting food handler during buffet preparation in catering. Tomato is infrequently in focus of investigations, but Stals *et al*. (2011) detected norovirus in 7 out of 30 samples of cherry tomatoes of which in two samples both norovirus genogroup I and genogroup II signals were present. Furthermore, Yilmaz *et al*. (2011) analyzing tomatoes from salad bars and restaurants in Istanbul found single sample contaminated with norovirus GII out of the 95 tested. Neither of these investigations has been connected to outbreaks.

For all of these reasons, it is needed to investigate each type of food surfaces/matrices, in order to have a better perspective on possibilities to detect noroviruses and especially on potential measures to inactivate or remove them. For example, because human norovirus GII.4 strain attach efficiently to the Romaine lettuce leaves and roots and green onion shoots, washing with PBS or 200 ppm of chlorine remove less than 0.4 log of viral RNA copies from the tissues and hence is ineffective for treatment of these produce (DiCaprio *et al*., 2015).

CONCLUSIONS

Virus detection from food includes diverse challenges, such as typically low viral load, extreme genetic heterogeneity, presence of food components that inhibit molecular assays;

the adsorption of viruses to food surfaces depending on complexity, binding characteristics, different physicochemical factors, varieties of localization patterns, etc. In this study tomato had no inhibitory effect on norovirus GII detection, followed by approximately one log decrease of RT-PCRU due to properties of green onions, and two log decrease for lettuce and deli ham slices. The need to investigate each type of food surfaces/matrices in order to have a better perspective on possibilities to detect noroviruses and especially on potential measures to inactivate or remove them is evident.

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CYPRODINIL AND FLUDIOXONIL FUNGICIDE RESIDUES AND DISSIPATION IN LETTUCE

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ABSTRACT

According to significance and frequency of their occurrence in our agro-ecological conditions, the most important lettuce pathogens are *Botrytis cinerea* and *Sclerotinia sclerotiorum*, and choice and dynamic of fungicide application in plants such as lettuce are additionally hardened by short vegetation period. Recently, in protection against the named pathogens are introduced fungicides from groups of anilinopyrimidines (cyprodinil) and phenylpyrrole (fludioxonil). With the aim of monitoring cyprodinil and fludioxonil degradation in lettuce, in the period of their use until the expiry of the pre-harvest interval (PHI), a treatment by fungicide SWITCH 62.5-WG (cyprodinil + fludioxonil, 375 g/l + 250 g/l) was performed at a recommended rate. Lettuce sampling was performed nine times – after two hours and 2, 4, 6, 8, 10, 12, 14 and 15 days. Fungicides extraction from lettuce was performed by QuEChERS method, while determination was accomplished by HPLC-DAD using C18 column. The achieved values of validation parameters of the applied methods are completely in accordance with demands of the standard SANCO/1257/2013.
Immediately after fungicide application, cyrodinil concentration in lettuce reduced from 2.34 mg/kg to 0.16 mg/kg, while respective values for fludioxonil were 1.44 mg/kg and 0.36 mg/kg. After the expiration of 14 days of PHI, the content of the studied fungicides in lettuce samples was below Serbian and EU MRL for these active ingredients. Thus, the analysis confirmed that PHI checked in our production conditions is sufficiently long in terms of safe use of lettuce in nutrition.
***Keywords**: lettuce, cyprodinil, fludioxonil, residues, dissipation*

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is an important component of the human diet, due to the low content of calories and fat, and high content of protein, dietary fibre, iron, calcium and phytochemicals (Ko *et al.,* 2014). In many countries, lettuce is grown all year round, combining field and greenhouse production. However, lettuce production is obstructed by a greater number of predominantly, fungi diseases. According to significance and frequency of occurrence, the most important pathogen of lettuce in our agro-ecological conditions is *Botrytis cinerea*, the agent of grey mould disease. Besides this, the disease that regularly occurs during lettuce growing in greenhouses is white mould, whose agent is *Sclerotinia sclerotiorum*.

Recently, for the protection of lettuce against the named pathogens cyprodinil and fludioxonil fungicides are introduced. In the European Union, including neighbouring countries, for control of the described pathogens in lettuce is applied the product SWITCH 62.5-WG (cyprodinil + fludioxonil 375 g/l + 250 g/l; Syngenta; Figure 1), with defined pre-harvest interval (PHI) of 14 days. However, for this purpose, SWITCH 62.5-WG has still not been registered in the Republic of Serbia. The double action of this product is due to its containing active ingredients of two different families, anilinopyrimidine (cyprodinil) and phenylpyrrole (fludioxonil). The first inhibits the biological synthesis of methionine, one of the principal component of the fungus protein synthesis, while fludioxonil stimulates the synthesis of glycerol, which blocks the cell growth in the fungus (Roberts and Hadson, 1999).

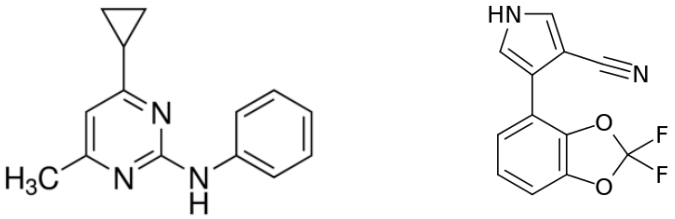

Figure 1. Chemical structure of cyprodinil and fludioxonil

Lettuce is a plant of short vegetation period, which additionally hardens choice and dynamic of fungicide application, due to the necessity to respect the PHI of the applied products. Many factors influence the fate of pesticides in plants, such as the climate conditions - temperature, humidity, light intensity, etc., crop species, nature of the chemicals, formulations and application methods (Garau *et al.,* 2002; Montemurro *et al.,*2002; Wang *et al.,* 2014; Lu *et al.,* 2014). In addition to this, lettuce is the vegetable that is consumed fresh, mainly just after harvesting, which increases the risk of pesticide residues. For better understanding of the possible risks of pesticide residues, dissipation studies are necessary to test whether the established application strategies are suitable for different crop species under the specific growing conditions (Lu *et al.,* 2014). This means that dissipation curves are only valid for a given crop in the specific conditions of each growing area (Marin *et al.,* 2003).

According to the Regulation of the European Union (Reg. No 2016/567) and the Republic of Serbia (25/2010), the maximum residue level of cyprodinil (MRLs) in lettuce is 15 mg/kg, while according to the regulation of EU (Reg. No 2016/1003), the maximum allowed quantity of fludioxonil in lettuce is 40 mg/kg and according to Serbian regulation, MRL is 15 mg/kg (25/2010). PHI of these fungicides in lettuce, prescribed by EU, is 14 days (Savčić-Petrić, 2015).

This study aimed to the behaviour of cyprodinil and fludioxonil fungicides in lettuce grown in the open field, including the dissipation rate, half-life values (DT_{50}) and PHI, for the fungicides in lettuce after application according to the recommended procedures.

MATERIAL AND METHODS

Field experiment

In Mačva region of Serbia, the field experiment was carried out in order to analyse dissipation kinetics of cyprodinil and fludioxonil residues in lettuce variety Seter. The experiment was conducted according to EPPO guidelines, with the aim of protection against grey and white mould diseases agents. The product SWITCH 62.5-WG (cyprodinil, 375 g/l, + fludioxonil 250 g/l) was foliar applied at a rate of 0.5-0.8 kg/ha with water consumption of 400 l/ha, recommended for use in EU.

Sampling

Samples were collected randomly, from more places within the experimental plot, with the aim of obtaining the representative sample. Lettuce samples were taken at 0 (2 h after application), 2, 4, 6, 8, 10, 12, 14 and 15 days period post-application. Until analysis, lettuce samples were stored at −24 °C. Every single analytical sample was considered in triplicates.

Chemicals and standard solutions

Analytical standards of cyprodinil and fludioxonil were obtained from Dr Ehrenstorfer, Germany, while acetonitrile (MeCN; HPLC grade) and acetic acid (CH_3COOH) were purchased from "J.T.Baker" (Darmstadt, Germany). Ultrapure water was obtained from TKA apparatus (Germany). The dispersive SP extraction (Cat. No. 5982-5650) and clean-up (Cat. No. 5982-5356) kits for QuEChERS sample preparation were purchased as ready-to-use from Agilent Technologies (USA). Stock solutions of both fungicides were prepared in MeCN at a concentration of ~100 µg/ml and stored at −20 ± 2 °C. Working standard solutions for chromatographic analysis were prepared diluting the stock solution in acetonitrile (0.02 to 40.0 µg/ml).

Fungicides extraction and HPLC-DAD analysis

Extraction of cyprodinil and fludioxonil from the lettuce was accomplished by previously developed and validated QuEChERS-based method (Anastassiades *et al*., 2003; Lazić *et al*., 2016). Pesticide residue analysis was performed with Agilent technologies 1100 Series high-performance liquid chromatographic system equipped with a diode array detector. The separation was performed on a C18 column (50 × 4.6 mm, 1.8 µm). The mobile phase was (0.5% acetic acid/MeCN, 40:60 v/v) with a flow rate of 0.9 ml/min and detection wavelength of 254 nm. Data analysis was performed using ChemStation software.

RESULTS AND DISCUSSION

Method validation was performed in our previous study (Lazić *et al*., 2016). The check of chromatographic conditions was carried out by linearity of the detector response, precision and accuracy of the method, and matrix effect, as well as determination of limits of detection (LOD) and quantification (LOQ) (Table 1). The results obtained in this study confirm that proposed method is easy and reliable for the determination of the analysed fungicides residues in lettuce.

Table 1. Validation parameters

Fungicide	Concentration range	R^2	LOD, mg/kg	LOQ, mg/kg	Recovery, %	RSD, %
Cyprodinil	0.02-0.40 µg/ml	0.994	0.02	0.05	87.1	0.80
Fludioxonil		0.995			85.9	0.38

The results of the field studies conducted in our research are shown in Figure 2 and 3. The dissipation kinetic was determined by plotting residue concentration against time. The half-life of pesticides calculated using the first order rate equation - $C_t=C_0e^{-kt}$, where C_t represents the concentration of the pesticide residue at time t, C_0 represents the initial concentration and k is the rate constant per day. Half-lives (DT_{50}) were determined from the k value, $DT_{50}=ln2/k$. Results show gradual and continuous reduction of cyprodinil and fludioxonil in lettuce, with degradation coefficient of 0.21, i.e. 0.10, respectively. The initial concentration of cyprodinil in lettuce was 2.34 mg/kg, the dissipation regressive equation was $y= 2.34e^{-0.21t}$ and the correlation coefficient (r) was 0.894 with a half-life of 3.3 days.

Dissipation curve of fludioxonil in the lettuce leaves under field condition is presented in Fig. 3. The mean value of fludioxonil residue determined with these samples was 1.44 mg kg to 0.28 mg/kg. The dissipation regressive equation of fludioxonil in lettuce samples was $C_t=1.44e^{-0.10t}$ and the correlation coefficient (R^2) was 0.855 with a DT_{50} of fludioxonil of 6.93 days.

Having in mind MRLs prescribed by both EU and Serbian authorities, the highest quantity of the existing cyprodinil and fludioxonil were significantly below these values. Further analysis established reduction in cyprodinil and fludioxonil content in sampled vegetable and after the

PHI, cyprodinil and fludioxonil quantity was reduced for 0.16 mg/kg and 0.28 mg/kg, respectively.

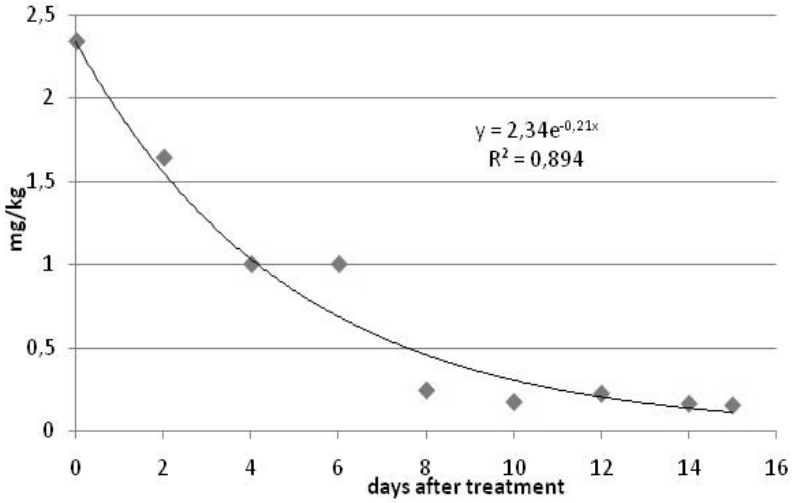

Figure 2. Dissipation curve of cyprodinil in lettuce samples

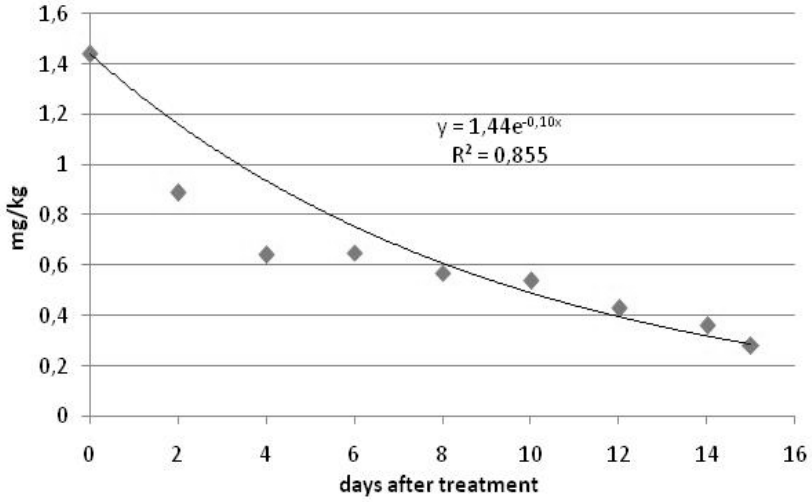

Figure 3. Dissipation curve of fludioxonil in lettuce samples

CONCLUSIONS

The results of this study indicate that cyprodinil and fludioxonil dissipate relatively rapidly in lettuce leaf grown in a field, as their half-lives were 3.3 and 6.93 days, respectively. After the expiration of 14 days of PHI prescribed by EU, the content of the studied fungicides in lettuce samples was below MRL for these active ingredients. Thus, the analysis confirmed that in out agro-ecological conditions from the aspect of pesticide residues, the product SWITCH 62.5-WG can be used safely in lettuce protection against *Botrytis cinerea* and *Sclerotinia sclerotiorum*, and that 14 days PHI is sufficiently long in terms of safe use of lettuce in nutrition.

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SAFE USE OF NEONICOTINOIDS IN SOME VEGETABLES IN TERMS OF RESIDUES

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ABSTRACT

Despite their high efficacy and increasing use in crop protection, data on the residual status and dissipation of the neonicotinoids in vegetables are limited. Therefore, the principal objective of this study was to generate data regarding the persistence and residue levels of neonicotinoid insecticides in selected vegetables. To investigate the dissipation of acetamiprid and thiacloprid in pepper and tomato fruits, field studies were done under controlled greenhouse conditions. Insecticides were applied in accordance with manufacturer's recommendations for Aphids control. Samples were randomly collected immediately after the application and every second day during the pre-harvest interval (PHI). For sample pre-treatment QuEChERS procedure was used, followed by HPLC-DAD analysis. In this study, MRLs of acetamiprid in pepper and tomato fruits achieved the second and sixth day after the insecticide application, respectively, while thiacloprid residue in pepper fruits were under MRL two days after the application. The half-life (DT_{50}) of insecticides fitted to a first order kinetic equation. DT_{50} of acetamiprid in pepper (3.9 days) and in tomato samples (4.3 days), as well DT_{50} of thiacloprid in pepper fruits (4.9 days), obtained in this study were similar. Finally, PHI for acetamiprid in pepper and tomato (14 days) and thiacloprid in pepper fruits (7 days), which has been established by Serbian authorities, are proved to be safe enough for application of these insecticides in greenhouse production of pepper and tomato.

***Keywords**: acetamiprid, thiacloprid, residues, pepper, tomato*

INTRODUCTION

Taking into account the susceptibility of cultivated plants to a greater number of diseases and pests, vegetable growing in greenhouse conditions demands regular chemical protection. For the protection of pepper and tomato against economically important pests such as aphids, insecticides from the group of neonicotinoids such as acetamiprid, imidacloprid, thiacloprid and thiamethoxam have been recently applied.
Acetamiprid, (E)-N^1-[(6-hlor-3-piridil)metil]-N^2-cijano-N^1-metilacetamidin, acts as an agonist of acetylcholine nicotinic receptor, causing disruption of nerve impulses and leading to insects dying. It has extremely expressed translaminar activity in plants and in pepper and tomato protection, products based on acetamiprid have been used for control of leaf aphids (*Aphididae*), at a rate of 0.25-0.4 kg/ha (Savčić-Petrić, 2015) with consumption of 400 l/ha of water.

The insecticide thiacloprid is widely used for control of economically highly important pests of pepper, such as aphids. Thiacloprid [(Z)-3-(6-chloro-3-pyridylmethyl)-1,3-thiazolidin-2 ylidenecyanamide] is the chloronicotinyl insecticide that acts selectively on the insect nervous system by inhibiting nicotinic acetylcholine receptors (Li *et al*., 2016). Thiacloprid is the second member of Bayer's chloronicotinyl insecticide (CNI) family (Jeschke *et al*., 2001) with a rising usage trend as it has been registered for use in many crops. Previous studies had indicated that thiacloprid could produce delayed lethal and sub-lethal effects on freshwater arthropods at low concentrations (Beketov and Liess, 2008). However, very little information is available on the behaviour of thiacloprid in plants and soils (Oliver *et al*., 2005; Wang *et al*., 2011).

Correct use of pesticides leads to increase in yield and higher product quality, while incorrect can cause increase in residues content in agricultural products and the environment, and it can also have negative impact to human health. Pesticide use in greenhouses demands special checking measures of their residues in agricultural products.

One of the basic conditions for assumption of danger posed by pesticide residues is existence of quantitative data on their presence in food. Norms used for regulation of amounts of pesticide residues in our country, i.e. their maximum residue level (MRL), are prescribed by the Regulations on the maximum allowable residues of pesticides in food and feed and of feed and food and animal feed which is determined by the maximum allowable amounts of residues of plant protection products (The Official Gazette of Republic of Serbia, No. 25/2010). Based upon this Regulation, the maximum residue level of acetamiprid in pepper is 0.3 mg/kg, and in tomato 0.2 mg/kg, which is in accordance with European regulations on MRLs (maximum residue levels) for these kinds of vegetables (EU pesticides database, 396/2005). The MRL for thiacloprid residues in pepper fruits, according to EU and Serbian legislation, is 1.0 mg/kg.

The principal objective of this study was to generate data regarding the persistence and residue levels of neonicotinoid insecticides in selected vegetables. Filed studies under controlled greenhouse conditions were carried out in order to investigate the dissipation of acetamiprid and thiacloprid in pepper and tomato fruits.

MATERIAL AND METHODS

Field experiments

The pepper and tomato plants were grown in a greenhouse. The trial was set up complying with the principles of good agricultural practice and OEPP/EPPO methods were used for trial design and data processing (EPPO, 2012), as well as the efficacy evaluation of the insecticide in control of aphids in vegetables (EPPO, 2004). All the experiments were conducted in three replications.

The insecticides were applied at the manufacturer's recommended rates for acetamiprid (in pepper and tomato) and for thiacloprid (in pepper) using a portable hand sprayer. The product was used in the ripening phase of vegetables (BBCH 81), at the beginning of aphids' colony formation. Samples of about 0.5 kg were randomly collected immediately after drying of the spraying mixture and every second day during the pre-harvest interval (PHI) of 14 (for acetamiprid in pepper and tomato), i.e. 7 days (for thiacloprid in pepper fruits) (Savčić-Petrić, 2015). Vegetable fruits were stored in individual polyethylene bags at -20 °C until extraction (Commission Directive 2002/63/EC).

Figure 1. Field trials in 2013 - locality Čelarevo (photo. S. Đurak)

Analytical procedure

Extraction and clean-up were done according to the method developed by Lazić *et al*. (2014, 2015). HPLC analysis was performed using an Agilent 1100 series liquid chromatography system (Agilent Technologies, USA) with a DAD detector and Zorbax Eclipse C18 column, 50 mm × 4.6 mm internal diameter, 1.8 μm particle size (Lazić *et al*., 2014; 2015).

Statistical analysis

The content of pesticide residues that remained in vegetable fruits at different intervals was fitted to first-order exponential equation (1), in order to obtain the half-life (DT_{50}).

$C_t=C_0e^{-kt}$ (1)

where C_t - the concentration after time, t (day), C_o - initial concentration, k - rate constant.

The half-life was computed using equation (2)

DT_{50}=ln2/k, 0.693/k (2)

where k is rate constant, in days.

RESULTS AND DISCUSSION

Pre-harvest interval for acetamiprid in tomato and pepper set by Serbian Regulations is 14 days. In pepper samples, 1 hour after application of the product based on acetamiprid, i.e. after drying of deposit, the content of residues amounted 0.47 mg/kg. Two days after the treatment in these conditions, acetamiprid residue level decreased by 50% (0.24 mg/kg), which is below MRL (0.3 mg/kg). Further on, during the trial, residue level of the studied insecticides gradually decreased, and after the expiry of the given waiting period, acetamiprid level was 0.08 mg/kg. In regard to the presented results, acetamiprid content in the analyzed samples of pepper, after the expiry of 14 days waiting period (Savčić-Petrić, 2015), was significantly below MRL prescribed by both our and EU Regulations.

The maximum residue level of acetamiprid in tomato samples was detected immediately after the application, 0.33 mg/kg. Two days after insecticide application, the loss was 5.54%, while on the fourth day it was 32.0%. Afterwards, six days after the treatment content of acetamiprid was at the MRL of 0.20 mg/kg, significantly shorter than recommended PHI of 14 days.

In this study, DT_{50} was calculated from the exponential equation (Table 1). The obtained DT_{50} values for acetamiprid in pepper and tomato were 3.9 and 4.3 days, respectively.

Table 1. The half-life of dissipation (DT_{50}) and values of acetamiprid parameters degradation in pepper and tomato fruits.

	Degradation constant	**R^2**	**DT_{50} (day)**
Pepper	0.18	0.864	3.9
Tomato	0.16	0.981	4.3

Last years, acetamiprid degradation was monitored in various plant products, such as mustard (Pramanik *et al*., 2006), tea (Gupta and Shanker, 2008), tomato and cucumbers (Shams El Din *et al*., 2012), watermelon (Wu *et al*., 2012), cherries and tomato fruits (Lazić *et al*., 2014, 2015).

In this study, dissipation of the insecticide thiacloprid, after its use at a recommended rate for Aphid control was also monitored in pepper fruits. Dissipation results of thiacloprid in pepper fruits are shown in Table 2. The highest residue levels were found in samples taken at the first sampling time 1 h after pesticide application, and the mean value was 1.136 mg/kg. Thiacloprid residue levels kept decreasing in the following period, reaching the level of 0.321 mg/kg with the dissipation of 72% only 2 days after application, with almost unchanged values on the 3rd day. Over the further three days, thiacloprid residue level was relatively stable, ranging from 0.276 mg/kg on the 4th day to 0.282 mg/kg on the 6th day. At the end of the pre-harvest interval of 7 days, dissipation of thiacloprid was 83%, and residue level in pepper fruits was 0.198 mg/kg. During the sampling time, thiacloprid content in pepper fruits was far below the MRL of 1.0 mg/kg.

Table 2. The half-life of dissipation (DT_{50}) and values of thiacloprid degradation parameters in pepper fruits

	Degradation constant	**R^2**	**DT_{50} (day)**
Pepper	0.14	0.682	4.9

Similar results had been reported by Sharma and Perihar (2013). They detected intensive thiacloprid dissipation (60.42%) on the 2nd day after application.

CONCLUSIONS

The results of this study indicate that in greenhouse conditions, acetamiprid and thiacloprid dissipate relatively rapidly in pepper, i.e. tomato fruits. DT_{50} of acetamiprid in pepper (3.9 days) and in tomato samples (4.3 days), as well DT_{50} of thiacloprid in pepper fruits (4.9 days), obtained in this study were similar. Finally, PHIs for acetamiprid in pepper and tomato (14 days) and thiacloprid in pepper fruits (7 days), which has been established by Serbian authorities, are proved to be safe enough for application of these insecticides in greenhouse production of pepper and tomato in our production conditions.

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EFFECT OF ACRYLAMIDE TREATMENT ON MORPHOMETRICAL PARAMETERS OF PANCREATIC BETA CELLS

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ABSTRACT

Acrylamide (AA) is a widespread industrial chemical with recognized adverse effects on living organisms. In the last few years, acrylamide has been in the focus of interest due to its considerable presence in various food commodities, including everyday staple foods. Since acrylamide is showing the variety of harmful effects on living systems, our study was oriented towards the observation of the potential adverse effect of acrylamide on the pancreatic beta cells of Wistar rats. Thirty male Wistar rats were divided into three groups, one control and two groups treated with 25 mg/kg and 50 mg/kg of AA respectively, from 23rd until 42nd postnatal day. Morphometrical analysis of beta cells was performed on 5 μm thick pancreas sections stained with immunohistochemical staining technique using insulin - mouse monoclonal antibody. Estimated parameters were: volume density, numerical density and surface density of beta cells, as well as their nucleocytoplasmic ratio. Numerical density and surface density of beta cells showed statistically significant decrease in both acrylamide treated groups ($p<0.05$) compared to the control. However, volume density and nucleocytoplasmic ratio of these cells, as well as the blood glucose level in both treatments, did not show any statistically significant differences compared to the control animals. Given results indicate that acrylamide has a potentially adverse effect on the pancreatic beta cells and our further study will be focused on changes of these cells on structural and molecular levels.

***Keywords**: acrylamide, beta cells, pancreas*

INTRODUCTION

Acrylamide (AA) is a widespread industrial chemical with recognized adverse effects on living organisms. It has been present in the environment for many years since acrylamide is an important monomer used for various industrial and laboratory purposes. Swedish scientists in 2002. (Tareke et al., 2002) found that AA is formed during thermal processing of a large number of foodstuffs at high temperatures, especially starch rich foods that are in everyday use. The formation of AA has been described in a wide variety of fried and baked potato products, but also in bread, coffee, cereals and various confectionery products. According to World Health Organization data, human consumption of acrylamide via food is about 1 μg/kg bw· day in general population (WHO, 2005).

It is believed that long – term intake of this rate could lead to disease (Tornqvist et al., 2005). That is the main reason why AA does not cease to be a subject of interest of many research groups. The formation pathways of AA is rather complex, and not yet fully clarified, but the Maillard reaction between amino acids and reducing sugars has been shown to be the most probable process for AA production at temperatures higher than 120°C (Mottram et al. 2002, Yayalan&Stadler, 2005). Cooking temperature and cooking time are most important factors which can vary and in this way influence the concentration of formed AA in observed foodstuffs (Romani et al., 2008).

Since acrylamide is showing the variety of harmful effects on living organisms, the aim of our present study is oriented towards the observation of the potential adverse effect of acrylamide on the pancreatic beta cells and on glucose levels in the Wistar rat serum.

Insulin secreting beta cells of the pancreatic islets of Langerhans, in rodents, are present predominately in the central core of the islet, making them it's most important part. Some pathophysiological conditions have shown to have an effect on islet architecture due to islet's high plasticity potential (Kim, 2009). Islet's potential to adjust to the newly created metabolic conditions, and the fact that insulin is one of the crucial metabolic regulatory hormones, made beta cells, and their potential alterations in volume or number, the main aim of our study.

MATERIAL AND METHODS

The experiment was carried out on 30 male Wistar rats aged 23 postnatal days at the beginning of the study. The animals were measured and divided into three experimental groups (n = 10) according to the body weight, one control and two acrylamide treated groups. The rats were kept under constant laboratory conditions with 22±2 °C temperature, with 12h light / dark periods, and had access to standard rat food and tap water *ad libitum.* First experimental group received acrylamide (99% purity, Sigma Aldrich Chemicals Co., St. Louis, MO, USA; Cas 79-06-1) dissolved in distilled water in concentration of 25 mg/kg bw day. Second experimental group received acrylamide in concentration of 50 mg/kg bw day. The control group received the distilled water. Both acrylamide and distilled water were administered *per os* during three weeks with the applications dynamics of 5 days a week with two days break, at the same time in the morning, and with no applications on the day of the termination of the experiment. Animals were sacrificed in anesthesia of diethyl ether vapor. The study was approved by the Ethical Committee on Animal Experiments of the University of Novi Sad (No .I-2011-03).

Following the termination of the experiment, the pancreata taken from the animals were kept in 10% formalin solution and subjected to standard procedure for paraffin embedding, cut into 5 μm thick sections and stained with immunohistochemical staining technique using the insulin - mouse monoclonal antibody. Stereological analysis of the pancreatic islets of Langerhans was performed using the "point counting technique" with Weibel's multi – purpose test grid (M42) (Weibel et al., 1979). The observed stereological parameters of beta cells were volume density (Vvβ), numeric density (Nvβ), surface density (Svβ) and nucleocytoplasmic ratio (Nβ/Cβ).

For determination of blood glucose level, blood was collected in plastic tubes from the body, after sacrifation of the animals. Samples were centrifuged for 10 minutes at 1500 rpm. The formed supernatant was carefully collected by an automatic pipette and serum samples were frozen at -20°C.

RESULTS AND DISCUSSION

Morphometric analysis was performed on 5μm thick pancreas sections stained with immunohistochemical staining technique using the Insulin - Mouse monoclonal antibody to display beta cells of the pancreas.

Results of the stereological analysis of pancreatic beta cells in control and acrylamide treated animals (Table 1) indicated that volume density of beta cells did not show any statistically significant difference between the control group and the either group treated with acrylamide. Numeric density of beta cells showed statistically significant decrease in beta cell number between the control group and the experimental groups treated with 25 mg/kg bw day of acrylamide, and 50 mg/kg bw day ($p<0.05$). The numerical density of beta cells between two experimental groups showed a slight increase in the group which received a higher

treatment, but the increase was not statistically significant. Surface density of beta cells also showed a statistically significant decrease in both acrylamide treatments compared to control animals ($p<0.05$). Comparing the surface density of beta cells between two acrylamide treated groups showed a small decrease in value of these parameters in animals treated with higher acrylamide treatment, but that decrease was statistically insignificant. Nucleocytoplasmatic ratio of beta cells did not show a statistically significant difference between the control group and both acrylamide treated groups.
Serological analysis which refers to the amount of glucose in rat serum did not show any statistically significant difference between the control group and both acrylamidde treated groups (Table 2).

Table 1. Volume density (Vvβ), numeric density (Nvβ), surface density (Svβ) and nucleocytoplasmatic ratio (N/Cβ) of beta cells (mean ± SE) of control rats and rats treated with acrylamide in 25 mg/kg bw day and 50 mg/kg bw day doses

Stereological parameter	**Control**	**25 mg/kg AA**	**50 mg/kg AA**
Vvβ	0.1860 ± 0.0067	0.1744 ± 0.0053	0.1688 ± 0.0048
Nvβ	424.09 ± 16.34	344.75 ± 11.68*	346.52 ± 15.94*
Svβ	13.89 ± 0.39	12.31 ± 0.51*	11.83 ± 0.34*
N/Cβ	0.58 ± 0.05	0.54 ± 0.04	0.53 ± 0.03

**p<0.05*

Table 2. Blood glucose level (mean ± SE) of control rats and rats treated with acrylamide in 25 mg/kg bw day and 50 mg/kg bw day doses

Serological parameter	**Control**	**25 mg/kg AA**	**50 mg/kg AA**
Blood glucose level	7.46 ± 0.17	7.15 ± 0.22	7.4 ± 0.29

Acrylamide and its potential adverse effect on living systems in general, as well as on specific biochemical and physiological processes in different organs has been a main subject for many research groups in the past decade. Even though there are a lot of studies dealing with this subject, data which explain the effect of acrylamide on pancreas, especially on its endocrine part, are very rare.

Nowadays, diabetes has epidemic proportions and researchers are intensively searching for the real cause of such high incidence of this disease. Streptozotocin induced diabetic rats have decreased numeric and volume density of beta cells in the islets of Langerhans (Abunasef et al., 2014; Akbarzadeh et al., 2007; Szkudelski, 2001; Ito, 1999). Results of this study showed a very significant reduction of the numeric density of beta cells in animals which received both acrylamide treatments in comparison to control animals.

Due to the fact that beta cells make up approximately 80% of the cells which form islets of Langerhans (Liu &Habner, 2009; Sato & Herman, 1981), a reduction of beta cells number may have an undoubted effect on the intensity of the secretion of insulin and thus the whole metabolism.

In addition to reduced number of beta cells in islets, their decrease in volume density is a typical occurrence during a toxic adverse effect. Even though animals exposed to acrylamide treatment had no significant influence on volume density of islets, results show a certain reduction in beta cells volume density of treated animals (Table 1). This result is in correspondence with findings in studies dealing with diabetes mellitus (Pirmoradi et al., 2016; Sakuraba et al., 2002; Novikova et al., 2013; Cnop et al., 2005).

Nucleocytoplasmic ratio is a parameter which is an indicator of the cell activity (Samsonidze, 1969). In circumstances where we want to reach a conclusion on the potential toxic effect of acrylamide on beta cell metabolism, determination of the ratio between area of the cells occupied by nuclei and area occupied with cytoplasm is of great importance. Our results showed a decrease in nucleocytoplasmic ratio, which is not statistically relevant.

This slight decrease may indicate a small decline in cell activity. The fact that there is no significant change in the blood – glucose level is in accordance with the fact that in type – 1 diabetes, almost 80% of beta cell mass is lost before the time first symptoms are visible (Cnop et al., 2005). The percentage reduction of numerical density of beta cells in our experiment is less than 19% which can explain why there are still no significant changes in blood – glucose level in animals of both experimental groups.

CONCLUSION

In conclusion, the results of the present study demonstrate that subchronic acrylamide treatment leads to a decrease in numerical and surface density of pancreatic beta cells but has no significant effect on volume density and nucleocytoplasmic ratio, even though it shows a decreasing trend in both of these parameters in both acrylamide treated groups. Blood glucose level remained unchanged compared to the control in spite of the reduced number of beta cells that we have noted probably due to the fact that remaining beta cells are still functional and capable to overcome the decrease in their number.

Taking into account that we gave the initial evidence of acrylamide effect on pancreatic beta cells, the focus of our future research will be the further analysis of structural and functional response beta cells have on acrylamide treatment.

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THE CONTENT EXPLORATION OF SOME FOOD ADDITIVES IN DIFFERENT SAMPLES OF INSTANT COFFEE 3in1 AND 2in1

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ABSTRACT

Additives are substances which are added to food products with the purpose of improving their characteristics. Food additives are substances of known chemical composition which are not substitutes for food. For easier identification, food additives are coded in E numbers. If an additive is coded in an E number that suggests that certain additive has been proven as safe for the health of the consumers. Nevertheless, many additives are considered a threat to human health if they are consumed in quantity higher than acceptable daily intake (ADI).
Instant or soluble coffee was invented and patented in 1938. It is made through the process called extraction which involves roasting and milling coffee beans and dissolving in the water afterwards. After drying coffee, emulsifiers (E471, E472e, E481), stabilizers (E331, E340, E451, E452) and anti-caking agents (E341, E551) are added to the coffee powder of granules.
The aim of this paper is to examine the content of emulsifiers, stabilizers and anti-caking agents in samples of Instant coffee – 3 in 1 and 2 in 1 of three different brands.

Keywords: *instant coffee, emulsifiers, stabilizers, anti-caking agents*

INTRODUCTION

There are records which show that people, even before mass food production, in the Bronze Age, wanting both to make food more delicious and to preserve it for longer periods of time, used salt for flavor, smoking for conserving meat and ferments in the making of bread, wine, beer and cheese. Until the second half of the XX century only raw materials were available on the market, but with the development of handicraft workshops for producing food for people in urban settlements, usage of chemical substances – food additives began.The word "food" in the syntagm "food additives" means that additives are used only in food products. Modern food industry cannot be imagined without the addition of these substances under precisely controlled conditions, with determined purpose.

The advantage of refined food products lies in a variety of different products available, which all have known composition and a nutritive value, as well as in producing whole range of goods whose composition and biological value have been adapted for people with special food diets. Food without the appropriate additives is not only unappealing for costumers but it also has negative effects on the competitiveness of the product and on the profit of the producer. Joint WHO/FAO Committee of Food Additives (JEFCA) make decisions regarding types of additives, their health safety, ways of preparations, acceptable daily intake and other questions concerning food additives on the global level. The positive list of additives which can be used in food industry is proposed by Codex Alimentarius whose members include WHO and FAO experts. Coding of food additives on positive lists is done according to the codes provided by the EU. Those codes are known as E numbers and they differ from one to another by numbers following the letter E. Different coding is used for different groups of additives as well.

Modern life and its tempo require not only different lifestyles, habits and needs but also various food products that can nowadays be found on the market. One of those products is instant coffee which is preferred because its preparation is simple and requires less time than the preparation of other types of coffee. During the 1930s, the Brazilian coffee industry encouraged research on instant coffee as a way of preserving their excess coffee production. The Nestlé company worked on this project and began manufacturing Nescafé in 1938. By 1950, Borden researchers had devised methods for making pure coffee extract without the additional carbohydrate component. In 1963, Maxwell House began marketing freeze-dried granules, which reconstituted into a beverage that tasted more like freshly brewed coffee. During the next five years, all of the major manufacturers introduced freeze-dried versions.

The Manufacturing Process:

1. Extraction: The manufacture of instant coffee begins with brewing coffee using highly efficient extraction equipment. Softened water is passed through a series of five to eight columns of ground coffee beans. The water first passes through several "hot" cells (140-180 °C), at least some of which operate at higher-than-atmospheric pressure, for extraction of difficult components like carbohydrates. It then passes through two or more "cold" cells (100 °C) for extraction of the more flavorful elements. The extract is passed through a heat exchanger to cool it to about 5°C. By the end of this cycle, the coffee extract contains 20-30% solids.

2. Filtration and concentration: After a filtering step, the brewed coffee is treated in one of several ways to increase its concentration. The goal is to create an extract that is about 40% solids. In some cases, the liquid is processed in a centrifuge to separate out the lighter water from the heavier coffee extract. Another technique is to remove water by evaporation before cooling the hot, brewed extract. A third alternative is to cool the extract enough to freeze water, and then mechanically separate the ice crystals from the coffee concentrate.

3. Recovery of aromatic volatiles: Part of the enjoyment of making and drinking coffee is smelling the aroma. During the several steps of the manufacturing process, volatile aromatic elements are lost; they must be returned in a later step to produce an attractive instant coffee product. Aromatics can be recovered during several stages of the manufacturing process.

4. Dehydration: Two basic methods are available for converting the liquid coffee extract to a dry form**:** spray drying and freeze drying. Spray drying is done at a higher temperature, which affects the taste of the final product, but it is less costly than freeze drying.

5. Aromatization: Volatile aromas that have been recovered from earlier steps in the manufacturing process are sprayed on the dry coffee particles. This may be done during the packaging operation.

6. Packaging: Instant coffee particles are hygroscopic,so they must be packaged under low humidity conditions in a moisture-proof container to keep the product dry until purchased and opened by the consumer. Also, to prevent loss of aroma and flavor, the product is packaged in a low-oxygen atmosphere (usually carbon dioxide or nitrogen).

Emulsifiers are food additives which include substances of specific chemical composition. Their function is to enable stability of emulsions, respectively forming and sustaining homogeneus blends of two or more phases that do not mix with one another.

Stabilizers are substances which enable homogeneity, maintain or amplify the existing color of food and substances which amplify the ability of forming bonds between different food

components. Specific function of stabilizers are improvement and stabilizing of texture, inhibition of cristalization, stabilization of foams and emulsions, reducing the glutinous/stickiness of glaze on pastries.

Anti-caking agents are food additives which prevent agglomeration of certain substances and secure homogenity of products.

MATERIAL AND METHODS

Determination of: E471 (Mono and diglycerides of fatty acids), E472e (Diacetyl tartaric acid ester of mono- and diglycerides), E481 (Sodium stearoyl-2-lactylate), E331 (Sodium salts of citric acid – this group includes monosodium citrate (E331(i)), **disodium citrate (**E331(ii)) and **trisodium citrate** (**E331 (iii))**, E340 (Potassium phosphates - this group includes monopotassium dihydrogen phosphate (E340 (i)), dipotassium monohydrogen phosphate (E340 (ii)) and tripotassium phosphate (E340 (iii)), E451 (potassium and sodium tri-phosphates), E452 – Polyphosphates - Sodium polyphosphate (E452(i)), Potassium polyphosphate (E452(ii)), Sodium calcium polyphosphate (E452(iii)), Calcium polyphosphate (E452(iv)) and Ammonium polyphosphate (E452(v)), E341 Calcium phosphates and E551 (Silicium dioxide) was done following procedures described in "Combined Compendium of Food Additive Specifications" by FAO/WHO Expert Committee on Food Additives.

RESULTS AND DISCUSSION

Table 1. contains data that indicate that the proportion of stabilizers differs within used samples. Sample A_1 contains the most of these substances (E340, E451, E452, E331). Sample B_1 and sample C_1 contain only two stabilizers (E340, E452) and both are used in these two samples. Samples B_1 i C_1 contain the same emulsifier (E471), while the sample A_1 besides E471 contains also one more type of emulsifiers (E472e). Sample A_1 doesn't contain anti-caking agents, while samples B_1 and C_1 contain identical substance (E341).

Table 1. The ingredients of analyzed samples of instant coffee 3in1 (A1, B1, C1 are used as a label of different brands

Ingredients/Sample	**A_1**	**B_1**	**C_1**
Stabilizers	E340, E451, E452, E331	E340, E452	E340ii, E452i
Emulsifiers	E471, E472e	E471	E471
Anti-caking agents	/	E341	E341

Table 2. The ingredients of analyzed samples of instant coffee 2in1 (A2, B2, C2 are used as a label of different brands)

Ingredients/Sample	**A_2**	**B_2**	**C_2**
Stabilizers	E340, E451, E452, E331	E340, E452	E340ii, E452i
Emulsifiers	E471, E472e	E471, E481	E471
Anti-caking agents	**/**	E551	E551

Content of used samples of instant coffee 2in1 is shown in Table 2. Sample A_2 contains largest amount of stabilizers (E340, E451, E452, E331), while samples B_2 and C_2 contain only two types of stabilizers (E340, E452). The same emulsifier is found in all three samples (E471). Sample A_2 contains also the substance E472e and sample B_2 E481. Sample A_1 doesn't contain anti-caking agents, while samples B_1 and C_1 contain the identical substance (E551).

Components of samples of instant coffee 3in1 differ from all of the samples of instant coffee 2in1. Samples A_1 and A_2 contain the same food additives. Samples $B_1$and B_2 contain the same stabilizers, but sample B_2 contains one more emulsifier compared to sample B_1 (E481). Different anti-caking agents are used in B_1 and B_2 samples. Samples C_1 and C_2 contain the same types of stabilizers and emulsifiers, but they differ by anti-caking agents used.

CONCLUSIONS

The used samples of instant coffee 3in1 contain less food additives (stabilizers, emulsifiers and anti-caking agents) then the samples of instant coffee 2in1. The sample A_1 contains the highest amount of stabilizers and emulsifiers, but it does not contain anti-caking agents.

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THE POULTRY MARKET IN UKRAINE: PROBLEMS, OBJECTIVE AND SOLUTIONS

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ABSTRACT

One of the segments of meat market in Ukraine which is developing at fast pace is poultry meat. This is because poultry is considered a quality, high-protein food low in calories compared to pork and beef. In poultry, collagen and elastin content is lower than in the flesh of cattle, and due to this increased content of valuable protein, poultry is absorbed more easily (96-98%) by the human body than meat of other farm animals, and is also different with regard to optimal quantitative ratio of essential amino acids. The composition of bird muscle also includes almost all water-soluble vitamins. Meat is one of the sources of B vitamins. Also, bird muscles are rich in macro- and micronutrients which are essential for metabolism.
New poultry industry growth trends demonstrate a stable and dynamic development. Despite the considerable scientific and practical potential of poultry production, the issue of determining its quality cannot be fully solved. In this work we examined a number of factors that affect the functional and technological properties of poultry meat. For example, for the same age gutted carcasses weight (the main feature of the breed) can vary significantly, and therefore, the quality of meat carcasses and parts will also be different.

***Keywords**: quality, poultry meat, aviculture, autolysis, maturation*

INTRODUCTION

Modern Ukrainian poultry industry is one of the most attractive areas of Ukrainian agriculture for investors. Thanks to private capital, introduction of new technologies and purchase of modern equipment was enabled enabled, which in turn provided increased production. Over the past 5 years, consumers have consumed 2 times more poultry meat and eggs, and buyers began to again give preference to domestic products. At the same time, many enterprises are not operating at full capacity or are even closed. This in particular applies to companies where previously turkeys, geese and ducks were grown. Nevertheless, poultry ranks first among other livestock industries by the time spent per unit of production and finished goods, namely meat. This is because it is virtually the only sector in livestock industry which is capable to radically improve the provision of population with cheap and high-quality meat during the recent years.

This branch brings together 680 poultry farms of different ownership forms, of which 102 are specialized in the production of pedigree products, 150 for the production of eggs, 80 are specialized in the production of poultry meat, while other farms deal with incubator-poultry and off-farm enterprises.The poultry industry is developed in 23 out of 25 regions of Ukraine.

Specialized management through a network of incubator-poultry organizations that are part of «Ukrainian poultry industry», provides the population with young birds, and is exerting a decisive influence on the development of poultry farms in the individual sector. At the same time, achievements in cross breeding poultry breeding farms and the top foreign companies are becoming available to a large farms and population. Specialists of «Ukrainian poultry industry» held a workshop on preparation and conducting of certification of pedigree poultry farms of Ukraine. As a result of certification of companies that have passed the competition,

we received certificates of pedigree breeding farm with the assignment status Plemreproductor. The most common chicken breeds which are used in the Ukrainian poultry industry today are the following:

- Egg chickens: Lohmann Brown, Shaver-576, Belarus-9 High Line white, brown hi-line, the Tetra-SL-Bovans Goldline, Isa Brown, Hajseks Brown, Lohmann-White, Borki-2M Borki- 117, Borki-Color, Sloboda, Rhodonite, Harco, Dominant, Iza White, Sloboda 2A, Ukraine;

- Broilers: Cobb-500 Arbor Aykres, Change, Hubbard-Isa, Ross 308, Starbro.

The average profitability of poultry production at the enterprises of Ukraine in the period 1999-2003 was gradually increased from -45.5% to the level of its payback. From 2004 to 2006, production of poultry meat has been profitable, with an increase in average margins from 3.8% to 12.1%, reaching a peak of 24.9% in 2005.

Hovewer, since 2006 the situation has began to change for the worse. Political instability, the processes of monopolization of the poultry market, the import of cheap products from abroad, deterioration of the means of production have led to a decrease in the profitability of most businesses and, as a consequence, to their bankruptcy. Therefore, during just one year (from 2006 to 2007 years) the profitability decreased from 19% to 12.1%. This was followed by the global economic crisis, which also had a negative impact on the state of the industry.

Despite all these factors, a linear trend has still shown a positive value in gain of almost 1.7% per year in the period from 2007 to 2012. This allows us to conclude that the profitability of enterprises for the production of poultry meat is gradually approaching the level of payback.

Analysis of the results showed that the Ukrainian poultry industry will continue to develop dynamically.

Average profitability, according to the production of poultry meat to Ukrainian enterprises of reporting period 2007-2012 years has shown a gain of 1.7% per year. With such growth rates, the level of recoupment of poultry meat production will be reached in 2017, and will increase to almost 5% in 2020.

According to the calculations of linear and polynomial trend obtained in the previous period, it is clear that, by 2020, the number of birds will increase by 20% compared to 2012. Total head of livestock poultry is presented in Figure 1.

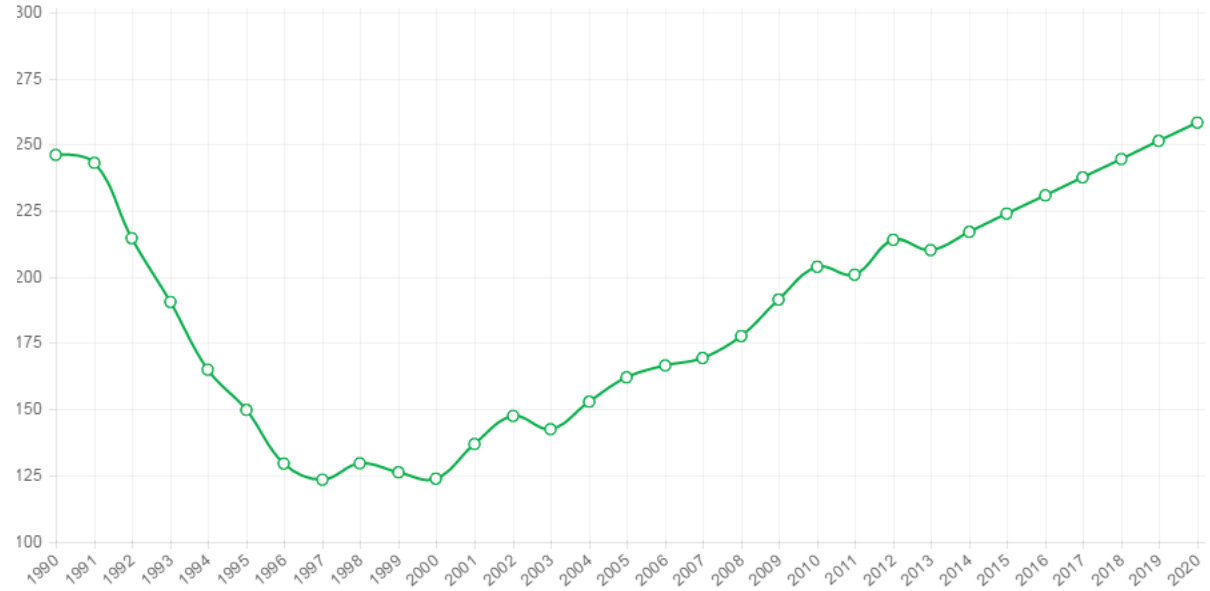

Figure 1 - The total head of livestock of poultry, million head

The urgency of the problem

Positive changes in the development of poultry meat in the world and Ukraine are caused by the creation and widespread introduction of highly cross-meat poultry, industrial cultivation technologies, content and processing. However, due to a significant increase in the production and processing of poultry, the impact of a series of natural, technical and organizational factors at all stages of production can lead to degradation of the quality, appearance of various product defects and related quantitative loss of meat.

Increasingly important work of domestic and foreign scientists, is aimed at studying the effect of these factors on the quality and safety of poultry meat.

Little attention is paid to the system interlinkages of technological, technical and organizational factors in the cultivation, processing and delivery of poultry with the quality of the finished product, while at the same time improving them is a more effective way to increase efficiency in comparison with other production factors.

The general concept of "quality food", includes poultry products, embedded set of properties that characterize the biological and energy value, organoleptic, structural-mechanical, functional-technological, sanitary and other features of the product, as well as their degree of severity.

The quality of poultry products depends on several factors, but is mostly determined by the quality of farmed birds to be processed, their age, body weight, slaughter yield, fatness, the ratio of muscle and bone tissue, as well as valuable parts of the carcass, organoleptic characteristics and other. The quality of poultry meat is influenced by a number of factors: lifetime, slaughter and post-mortem. Lifetime factors and the long-term of effect exposure have an impact on the bird until slaughter. These include genetics, physiology, nutrition, keeping conditions and diseases. Short-term impact factors are manifested during the last 24 hours of the bird life in the period of preparation of poultry for slaughter. These include hunger before slaughter, capture, loading, transportation, conditions and the period within the processing plant, discharge, suspension on the conveyor, immobilisation, stunning and slaughter.

Slaughter and post-slaughter factors, manifested during the slaughter and processing of poultry, can mainly be attributed to the short-term effects, except for the storage of poultry meat in refrigerated and frozen conditions.

Visible manifestation of negative factors are common to all external defects that can be detected by organoleptic methods, and hidden, for example, for such defects as the pH of meat or its contamination - detected only as a result of specific analyzes.

The factors include the negative impact which can be disposable and non disposable. These vary from removable attributed disadvantages in carrying out technology requirements such as the equipment, the organization of production, to non removable (in accordance with the current level of development of the breed) such as poultry genetic construct and its response to stressors.

Negative factors affect the quality of the final product directly or indirectly. The impact of negative factors on the quality parameters of poultry are classified in the following areas: food and biological value, acceptability to the consumer, food safety and functional-technological properties.

Biochemical changes in birds meat are not well understood, there is no consensus about the meaning of life and its maturation. It is known that the maturation process has a positive effect on the product quality, improving its organoleptic characteristics. However, it has not been studied with respect to poultry meat.

After the termination of life of an animal, processes take place wich are connected to the termination of the supply of oxygen, the absence of oxidative transformations and circulation, inhibition of synthesis and generation of energy accumulation in the tissues of the final product. Disorders of the osmotic pressure in the meat cells takes place, resulting in self-destruction of life-supporting systems and spontaneous development of enzymatic processes which maintain their catalytic activity for a long time. As a result of the disintegration of tissue components, meat quality characteristics (mechanical strength, the level of water-binding capacity, taste, color, flavor) and its resistance to microbiological processes vary considerably.

Autolysis of meat is the process of changes in the chemical composition, structure and properties of raw meat after slaughter under the influence of its own enzymes. Meat properties change occurs in a certain sequence in accordance with the basic stages of autolysis (fresh meat → completion of rigor mortis rigor mortis and ripening → deep autolysis), and its quality indicators become significantly different.

During the long maturation of meat there is a significant improvement in sensory and technological characteristics. In the early stages of autolysis meat has a pronounced taste and odor which, depending on the storage temperature appear only for 3-4 hours due to the formation of products of the enzymatic breakdown of proteins and peptides (glutamic acid, threonine, sulfur-containing amino acids), nucleotides (inosine, hypoxanthine etc.), carbohydrates (glucose, fructose, pyruvic and lactic acid), lipids (fatty acids, low molecular weight) and creatine, creatinine and other nitrogenous compounds.

The question is now directed toward use of raw materials, taking into account the progress of autolysis is of particular importance, since the share of animals arriving at the processing of industrial complexes is substantially increased. At the same time, due to post mortem processes in the muscle tissue, significant deviations from the normal development of autolytic processes are found. Accordingly, one should distinguish meat with high final pH (DFD) from meat and exudative (PSE) with a low pH. Most companies exclude maturation of poultry meat, which leads to the appearance of meat markings, etc.

CONCLUSIONS

Analysis of the development status of poultry meat in the country proves that it is the basis for the production of poultry meat. The effectiveness of poultry feed has increased and the volume of processing that needs to be improved. Thus, the problems highlighted by the quality of poultry meat, allows one to find ways to solve them. It is necessary to pay attention to the influence of a number of natural, technological and organizational factors, at all stages of production, which can lead to lower quality, the appearance of a variety of product defects and related quantitative loss of meat.

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QUALITY OF BEVERAGE OF HYDROLYZED MILK PERMEATE

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ABSTRACT

The aim of the study was to investigate the quality and stability of the beverage with addition of orange/carrot base produced from hydrolyzed milk permeate during 60 days of storage. The following characteristics of the produced orange/carrot beverage were tested: pH, dry matter, milk fat, total protein, sugar content, content of vitamin C, ash, antioxidant potential and energy value. Produced beverage is fat-free and lactose-free. The average pH value was 4.08 ± 0.01. It was found that the produced beverage contains an average of: 6.52 ± 0.07 g / 100 g dry matter, 0.18 ± 0.01 g / 100 g of total proteins and 0.52 ± 0.03 g / 100 g of ash. More than half of the total sugar content in beverage is glucose - 50.42%. After the production, the content of vitamin C in orange/carrot beverage was 0.20 mg/100g. The content of polyphenolic substances during storage period decreased by 8%. During the storage period a reduction of DPPH values (%) from 11.61 to 9.68 was observed. Energy value of 100 g of beverage was 88.295 kJ and as such can be classified into a group of low-energy drinks. According to obtained results, produced beverage is suitable for use in energy-restricted diets. By consuming 250 mL of orange/carrot beverage 22% of daily needs for calcium in children and 11% in adults can be satisfied.
Overall, the beverage obtained with the use of hydrolyzed permeate has high nutritional value, low energy value and excellent sensory characteristics (color typical of aromatic supplement, peculiar smell, pleasant and refreshing taste).

Keywords: *beverage of hydrolyzed milk permeate, quality, chemical composition*

INTRODUCTION

Valorization of by-products of the food industry is one of the most important priorities today. The group of by-products of the dairy industry includes whey and permeate obtained after ultrafiltration of milk. One possibility for the valorisation of these products is to process them into beverages based on milk components (Carić and Milanović, 1995; Girsh, 1999)**.** Numerous efforts have been made to transform permeate into a product suitable to be used as human food and develop beverages with acceptable sensory qualities, especially in terms of taste (Bangert, 1975; Remer, 1982; Đurić *et al*., 2004; Koffi *et al.,* 2005).

Authors Geilman *et al*., (1992) and Beucler *et al.*, (2005) reported enzyme hydrolysis of lactose in the production of beverages, reducing digestive problems of consumers and increasing the sweetness of beverages. Fruit supplement, significantly affects sensory qualities of the end product, such as colors, taste and odor (Koffi *et al*., 2005). The invention of the optimal normative for the mixture of fruit concentrates and/or other additives with fresh permeate in order to produce a beverage with acceptable sensory qualities is still a challenge.

Some authors suggest adding metal-gluconate (Remer, 1982), citric acid or various sweeteners such as fructose, sucrose or hydrolyzed lactose for adjusting odor and flavor. Beverages obtained from permeate are clear, transparent liquids. Most of them are saturated with carbonic acid and have a distinctive refreshing taste. The use of permeate as a deproteinized product reduces the specific taste of whey (Renner and Abd El-Salam, 1991). Permeate has a potential to be produced as a beverage similar to sport drinks for athletes

due to similar electrolyte content. Also, it contains a high concentration of minerals, such as calcium and potassium, which may be relevant for maintaining good health (Geilman *et al*., 1990).

The aim of the study was to produce a beverage based on hydrolyzed permeate with the addition of orange/carrot fruit base and to establish parameters of quality and its durability during 60 days of storage period.

MATERIAL AND METHODS

Permeate was obtained, during the manufacture of feta cheese by ultrafiltration of milk with 3.7 g/100g fat (manufacturer "DAIRY Šabac'', Serbia). Device for UF process (with polysulfone membrane) had capacity of 5000 L of milk/h. Enzyme Maxilact® LG5000 - β-galactosidase derived from the yeast *Kluyveromyces lactis* was used for permeate hydrolysis. Enzyme preparation was added to permeate at the temperature of 40° C in concentration of 0.1 g/100g. For the production of beverages, orange/carrot fruit base was used (30 g/L), of the manufacturer Frutarom Etol, d.o.o. Slovenia. Hydrolyzed permeate was mixed with 3% orange/carrot fruit base. The pH of the mixture was adjusted to pH 4.0. Samples were homogenized, pasteurized at the temperature of 90° C for 10 min, packed and stored in refrigerator at 4 ±1 °C.

Application of standard analytical methods (Carić *et al*., 2000) in samples of hydrolyzed permeate, orange/carrot base and obtained orange/carrot beverage resulted in the determination of: dry matter (ISO 6731, IDF 21: 2010), milk fat (IDF 105:1985), total proteins (IDF 20:1962), ash (IDF 90:1979), pH value using a pH-meter (EcoScan pH 6 Eutech Instruments, The Netherlands), energy value: EV=(% protein x 4,4 + % milk fat x 9,3 + % total sugar x 4,1) x 4,186 [$kJ100g^{-1}$]. Content of minerals: Ca, K and Na, using ICP-MS method with microwave-assisted. Phosphorus was analyzed using spectrophotometric method, at 720 nm.

Sugar content was analyzed by Liquid Chromatograph Agilent Technologies 1200 Series with ELSD and Zorbax Carbohydrate Column (4.6x250mm. 5 μm). The flow rate was 1.000 mL/min, at ambient temperature and run time was 15 min. The mobile phase with isocratic flow was a cetonitrile/water (70/30. v/v). Content of vitamin C was determined using HPLC method (High Performance Liquid Chromatography (Malbaša *et al.,* 2009). Antioxidant activity was tested using test of radical inhibition (DRI) with 2,2-diphenyl-1- picrylhydrazyl (DPPH) (Amirdivani and Baba, 2011). Polyphenolic content was determined using the Folin–Ciocalteu method (Shetty *et al.,* 2005). Sensory analysis of sample was carried outlying expert committee (trained panelists selected from University staff members). The five point system and the descriptive method with assessing the scoring range from 1 to 5. Statistical analysis of results was carried out with the computer software program "Statistica" and were expressed as average of values obtained at three independent experiments.

RESULTS AND DISCUSSION

Chemical composition

From the aspect of sustainability and stability of the product during the storage period, as well as sensory qualities, physico-chemical characteristics of the product are a significant quality parameter. The average chemical composition of hydrolyzed permeate, fruit base and produced orange/carrot beverage from hydrolyzed permeate is shown in Table 1. Hydrolyzed permeate used for beverage production had a pH of 6.28 and dry matter of 5.41 g/100g. Energy value of 100 g hydrolyzed permeate was low (84.32 kJ), which allows its wider implementation as a dietary product.

Dry matter of the orange/carrot beverage after the production was 6.52±0.07 g/100g. Similar values of the dry matter were obtained by Hattem *et al.,* (2011) and Đurić *et al.,* (2004), while

the dry matter of beverages according to Sady *et al.,* (2013) had much higher value (12.01 g/100g). During the storage period, there was a slight variation of the dry matter content. The produced orange/carrot beverage was fat-free and lactose-free, which was expected due to the chemical composition of raw material for the production. The protein content was not changed compared to hydrolyzed permeate. The pH value of the orange/carrot beverage slightly decreased during the storage.

Table 1. Characteristics of the hydrolyzed permeate, the orange/carrot base and the produced orange/carrot beverage

Component	**Hydrolyzed permeate (g/100g)**	**Orange/carrot base (g/100g)**	**Orange/carrot beverage**	
			Day of storage	**Content (g/100g)**
Dry matter	5.41 ± 0.01	43.05 ±0.06	0	6.52±0.07
			60	6.54±0.02
Milk fat	0.00 ± 0.01	0.00	0	0.00
			60	0.00
Total proteins	0.18 ± 0.01	0.67 ± 0.01	0	0.18±0.01
			60	0.22±0.01
Total sugars	4.23 ± 0.01	32.93 ± 0.01	60	6.33± 0.01
Ash	0.51 ± 0.01	0.84 ± 0.01	0	0.52±0.03
			60	0.47±0.003
pH	6.28	3.05	0	4.08±0.01
			60	4.03±0.01
Energy value kJ/100g	84.32	-	88.295	

The analysis of sugars present in hydrolyzed permeate confirmed the presence of monosaccharides glucose (2.12 %) and galactose (2.11 %) obtained by decomposition of lactose (Table 2).

Table 2. The level of carbohydrates in the hydrolyzed permeate, the fruit base and the orange/carrot beverage after production

Content	**Hydrolysed permeate**	**Orange/carrot base**	**Orange/carrot beverage**
Glucose (g/100 g)	2.12	12.50	1.78
% of total sugars	50.12	37.96	50.42
Fructose (g/100 g)	0.00	10.10	0.15
% of total sugars	0.0	30.67	4.25
Galactose (g/100 g)	2.11	0.00	1.38
% of total sugars	49.88	0.0	39.09
Sucrose (g/100 g)	0.00	10.33	0.22
% of total sugars	0.0	31.37	6.23

The fruit base mostly consisted of fructose and glucose originating from fruit which is a component of fruit base and sucrose. The proportion of sugars in the dry matter of orange/carrot fruit base was 76.49 %. Out of the total sugars in the beverage after production, more than a half was glucose (50.42 %), similarly with the initial raw material. The storage period have shown that there was a slight change in the proportion of glucose and galactose in the orange/carrot beverage. According to literature data, glucose content in orange juice may vary between 1.75 and 3.23 g/100g (Kelebek *et al.,* 2009; Farnworth *et al.,* 2001; Sanz *et al.,* 2004). Glucose content in beverage is in accordance with the cited values. Glucose is preferred energy source for the body that is easily absorbed and therefore gives rise to reduced fatigue and faster recovery following exercise (Anderson *et al.,* 2002). The produced hydrolyzed permeate beverage is lactose-free, which enables lactose intolerant people to use it. Energy value of 100 g of beverage is 88.295 KJ and as such, it can be classified into a group of low-energy drinks suitable for use in energy-restricted diets.

Minerals

The content of mineral matter was not significantly different in hydrolyzed permeate compared topermeate (Ilić-Udovičić *et al.,* 2013). The dominant mineral in all samples is potassium. In the fruit base, the portion of this mineral component is 1971.9 mg/kg (Fig. 1).

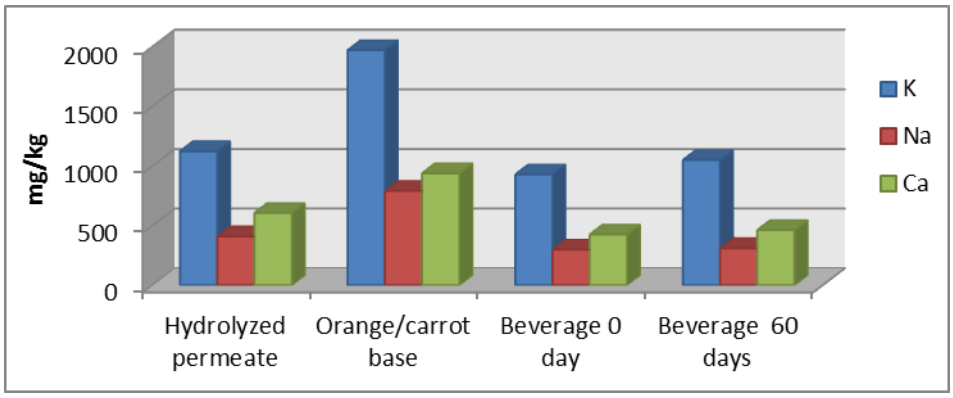

Figure 1. Mineral content in the hydrolyzed permeate, the orange/carrot and orange/carrot beverage during the storage

The second most abundant mineral is calcium without significant deviation until the end of the storage period, followed by sodium. After 60 days of storage, there were no significant changes to the mineral content in the hydrolyzed permeate beverage (Figure 2). The consumption of 250 ml of the beverage can satisfy 22 % of daily needs for calcium in children and 11 % in adults.

Vitamin C

Orange/carrot base contained 13.50 mg/100g of vitamin C (Fig. 2). Hydrolyzed permeate had a total of 0.43 mg/100g of vitamin C, which is approximately equal to the value found in the permeate (0.45 mg/100g). The composition of permeate, the type and composition of fruit bases and technological parameters are the most important factors affecting the concentration of vitamins in beverages (Tamime and Robinson, 2004). After 15 days of storage at the temperature of 4°C, the concentration of vitamin C in the beverage was reduced by 0.3 %, and after a month of storage, the changes in the content of vitamin C were 1 %.

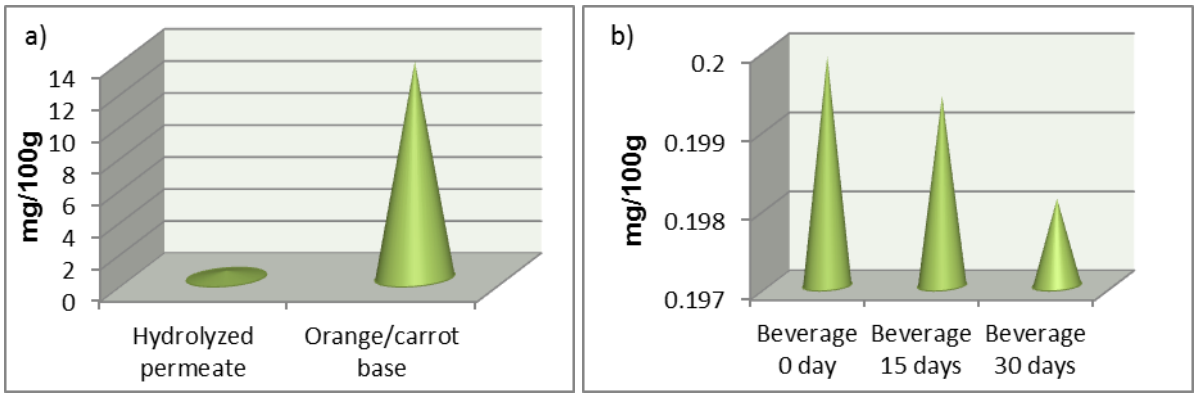

Figure 2. Vitamin C content in: a) the hydrolyzed permeate and orange/carrot base, b) the orange/carrot beverage during storage

Antioxidant activity

The results of antioxidant activity ($AA_{DPPH,\ \%}$) of samples on DPPH radicals and the content of polyphenol are shown in Table 3. The addition of fruit content to hydrolysed permeate caused an increase in antioxidant activity due to the high content of anti-oxidant components in fruit.

Table 3. Radical scavenging activity measured by DPPH (2,2-diphenyl-1picrylhydrazyl) method and polyphenol content of analysed samples

	Hydrolysed permeate	**Orange/carrot base**	**Orange/carrot beverage**	
			Day of storage	**Content**
$AA_{DPPH\ (\%)}$	1.29	59.15	0	11.61
			60	9.68
Polifenol (mg GAE /L)	12.53	1919.75	0	55.97
			60	51.59

Time of storage influences the change of polyphenol content in the beverage, and after 60 days, the value decreased by 8 %. A significant decline in the level of polyphenol in orange juice during relatively short period of storage was reported by del Caro *et al*., (2004), while the research of Calisanturk *et al*., (2011) found subtle changes to the content of polyphenol. During the storage period, there was a decrease of $AA_{DPPH\%}$ value by 17 % in the orange/carrot beverage. The decrease of antioxidant activity after 60 days can be explained by degradation of vitamin C and polyphenol that happened during storage period.

Sensory analysis

Sensory qualities of the beverage (appearance, color, sediment, flavor and taste) after production were characteristic of the group of products and typical for the type of aromatic additive. The evaluation of the orange/carrot beverage is presented in the Figure 3. The taste is distinctive, distinguishing, slightly acidic (due to the addition of the fruit base and its composition, the content of sugar and acid) and refreshing. The beverage is without sediment, the color and the consistency of the sample are uniform, the flavor is pleasant, mild and typical of the used aromatic additive.

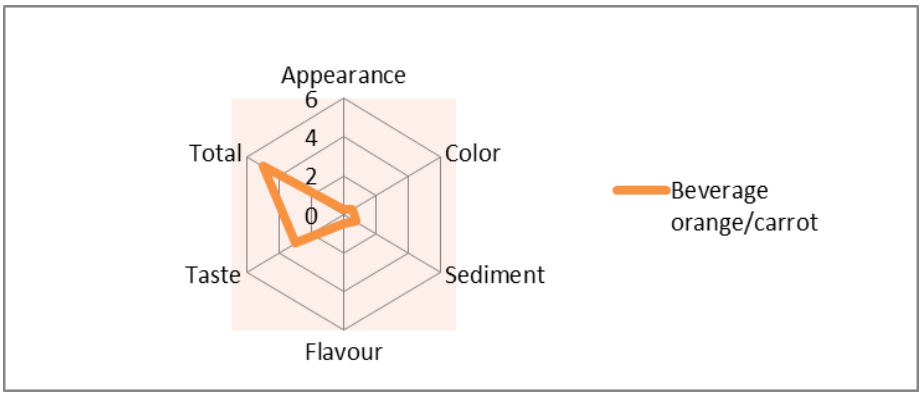

Figure 3. Sensory analyses of beverage after production

CONCLUSION

The obtained results showed that the applied technological process is suitable for production of hydrolyzed UF milk permeate beverage. The produced beverage had stable physicochemical composition during 60 days of storage period, and high nutritional and low energy value. The beverage was delactosed and did not contained milk fat. Out of the total sugars in the beverage, the content of glucose was more than 50 %. The beverage is an

excellent source of minerals potassium and calcium, while the content of sodium satisfies the body's needs. Given that glucose is a preferable energy source for the body and due to the presence of water and electrolytes, the beverage can be successfully used for rehydration of the body and the compensation of minerals after strenuous exercise. The beverage contained vitamin C and polyphenols, which contributes to increased daily intake of these substances with high antioxidant properties. The proposed procedure for processing permeate solves the problem of permeate disposal by converting it into a product intended for human consumption which would completely satisfy the criteria of the lactose intolerant consumers.

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CADMIUM IN BEEF, PORK AND CHICKEN MEAT

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ABSTRACT

Due to industrial development, large amount of various harmful substances are emitted in the environment, including heavy metals. As a result of their accumulation in the biosphere humans and animals are introducing them into their bodies, which causes various diseases because of toxic effect they have on the human body. While heavy metals, among others, cadmium, pose potential risks in the production of quality food, we examined the amount of cadmium in different types of meat. In order to determine the cadmium content in beef, pork and chicken meat placed on the Serbian Republic of Bosnia and Herzegovina market, extensive research was conducted. Part of this research is presented in this paper.
Cadmium was measured bygraphite furnace atomic absorption spectrometry (GFAAS) and the measured concentrations are shown in mg/kg of meat. The study included 237 samples of beef, pork and chicken meat. Of the total of 237 samples, in 133 samples (56.12%), the amount of cadmium was at or below the established limit of quantification (≤0.001 mg/kg). Among these samples mostly beef had this low cadmium content (58.89%). The largest amount of cadmium was found in pork samples at a concentration of 0.025 mg/kg, followed by beef at concentrations of 0.010 mg/kg. The maximum amount of cadmium in poultry meat was 0.008 mg/kg which is the lowest compared to the concentrations determined in the other two types of meat. When comparing the results obtained with the maximum allowed level of this element it can be concluded that consumption of examined meat, does not pose a risk to human health.

***Keywords**: cadmium, heavy metals, residues,* beef, *pork and chicken meat*

INTRODUCTION

Metals having a bulk density greater than 5 g per cm-3 belong to the group of heavy metals. Heavy metals are widespread in the environment (Walker et al., 1995). Their representation as pollutants in the working environment poses a serious health and environmental problem because they are toxic, non-biodegradable, have a very long half-life in soil (Ram Isar, 2000) and accumulate in living system through active human food chain (Merian 1991, ATSDR - Agency for Toxic Substances and Disease Registry, 2008, Mariam et al, 2004).

Toxic effect of heavy metals is that they stimulate the formation of free radicals and reactive oxygen species and biomolecules in the organism which causes oxidative stress and leads to membrane lipid peroxidation which disrupts its functionality and selectivity in transporting substances. On that way they can cause damage to cells, the function of the enzyme, or genetic material (DNA) (David, 2001). It is believed that heavy metals pose a continuing danger as carcinogenic for human's organism (Kokilavani, 2005).

In normal conditions, there are three ways of entering the heavy metal in the body: through the skin, the gastrointestinal tract and through the respiratory tract. Although discovered in the early nineteenth century, cadmium is now classified as a group of the most important metals in terms of professional and ecotoxicology. It is known that this metal, with a strong cumulative effect (Demirezen, 2006) causes harmful effects in the body especially at the level of the kidneys, lungs and skeletal system, and may be the cause of lung and testicular cancer (Matovic et al., 2004). The largest source of inhalant intoxication of cadmium is

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