CEFood Congress

Novi Sad, Serbia
23 - 26 May, 2012

PROCEEDINGS

of 6th Central European Congress on Food

SUPPLEMENT

IUFoST International Union of Food Science and Technology

EFFoST European Federation of Food Science and Technology

CEI CENTRAL EUROPEAN INITIATIVE Central European Initiative

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CONTENT

Keynote Lecture

EMERGING ISSUES IN CHEMICAL FOOD SAFETY: ADVANCED STRATEGIES IN LABORATORY CONTROL

Jana Hajslova

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Various contaminants / toxicants, both natural and environmental, may occur in human food chain, some of them have emerged only recently. Also food/feed adulteration cases, number of which has grown in the recent decade, is associated not only with economic fraud but may pose a serious food safety issue. To protect consumers´ health, besides of other measures, effective analytical strategies have to be available in laboratories concerned with food control. In addition to meeting criteria established for performance characteristics, also sample throughput, cost and labour demands are important parameters. Following examples will be used in this presentation to demonstrate recent analytical challenges in food quality, authenticity and safety control:

- Novel simple and fast sample preparation strategy enabling simultaneous analysis of three groups of environmental contaminants in fish and sea food: (i) polychlorinated biphenyls (PCBs), (ii) brominated flame retardants (BFRs) including ´emerging´ ones, and (iii) polycyclic aromatic hydrocarbons (PAHs) employing two-dimensional gas chromatography (GCxGC) coupled with fast time of flight mass spectrometry (TOF) and/or tandem mass spectrometry (MS/MS] for quantification.
- Multidetection method employing high resolution mass spectrometry (HRMS) for rapid screening of mycotoxins and their metabolites (´masked´ forms) in cereals and products thereof. Illustration mycotoxins changes during cereals processing. Documentation of the use of immunoaffinity cartridges with cross-reactive antibodies for pre-concentration/purification in masked mycotoxins.studies
- Implementation of a novel strategy for tracing of the sources of ´emerging´ contaminants perfluorinated/polyfluorinated alkylated substances (PFAS) in human food chain. The use of ambient mass spectrometry employing Direct analysis in Real Time (DART) ion source for examination of food contact materials for the presence of their precursors, polyfluorinated surfactants (PFS)
- Metabolomic fingerprinting/non-target screening employing DART ion source coupled with orbitrap MS followed by advanced chemometric generated data processing strategies as the challenging tool for recognition of (i) lipids adulteration and (ii) prediction of processing contaminants formation in relation to the biscuits recipe

The research presented has been funded within 7th Framework EU program and supported by Ministry of Education

RESVERATROL RELAXES SMOOTH MUSCLES OF RAT UTERUS

Novakovic Radmila[1], Protic Dragana[1], Radunovic Nebojsa[2], Heinle Helmut[3], Vladimir Kanjuh[4], Ida Leskosek-Cukalovic[5], Slobodan Jovic[5] and Gojkovic-Bukarica Ljiljana[1]

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Introduction: Resveratrol (RSV) is a phytoalexin produced by grapevines. The benefit of resveratrol to health is widely reported. Resveratrol has been found to promote vascular relaxation but its mechanism of action is unclear. The data about influence of RSV on the contractility of smooth muscles of uterus are not available. The aims of our study were to investigate the effects of RSV on the contractility of rat uterus and to investigate the involvement of K^+ channels in effect of RSV on the spontaneous contractions (SC) and contractions provoked by oxytocin.

Methods: Uterine strips were obtained from virgin female Wistar rats in oestrus. Strips were mounted into organ bath for recording isometric tension in Krebs-Ringer solution. Experiments followed a multiple curve design. In order to test the involvement of K^+ channels in a mechanism of action of RSV, a selective blocker of K_{ATP} channels, glibenclamide (GLB), a selective blocker of inwardly rectifying $BaCl_2$ (1 mM) as well as 4-aminopiridine (4AP), a non-selective blocker of voltage-gated K^+ channels and tetraethylamonium (TEA), predominantly blocker BK_{Ca} were used.

Results: RSV induced a concentration-dependent relaxation of SC with EC_{50}=19.52μM and Emax=94% and contractions provoked by oxytocin with EC_{50}=21.88μM and Emax=95% ($P<0.05$). GLB (10 μM), 4AP (1 mM), TEA (1 mM), $BaCl_2$ (1 mM) antagonized the response to RSV in both, oxytocin induced contractions and SRC. Relaxation achieved by concentration of 100 μM RSV was insensitive to K^+-channels blockers.

Conclusions: RSV is uterine relaxant and can be use in tocolysis. The antagonism of RSV effect by different K^+-channels blockers suggests that K^+-channels are involved in resveratrol action on the contractions of rat uterus. It seems that RSV, when applied in high concentration, may exert an additional mechanism of action.

Funding: Our work has been supported by Scientific Research Grants TR 31020 from the Ministry of Science (Serbia).

THE EFFECT OF RESVERATROL ON THE HUMAN UMBILICAL VEIN WITHOUT ENDOTHELIUM

Protic D[1], Novakovic R[1], Spremovic-Radjenovic S[2], Radunovic N[2], Heinle H[3], Petrovic A[4], Despotovic S[4], Kanjuh V[5], Gojkovic-Bukarica Lj[1]

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Introduction: Resveratrol induces vasorelaxation through both endothelium-dependent and -independent mechanisms. The effect of resveratrol on human umbilical vein (HUV) is not known. Therefore, the aim of our study was to define the role of K^+ channel in the vasodilatation of HUV induced by resveratrol.

Materials and Methods: Serotonin (5-HT) or 100 mM K^+ were used for precontraction of the HUV without endothelium. The cumulative concentration-response curves were obtained by adding increasing concentrations (1-100 μM) of resveratrol. K^+-channel inhibitors were added in the bath before resveratrol in order to tested the role of vascular K^+ channels in its effect.

Results: Resveratrol induced concentration-dependent vasodilatation (EC_{50} = 16.5 μM). A selective blocker of K_{ATP} channels, glibenclamide (10 μM) and 4-aminopiridine (4-AP, 1 mM), a blocker K_V channels, induced significant shift to the right ($P < 0.05$) of the concentration-response curves for resveratrol. Tetraethylamonium (TEA, 10 μM), which predominantly inhibits K_{Ca} channels and barium-chloride ($BaCl_2$, 1 mM), a blocker of K_{ir} channels, antagonized the response to resveratrol. The high concentration of resveratrol (> 30 μM) relaxed HUV bathed by a medium containing 100 mM K^+, with maximum response of 94 % and EC_{50} of 47 μM, $P < 0.05$).

Conclusions: Results suggest that resveratrol induced endothelium-independent vasorelaxation of HUV. The glibenclamide-, 4-AP, TEA- and $BaCl_2$-sensitive K^+ channels are involved in resveratrol vasodilatatory effect. It seems that resveratrol has additional K^+-channel independent mechanism of action.

Funding: Our work has been supported by Scientific Research Grant (TR31020) from the Ministry of Science (Serbia).

MULTIEXPOSURE TO BACTERIAL PATHOGENS AND TOXINS IN FOOD: CASE OF *BACILLUS CEREUS* AND *STAPHYLOCOCCUS AUREUS*

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ABSTRACT: Some of the foodborne pathogens are characterized by the ability to produce toxins. These toxins play an important role in array of virulence factors being often the principal mechanism by which these pathogens cause a disease. The strains of *Bacillus cereus* can cause diarrheal and emetic (vomiting) food poisoning and are commonly found in a wide range of foods and environments. The diarrheal type of *B. cereus* food poisoning is caused by protein enterotoxins such as haemolysin BL, non-haemolytic enterotoxin, enterotoxin FM and cytotoxin K (cytK), all upon the production in small intestines. This scenario, often called toxin-mediated infection or toxicoinfection, requires ingestion of bacterial cells or spores. Contrastingly, by growth of *B. cereus* in food, an emetic peptide-like toxin (cereulide, CER) is produced inducing intoxication *sensu stricto* known as emetic food poisoning. Staphylococcal enterotoxins (SEs) are extracellular proteins, produced mainly by *Staphylococcus aureus*, causing food intoxication when ingested. To date, 21 SEs, excluding variants, have been identified. These enterotoxins are similarly to emetic toxin of *B. cereus* causative agents of intoxication.
Incomplete heat inactivation of bacterial spores and possible post-contamination are plausible scenarios in the production of ready-to-eat foods (such as premade lasagne) that could result in co-presence of different pathogens in the same food. Their behaviour and risks they carry for the consumer, such as growth and production of toxins, are important aspects to investigate. In this study, two *B. cereus* diarrheal strains and two enterotoxin (SEA) positive *S. aureus* strains were tested. Spores of *B. cereus* were heat treated and used for co-culture inoculation with *S. aureus* of food samples that were subsequently packaged in modified atmosphere (MAP 8% O_2 and 92% N_2). Such samples were stored at 12 and 22°C, to mimic temperature abuses. Results showed that both pathogens were able to grow and produce respective enterotoxins in the same ready-to-eat lasagne sample at both storage temperatures, regardless of MAP conditions and the mutual competition. These results confirm our hypothesis that multitoxin and multipathogen exposure is a plausible scenario.

Keywords: *toxins, B. cereus, S. aureus, ready-to-eat, multi exposure*

INTRODUCTION

Number of substitute technologies rose up to replace heat treatments in attempt to satisfy modern trends of a food consumer. The main change in terms of microbial food safety is that sterilization and pasteurization as we knew them are in great extent replaced by mild(er) heat treatments. These treatment differ for different food products, but are in general characterized by a partial microbial inactivation (certainly spore remain active and often only part of vegetative population is inactivated, while part remains injured or stressed). This is usual situation in ready-to-eat meals or in refrigerated foods of extended durability (Rajkovic et al. 2010; Rajkovic et al. 2009; Smigic et al. 2009; Del Torre et al. 2004; Del Torre et al. 2001). As these meals are composed of different ingredients and food components it is to be expected that they may contain different pathogens at different stages of the product life. It is therefore highly relevant to investigate the possibility of mutual growth and toxin production of *Bacillus cereus* and *Staphylococcus aureus*, two toxigenic organisms with great prevalence and public health relevance (Logan 2012; EFSA 2011; Hennekinne et al. 2011; Taormina 2010; Newell et al. 2010; Oh and Cox 2009; Larkin et al. 2009; Arnesen et al. 2008; EFSA 2005).The aim of this study was to investigate pathogen outgrowth and toxin production of two foodbrone pathogens, *B. cereus* and *S. aureus* in one ready-to-eat product.

B. cereus can cause emetic intoxication and diarrheic toxico infection. For the later one food poisoning is caused by production of several enterotoxins such as non-haemolytic enterotoxin (Nhe), haemolysin BL (Hbl, cytotoxin K (CytK) and enterotoxin FM and virulence factors such as hemolysins (HlyII and HlyIII), collagenases, phospholipases C and cereolysins (Ceuppens et al. 2011). Multiple methods for enterotoxin detection exist, namely biological assays such as the vascular permeability reaction, rabbit ileal loop and cytotoxicity assays, mass-spectrometry and antibody-based immunological detection. The relatively fast, easy and cheap immunological detection of enterotoxins make it suitable for routine analysis, while it can remain highly specific for a particular toxin component, depending on the antibody quality. Commercial detection methods for *B. cereus* enterotoxins are available in the form of immunological kits for components Nhe-A, Nhe-B and Hbl-L2, but currently none exist for CytK or other virulence factors. With the *Bacillus* diarrhoeal enterotoxin visual immunoassay (BDE VIA™) kit (3M Tecra) the Nhe-A component is detected in 5 h, 200 µL samples are required, the detection limit is < 1 ng/mL (according to the manufacturer). The *Bacillus cereus enterotoxin* reversed passive latex agglutination (BCET-RPLA) kit (Oxoid) requires 50 µL samples for Hbl-L2 detection after 20 to 24 h, the detection limit is 2 ng/mL (according to the manufacturer). Due to the use of dilution series the BCET-RPLA kit is semi-quantitative. The Duopath® Cereus Enterotoxins (Merck) simultaneously detects

the Nhe-B and Hbl-L2 components with detection limits of 6 and 20 ng/mL respectively (Krause et al. 2010), in a 150 µL sample after 30 min.

The most used methods for determination of staphylococcal enterotoxins in foods are based on different formats of immunological assays. Commercially available immunological kits have been developed according to three different principles: enzyme linked immuno sorbent assay (ELISA), enzyme linked fluorescent assay (ELFA) and reverse passive latex agglutination (RPLA). Some of these tests are able to differentiate only six or seven classical types of SEs (SEA to SEE), while the others do not differentiate them. None of these assays is currently able to detect other SEs (from SEH to SEIU). The Vidas SET2 is a rapid and fully automated kit detecting, without differentiation, the SEA to SEE, using a cone coated with antibodies specific for SEA, SEB, SECs, SED and SEE. An immune complex is formed between (i) the coated antibodies, (ii) the toxins in the concentrated extract and (iii) the anti-SE antibodies conjugated with alkaline phosphatase. All reagents are included in the wells of the strip used (Hennekinne et al. 2007), making it very user friendly and reproducible within routine testing.

MATERIAL AND METHODS

Microorganisms, culture conditions and food

The stock cultures of four bacterial strains (all food isolates and all originating from LFMFP culture collection) used within the experimental setup were stored at -75 °C and were activated in Tryptone soya broth (TSB) (Oxoid, Hampshire, UK) by overnight incubation at 30 °C for two *B. cereus* and 37°C for two *S. aureus* strains. The enumeration was done on the selective media: M.Y.P Agar Base (Oxoid, Hampshire, UK) for *B. cereus* and Baird-Parker Agar Base (Oxoid, Hampshire, UK) for *S. aureus*. Lasagne samples that served only as an example of ready to eat foods that were implicated in food poisoning with *B. cereus* and *S. aureus*, were inoculated with 2 ml cultures to obtain inoculum level of 100 CFU/g for *S. aureus* and 100 spores/g for *B. cereus*. Spores used for inoculation were prepared using an in-house protocol of ISO 17025:2005 accredited laboratory. Prior to inoculation into food the spores were heat treated at 90 °C for 10 minutes (most often used treatment in the production line – data from interviews with industry).

pH and aw measurements

Water activity determinations were done using AWK-20 aw-cryometer (AWK-20, NAGY Messysteme GmbH, Gäufelden, Germany), making sure that a_w with inoculation did not change. A Mettler Toledo, S20 SevenEasy™ (Mettler Toledo, Colombo, USA) pH meter was employed to measure the pH of food samples. Both of the measurements were done in triplicate.

Modified atmosphere packaging

Selected gas mixture (8% O_2, balanced by 92 N_2%, chosen on the basis of industrial screening performed on more than 1000 samples of different ready-to-eat foods) was used to package inoculated lasagne samples in packaging system (MECA 900, DECA® technic, Herentals, Belgium). The packaging trays used consisted of polypropylene and EVOH warranting good gas barrier and the top film sealed on the trays was Opalen HB65AF PP. Each 10 packages the gas mixture was controlled with Checkmate 9900 (PBI Dansensor, Checkmate 9900, Gullimex BV, Ringsted, Denmark).

Enterotoxin tests

Enterotoxins produced by *S. aureus* were tested by VIDAS SET2 (bioMérieux, VIDAS, bioMérieux, Marcy, France) (Hennekinne et al. 2007) and the entrotoxins (HBL and NHE) of *B. cereus* were detected using latteral flow immuno assay Duopath® (Duopath® Cereus Enterotoxins, Merck KGaA, Darmstadt, Germany) following manufacturers' instructions (Krause et al. 2010).

RESULTS AND DISCUSSION

Figure 1 shows growth of *S. aureus* and *B. cereus* and an onset of the detectable enterotoxin production in the same lasagne sample packaged in MAP and stored at 22°C. It can be noted that at such temperature abuse both pathogens can grow and produce respective enterotoxins under the MAP conditions where oxygen is available. Although *S. aureus* exhibited somewhat slower growth in comparison to *B. cereus* its enterotoxins were earlier to be detected than HBL and NHE of *B. cereus*. At 12°C slower growth and lower final cell density was found for *S. aureus*, while growth of *B. cereus* did not defer much between two temperatures (Figure 2). However, delayed enterotoxin production was found for both pathogens, with extent of delay being higher with staphylococcal enterotoxins. This is not surprising when more mesophilic properties of *S. aureus* in comparison to diarrheal strains of *B. cereus* is taken into account (ICMSF 1996a; ICMSF 1996b).

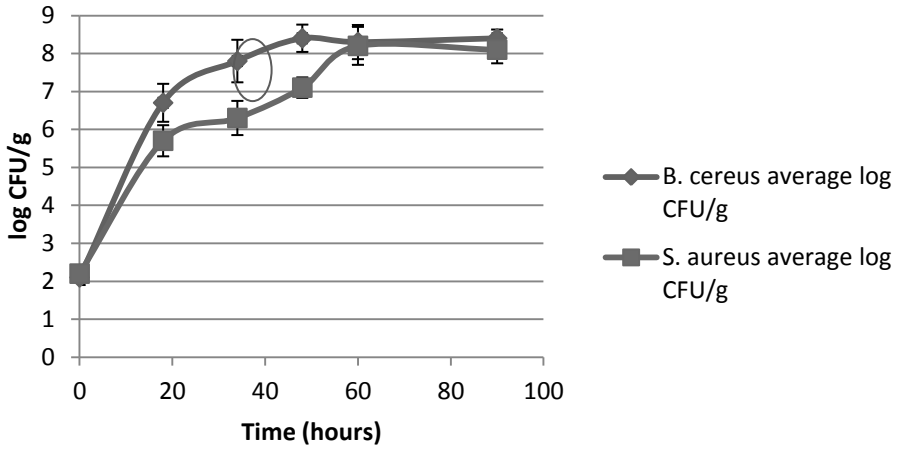

Figure 1: Growth and onset of enterotoxin production by *B. cereus* and *S. aureus* in the same lasagne sample at 22°C under MAP (8% O_2 and 92% N_2). The eclipse represents the earliest day at which detectable amounts of staphylococcal enterotoxins were found; the rectangle represents the earliest day at which detectable amounts of *B. cereus* enterotoxins were found.

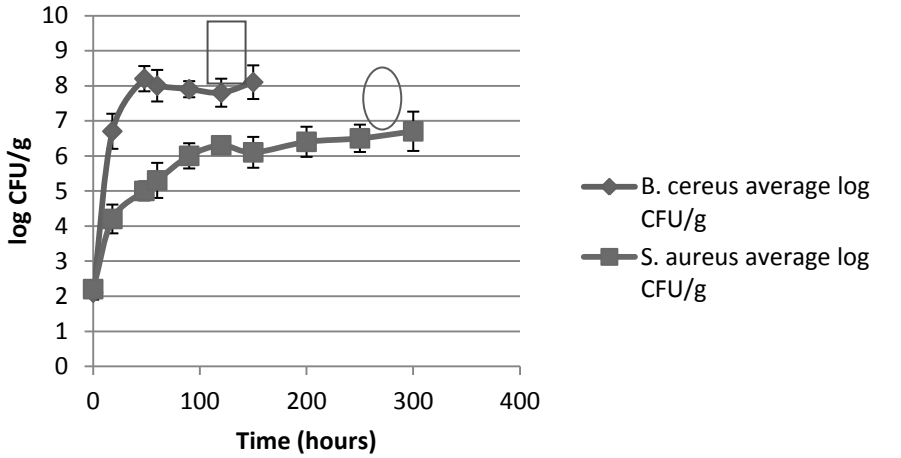

Figure 2: Growth and onset of enterotoxin production by *B. cereus* and *S. aureus* in the same lasagne sample at 12°C under MAP (8% O2 and 92% N2). The eclipse represents the earliest day at which detectable amounts of staphylococcal enterotoxins were found; the rectangle represents the earliest day at which detectable amounts of *B. cereus* enterotoxins were found.

In additional experiments (data not shown) it has been seen that tested *B. cereus* strains were able to supress *S. aureus* more than the other way around, although in all occasions *S. aureus* reached counts higher than 7 log CFU/g and produced enterotoxins.

The effect of heat treatment tested here had usual activation effect on *B. cereus* spores. While eliminating present vegetative microflora such treatment can create opportunity for spore forming pathogens to germinate and grow under favourable conditions. In post-heat treatment activities, such as filling of packages or even home preparation post-contamination with human associated pathogens, like *S. aureus* is a well-documented food safety issue. Therefore, temperature abuse must be avoided and more stringent MAP (complemented with intrinsic hurdles) must be used to warrant desired level of safety when heat sterilization in the final package (or aseptic hot filling) is not an option.

During gastric passage, the majority of preformed *B. cereus* enterotoxins and vegetative cells are inactivated in healthy hosts, while the spores remain active. In the proximal small intestine, the surviving vegetative cells are inactivated by the high bile concentration, enterotoxins are degraded by the digestive host secretions and the spores slowly germinate. Moreover, the distal small intestine harbours an indigenous intestinal microbial community which inhibits the vegetative outgrowth of *B. cereus* in the intestinal lumen. Although the course of *B. cereus* diarrhoeal food poisoning probably follows ingestion of food contaminated with *B. cereus* spores and less than that of vegetative cells and/or enterotoxins *B. cereus* the concern exists that at compromised individuals and under certain food consumption conditions also vegetative cells and in-food formed enterotoxins can contribute to the illness. For *S. aureus* enterotoxins is known that their mode of action is based on their pre-formation in food and thanks to their pH and photolytic stability the damage is inflicted upon the food consumption. So far no modelling studies or risk assessment approaches were undertaken to interpret the risks associated to dual hazard exposure: both enterotoxins present in the same food, and spores and vegetative cells of both organisms present in the same food sample. This exposure seems likely even with microwave preheating of contaminated lasagne (only 52 °C were achieved in 2/3 of lasagne if preheating prescriptions by the manufactures were followed, data not shown).

CONCLUSIONS

In the context of mounting concern on the exposure to multiple microbial toxins via versatile vectors coming from the food, environment and direct human activities it has to be realized that (re)emerging risks need a continuous assessment for the level of threat they pose to the public health. Official data of EFSA show that a total of 558 foodborne outbreaks, constituting around 10 % of all reported food-borne outbreaks in EU in 2009, were caused by bacterial toxins, namely toxins of *Bacillus*, *Clostridium* and *Staphylococcus* species found in almost all types of food. The development and application of continuously improving analytical methods will clarify the grounds of the reported

epidemiological findings of measurable human exposure. Since for the vast majority of these toxins, identification and characterization information is absent or limited the assessment of their health significance is complex. Only scarce data exist to evaluate the risk related to public health originating from the exposure to multiple foodborne hazards. Even less is known on the combined exposure to multiple bacterial toxins that for many pathogens are primarily virulence factors. Current data shows that such scenario is realistic. Even emetic toxin of B. cereus can be formed at the same time in the same food sample together with investigated enterotoxins (data not shown here).

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CONTEMPORARY FEED PRODUCTION AIMED AT INCREASING COMPETITIVENES QUALITY AND SAFETY

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ABSTRACT: The concept of animal nutrition undergoes constant changes, similarly to the concept of human nutrition. In addition, to enhancing animal performance, optimal animal feed is also expected to have positive effect on human health. Animal health represents the basis for quality, safety and utility of food of animal origin used in human nutrition. In order to develop this new concept of nutrition, it is necessary to investigate links between bioactive components in feed and food, as well as an influence of individual food components and complete diets on human health. It is also important to optimize technological processes in animal feed production in order to ensure that all ingredients of the formulated mixture maintain their prescribed concentrations, activities and other characteristics when these processes are completed. Furthermore, for optimal feed utilisation, it is necessary to ensure a proper physical form, consistency and stability of feed during processing. Technological processes used in feed industry impact all above mentioned aspects. Their permanent development and improvement is necessary for further progress. Challenges to feed industry also represent challenges to science, research and development in the whole feed to food chain. In order to achieve the nutritional content, functionality, palatability, taste, smell and texture, animal feed must be carefully formulated. It is also necessary to define parameters for milling, mixing, liquid component application, pelleting, expanding, extruding, drying, cooling, vacuum coating etc. based on research results. Scientific approach must contribute to economically, socially and environmentally sustainable development of feed industry by defining practical and acceptable methodologies in the food production chain. This approach is important not only for manufacturers, but also for attaining better production parameters, while securing well-being of animals and fulfilling requirements of milk, meat and eggs consumers. The industry must recognise that it needs to adopt the rules of good manufacture and hygiene practice in order to achieve the necessary level of feed and food competiveness, quality and safety.
Key words: *feed, technology, production, quality, safety*

INTRODUCTION

Feed industry is of great economic importance in the EU and an important part of food chain. It play a crucial role in the meaning of sustainability and careful use of resources (Cardy-Brown 2006, Sredanović et al. 2008). Sustainable development is strategy, and development in sustainable feed technologies must involved different aspects such as:

- Feed production
- Animal feeding
- Feed safe for animals, people and environment
- Feed control and evaluation
- Quality of food of animal origin
- Environmental impact (Barletta, 2005).

Technological processes used in Feed industry have unavoidable impact in all these aspects, and their permanent development and improvement is necessary for future challenges. These challenges are changing during the time and were targeted to different aims:

- Best yield at least cost (1970-1980)
- Quality of products - milk, meat, eggs (1980-1995)
- Feed & Food Safety (1996-2000)
- Feed for Food (2000 - 2010) (Ferrary 2011)

In addition to enhancing animal performance and quality of edible animal products, optimal animal feed is now expected to have positive effect on human health also (Pinotti 2009, Adams 2005). In this „Feed for Food“ and „Feed for Health“ aproach research should be directed at:

- Improving food safety
- Strengthening consumer confidence in traceability
- Creating unique foods through feed (ω3-eggs, Se-milk etc.)
- Collaboration with Human Nutritionists
- Nutrigenomics - Functional foods to prevent food related pathologies (heart diseases, cancer, obesity, osteoporosis, diabetes) (Ferrary 2008).

In order to develop this new concept of nutrition, it is necessary to investigate links between bioactive components in feed and food of animal origin, as well as an influence of individual food components and complete diets on human health. The nutritional value, taste, texture and safety of food products such as meat, fish, dairy products and eggs are directly influenced by the nutrition of the animal concerned (Pinotti 2009, Kaushik 2006, Kersten et al. 2005).
Furthermore, for optimal feed utilisation, it is necessary to ensure a proper physical form, consistency and stability of feed (Kersten et al. 2005, Đuragić et al. 2007). The challenge for the compound feed manufacturer is to make the best use of available resources without compromising on the high safety standards established in the EU ((Doring 2003).

To achieve the nutritional content, functionality, palatability, taste, smell, texture etc. animal feed must be carefully formulated and advanced technological processes must be used.

FEED FORMULATION

Feed directly contribute to the quality of meat, milk and eggs in possitive and negative direction. Throught feed diet content it is possible to manipulate the animal products quality and it is possible to achieve different nutritional, sensoric, physical and chemical characteristics (Chae and Han 1998). Also, the different contaminants may be transmit to animal products through feed. This indicates the necessity of research related to determining the impact of feed on animal products quality and following the quality of animal products depending on the composition of diets consumed by animals (Clayton 2001).
Feed formulation should be based on the animal reqiurements or required performances according to:

- Genetics
- Species
- Physiological needs (maintenance, growth, reproduction, work/lactation)
- Environment

For succesfull feed constitutent utilisation it is important to evaluate nutritient availability for the different animal species and categories on the right way. Contemporary metods (Moughan et. al. 2000) include following characteristic as the main determinants of the nutritional value of a feed ingredient:

- Total nutrient content
- Nutrient availability
- Content of antinutritional factors
- Physico-chemical properties
- Ingredient-specific effects on the utilisation of absorbed nutrients
- Effects on voluntary feed intake and
- Effect on final animal product quality (meat, eggs, milk, manure, etc.)

Knowledge about composition is necessary to assure consistent quality of products as the starting point for future research and understanding the nutrient supply from ingredients to nutrients (Larsson 2006). Research and development in Feed industry must be targeted on raw materials their availability, consistency and impact on animals, people and environment.

It is important to take care about by-products from new industries especially from biofuel production. Kyoto Protocol ratification commits development countries to an 8% reduction in GHG emission by 2010 which will significantly increase the quantity of these products. Biofuel production pulls the carbohydrates and lipids (energy) out of the raw material leaving unused protein to be put back into the feed. Investigation concerned on upgrading of biofuel by-products feeding value may have significant contribution on feed industry

development (Pinotti and Del'Otro 2011).

Besides raw materials, feeds utilized in intensive farming require the use of a diverse range of feed additives. As laid down by the EC regulation (Directive No 1831/2003 EC) they are a large and heterogeneous group of compounds added to feeds due to their nutritional (vitamins, trace elements), zootechnical (such as growth promoters, coccidiostats and anti-blackhead compounds), sensory (colourants and flavours) or technological (antioxidants, preservatives, emulsi-fiers, etc.) role; moreover, the increasing importance of enzymes and microorganisms as probiotics should be taken into account. Such a diverse range of compounds entrain a number of specific issues besides the general objective of ensuring that possible residues in animal products would not pose any appreciable risk to consumers.

It is important to optimize technological processes in animal feed production in order to ensure that all ingredients of the formulated mixture maintain their prescribed concentrations, activities and other characteristics (Rey 2004, Kempen et al. 2001, Behnke 2001). To accomplish that goal it is of utmost importance to develop and improve scientifically-based technological processes ensuring production of quality, safe and environmentally friendly feed.

QUALITY ASSURANCE

The quality of animal feed must be such, that the health and safety of animals and humans as the consumer of meat, milk and eggs is sufficiently guaranteed. To consider consumer confidence about feed and food safety and feed for food quality it is necessary to adopt and promote GMP, GMP$^+$, GHP, HACCP, EFMC, FAMI-QS or some other eficient quality assurance system (Wesselink 1998, Feil 2003, Annon 2007, Hartog 2003 and 2004). Compound feed manufacturers have an important role to play in providing feeds that are nutritious and safe, thereby contributing to the supply to consumers of safe food of high and consistent quality (Ferrary 2011). Feed safety is intended not only in terms of presence of specific contaminants in feed ingredients (such as mycotoxins, plant-produced toxins and residues, as well as heavy metals and other harmful biological agents (particularly pathogenic bacteria), but also in terms of presence of plant secondary metabolites that can affect animal health and performance (Annon 1986, Clayton 2001). Regular quality control must include the determination of chemical composition, specific contaminants and antinutritional factors also.

Apart from material testing, whether processed, semi-processed or raw, condition of equipment and processing machinery is yet another essential prerequisite for successful and cost effective quality assurance throughout the production process (Annon 1986). Irrespective of their size and capacity, feed

processing plants must meet following key requirements to keep the entire process under control (Annon 2002):

- Optimum traceability system
- Prevent contamination of the product by external sources
- Isolation -«Quarantine» of critical or suspected raw material and final products
- Minimize number of sites within the processing plant which can produce conditions favorable to water vapour development, bacteria growth etc.
- Strict control of hygiene and process conditions.

Any piece of equipment, process, or production line or entire feed plant can be the potential source of contamination in feed processing technology threatening quality of final products. Conveyors, separators, switches, cutters, bins and hoppers, mills, scales, weighing devices, devices for thermo-mechanical treatments (conditioners, pelleting machines, extruders, expanders, sanitation devices...), coolers, dryers and other equipments may become critical points in the production process, either due to inadequate design for intended use, defects, damages or as the result of inadequately trained personnel operating the equipment (Gadient 1997, Van der Aar 1998, Đuragić et al. 2007,Sredanović et al. 2005, Lević et al. 1998 and 2000, Vukmirović et al 2010).

PROCESSING

All technological processes must be under active research attention and they are usualy dictated by: animal response, regulatory guidelines or health concernes. Advanced processing technologies such as fine grinding, pelleting, high share conditioning, together with optimum mixing uniformity, can greatly improve feed utilization and subsequently animal performance. When properly processed feed will further enhance growth and feed efficiency as well as product quality (Koster 2003, Behnke 2001, Peisker 2006). The industry must recognise that it needs to adopt the rules of good manufacture and hygiene practice in order to achieve the necessary level of feed and food competiveness, quality and safety (Hartog 2003[a], Lević and Sredanović 2009). Of all the technological processes used in feed production grinding, mixing and hydrothermal or hydrothermal mechanical treatments are of the greatest influence on animal performance and feed quality (Behnke 2002) .

Grinding

Grinding is a major function of feed manufacturing and is by far the most common method of feed processing (Behnke 2001). Particle size is the characteristic that is prescribed and could be adjusted by grinding in the feed mill. Very important is effect of particle size on nutritional quality and for further technological processes (transport, storaging, mixing, pelleting, extruding etc.). Grinding increased surface area of the diet. This improved rate of digestion, influenced digestive passage, decreased segregation and mixing problems and

facilitate further processes such as extrusion and pelleting but also may increased flovability and provoked sticking and feed remaining in transporters, silo bins and other equipments (Koster 2003).

Mixing

Formulated ingredients must be combined through a mixing process to be fed as a complete diet. Making a quality dry mixture with uniform dispersion of highly concentrated microingredients is a major challenge for the feed manufacturer (Kersten 2005). Uniformity is one of the most important quality aspects in feed production. Mixer is a heart of feed production and has to be carefully selected to facilitate thorough mixing of all ingredients. Mixers must be appropriate for the range of weights and volumes required to obtain homogeneous mixtures. Mixer design affects the aggressiveness of the mixing process, and the time needed to achieve desired mix uniformity. Feed mixers of any possible size, shape, design and configuration must achieve a coefficient of variation (CV) of 10% or less as the indicator of mixing abbility and proper functioning. CV is determined for the distribution of a specific nutrient or marker within the feed (Đuragić et al. 2008). For reaching optimum efficiency, mixers must be routinely tested (at purchase and during production from time to time), filled up to certain level, regularly cleaned and maintained. Mixing efficiency may be improved by extension of mixing time, change of filling levels, improvement of physical characteristics of mixer components and elimination of defects and faults caused by improper closing, vibrations during operation, distortion of mixing shaft or tools. Trends in mixer manufacture are evidently directed towards shortening of mixing time, dicreasing height/diameter ratio, elevating filling level and liquid addition (Heidenreich and Strauch 2000). Generally speaking, overall tendency in mixer manufacture is to reduce time needed for individual process in order to increase capacity and cost effectiveness. Newly designed mixers may produce blends with coefficient of variations less than 5% per 90 seconds. Installation of these mixers in existing plant shall result in mixing 20 batches per hour, without additional funds for the reconstruction of entire production line (Sredanović et al. 2005[a]). Capacity of modern feedmills is measured in terms of processing speed and flexibiliy, not the mixer size. Yet another novelty in mixer design is the opening aspect tending towards immediate opening of the mixer and material emptying through the widest possible opening. In that way mixer residuals and their carryover between batches are substantially reduced (Heidenreich and Strauch 2000).

Mixing of dry and liquid components in the same mixer is a critical process due to material build-up and mixer residuals and may result in cross contamination between batches, moisture development and growth of microorganisms. Two parallel mixers are ideal solution when specific components are used for certain mixture types (meat meal, for example), in which case one mixer is operated for the specific use to avoid cross contamination and doubling (Sredanović et al. 2005).

Hydrothermal and hydrothermal mechanical treatments

Interest in hydrothermal and hydrothermal mechanical treatments, other than pelleting, such as steam conditioning, roasting, toasting, micronizing, compacting, expanding and extruding has increased in recent years (Riaz 2007, Lević 2010). These processes are applied for:

- reducing the content of harmful substances,
- improving feed hygiene
- improving feed functionality (Koster, 2003).

Optimal results of these processes must be achieved with sometimes conflicting objectives such as improvement of nutritive value and decontamination of patogenic microorganisms (.

Feed safety, necessary prerequisite for food safety and feed hygiene requirements, dictate decisions concerning which technology for feed preserving to adopt (Van der Aar 1998). Real-time control of microorganisms in raw material is not applicable for feed manufactures. Therefore, preventive technologies with defined decontamination effect are recommended. The efficiency of each decontamination and therefore the energy input of different technological measures are influenced by:

- the level of microbiological infection
- temperature level
- level of moisture content
- addition of mechanical stress
- treatment time
- structure and composition of feed (Heidenreich 2002, Lević et al. 2004).

For safe feed production, various hydrothermal and mechanical treatments are used. More aggressive and longer treatment and higher moisture produce better results (Ziggers 2002). Long-term conditioning, two-fold pelleting, expanding, extruding and higher moisture, and equipment under brand names “BOA compactor”, “SIRT”, “APC”, etc., and their numerous combinations are some of possible solutions.

These decontaminating processes does not prevent recontamination. It is much easier to achieve than to maintain microbiological safety. Condensation, feed residuals in conveying systems and other parts of equipment, heating during some processes, conditions in coolers etc. favour microbial re-growth (Sredanović et a. 2005, Lević et al. 2010). Regulation of moisture, temperature, pH value and addition of acidifiers or some other chemicals is useful for feed preservation during the storage (Dibner 2001, Luckstadt 2005). Contamination levels in the final product must be continuously checked and corrective actions taken if they are exceeded.
Some of hydrothermal and hydrothermal mechanical treatments have adverse effect on the digestibility of some components and can be critical for some

additives of low thermal and mechanical stability (Riaz 2007 and 2009). Possible options for preserving activity of some aditives are: reducing of processs aggressiveness; increasing heat stability of additives; overdosing; application after stressful processs.
The industry must recognise that it needs to adopt the rules of good manufacture and hygiene practice in order to achieve the necessary level of feed and food competiveness, quality and safety.

CONCLUSIONS

There is no doubt that consumers expect high quality and safety along the entire feed and food production chain and that feed industry as the link in this chain must be under active research attention in order to meet all required demands. Feed is now required to be equivalent to foods in terms of nutritional quality, technical aspects, safety etc. and to have positive effect on human health. Challenges for feed industry are also challenges for science, research and development in feed industry and whole feed to food chain. Compound feed manufacturers have an important role to play in providing feeds that are nutritious and safe, thereby contributing to the supply to consumers of safe food of high and consistent quality.
Permanent development and improvement of technological processes is necessary for further progress in feed industry.

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BAKING POTENTIAL OF SPELT CULTIVARS FROM ORGANIC FARMING SYSTEMS IN SERBIA

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Abstract

Introduction
Spelt (*Triticum aestivum* ssp.*spelta*) is an ancient bread-making crop which production declined over time as it has been displaced by modern wheat. Recently, however, it has undergone renewed interest as an "eco-alternative" to common wheat owing to its low nutritional requirements and suitability for cultivation in low-input organic systems. In Serbia, spelt grain production has no traditional base but it has been gradually increasing along with the spread of organic production. This paper examines several spelt cultivars available for Serbian farmers.
Materials and methods
Three cultivars of spelt wheat grown in organic farming in the Province of Vojvodina (Serbia), harvested in 2010, were evaluated for breadmaking potential. The assessment of baking potential included the evaluation of indirect (protein content, gluten content, gluten index, falling number, rheological parameters) and direct indicators (bread and volume yield, crumb hardness and resilience).
Results and discussion
The spelt cultivars had high protein contents (15.5-17.0%), very good wet gluten content (40-45%), and optimal falling number for bakery applications. Two cultivars showed high gluten index. However, one cultivar exhibited good rheological properties (A2 quality group) and high deformation work whereas others were inferior and showed lower dough stability and higher softening. Bread yields were in the range usual for standard wheat breads (132.32-135.54 g). Volume yields ranged between 322.29-486.32 cm^3 and were lower than for standard breads. The spelt cultivar with better rheological behaviour gave bread with satisfactory volume, softer and elastic crumb, good porosity and pore fineness.
Key words: *spelt, rheological properties, bread, quality*

INTRODUCTION

Spelt wheat (*Triticum aestivum* ssp.*spelta* L.) is one of the hexaploid wheats which has many similarities to bread wheat (*Triticum aestivum* ssp. *aestivum* L.).

However, there are few but distinct differences between them: spelt is taller, not free-threshing, hulled grain, has a long straw with a tendency to lodge, and gives lower yields (Campbell, 1997). It was a dominant crop for centuries until the medieval times but its cultivation gradually declined and it has been replaced by modern wheat. Its production as a relict crop survived mainly in Central Europe and some adjacent areas.
In the recent decades, spelt wheat has been receiving increasing interest as a potential "eco-alternative" to common wheat owing to its low nutritional requirements and suitability for cultivation in low-input organic systems (Grela, 1996). Spelt wheat gives good yields at unfavourable environmental conditions and low fertilization and has a better mineral uptake in comparison to modern wheat (Bojňanská and Frančáková, 2002).
In Serbia, cultivation of spelt has not been a part of traditional practise but the popularity of this cereal has been growing since the promotion of its "organic" performance and suitability for production of health food. For this reason, little information is available on the adaptability, yields and technological features of spelt cultivars available for cultivation in Serbia (Bodroža-Solarov et al. 2010). This paper is aimed at filling the existing gaps in data by investigating the breadmaking potential of spelt cultivars currently available for Serbian farmers engaged in organic systems.

MATERIALS AND METHODS

Three cultivars of spelt wheat (*Triticum aestivum* ssp.spelta) which are currently at disposal to organic farmers in Serbia were analyzed. The cultivars were of different origin, namely, Eko-10 (Hungary), Nirvana (Serbia) and one, yet non-identified, coming from Austria or Germany. The spelt wheat samples were grown in an ecological system of the region of Bačka, Province of Vojvodina, Serbia, during 2009/2010 vegetation period.
The samples were dehulled in a dehuller (Heger type DS I 400S, Herrensberg D-7033, Germany) and milled in an ecological mill (A 500 MSM KOMBI Getreide Mühle, Osttiroler Getreidemühlen, Stribach-Dölsach A-9991, Austria) to flour ash content 1.15%.
Total protein (Kjeldahl) (Pravilnik, 74/1988) and wet gluten content (ICC № 106/2), falling number (ICC № 107/1) and gluten index (ICC № 155) were determined according to standard methods.
Rheological properies were determined on a Brabender farinograph (Pravilnik, 74/1988) and a Chopin alveograph (according to standard ICC method № 121).
Baking trial for spelt wheat was modified in comparison to standard procedure. It was necessary to introduce shorter mixing time, lower dough yields, addition of ascorbic acid (5 g/100 kg), and longer duration of dough ripening at lower temperature (25°C) with rounding every 20 minutes. The loaves were baked for 20 min in an oven at 230°C.
Loaf volume was determined by millet seed replacement method. Specific volume, volume yield and bread yield were calculated according to formulas given in Kaluđerski and Filipović (1999).

Crumb textural properties were determined on a TA.XT2 Texture Analyzer (Stable Micro Systems, Surrey, UK), using a 36 mm flat-end compression disc (probe P/36R). Bread firmness and resilience were measured according to a modified 74-10A AACC method.

RESULTS AND DISCUSSION

The results of the research on spelt grains cultivated on certified organic farm in Serbia are given in Table 1.

Table 1 Indirect indicators of baking quality of spelt wheat

Parameters	Cultivars		
	Cultivar non-identified	Eko-10	Nirvana
Protein content (% d.m.)	15.81	15.42	17.02
Wet gluten content (%)	40.8	41.9	44.6
Gluten index	92	54	82
Falling number (s)	282	227	266

The spelt cultivars were characterized with protein contents higher than those usual for bread wheats with an average figure of 16.08% d.m. This is in line with the results of Lacko-Bartošová and Rédlová (2007) who reported an average protein content of 16.8% for 5 spelt cultivars. Wet gluten contents were high and ranged from 40.8% to 44.6%. This is the basic parameter of technological quality; for bread wheat wet gluten content over 27% is considered very good and usually characterizes bread wheat of premium quality. Gluten index and Falling number of two cultivars were in the range optimal for bakery production whereas one cultivar showed gluten index less than 60 which is considered too weak for breadmaking (Grootenboer, 1989) and somewhat lower Falling number. The optimal gluten index values for bread wheat range between 79-90% according to Ćurić and co-workers (2001).
Farinograms, alveograms and photographs of bread slices of the tested spelt cultivars are shown in Figs. 1-3.

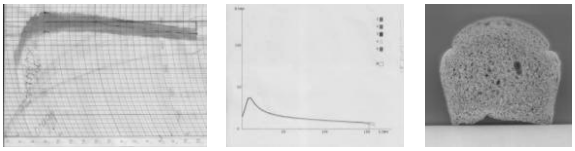

Fig. 1 Baking quality of non-identified spelt cultivar

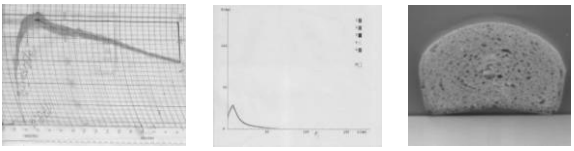

Fig. 2 Baking quality of Eko-10 spelt cultivar

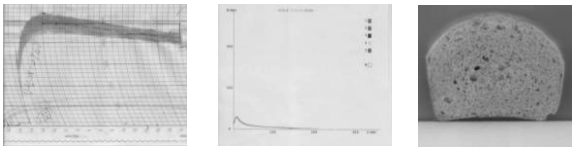

Fig. 3 Baking quality of Nirvana spelt cultivar

Considering their rheological properties, the best performance was shown by the non-identified cultivar which had the highest dough stability (4.5 min) competitive to premium cultivars of common bread wheat and low values of 15-min drop i.e. dough softening, the highest deformation work and belonged to high A_2 quality class. Nirvana and Eko-10 had weaker rheological properties characterized by lower dough stability,higher dough softening and low deformation work. All in all, Nirvana performed better and could be classified to B_1 class whereas Eko-10 belonged to C_1 quality class.

Results of baking trials are displayed in Table 2.

Table 2 Direct indicators of baking quality for spelt cultivars

Parameters	Cultivars		
	Cultivar non-identified	Eko-10	Nirvana
Bread yield (g)	135.54	133.92	132.32
Volume yield (cm^3)	486.32	322.29	387.33
Specific volume (cm^3/g)	3.59	2.41	2.93
Crumb hardness (g)	512.96	1497.68	838.40
Crumb resilience (%)	24.30	17.10	19.37

Bread yields were in the range of usual for standard breads but volume yields were lower. The highest specific volume was shown for the non-identified spelt cultivar. Bojňanská and Frančáková (2002) consider specific volume less than 2.1 ml/g unsatisfactory. Thus, bread loaves made from the examined spelt cultivars were satisfactory. However, there was considerable difference in the crumb quality. Spelt cultivar Eko-10 gave significantly firmer and less elastic crumb.

CONCLUSION

This research showed that the spelt cultivar of non-identified origin had the best bread-making potential either from the viewpoints of indirect and direct parameters. Unexpectedly poor results were found with Eko-10. In general, spelt cultivars had high protein and gluten contents, low bread volume yields but gave loaves of satisfactory specific volume. The major difference between good and

poor performing cultivars was in dough stability, softening and deformation energy which was reflected on bread volume, crumb firmness and elasticity.

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Pravilnik o metodama fizičkih i hemijskih analiza a kontrolu kvaliteta žita, mlinskih i pekarskih proizvoda, testenina i brzo smrznutih testa. Službeni list 74/88.

EFFECT OF VACUUM PACKAGING ON THE COLOR OF TRADITIONAL DRY FERMENTED SAUSAGE (*Petrovská klobása*) DURING STORAGE

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ABSTRACT: The aim of this study was to examine influence of vacuum packaging on the color characteristics of *Petrovská klobása* during storage. Sausages were manufactured in two rural households from hot deboned (A samples) and cold meat (B samples), stuffed into collagen casings and subjected to the processes of smoking, drying and ripening in the traditional way. After completing the drying process (90 days) sausages were stored non-packed (AN; BN) and packed in vacuum (AV; BV).
Color and color maintenance on the cut surface of sausages were sensory evaluated. Also, color characteristics were determined instrumentally using Minolta CR-400 and expressed by CIE *L*a*b** system: lightness (*L**), redness (*a**) and yellowness (*b**). Samples for the analyses were taken at: 0th, 30th, 120th, 180th day of storage.
After 30 day of storage sausages of B group retain optimal score (BN-5.00; BV-5.00), while sausages of group A had significantly better score for sensory evaluation of color (AN-4.52; AV-4.58) comparing to 0th day (A-4.00; B-5.00). Differences in sensory evaluation and instrumental characteristics (*L**; *a**; *b**) of color were not significant (P>0.05) between non-packed and vacuum packed samples at 30th day of storage. Decline in sensory evaluation of color for non-packed sausages was determined after 120 days of storage (AN-3.86; BN-4.75), while for vacuum packed sausages decline was determined after 180 days (AV-3.64; BN-4.88).
During the storage period (120th and 180th day of storage) samples of sausages packed in vacuum had highly significantly higher (P<0.01) score for sensory evaluation of color comparing to non-packed sausages.
The results obtained in this study showed that fermented sausages packed in vacuum, in general, had higher values of color characteristics (*L**; *a**; *b**) compared to the non-packed sausages, and that could be stored without significant losses in sensory quality of color for 120 days after finishing the drying process.

Key words: *Petrovská klobása, traditional product, color, packing, vacuum*

INTRODUCTION

Color is by far the most important sensory attribute in relation to the visual appearance and acceptability of most food items. Dry fermented sausages are not an exception to this; appealing red color of cured meat strongly determine product acceptance and purchase decision (Toldrá, 2007). In fermented sausages, the pigment responsible for the characteristic cured color is the bright red nitrosylmyoglobin ($MbFe^{II}NO$) (Møller and Skibsted, 2002). Nitrosylmyoglobin is formed, under mildly acidic conditions, by reaction of myoglobin with nitrites (Papadima and Bloukas, 1999). In products where no nitrites are added, as in the case of traditional sausages, nitrites could be produced from nitrates by the action of nitrate reducing bacteria (Wirth, 1987). According to Wirth (1987) traces of nitrates were detected in the sausage mixture on the day of preparation, meat itself contained 2 ± 8 mg/kg nitrates and the spices 50±2000 mg/kg (Wirth, 1987). Published date (EFSA Journal, 2008) reported average nitrate content in paprika and chilli peppers of 108 mg/kg and 67 mg/kg, respectively.

The major quality changes that limit the shelf life of fermented meat products include discoloration, lipid oxidation, rehydration, dehydration, and microbial spoilage (Toldrá, 2007). Oxidative discoloration of fermented sausages is characterized by conversion of $MbFe^{II}NO$ to nitrate and the brown derivative metmyoglobin ($MbFe^{III}$) (Gotterup et al., 2008). In addition to selecting suitable packaging materials, appropriate packaging systems should be applied in order to retard or prevent those unfavorable quality changes in the products during storage and distribution. Vacuum packaging is the primary packaging system used for fermented meat products to delay or prevent the quality deterioration by oxygen (Toldrá, 2007). A number of studies investigated color characteristics of fermented dry sausages (Pẻrez-Alvarez et al., 1999; Gimeno et al., 2000; Casaburi et al., 2007; Elías et al., 2010;) but little attention has been paid to the effect of packing conditions on color of fermented sausages.

Petrovská klobása, a traditional dry fermented is produced following original recipe, which includes only natural spices, without addition of synthetic additives. This product is protected with designation of origin (PDO) according to Serbian legislation because of its specific and distinctive quality (Petrović et al., 2007).

Therefore, the aim of this study was to examine influence of vacuum packaging on the color characteristics of *Petrovská klobása,* manufactured from hot deboned and cold meat, during storage.

MATERIALS AND METHODS

Petrovská klobása sausages were manufactured in traditional manner in two rural household from hot deboned (A samples) and cold meat (B samples). All sausages were produced from a mixture of lean minced pork (80 %) and pig fat (20 %). The other added ingredients were: red hot paprika powder (2.5 %), salt (1.80 %), raw garlic paste (0.20 %), caraway (0.20 %) and sucrose (0.15 %). After grinding meat and fat to a size of about 10 mm, spices were added and

raw materials were manually mixed. The mixture was stuffed in collagens casings. The sausages were stored in a cold room (0 – 4 °C) for 24 h, and after a resting day were processed to smoking. Samples of sausages were cold smoked in a traditional way with specific kinds of wood (mixture of cherry and apricot) during 10 days. After smoking, drying and ripening processes continued till achieving desired moisture content, what was after 90 days. After completing the drying process sausages were stored non-packed (AN; BN) and packed in vacuum (AV; BV). Samples for the analyses were taken at: 0^{th} (at the day of packing), 30^{th}, 120^{th} and 180^{th} day of storage.

Sensory evaluation of color

Color of the samples was sensory evaluated by a trained sensory panel. The six members of the panel were asked to judge the appearance of color by using a 5 point scale in which 0 stood for atypical color of products and 5 for a dark red color of products (optimal quality level).

Color measurement

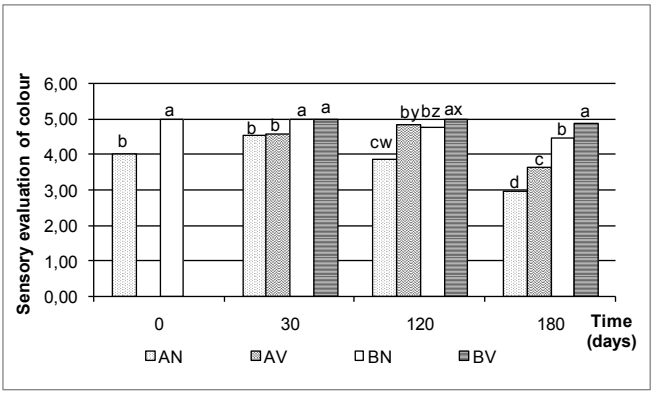
Color measurements were taken immediately after cutting the samples in order to prevent color degradation as a result of the action of light and oxygen, in accordance with the recommendations for color determination of the American Meat Science Association (Hunt, 1991). Sample thickness was 3 cm. Color was studied in the CIE *L*a*b** color space and described by coordinates: lightness (*L**), redness (*a**) and yellowness (*b**). The CIE *L*a*b** color coordinates were determined on the sausage core using Minolta Chromo Meter CR-400 with Light Protection Tube CR-A33b (Minolta, Osaka, Japan). Lighting D-65, standard observer angle of 2° and aperture of 8 mm were used. Before each set of measurements, the instrument was calibrated using a white ceramic tile (CR-A43).

Statistical analysis

All determinations were done for three samples in two parallel, and the results were presented as mean values. Analysis of variance (Duncan test) was used to test the hypothesis about differences among obtained results. The software package STATISTICA 8.0 (STATISTICA, 2008) was used for analysis.

RESULTS AND DISCUSSION

Results of sensory evaluation of color of *Petrovská klobasá* for non-packaged sausage (AN; BN) and sausages packaged in vacuum (AV; BV), during storage period are shown in Figure 1. At the beginning of storage period the sausages of BN group retained optimal score for sensory evaluation of color (5.00), while sausages of AN group had highly significantly lower ($P < 0.01$) score (4.00) compared to BN group.



ns Means are not significantly different (P > 0.05); xyzw The differences are statistically significant with 95% probability (P < 0.05); abcd The differences are statistically significant with 99% probability (P < 0.01)

Figure 1. Sensory evaluation of color during storage period of non-packed sausages (AN; BN) and sausages packed in vacuum (AV; BV)

Furthermore, after 30 day of storage the score for sensory evaluation of color increased for sausages of group A (AN-4.52; AV-4.58), while scores for sausages of B group remained unchanged, accordingly optimal (BN-5.00; BV-5.00) comparing to 0th day. Decrease in sensory evaluation of color for non-packed sausages was determined after 120 days of storage period (AN-3.86; BN-4.75). For vacuum packed sausages an increase in sensory evaluation of color for sausages of AV group (4.83) was determined, while scores for sausages of BV group remained unchanged (5.00) at 120th day of storage.

Decrease in sensory evaluation of color for vacuum packed sausages was determined after 180 days (AV-3.64; BN-4.88). During the storage period (120th and 180th day of storage) samples of sausages packed in vacuum had highly significantly higher ($P < 0.01$) score for sensory evaluation of color compared to non-packed sausages. Also, the sausages that were made from cold meat during storage period had highly significantly higher ($P < 0.01$) scores for sensory evaluation of color compared to sausages made from hot deboned meat.

Average values of lightness (*L**) during storage period of non-packed sausages and sausages packed in vacuum are shown in Figure 2. At the beginning of storage period (0th day) the samples of AN group (*L**- 34.43) had highly significantly higher ($P < 0.01$) values of lightness (*L**) compared to sausages of BN group (*L**- 30.50). From the results shown in Figure 2 it can be observed that after 30 days of storage there were no significant differences ($P > 0.05$) in *L** value for vacuum packaged and non-packaged sausages. Furthermore, at 120th day of storage *L** values ranged from 26.72 (AN) to 34.82 (BV), and at 180th day of storage it ranged from 28.59 (AN) to 34.37 (BV).

During the storage period (120th and 180th day of storage) samples of sausages packed in vacuum had highly significantly higher ($P < 0.01$) values of lightness (*L**) compared to non-packaged sausages. The exception was for AN and AV samples of sausages where significant differences ($P > 0.05$) in values of lightness were not determinate at 180th day of storage.

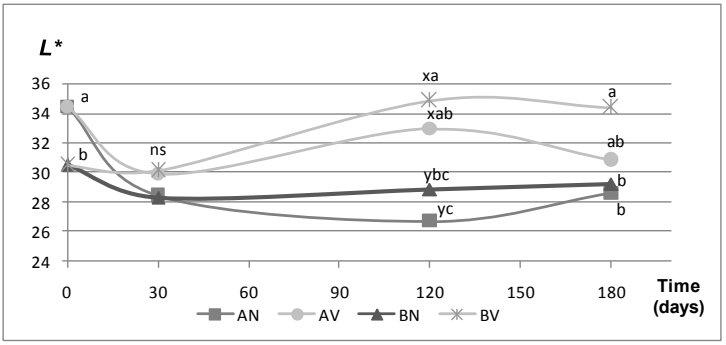


Figure 2. Average values of lightness (*L**) during storage period of non-packed sausages (AN; BN) and sausages packed in vacuum (AV; BV)

Papadima and Bloukas (1999) studied the effect of storage conditions on quality of traditional Greek sausages. They found that sausages with a higher weight loss had lower values of lightness (*L**). Liaros et al. (2009) found that sausages packed in vacuum during ripening had higher values of lightness (*L**) compared to non-packaged sausages what was also confirmed in this study.

*L** value obtained for *Petrovská klobása* were lower in comparison with the same values determined for Spanish (Pẻrez-Alvarez et al., 1999; Gimeno et al., 2000) and Portuguese traditional fermented sausages (Elías and Carrascosa, 2010, Casquete et al., 2011). The similar values were obtained for Croatian (Kovačević et al., 2010) and Italian traditional fermented sausages (Casaburi et al., 2007). *Petrovská klobása* sausages were darker than other traditional sausage primarily due to the specific recipe, which includes usage of larger amounts red pepper (2.5 %). Also, the differences in *L** values can be explained by the longer ripening period of *Petrovská klobása* (4 months) comparing to Spanish and Portuguese traditional fermented sausage.

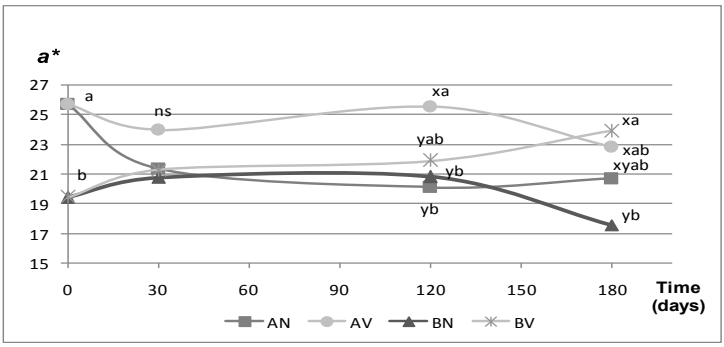


Figure 3. Average values of redness (*a**) during storage period of non-packed sausages (AN; BN) and sausages packed in vacuum (AV; BV)

Redness (*a**) is often used as an indicator of meat and meat products color stability and it is an important indicator of color changes during storage (Rubio et al., 2008). Average redness (*a**) and yellowness (*b**) values for non-packed and vacuum packed sausages during storage are shown in Figure 3 and 4. At the beginning of storage period the samples of AN group (*a**-25.72; *b**-23.09) had highly significantly higher ($P < 0.01$) *a** and *b** values compared to sausages of BN group (*a**-19.42; *b**-13.97). During the storage period the samples of sausages packed in vacuum had higher *a** and *b** values compared to those values of non-packaged sausages. After 120 days of storage the sausages of AV group (*a**-25.54; *b**-22.23) had highly significantly higher ($P < 0.01$) *a** and *b** values compared to those of non-packaged sausages (AN = *a**-20.12; *b**-15.00). For the sausages of B group after 180 days of storage it was determined that sausages packed in vacuum (BV) had significantly higher values *of redness* (*a**-23.89 ($P < 0.01$)) *and* values of yellowness (*b**-19.83 ($P < 0.05$)) compared to values of non-packaged sausages (BN = *a**-17.55; *b**-15.11).

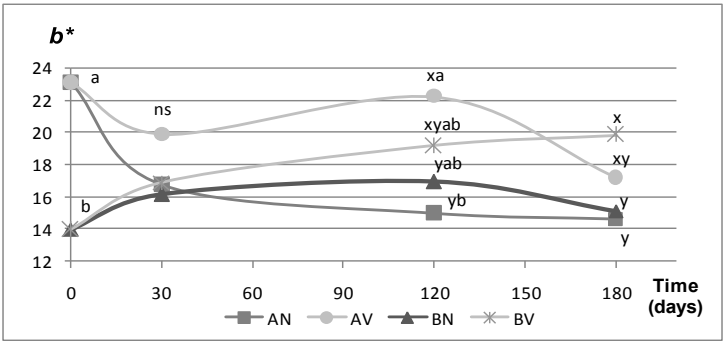


Figure 4. Average values of yellowness (*b**) during storage period of non-packed sausages (AN; BN) and sausages packed in vacuum (AV; BV)

Papadima and Bloukas (1999) explained that in traditional Greek sausages the decrease of a* value was caused by oxidation of nitrosylmyoglobin. This process was accelerated by increased salt content of the product because the salt acted as pro-oxidant (Savic and Savic, 1996). Liaros et al. (2009) found that longer ripening times under vacuum resulted in higher *a** value, probably due to the decrease of oxidative discoloration. Also, they determined that the fermented sausages with the longest period of ripening at vacuum packaging had the highest values of yellowness (*b**), what was in accordance with results of our work.

Values of redness *(a*)* and yellowness (*b*)* obtained for *Petrovská klobása* were higher in comparison with the same value determined for Portuguese traditional fermented sausages (Elías and Carrascosa, 2010, Casquete et al., 2011). The similar values were obtained for Croatian (Kovačević et al., 2010) and Italian traditional fermented sausages (Casaburi et al., 2007). Spanish traditional fermented sausages (Gimeno et al., 2000) had similar values of redness (*a**), but lower values of yellowness (*b*)* than those for *Petrovská klobása.* Higher values of redness and yellowness for *Petrovská klobása* were the consequence of the presence of the characteristic ingredients of this product – domestic paprika. The color of red paprika is controlled by several carotenoids (capsanthin, capsorubin, and xanthophyllys for the red color and β-carotene zeaxanthin for the yellow–orange color) (Ittah et al., 1993; Mínguez-Mosquera and Hornero-Mẻndez, 1994). Color of fermented sausages depends on the amount of paprika added to this product, as well as the quality of the paprika.

CONCLUSIONS

Based on the obtained results it can be concluded that scores for sensory evaluation of color at 30th day of storage were not significantly different ($P > 0.05$) between non-packed and vacuum packed sausages. At 120th and 180th day of storage the samples of vacuum packed sausages had highly significantly higher ($P < 0.01$) score for sensory evaluation of color compared to non-packed sausages. Also, during storage, the sausages made from cold meat had highly significantly higher ($P < 0.01$) scores for sensory evaluation of color compared with sausages made of hot deboned meat.

The results obtained in this study showed that fermented sausages packed in vacuum, in general, had higher values of color characteristics (*L**; *a**; *b**) compared to the non-packed sausages, and that could be stored without significant losses in sensory quality of color for 120 days after drying process.

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ECO-SUSTAINABLE FOOD PACKAGING BY NANOMATERIALS

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ABSTRACT: For long period of time traditional polymers derived from fossil fuels have played the major role in common packaging material because of their several desired properties like softness, lightness and transparency. However, traditional polymers are non-biodegradable and impractical for recycling so their increased use has led to ecological problems. New demand for eco-friendly sustainable food packaging put the more attention to the biobased polymer materials as possible alternative. However, these polymers still have some disadvantages considering performance, processability and cost. Recently, polymer nanotechnology brings new opportunities and innovative solution to improve their performance. This article gives short reviews of different type of biobased polymer nanocomposites for food packaging application.

Key words: *biobased polymer, polymer nanocomposites, sustainable food packaging*

INTRODUCTION

The basic function of the food packaging is to protect food from physical damage, to maintain freshness, to protect from light gases and vapor. Additionally, food packaging must enable to display informations about the content, the brand and stockability. Packaging is also an important factor in the buying decision process, so it must be attractive in activate visual stimulation.
Packaging materials and technologies have been developed over the centuries, but today's demands are looking for new improvement and innovation (Bradley et all 2011).

It is well known that synthetic conventional polymer materials (made from fossil fuels) have a number of advantages as food packaging materials over the traditional materials such as metal, alloys or ceramics because of their availability at relatively low cost, good mechanical, thermal, barrier properties and good processability. Using of polymers in food packaging industry has increased enormously in the last decades. In the polymer global market, that has increased from 5 million tons in 1950 s to nearly 100 million tons today, the 42% is covered by packaging, figure 1. (Silvestre et. all 2011). The most used conventional polymers as food packaging are: polyolefines, (polyethylene PE,

polypropylene PP), polystyrene PS, polyvinylchloride PVC, polyurethane PU, polyethyleneterephtalate PET, polyamide PA, epoxy resins.

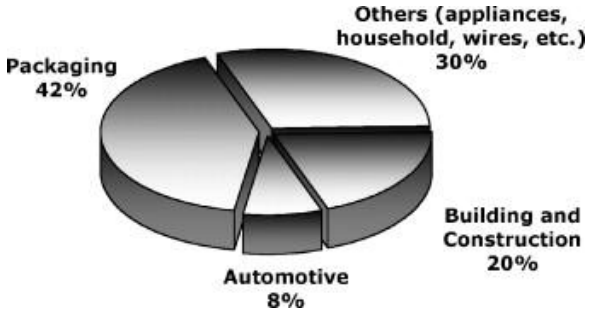


Figure 1. Polymer global market

However, recycling of conventional plastic food packaging materials is impracticable because of being food contaminated, they are not totally recyclable and biodegradable. Recently, growing environmental awareness followed by possible lack of fossil fuels has led to new packaging material trends: sustainability, eco-efficiency and biodegradability. New demands have stimulated the researchers for developing and using biodegradable and biobased materials as alternatives to conventional non-degradable polymers for food packaging. Up to now, broad application of biodegradable and biobased polymers has been limited due to many problems which must be solved in the future. The major problems are: performance, processability and cost. The application of nanotechnology and incorporation of nanoparticles to these polymers may help.

Polymer nanotechnology is a broad interdisciplinary area of research that can provide innovative solutions to increase the performance of the bio-polymers further adding safety, economical and environmental advantages.

This paper provides a short review about new trends in using biobased polymer nanocomposites for food packaging applications.

BIODEGRADABLE AND BIOBASED POLYMERS

Sustainable materials are made from renewable resources. They are recyclable, biodegradable, economically viable and environmentally acceptable. According to definitions, (Weber J.C., 2000), biodegradable material is one that can be broken down as the results of being exposed to naturally occurring micro-organisms such as bacteria, fungi and algae into biogases and biomass (CO_2 and H_2O). Biobased materials are materials derived from renewable sources and are biodegradable. Biobased polymers can be divided in three categories based on their origin and production, figure 2:

1. Polymers directly extracted /removed from biomass – such as the polysaccharides, starch, cellulose and proteins like casein and gluten

2. Polymers produced by classical chemical synthesis using renewable biobased monomers such as polylactic acid and vegetable oils monomer such as different polyols. The monomers may be produced via fermentation of carbohydrate feedstock.
3. Polymers produced by microorganisms or genetically modified bacteria such as polyhydoroxyalkonates and polypeptides.

It is also possible to obtain biodegradability is for non biobased materials making mixtures with biobased materials. Biodegradability can help reduce plastic waste. Biobased sustainable materials (also called biopolymers or natural polymers) are currently considered as the only alternative in replacing the traditional fossil fuel materials in food packaging, even that production of bio-plastic can potentially lead to competition with agricultural resources for foods. However, in spite of their significant potential, those materials still have some disadvantages for the applications in food packaging. They have lower mechanical, thermal and barrier properties to gasses and vapors, strong water sensitivity, lower shelf life stability due to ageing and number of processability issues.

Polymer nanotechnology brings in significant opportunities to overcome the above mentioned disadvantages of biobased polymers.

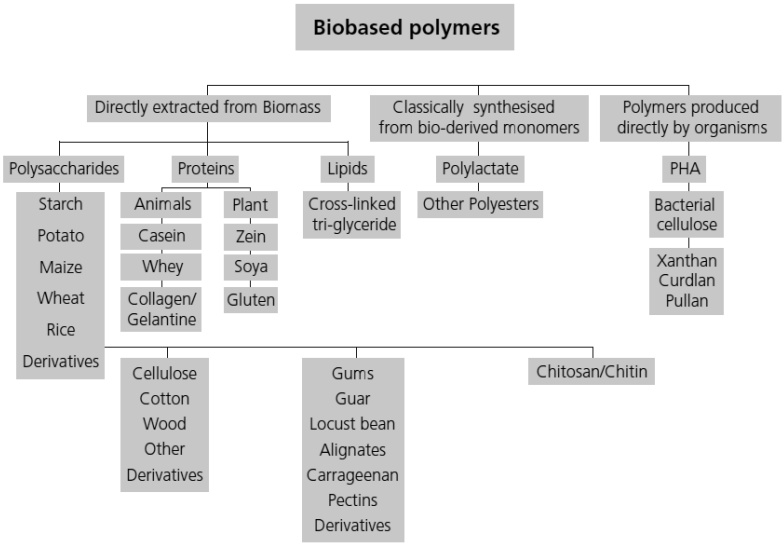


Figure 2. Schematic presentation of biobased polymers based on their origin and method of production (Weber J.C. 2000),

BIOBASED POLYMER NANOCOMPOSITES FOR FOOD PACKAGING

Polymer nanocomposites are a novel class of composites, derived from ultrafine inorganic particles with large surface area per volume with typical range of 1-1000 nm dispersed in the polymer matrix. Organic-inorganic composite materials

combine the advantages of the inorganic materials such as rigidity, hardness, durability, thermal stability and those of organic polymers (flexibility and processability). They can be prepared by different methods: solution casting, in situ-polymerization, melting processing. However, for obtaining the enhanced properties it is necessary to have good disperseability of nanoparticles and their good interaction with polymer matrix. This is often one of the major challenges for the researcher in developing new polymer nanocomposites. Du to unique properties, these types of materials have attracted strong interest in many industrial processes. Although the large amount of research being undertaken in industry and academia, polymer nanotechnology for food packaging is still in development stage (Silvestre et. all 2011).

The main driver for application of nanomaterials in food packaging is innovation and new product development. New products can give greater consumer choice and connivance (Bradley et all 2011).

For example, polymers nanostructured materials give the possibility for developing improved food packaging with functional properties. Incorporation of nanoparticles in the polymeric matrix enables food packaging production with improve mechanical, gas barrier properties, temperature/moisture stability, durability providing the extended shelf life of the food. The nanoparticles with antimicrobial or antioxidant properties give the active packaging concepts and allow packaging to interact with food and environment. Using nanosensors it is possible to monitor and report the food conditions and to develop intelligent food packaging.

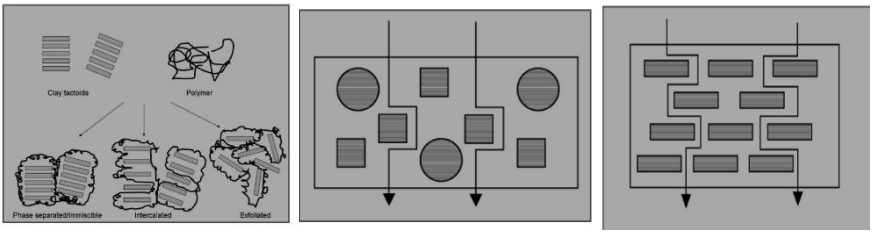
It is convenient to define polymer nanocomposites that have been currently used In the food packaging industry as polymer matrix with nanoditive. Nanoaditives can be divided as nanoclay, nanofibers, nanoparticles, nanotubes.

Up to now, the most studied biodegradable polymers used as polymers matrix suitable for wide range of packaging applications are: starch and derivates, polylactid acid (PLA), poly(buthylenesuccinate) (PBS), polyhydroxybutyrate (PHB), aliphatic polyestar as polycaprolactone (PCL), polyhydroxyalkanoate (PHA), etc.

Nanaoclay and their biopolymers composites

The polymers composites based on clay nanoparticles are among the first that have attracted more attention in combining with biopolymers and that have been emerge on the market as improved materials for food packaging. The nanoclay generally used is montimorrillonite – hydrated alumina-silicatelayered clay, widely available natural clay derived from volcanic ash and rocks. (www.nanowiki.no/wiki/Applications_of_nanotechnolofy_for_the_food_sector). It has been reported (Lagaron et. all 2011) that loading nanolayered clay particles to biopolymer can improve mechanical properties, thermal stability, barrier properties for oxygen, vapor and UV protection of virgin biopolymer. To obtain the above mentioned improvements, a small amount of clay must be incorporated in polymer matrix. This process is often called layer dispersion in

polymers and involves two major steps: intercalation and exfoliation. In the intercalation step, polymer chains or monomers molecules diffuse into the clay galleries. In exfoliation, the clay particles are dispersed in the matrix polymer with no apparent particle interaction (www.nanowiki.no/wiki/Applications_of_nanotechnolofy_for_the_food_sector). However, the main advantage of using nanoclays is achieved in barrier properties to gas and water. When nanoclay is well dispersed in the matrix, limits the permeations of gases. This phenomena is explained by Nielsen theory (Nielsen L.E. 1967) which focuses on a tortuous path around the clay plates forcing the gas permeate to travel longer path to diffuse through the film, figure 3. These improvements have led to the development of nanoclay polymer material for potential use in a variety food packaging applications such as processed meats, cheese, confectionery, cereals, boil-in-the-bag foods as well as in extrusion coatings, application for fruit juices and dairy products, or co-extrusion processes for the producing bottles for the beer and carbonated drinks (Silvestre et. all 2011). Many studies have reported the effectiveness of nanoclay in decreasing oxygen and water vapor permeability of several polymers (Bgradwaj et. all 2002, Mangiacpra et.all 2006).



a) b) c)

Figure 3. a) Creation of clay nanocomposites, b) diffusion conventional composites, c) diffusion nanoclay

One of the most promising biopolymer and already the most commercialized is polylactic acid (PLA). Lactic acid, monomer of polylactic acid may be easily produced by fermentation of carbohydrate feedstock, corn etc. which are renewable resources. (Cabedo at al.,2006). It is versatile polymer recyclable and compostable, with high transparency, high molecular weight, good processability and water solubility resistance. However, the low deformation at the break and quite expensive price limit its application. The properties of the PLA are highly related to the ratio between the two forms (L or D) of the lactic acid monomer. L-PLA is a material with a very high melting point and high cristallinity whereas a mixture of D and L –PLA results in amorphous polymer with low glass transition temperatures. Considerable efforts have been made to improve mechanical properties of PLA, so it can compete with commodity polymers by incorporating nanoparticles. Many papers reported the improvement of PLA mechanical, thermal and barrier properties by nanoclay (Zhang et.all 2008, Rasal et.all, 2010,

Pluta, 2004). Currently PLA is used in food packaging applications only for short shelf-life products.

Combination of nanoclay and natural polymers such as sugar and proteins can create potentially non-toxic biodegradable and biocompatible materials so called „green nanocomposites". Starch is a natural material, completely biodegradable and can promote the biodegradability of non bio-degradable plastics when blended and is considered as promising candidate for developing sustainable packaging materials. But, as a packaging material, starch alone does not posses the desired mechanical and thermal properties and therefore must be modified by making blends or composites with different materials. It has been shown that incorporation of small amounts (less then 5%) of sodium montmorillonite nanoclay particles provide improvement of the tensile strength and elongation at the break of thermoplastics starch. (Park et. all 2002). Also, mechanical and testing results show an increase of modulus of starch/clay nanocomposite films obtained by dispersing monmorillonite nanoclay via melting processing techniques (Avella et. all 2005).

Polycaprolactone (PCL) is a linear polyester produced by ring-opening polymerization of ε-caprolactone. It is a semicriytalline polymer with high elongation at the break and low modulus. Better physical properties are enhanced by preparation of PCL/organoclay modified layered silicate (OMLS), (Gorrasi et. all, 2004).

Polyhydroxybutyrate is polyester suitable for industrial application. It is highly crystalline and has low water permeability, but mixing with nanoclay overcome its disadvantages (Chen et. all., 2004).

Chitosan and casein are biopolymer that exhibit improved mechanical and barrier properties mixing with nanoclay (ObservatoryNANO briefing July 2010, briefing No. 1).

Other nanoparticles

Besides nanoclay, other nanoparticles found their application in preparing bio nanaocomposites for food packaging such as metal and oxide nanoparticles, carbon nanotubes. Due to their antimicrobial properties and UV resistance properties, metal nanocomposites have been studied to develop active antimicrobial packaging. Silver, gold and zinc are the most studied. Silver has been used in medical care for ages. Currently, silver nanoparticles being used in a wide range of consumer products including refrigerate and socks. The silver nanoparticles in contact with bacteria and fungus will adversely affect cellular metabolism and inhibit cell growth, (Silvestre et. all 2011). Recently, a comprehensive performance study of PLA biocomposites has been reported, congaing a novel silver based antimicrobial layered silicate additive for use in active food packaging application. The silver based nanoclay showed strong antimicrobial activity against gram negative Salmonella spp. (Lagaron et. all 2011). Titanium dioxide (TiO_2), zinc oxide (ZnO), silica (SiO_2), magnesium oxide (MgO) are the most studied oxide nanoparticles for their ability to be UV

blockers and photo catalytic disinfecting agents (Silvestre et. all 2011). Further, reinforcing effectsit is possible to achived by spherical silica nanoparticles. Because of their great natural abundance, low cost and high thermal resistance and surface functionality, they are very suitable for various applications. (Wen et. all 2011). Carbon nanotubes are also widely used not only for improving the mechanical and thermal properties of polymer matrix, but also for their antibacterial properties.

Biobased nanofibres

Many biopolymers such as chitosan, cellulose, collagen, zein (derived from corn) have been made as nanofibres via the new elektrospinning techniques (ObservatoryNANO briefing July 2010, briefing No. 1). These fibers in some cases have superior properties to the traditionally polymers and can be used both as material for food packaging or for encapsulation in polymer matrix for obtaining additional functionality.

Edible nanocomposites based material

Edible films are defined as thin continuous layers used to coat food or as barrier between food and other materials or environments. Those films may be applied with a paintbrush, by spraying or fluidizing. It can be noticed that edible coatings form an integral part of the food product and hence should not impact the sensory characteristics of the food. Components of edible films and coatings can be divided in two categories: water soluble polysaccharides (hydro-colloids) and lipids. Suitable polysaccharides include cellulose derivates, alginates, pectines, starches, chitosan. Suitable lipids are waxes, acylglycerols and fatty acids. Polysaccharide films are low cost but exhibit low moisture barrier properties. Protein films have advantages functional properties as plasticity, elasticity and good oxygen barrier but poor water barrier properties. Lipid films have good moisture barrier but pure oxygen barrier and mechanical properties. (Sorrentino et all 2007). Same as to other food packaging application, incorporation of nanoparticles in edible film components can improve mechanical and physical properties. Furthermore, edible films can serve for incorporating food additives and other substances in order to enhance product color, flavor and texture and to control microbial growth. Here, nanoparticles may have an important role as carrier.

Figure 4. shows the indication of the status of the nanotechnology research and development in food packaging according to a five step Technology Readiness Level (TRL) system for having a quick reference developed by Observatory NANO.

CONCLUSIONS

Bionanocomposites represent an exciting field with a number area of applications. Many efforts have been made to connect research consortia and institutes with food packaging industry. Nanotechnology adds value to the currently socially desirable bioplastic paradigm by making them stronger, water and gas impermeable and adding functionalities such as antimicrobial effects.

However, for the benefits to be harvested there are still many open questions like: safety of nanomaterials, regulatory, composting capacity, balancing biomass production between materials and food. Anyhow, nanoprocessing and bionanocomposites promise competitive and eco-sustainable food packaging solutions.

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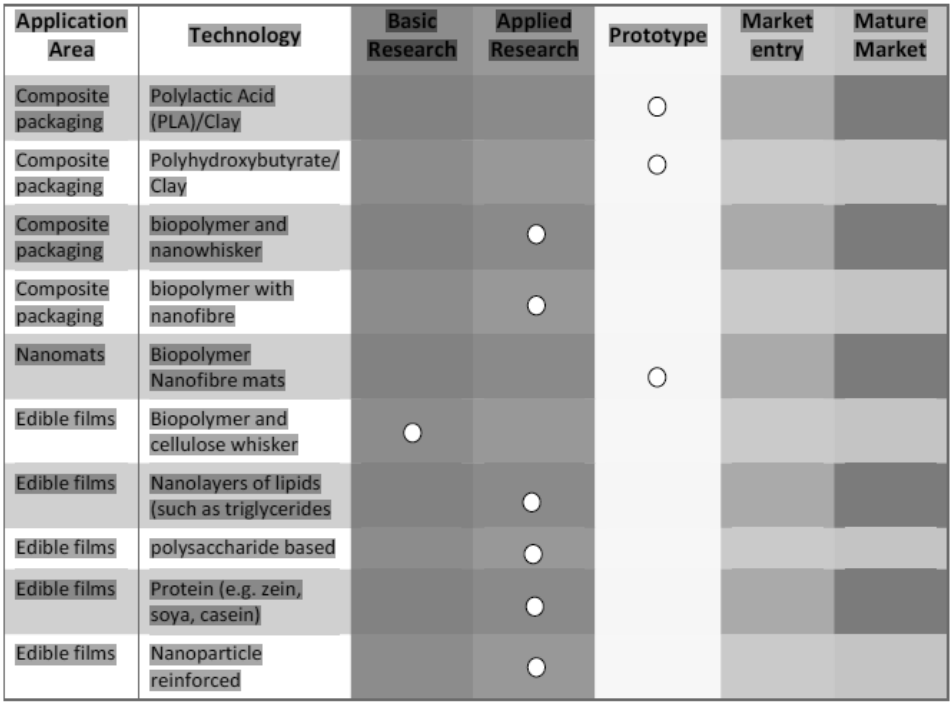

Figure 4. The indication of the status of the nanotechnology research and development in food packaging

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