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Keynote Presentations

Major Trends in Flavor Research

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Abstract

Flavor research has been a key activity in academia as well as in the flavor and food industry. Many key aroma and taste compounds have been identified, their sensory characteristics described, their formation mechanisms studied using thermal and/or bio-assisted approaches and means for protection against instability developed. For a long time, discovery of new molecules has been the focus, using analytics and synthetic chemistry, and recently new high throughput receptor-based assays have been designed for the screening of flavor-active components.

Given the global trends, i.e. natural, organic, authentic, “clean label”, it has become essential to revisit the way flavor is used in foods and beverages. Adding flavors is not anymore, the standard and often not the most preferred approach. This may even apply to “natural flavors”, depending on the product category. Hence, the food industry requires alternative concepts responding to consumers’ needs, inspired by home-style cooking, natural flavor extracts, and ingredient-based flavoring. The talk will elaborate on a few current topics and how challenges can be transformed into opportunities. This, however, needs to be supported by academic research adapted to the new trends.

21st Century Challenges and Opportunities in Food Risk Assessment

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Abstract

The methods of food risk assessment developed in the 20th century have been superseded by a new generation of tools not yet fully integrated into the regulatory system.

For chemical risk assessment, advances in exposure science have complemented increasing knowledge of biological processes and toxicological hazard identification to build better models of toxicity. For example, in the area of dietary studies, new tools such as urinary biomarker panels, are helping to generate more precise estimates of food intakes than classical food questionnaires alone. High-content cellomic and organ-on-chip platforms have expanded the repertoire of toxicology tools enormously. Some *in-silico* models have achieved similar or better predictive power to conventional animal bioassays. However, the development of new scientific tools in toxicology and microbiology have outpaced our ability to consistently interpret the large amounts of data generated. Equally, while the previous generation of tools benefited from a long history of experience and precedent in regulatory applications, many of the new tools are poorly understood by regulators and risk assessors alike. Furthermore, there is growing consensus that the gut microbiota may influence risk associated with some dietary exposures, but there is still no standard approach to incorporate such considerations in safety evaluation. All of the above argue for the development of transparent, evidence-based rules and standards for risk assessment that support both consumer protection and commercial safety assessment. Data science has started to play a major role in the risk assessment process as large volumes of

data need to be curated, processed, and modelled. This presentation will review the evolution of risk assessment science and will propose frameworks to support successful integration into decision making.

Food Authentication

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Abstract

Worldwide, food fraud most commonly affects goods such as olive oil, fish and organic foods, as well as commodities such as spices, tea, cocoa, coffee or nuts. Globally, revenue from counterfeit or adulterated raw materials and foods amounts to tens of billions of Euros every year. This figure highlights the fact that the quality control strategies practiced to date are unequal to the problem. Compared to centuries past, contemporary challenges are therefore considerably more complicated and, due to the global material cycles now in place, include determining the commodity type (e.g. variety), identifying the exact geographical origin (e.g. to verify a product as a regionally-protected food) and distinguishing between specific types of production (ecological and sustainable vs. conventional agriculture).

The talk is going to give insights on state-of-the art approaches to cover the originality of food starting materials.

Foods High in Folate Vitamins – Analytical Confirmation and Biofortification

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Abstract

Folates are a group of water-soluble vitamins the intake of which is considered to be well below dietary recommendations. This particularly applies to countries, which do not fortify staple foods. However, mandatory fortification is still under discussion due to potential risks for some parts of the population. Therefore, there is an emerging search for natural folate sources. Besides, green vegetables available in developed countries, there is an additional variety of other promising sources from regions with high biodiversity of plants suitable for food use. For evaluating these sources, accurate methods for folate analysis and assessing their bioavailability are required. In this presentation, the use of stable isotopes to achieve this goal will be presented as well as recent results on promising folate sources from parts of the world with high biodiversity. Moreover, options for biofortification and for assessing folate bioavailability will be presented.

Risk on the Table? - Perspectives in Chemical Food Safety

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Abstract

Safety assessment of chemical compounds present in food is focused on the risk of the occurrence and levels of defined chemical entities to humans. Chemicals can either occur as natural constituents, as unwanted contaminants or as residues of the treatment of plants or animals used in food production. The risk assessment process can be separated into four steps, i.e., the exposure assessment, hazard characterization, mode of action assessment, and risk characterization. It is the aim of these steps to classify the chemical in particular with respect to its carcinogenic, genotoxic and teratogenic/developmental effects. The data used comprise analytical data, exposure assessments, biochemical data, toxicological studies in laboratory animals and observations in humans. Furthermore, structure-based *in silico* and read-across data are more and more used. In particular, the differentiation between genotoxic carcinogens and non-genotoxic and/or non-carcinogenic compounds is of crucial importance. In the final risk assessment, the methods used comprise the calculation of a Margin-of-exposure (MoE) for genotoxic carcinogens versus the derivation of a health-based guidance value (e.g. a Tolerable Daily Intake; TDI) for non-genotoxic and/or non-carcinogenic compounds. Both are usually based on No-observed-adverse effect levels (NOAELs) or Benchmark dose (BMD) modeling from animal studies resulting in relevant adverse dose levels called Point of Departure (POD). This is either divided by a safety factor to yield a TDI or compared to the exposure in relevant populations to yield a MoE. For compounds without relevant

toxicological data, a structure-guided read-across based concept, the TTC (Threshold of Toxicological Concern) approach is used.

Reduction of Saturated Fatty Acids in Structured Lipid Phases

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Abstract

Given that saturated and trans fatty acids are known to increase the risk of cardiovascular diseases by raising the blood cholesterol level, it is recommended to reduce their daily intake to a minimum (WHO draft guideline 10% of energy intake max). Fat compositions like palm or milk fat, which consist i.a. of crystallized triacylglycerol (TAG) fractions, contain considerable amounts of saturated fatty acids, but provide unique texture and rheology to foods.

Sources of mono- and polyunsaturated fatty acids cannot match these properties in their native state. It is hence, necessary to transform an oil into a gel with the help of structuring agents. The field of oleogelation tries to address this need. In the past decade a vast amount of different structuring systems has been identified. The level of understanding of the mechanism of action and depth of study differs widely between the various systems. In short, the structuring agents that have been found range from proteins to ethyl cellulose, from waxes to monoglycerides, and from combinations of tocopherols with lecithins to sterol/sterol ester combinations.

Despite the fact that most of the scientific contributions show that oleogelation is successful and suggest product applications practically no products are on the market currently. This contribution aims to formulate the hurdles to overcome and requirements to be met for a further implementation of oleogelation. The status of this emerging field with respect to the current state of technology and future prospective of the various approaches to reduce SaFA in structured lipid phases will be discussed.

Chemistry of Singlet Oxygen Induced Oxidation in Foods

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Abstract

Singlet oxygen is an excited state, electrophilic, non-radical molecular oxygen, which is different from triplet oxygen (ordinary molecular oxygen) in its electron arrangement. Singlet oxygen can be easily formed from triplet oxygen in the presence of light and photosensitizers. The lifetime of singlet oxygen is extremely short (microseconds), but still long enough to react with food components. The reaction rate of singlet oxygen with food components is extremely high as compared with that of triplet oxygen. Thus, the production of singlet oxygen in foods induces the highly fast oxidation of food compounds, producing a range of undesirable compounds in foods during processing and storage. Several important chemical reactions in foods such as the sunlight strike off-flavor in milk products and the fast lipid oxidation of edible oils under light storage can be explained by the singlet oxygen-involved oxidation. Singlet oxygen quenchers such as carotenoids and tocopherols in foods can minimize singlet oxygen oxidation. The scientific knowledge on the formation, reactions, quenching mechanisms, and kinetics of singlet oxygen in foods will be discussed.

Food, Microbiome and Nutrition - Causal Links Influencing the Health of Patients

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Abstract

Microbiological diseases usually correspond to specific causative agents and symptoms. However, various statuses of the

microbiome, associated with human health or disease, as well as complex relations between man and his microbes, have been recently analyzed by increasing depth. Molecular methods and learning systems provide tools for these studies.

In our laboratory, microbes isolated from stool samples have been screened against patient sera in order to understand microbial attachment, penetration and intrusion into the body system. These immunogenic traits together with information on the overall composition of the host microflora, help in the diagnostics of diseases, and in evaluation of the risks for their onset. While beneficial microbial composition can prevent the development of serious diseases, such as cancer or stroke, imbalanced microbiome may provoke e.g. lower back problems, headache or fatigue.

In numerous countries more than ten percent of the population suffer from the Irritable Bowel Syndrome (IBS). This imbalanced microbiome makes a huge toll by lost working days, stress-related illnesses etc. Many pathogenic conditions caused by e.g. *Bacillus*, *Salmonella*, *Listeria* strains and *staphylococci* or *streptococci* can be demonstrated by studying the intestinal isolates. These strains or their toxins potentially cause hazardous infections also elsewhere in the body system. The developments on the microbiome level reflect the nutrition, which in turn is dependent on the microbial activities within the alimentary tract.

To quantify the probiotic effect and for characterizing the balance, we have introduced the concept of Bacteriological Intestinal Balance (BIB) that can be determined by the Portable Microbe Enrichment Unit (PMEU). This BIB could be influenced by pre- and probiotic treatments, which mitigate the syndromes related to the imbalanced intestinal microbiota.

Special Talks

Hazelnut Non-Dairy Beverage: Additive-Free Stabilization and Culinary Application

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Abstract

In the last years, the demand for non-dairy plant-based milk substitutes has increased worldwide due to different medical reasons (lactose intolerance and cow's milk allergies) or by lifestyles choices (flexitarian, vegetarian and vegan diets). Plant milk substitutes are colloidal suspensions or emulsions comprising dissolved and disintegrated plant material. The present study has established a new protocol for the elaboration of hazelnut milk (free of additives and added sugar). Their stability and main physico-chemical properties (pH, viscosity, density and total soluble solid) have been determined. As expected, the insoluble particles contained in the hazelnut beverage sedimented and lipids creamed. The intensification of the homogenization parameters improved the stability of the beverage by disrupting aggregates and diminishing the lipid droplets size. Thus, the sedimentation, flocculation, coalescence and creaming processes were significantly delayed. It has been determined that the combination of an ultra-turrax and focalized ultrasound homogenization process allow to obtain a stable hazelnut beverage.

In addition, as an application in food systems, the developed non-dairy beverage was used as a substitute for milk in the preparation of cakes with a high degree of consumer's acceptance. The characteristic hazelnut flavor conferred to the cakes, also allowed to reduce up to 50% the amount of added sugar to its formulation without consumers detecting the difference.

Nanoclays and Carvacrol to Improve and Lengthen the Antimicrobial Activity of Chitosan Edible Films

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Abstract

Chitosan is among the most investigated polysaccharides for antimicrobial films development due to its inherent antimicrobial and antifungal properties and film forming ability. Carvacrol (2-methyl-5-(propan-2-yl) phenol) is currently being incorporated into chitosan-based films to enhance their antimicrobial or antioxidant properties. However, the carvacrol incorporation into the water-based chitosan film forming solutions presents several difficulties because of its strong hydrophobicity and volatility. In order to reduce the carvacrol release rate from chitosan films, different nanoclay types and concentrations were incorporated

to chitosan films. Both the carvacrol release rate and the antibacterial activity (against *Listeria innocua* and *Vibrio alginolyticus*) of the resulting films was determined. Developed films presented antimicrobial activity against the 2 bacteria tested, being *L. innocua* more sensitive than *V. alginolyticus* to the carvacrol action. The release rate of the carvacrol contained into the chitosan films was significantly affected by the nanoclays incorporation. The higher the nanoclay concentration, the slower the carvacrol's release rate. The incorporation of low amounts of Dellite nanoclay into carvacrol-chitosan films did not diminish their antimicrobial activity against *L. innocua* and *V. alginolyticus* and improves its physical, mechanical and barrier properties. The Dellite nanoclay treatment with citric acid as expander should produce a better exfoliation and interaction between chitosan and nanoclay resulting in a significant decrease of the carvacrol release rate.

Hemp (*Cannabis sativa* L.) flour: An Active Ingredient for the Formulation of Nutritious, Flavorful and Affordable Foods

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Abstract

Hemp (*Cannabis sativa* L.) flour is obtained as a byproduct of hemp oil processing. Considered so far, a food waste, it represents a significant source of nutritionally relevant compounds. Actually, besides being high in fibers and micronutrients, hemp flour is characterized by a peculiar protein content, devoid of prolamins, which allows it to be tolerated by people who suffer from celiac disease or by those who are sensitive to gluten. Moreover, the lipid profile includes high and well-balanced omega-6 and omega-3 fatty acids and the calorie content is lower if compared to the more refined wheat flour. Significantly, hemp flour is high in polyphenolic compounds, which provide the product with a relevant anti-oxidant efficacy. Altogether, the chemical composition makes this flour a well balance ingredient having the potential to be used as an active component for the formulation of foods.

Discovery of Novel Fungal Enzymes for Food Applications

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Abstract

Fungi are the main decomposers of plant biomass in nature, due to their extensive repertoire of enzymes that can break the various linkages in plant polysaccharides and other components. Because of this, these enzymes have a long history in food applications, such as in baking, dairy and juice processing. The availability of an exponentially increasing number of fungal genome sequences has brought to light the true wealth of enzymes that fungi can produce and has opened up the doors for better and novel applications in many industrial sectors, including food industry.

However, the selection of the right enzyme for a certain application has become no less challenging than before the availability of fungal genomes. Enzymes need to have a certain specificity, temperature and pH range under which they are active and stable, sensitivity to salts and other components, etc. The variability in these properties is huge and therefore we need new ways to select enzymes for applications that involve smart, rather than bulk screening approaches.

In this presentation, I will present some of the screening approaches we perform at Westerdijk Institute for discovery of novel enzymes for (food) applications, including classical, omics-based and fungal-biotope related aspects.

Current Developments on Emerging Technologies for Food Industry

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Abstract

Several technologies like pulsed electric fields (PEF), high-pressure processing (HPP), ohmic heating haven been introduced

in the food industry in the last years. However, economic efficiency and sustainable production processes aiming at increasing the shelf life, while maintaining quality and nutritional values as well as minimizing energy and material consumption, and recovery of high-value substances in an acceptable quality still need to be improved.

Among the newest emerging technologies, a non-thermal process for pasteurization of liquid foods and cell disintegration for downstream processing using pressure change technology-PCT is being investigated. This technology can be applied in different operational modes (continuous or in batch mode). Good results were achieved when applied for the pasteurization of orange juice or stop of wine fermentation or for cell membrane disruption using different process gases (Ar or N₂) [1, 2].

Subsequently to recover or extraction of high-value components, electro-membrane filtration (EMF) technology has been developed. A higher degree of selectivity combined with low energy consumption was achieved using this technology process for the electrophoretic separation or fraction based on the electric charge and the size of the molecules to be recovered. The EMF device and application were studied with samples from food industry or downstream processing for fractionation of otherwise inseparable materials like valuable proteins or peptides, e.g. casein macro-peptides (CMP), β -lactoglobulin, lactoferrin [3]. All of these techniques could be used for downstream processing in biotechnology as well.

This overview of current developments on emerging technologies for food industry was demonstrated as new technology solutions for example as well a promising veritable non-thermal alternative to conventional heat pasteurization and cell-disintegration as targeted separation for different inseparable material from food industry.

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Session - I: Chemistry of Food

Potentially Health-Promoting Phytochemicals of the Benzoxazinoid Group, Abundant in Cereal Grains and Food Products

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Abstract

Ten years ago, we discovered that mature grains of rye and wheat contain bioactive benzoxazinoid phytochemicals (BXs). Wholegrain bread baked in our lab from rye or hydrothermally processed wheat showed to maintain substantial amounts of BXs. Thus, the understanding of the impact on consumer's health upon consumption of BX-containing bread came into our focus. The composition and quantity of BXs in food products depended much more on the food preparation process than on the cultivar. BXs were taken up by pigs, rats and humans after consuming rye-based food. BXs have a range of potential pharmacological properties (antibacterial, anti-prostate-cancer, immunoregulatory, CNS stimulating). We showed that when bacteria induce the production of inflammatory cytokines in innate immune cells, a previous diet high in BXs enhances this production, indicating an immune-modulating effect of the BXs. With a highly sensitive analytical method we documented the uptake of 6 BX compounds in human prostate tissue after one week's high-BX diet.

Intake of BXs by humans, pigs and rats is not restricted to take place in experimental trials with specially produced food items. Our recent analyses of 25 commercial cereal food products, purchased in Danish supermarkets, showed concentrations of BXs from not detected to >500 $\mu\text{g/g}$ dry weight. Most products high in rye content had high concentrations of BXs.

We continue to focus on the possible health protecting effects of BXs for future development of functional food products for humans and of feed for animals. Elucidation of the mechanisms behind the effects is needed.

Chemically Defined Flavoring Substances in Food: The EFSA Evaluation Procedure and its Status

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Abstract

In 1996, the European Union put in place a programme for the safety assessment of approximately 2800 flavoring substances, available on the EU market at that time. It was based on a pragmatic group-based approach developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and further improved by the European Food Safety Authority (EFSA). This approach integrates information on intake, structure-activity relationships, metabolism and toxicity.

The experience gained from the above 'old' procedure was used in the development of the new procedure 2,3 for the evaluation of flavoring substances by EFSA⁴. The risk assessment process and current status of the evaluations will be presented.

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Extraction of High Quality Protein from Green Biomass

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Abstract

Protein was extracted from four different green plants; red and white clover, lucerne, and ryegrass. When extracting protein from green plants, the protein yield may be compromised by indigenous enzymes. Polyphenol oxidases (PPOs) are a group of enzymes that are widely distributed in forage crops, with more PPOs in red clover than in other investigated species. PPOs are copper metalloenzymes, which catalyze o-hydroxylations and oxidation of monophenols to o-dihydroxyphenols and oxidation of o-dihydroxyphenols to o-diquinones that reacts with specific amino acids side chains, resulting in browning and crosslinking of proteins.

Therefore, to avoid enzymatic browning we introduced an antioxidant during extraction of the soluble protein fraction. The addition of sulfite inhibited the PPO activity, increased the recovery of polyphenols and more importantly increased the protein yield and quality significantly. SDS-page of soluble protein in untreated juices showed very weak protein bands for all investigated species. However, in the presence of antioxidant, clear intense bands were observed for all investigated plants dominated by the rubisco band. Size exclusion supported the effect of the used antioxidant. Furthermore, the digestibility of the extracted and acid precipitated proteins from lucerne and ryegrass increased significantly with ~5% in rats, but for red clover no effect of the antioxidant was observed.

In conclusion, the PPOs in green plants must be inhibited during extraction to secure high protein yield as well high-quality protein with high digestibility.

Analytical Strategies to Study the Migration of Selected Chemical Contaminants into Dry Foodstuffs

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Abstract

Several alerts for food contamination caused by photo-initiators, which are added to UV-curable inks to cure the ink onto the substrate, occurred in the past. In 2005, Italian authorities withdrew thirty million liters of infant milk from the market due to the presence of 2-Isopropylthioxanthone. Since then, other photo-initiators have also been found in foodstuffs.

Evaluation of the migration of photo-initiators in foodstuffs is challenging due to the complexity of the matrix and the wide variety of foodstuffs that need to be analyzed. Therefore, migration studies can be carried out using the official simulant for dry foods: Tenax[®]. In this contribution, the performance of Tenax[®] as a simulant for the migration of photo-initiators from cardboard packaging was evaluated. Therefore, the migration simulation was compared to the real migration conditions for dry foodstuffs. Important migration features were studied, supporting the suitability of Tenax[®] as a simulant for the migration of photo-initiators towards cardboard from a consumer safety point of view.

Unfortunately, the use of the Tenax[®] powder as a simulant is inconvenient since the powder has to be entirely collected in a recipient prior to contaminant extraction. Therefore, easy applicable Tenax[®] films were synthesized and their performance was compared to the performance of the Tenax[®] powder for a selection of model contaminants.

In conclusion, the performance of Tenax[®] as a simulant for the migration of photo-initiators from cardboard towards dry foodstuffs was illustrated. However, the use of easy applicable Tenax[®] films can open new perspectives in the domain of testing food contact materials intended for contact with dry foodstuffs for compliance.

Small Brazilian Wild Fruits: Nutrients, Bioactive Compounds, Health-Promotion Properties and Impact in Food Industries

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Abstract

As we know, Brazil covers about 20% of the world biological diversity, being represented mainly by Amazon forest, Brazilian Savanna and Atlantic Rainforest Biomes. In this context, the Brazilian wild berries have been subject of prospective and technological studies with regards to obtain bioactive substances that can be applied for several purposes besides to represent an important innovation domain for the food industry, particularly functional foods or nutraceutical fields. The main objective of this lecture is to mention the panorama of the Brazilian fruticulture and to describe the physicochemical, nutritional and biological aspects of six Brazilian small native wild fruits such as acai, camu-camu, jaborcaba and murici, highlighting their sensorial properties, nutritional value and biological properties related to antioxidant, anti-lipidaemic, anti-inflammatory, antiproliferative, and antigenotoxic potential among others. Will be also discussed some relevant topics with regard to the industrial applications that could improve the local market as well as the economy where these fruits are found.

Beetroot Supplementation and the Effects of its Bioactive Compounds in Health and Disease

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Abstract

Beetroot (*Beta vulgaris* L.) is considered one of the main dietary sources of nitrate. Nitrate supplementation has attracted physiological, nutritional and therapeutic interests, particularly due to its beneficial effects on the cardiovascular system, explained by the transformation of ingested nitrate to nitric oxide in the stomach. To increase adherence to nitrate supplementation, beetroot administration can be performed using different formulations, including juice, gel or even beetroot-cereal bars. Herein, the effects of nitrate supplementation were evaluated on the NO synthesis and biochemical and hemodynamic parameters of healthy and physically active individuals and in individuals displaying risk factors for the development of cardiovascular diseases. Nitric oxide synthesis was increased in 40 healthy volunteers after acute 100 mL beetroot juice ingestion and in 25 healthy runners after acute 100 g beetroot gel ingestion when compared to the administration of a beetroot juice or gel depleted in NO_3^- . Furthermore, after chronic ingestion of 60 g of a beetroot-cereal bar, nitric oxide synthesis increases (≈ 15 - and ≈ 7 -fold plasmatic nitrate and nitrite, respectively), endothelial function improvement, arterial stiffness reduction and arterial blood pressures decreases (by ≈ 14.0 mm Hg in systolic and ≈ 6.5 mm Hg in diastolic pressures) were observed in patients displaying three risk factors for the development of cardiovascular diseases. Increased dietary nitrate intake from the designed beetroot formulations may be an effective strategy to improve nitric oxide synthesis and cardiovascular parameters in healthy and physically active individuals and patients presenting risk factors for the development of cardiovascular diseases.

Molecular Mechanism of Radical Scavenging for Metal-Flavonoid Complexes as Antioxidants

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Abstract

Flavonoids, as secondary plant metabolites of polyphenols family, have important biological activities such as antioxidant, anti-inflammatory, and anticancer. Recent studies show that most metal-flavonoid complexes exhibit higher antioxidant effects than their parent flavonoids. However, molecular mechanisms of flavonoids binding with metal ions and of antioxidant activity of metal-flavonoid complexes are poorly understood. In our recent studies, binding of flavonoids with metal ions, including redox-active Cu (II) and redox-inert metal Zn (II) and alkaline-earth metals, and antioxidant activities of corresponding complexes were investigated in detail. Metal ion effect, acidity and reaction type related to antioxidant structure-activity relationship of metal-flavonoid complexes are elucidated.

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Using Chemical Sensors to Detect Fraud, Contaminants, or Changes in Rheological Properties During Food Processing

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Abstract

Analytical technology aims to control raw materials, final products, but also the intermediates in the process. The analysis in the process demands sensors for the "on-line" and "in-line" analysis. Information in real time is essential to actuate over the system during the production.

Consumers and inspectors are also very interesting in having access to easy to use affordable handle instruments that allow the detection of food contamination as well as frauds.

There is a market for such instruments. Surprisingly, the offer is scarce. The common citizen does not understand the reason why physical detectors of temperature, conductivity, and pressure oblique and the number of equivalent instruments for chemical compounds are that threaten their health is small.

The answer relies, in part, in the difficulty of finding a stable sensitive layer that should interact specifically with the target compound, preferably in a reversible manner. Reversibility would allow sensor calibration, but restricts the interaction to physical adsorption, excluding chemical bonding, difficult to break after the analyte removal.

Literature is rich in sensors for various applications. These include the quantification of potential hazardous compounds in food, sensors to follow milk coagulation in real time, to detect changes in starch rheological properties during temperature change, or after pressure application, and arrays of sensors, currently named electronic noses, to detect spoiled fruit or to discriminate cheese variety by inspecting the volatiles. Examples [1-7] will show the potentiality of the techniques, their stage of development and their weaknesses.

Influence of Diet Pattern on Bioaccessibility and Absorption of Heterocyclic Aromatic Amines and Polycyclic Aromatic Hydrocarbons

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Abstract

Introduction: Cooking/smoking improves meat digestibility and palatability, but also results on the formation of heat generated carcinogenic compounds, namely, heterocyclic aromatic amines (HAs) and polycyclic aromatic hydrocarbons (PAHs). Variable amounts of HAs are found in fried, grilled and barbecued red meat, and poultry, whereas PAHs are usually detected and quantified only in barbecued/smoked samples. More than 2 µg/100g of HAs and PAHs can be taken in a single meal of barbecued meat, which can damage DNA and cause cancer. The goals of this work were to assess the influence of three dietary patterns (WD - Western diet; MED - Mediterranean and VEG - vegetarian) on the bioaccessibility and absorption of most abundant HAs, namely, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-amino-9H-pyrido[2,3-b]indole (AαC), and relevant PAHs, benzo[a]pyrene (BaP) and benzo[b]fluoranthene (BbF).

Methods: Whole meals (WD, MED, VEG) were prepared based on the % of protein originated from different groups of foods as stated by Tilman and Clark. PhIP, AαC, BaP and BbF were spiked at 100 µg/L. A standardized static *In vitro* digestion method described by COST Infogest network was applied. HAs and PAHs were quantified by high performance liquid chromatography/FLD. Bioaccessibility was calculated as the % of spiked compounds remaining in the intestinal digests. Transport experiments were performed using Transwell inserts and Caco-2 human colon cancer cell line.

Results and Discussion: The bioaccessibility of PHIP was 2-folds higher than the bioaccessibility of AαC, BbF and BaP. No significant differences were observed on the bioaccessibility and absorption of the compounds under study when Mediterranean and vegetarian dietary patterns are compared. Nevertheless, Western diet pattern increased significantly the bioaccessibility of BbF and BaP, whereas no significant effect was noted on HAs bioaccessibility and absorption. The impact of Western diet on bioaccessibility and absorption of carcinogenic compounds is of major relevance for risk assessment.

Impact of Wheat Bread Fortified with Fibre Enriched Extracts on Mineral Composition and Bioaccessibility

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Abstract

Introduction: Bread is consumed on a daily basis, which makes it an interesting vehicle for nutritional enrichment through extracts recovered from brewing by-products and fruit. In this context, fibre enriched extracts recovered from agroindustry by-products (elderberry, EE; orange, OE; pomegranate, PE; and yeast, YE) were used to fortify wheat bread. The impact of this fortification on total and bioaccessible mineral composition of wheat breads, and estimated daily intake was evaluated.

Methods: Four different bread formulations were produced with addition of fiber extracts (% wheat flour): EE (7.0%), OE (2.5%), PE (5.0%), and YE (2.5%). For control (C) bread no extract was added. Simulated *in vitro* digestion was carried out according to the internationally standardized method described by COST Infogest network. Mineral determination was carried out by flame atomic absorption spectroscopy (macrominerals) and inductively coupled plasma mass spectrometry (trace elements). Statistical models to discriminate the different bread formulations based on the mineral composition and bioaccessibility were developed.

Results: Minerals content differed ($p < 0.050$) between bread formulations, with C bread presenting lower values and PE the highest. PE, OE, and EE breads contribution to minerals requirements is important, but YE bread contribution was not as noticeable. Mineral bioaccessibility also differed ($p < 0.050$) between bread formulations. Contrary to the observed for total mineral content, bioaccessibility in YE bread was significantly higher than in the other bread formulations, while the opposite was observed for PE bread. To discriminate between bread formulations Cu, Se, Zn, Mg and Mn had an important role for element content, whereas Cu, Mn and Ca had for bioaccessibility.

Discussion: Although promising results were obtained for total mineral content, the amount of bioaccessible mineral decreased, depending on the wheat bread formulation. We concluded, that the origin of fiber rich extract must be carefully selected, to avoid potential negative impact on minerals bioaccessibility.

Antiproliferative Interactions Between Anthocyanins, Phenolic Acids and Flavonols in Gastric and Intestinal Cancer Cells *in vitro*

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Abstract

The assessment of the bioavailability of foods is challenging due to the different mechanisms involved from digestion to final physiological effects. These are additionally affected by interactions at targets between concurring compounds ingested simultaneously being their study of paramount importance for the evaluation of foods bioactivity. Interactions occur in mixtures as their effects can be higher (synergism) or lower (antagonism) than the sum of individual compounds (addition). There is nowadays scientific evidence suggesting that the benefits of plant foods are enhanced by interactions. However, data on those effects is sparse.

Most of plants contains several classes of compounds that can potentially interact. An example is blackcurrants berries (*Ribes nigrum*) that present bioactivity at several levels including inflammation and cancer. Here, in three major bioactive compounds from blackcurrant from the classes: anthocyanins (delphinidin-3-O-rutinoside - D3R), phenolic acids (chlorogenic acid - CA) and flavonols ((-)-epicatechin - EC) were chose to evaluate their interactions.

Human cancer cell models (NCI-N87, Caco-2) were exposed during 72 hours to solutions of D3R, CA and EC at different concentrations (1 to 600 μ M), after which cell viability was measured. Combinations of 2 compounds in different concentrations were assayed maintaining the EC50 ratios using the Chou-Talalay method.

The bioactivity efficiency of individual compounds can be affected when these are ingested simultaneously with other molecules situation that occurs most of the times when substances are ingested in their natural matrices, as fruits, or in either more complex matrices, as whole meals. There is thus the need to thoroughly know compounds interactions to adopt a smart strategy to increase food bioactivity by optimal combination of food items.

Vaccinium meridionale Pomace as Ingredient for the Development of Functional Greek Yogurt

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Abstract

Colombian bilberry (*Vaccinium meridionale* S.) pomace is an excellent source of polyphenolics including anthocyanins, cinnamic acids, flavonols, and A and B-type procyanidins). Greek yogurt was fortified with pomace before (T1) and after

pasteurization (T2). The total phenolic content (TPC), antioxidant activity, kinetics of anthocyanin degradation, sensory and physicochemical properties of the products were evaluated during 3 weeks of storage at 4 °C. The results showed that T1 presented significantly higher TPC (46.6 ± 1.2 mg GAE/100 g yogurt) and antioxidant activity (3.70 ± 0.03 $\mu\text{mol TE/g}$ yogurt) than T2 (42.1 ± 0.8 mg GAE/100 g yogurt and 3.50 ± 0.03 $\mu\text{mol TE/g}$ yogurt, respectively) and the non-fortified control (13.4 ± 0.5 mg GAE/100 g yogurt and 1.6 ± 0.1 $\mu\text{mol TE/g}$ yogurt, respectively). Anthocyanin concentration was higher in T2 samples (9.49 ± 0.21 mg cyn-3-glu/100 g yogurt) as compared to T1 samples (3.20 ± 0.11 mg cyn-3-glu/100 g yogurt). T2 samples exhibited significant higher values for water holding capacity, acidity, chroma and hue angle. Overall, T2 samples presented the best scores in all evaluated sensory attributes. These results indicate that the pomace of *V. meridionale* is a potential ingredient in the manufacture of naturally-colored, highly-accepted functional Greek yogurt.

Effect of the Particle Size on the Technological Properties of Apple Dietary Insoluble Fiber Obtained by Using Different Drying and Milling Techniques

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Abstract

In this work, the effect of the different particle size on the technological properties such as water and oil holding capacity (WHC, OHC) of the apple fiber was studied by using different drying (tray dryer, 50-80 °C and microwave oven, 260, 300, 460 W) and milling methods (ball mill and blade mill). After drying with two methods, particle sizes of the samples were reduced to $0 < x < 36$ μm by using ball mill, and the technological properties and color parameters of the samples were determined. In result, it was observed that the best WHC and OHC with 1 g/11.5 g water and 1 g/3.06 g oil is for tray dryer, 70 °C temperature. After seeing the optimum drying temperature, the particle size was divided into three groups by reducing the size to $0 < x < 75$ μm , $75 \mu\text{m} < x < 180 \mu\text{m}$, $180 \mu\text{m} < x < 300 \mu\text{m}$ with ball mill and blade mill. It was determined that the WHC of the samples from reduced with the ball mill is higher than that of the blade mill samples, a particle of $180 \mu\text{m} < x < 300 \mu\text{m}$, with 1 g/28.4 g water. On the other hand, the value of the OHC was higher for the blade mill, a particle of $0 < x < 75 \mu\text{m}$ with 1 g/3.29 g oil. It may be said that as the particle size decreases, WHC of the samples decreases and the OHC was decreased to a certain range with $75 \mu\text{m} < x < 180 \mu\text{m}$ and then the value of OHC was increased with $0 < x < 75 \mu\text{m}$.

Optimization of the Production of Liquid Coffee Creamer by Using Different Ingredient: Taguchi Methodology

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Abstract

This study presents the determination of the optimum values of design parameters for a model liquid coffee creamer prepared with whey protein concentrate (35%), vegetable fat (palm kernel oil), sugar, emulsifiers and stabilisers. The effects of the performance parameters such as visual appearance and whitening ability (L^*), saturation index, viscosity and bulk density were determined by using Taguchi method as independent variables. An L16(4⁵) orthogonal array was chosen as experiment plan for optimization by using Taguchi. Consequently, it was observed that the L^* value of sample was 91.35, saturation index value was 1.51, the apparent viscosity was 7 MPa.s, and bulk density was 1.022 gml⁻¹ for optimum formulation. The model liquid creamer, an oil-in-water emulsion, had better lightness ($L^* = 88-92$) than a commercial liquid creamer ($L^* = 85-83$). The model creamer was more stable than commercial creamer to droplet aggregation and creaming from pH 3.5 to 7. Addition of model creamer at different volumes of hot black coffee (85 °C) produced white coffee drinks had the similar visual appearance as those produced by the commercial creamer. No feathering was observed in the model coffee systems indicating that the model creamer was stable after addition to hot coffee. The sensory evaluation showed that the model creamer was found more acceptable than commercial creamer. These results proved that coffee creamers can be formulated with whey protein concentrates and vegetable fat.

Effects of Cobalamin on Inflammatory Bowel Disease and the Intestinal Microbiota Composition in Mice

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Abstract

In this study, we aimed to investigate the relationship between cyanocobalamin (CNCBL) or methylcobalamin (MECBL) ingestion and inflammatory bowel disease (IBD) symptoms. Experiments in 60 mice revealed that a high concentration of CNCBL, but not MECBL, supplementation worsened the damage of colon mucosal tissue, led to severe neutrophilic infiltration, and obviously aggravated IBD. Our data suggested that CNCBL raised the proportion of *Enterobacteriaceae*, which could aggravate IBD and had distinct preferences for cobalamin. In cobalamin-dependent enzymes from *Enterobacteriaceae*, CNCBL had a higher activity in the adenosyl-cobalamin system than MECBL, and vice versa in the methylcobalamin system. Furthermore, CNCBL, but not MECBL, had a strong inhibitory effect on all riboswitches, especially on the *btuB* and *pocR* riboswitches from *Enterobacteriaceae*. Our results suggested that CNCBL aggravated IBD via enhancing the proportion of *Enterobacteriaceae* organisms through riboswitch and enzyme systems. The present study provides a critical reference for offering a suitable amount of cobalamin during a symbiotic condition.

Mathematical Index to Evaluate the Proteolytic Susceptibility of Food Proteins

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Abstract

The a/b ratio was proposed as a mathematical index to evaluate and quantify the proteolytic susceptibility of food proteins. The kinetic constants a and b are estimated from the logarithmic equation $P = 1/b \ln(abt+1)$, where P is the concentration of amino groups released and t is the reaction time. The experimental methodology consisted in the hydrolysis of whey proteins, salmon muscle proteins and keratin feathers by subtilisin at pH 8.0 and 50 °C at different initial concentrations of subtilisin. The reaction progress was recorded by pH-stat technique to obtain P vs t plots to estimate a and b. The results indicated that the ratio a/b linearly depends on the enzyme concentration. The plots for a/b ratio against the subtilisin concentration yield the quantified susceptibility for the different protein sources. The highest yield was obtained with the whey proteins, followed by the salmon muscle proteins and the feathers keratin. The values for the slope of a/b ratio versus subtilisin concentrations were 14.8, 8.4 and 0.16 mM²-g/(min-mAU) respectively. The low susceptibility of feathers keratin is explained by the high degree of compaction and linkage of the polypeptide fibers. In the middle is the salmon muscle proteins which are composed of a combination of globular and fibrous proteins. The higher susceptibility was found in whey proteins which are globular and very soluble proteins with a high degree of accessibility for the proteolytic action of subtilisin. In conclusion, the a/b ratio is a valid index to evaluate the proteolytic susceptibility of proteins.

Raman Spectroscopy for Carotenoids Analysis in *Bunchosia glandulifera* Pulps

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Abstract

Bunchosia glandulifera has been considered an important fruit with high bioactive potential due to its high carotenoids

content. In the scope, of characterization and quantification of these microconstituents in foods, Raman spectroscopy has been studied since it is a rapid and non-destructive analytical technique. Thus, *B. glandulifera* pulps were dried in hot air at 65 and 85 ° C for different drying times to evaluate losses of carotenoid content due to exposure to heat. The monitoring of the degradation profile of the molecules over time was performed by the Raman spectroscopy with the pure samples and with samples homogenized with an internal standard (TiO₂). Both methodologies were validated with the conventional analysis of carotenoid extraction. The reproducibility of the analysis was evaluated by PCA, which demonstrated better classification of the analyzed samples without addition of an internal standard. For this set, models with R² = 0.96 of calibration were achieved.

Simultaneous Determination of 20 Monosaccharide Using High Performance Anion-Exchange Chromatography Coupled with Pulsed Amperometric Detection

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Abstract

Monosaccharide analysis is a critical way to profile the composition of complex carbohydrates. In this report, a simple, accurate, and highly sensitive method was developed for the determination of 20 carbohydrates in foods based on high performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD) method. Samples were extracted with deionized water using ultrasonic-assisted extraction (UAE), then the extracted polysaccharide was hydrolyzed by adding 1 mol/L trifluoroacetic acid (TFA) before determination by HPAEC-PAD. The HPAEC-PAD method was performed on a CarboPac PA1 column by gradient elution using deionized water, 0.2 mol/L sodium hydroxide solution (NaOH) and 0.2 mol/L sodium acetate solution (NaAC) as the mobile phases. Good linearity was observed in the range of 0.05-10 mg/L. The average recoveries were ranged from 80.7% to 121.7%. The limits of detection for the analytes were in the range of 0.02-0.10 µg/L. The limits of quantification for the analytes were in the range of 0.2-1.2 µg/kg. The developed method has been successfully applied to the real samples, and the result indicated that HPAEC-PAD could provide a rapid and accurate method for the simultaneous determination of monosaccharide.

Poster Presentations

NMR Profiles and UHPLC-ESI/MS Analysis of Phenolic Compounds Coupled with Chemometric Approach for Botanical Origin Classification of Romanian Honey

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Abstract

Tracing the botanical and geographical origin of high value products including honey has become an important issue in the extending global market context. For this, advanced analytical techniques like NMR, HPLC-MS, GC-MS and IRMS have been proposed as viable tools for honey authenticity assessments. The current study investigated the potential of ^1H and ^{13}C NMR profiles coupled with flavonoids and phenolic acids analysis by UHPLC-ESI/MS and multivariate statistical analysis to differentiate honeys according to their floral origin. Specific markers from purified organic extracts of monofloral (linden, acacia, sunflower, rape, honeydew) and polyfloral honeys were identified in order to create a database in which each type of honey can be associated with one or more specific phytochemical. Additionally, certain aliphatic and aromatic compounds were quantified from the NMR data using TSP as internal standard and the data were compared with the phenolic compounds profiles determined by UHPLC-ESI/MS. Using statistical analysis of the data, the investigated honeys have been discriminated according to the floral origin of melliferous plant.

Honey Rheological Parameters Prediction Using Artificial Neural Networks

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Abstract

The aim of this study is to evaluate the potential of artificial neural networks for the prediction of honey rheological parameters (loss modulus G'' (Pa), elastic modulus G' (Pa) and complex viscosity η^* (Pa·s)) in function of some chemical parameters as fructose, glucose, sucrose and moisture content. Honey rheological parameters are important characteristics because they affect the quality and they are useful for the processing equipment design (Anupama, Bhat, & Sapna, 2003). The artificial neural network (ANN) is a system of information processing, which was inspired by the biological nervous system (brain). The purpose of ANN is to calculate the output values from the input values using some internal calculation (Khajeh & Barkhordar, 2013, Oroian 2015). In this study were used 25 honey samples of different botanical origin (5 acacia honeys, 5 tilia honeys, 5 polyfloral honeys, 5 honeydew honeys and 5 sunflower honeys respectively). The input parameters (moisture content, fructose, glucose and sucrose) were used for the ANN modelling of the output parameters (loss modulus, elastic modulus and complex viscosity) using the multilayer perceptron (MLP), probabilistic neural network (PNN) and modular neural network (MNN). The models which predicted better the rheological parameters were: MLP-1 hidden layer for loss modulus and complex viscosity and MLP-3 hidden layers for elastic modulus, respectively.

Detection of Food Adulteration – Differentiation Between Wild Boar and Domestic Pig by Targeting Two Gene Loci by Real-Time PCR

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Abstract

People consume game meat because of its characteristic taste and tenderness. Additionally, it is appreciated for its health promoting properties, in particular to its favorable ratio of omega-6 to omega-3 fatty acids, and the lack of drug residues. Because game meat is more expensive than meat from domesticated animals, food producers might be tempted to substitute meat from wild boar by meat from domesticated pig. This kind of food adulteration can only be detected with selective analytical methods. To be applicable, the methods should also yield quantitative information, because according to the Codex Alimentarius

Austriacus, at least 38% of the meat content of a “game” sausage must originate from game.

The differentiation between wild boar and domesticated pig is, however, a difficult task. Due to several back-crossings and hybridizations between wild boar and domesticated pig, the genomes contain only few subspecies-specific bases. In addition, there exists intraspecies variability.

Here, we present two real-time PCR assays, which in combination enable the differentiation and quantification of the content of wild boar and domesticated pig in food. One real-time PCR is based on the NR6A1 gene coding for the germ cell nuclear factor [1]. The primers of the second real-time PCR assay were designed to target a SNP which had previously been found to have high discriminating power [2]. The real-time PCR assays were validated with regard to the most important analytical parameters. Only for a few samples, the results were ambiguous.

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Changes in Organic Acids of Dried Apricots Containing SO₂ at Various Concentrations During Storage

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Abstract

Changes in profiles and contents of organic acids (OAs) in dried apricots (DAs) containing SO₂ at various concentrations (0, 451, 832, 1594, 2112 and 3241 mg/kg) were investigated during storage at 4, 20 and 30 °C for 379 days

Before storage, OAs in all samples were identified as oxalic (OXA), malic (MA), citric (CA) and succinic (SA) acids. However, SO₂ concentration in sulfured-DAs had important effects on OA profile and contents in samples (p<0.05). Major OA was MA (11.1–15.7 g/kg dw) for the samples containing higher than 1594 mg SO₂/kg, while major OA was SA (13.9–31.8 g/kg dw) for the samples containing less than 1594 mg SO₂/kg. Strong negative correlation (r = -0.884) was found between SA and SO₂ concentrations in samples. This may be due to SA formation during oxidation of levulinic acid (LA) resulting from decomposition of hydroxymethylfurfural (HMF). As SO₂ concentration increased, the contents of OXA (r = 0.880) and CA (r = 0.956) in DAs also increased. Moreover, MA contents of sulfured-DAs were considerably lower (10-36%) than that of unsulfured-DAs. The highest stabilities of MA and SO₂ were determined in SDAs containing 1594 mg SO₂/kg at 4 °C.

The effect of SO₂ concentration on the individual OAs might result mainly from effect of SO₂ concentration on the enzymes responsible for respiration in Krebs cycle and from browning reactions. If dried apricots in plastic films are stored, we suggest using 1594 mg SO₂/kg and storing SDAs at 4 °C to protect OAs.

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Exposure of Oversulfated Dried Apricots to Hot-Air Flow for the Removal of Sulfur Dioxide

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Abstract

Dried apricots (DAs) lose their golden yellow color during storage due to browning reactions. To retard browning, sulfuring is carried out. After sulfuring, DAs may contain as high as 6000 mg SO₂/kg, exceeding legal limit of 2000 mg SO₂/kg. Therefore, it may become necessary to decrease final SO₂ level.

In this study, DAs were exposed to hot-air at 40°–60° C and removal of SO₂ and formation of brown color were observed. First-order rate constants (k₁) for SO₂ removal were 0.00081, 0.0023 and 0.01135 h⁻¹ at 40°, 50° and 60 °C, respectively. At the respective temperatures after 96 h of exposure, 19.6, 27.6 and 64.2% of SO₂ were removed. Therefore, the higher the temperature the more SO₂ could be removed. However, increasing temperature also increased browning. For example, k₁ values for browning were 0,00177, 0.00396 and 0.02124 h⁻¹ at 40°, 50° and 60 °C, respectively. A420 vales (showing brown color formation) did never exceed limit value (A420 = 0.3) at 40° and 50 °C, but exceeded after 64 h at 60°C. Q₁₀ for SO₂ removal was 2.84 at 40°–50 °C and 4.93 at 50°–60 °C. Q₁₀ for browning was 2.24 at 40°–50 °C and 5.36 at 50°–60 °C. High Q₁₀ values especially at 50°–60 °C clearly showed the temperature dependence of both SO₂ removal and browning formation.

In conclusion, hot-air exposure was very effecting in reducing SO₂ from excessively-sulfated DAs. Critical factors for hot-air application are choosing appropriate temperature and time without excessive formation of brown color. Hot-air application at especially 40° and 50°C is highly recommended.

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Effect of SO₂ Concentration on Maillard indicators in Sulfured-Dried Apricots During Storage

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Abstract

Effect of SO₂ concentration (0, 451, 832, 2112 and 3241 mg/kg) on indicators [ϵ -N(L-furoylmethyl)-L-lysine (furosine) and 5-hydroxymethylfurfural (HMF)] of Maillard reaction (MR) in sulfured-dried apricots (SDAs) during storage at 4, 20 and 30 °C for 379 days was determined.

Before storage of non-SDAs, while furosine was detected, HMF was not detected. Thus, first stage of MR occurred in the non-SDAs during drying but intermediate stage had not yet completed. Different from that in non-SADs, even the lowest SO₂ concentration (451 mg/kg) was sufficient to prevent the formation of furosine and HMF during drying of apricots. Therefore, MR did not occur in SDAs during drying. However, furosine formation in all samples during storage at 4, 20 and 30 °C for 379 days also indicated that SO₂ could not prevent the occurrence of first stage of MR in SDAs during storage. In fact, SO₂ caused the increase in the rate of first stage of MR in SDAs stored at 30 °C for 90 days, although it caused the reduction in the rate of intermediate stage of MR after 135 days of storage at 30 °C.

Main prevention effect of SO₂ on browning in dried apricots occurred during drying, not during storage. Moreover, HMF formation in SDAs resulted from the conversion of sucrose to 5-HMF depending on Lewis acid formation due to the solution of SO₂ in water present in apricots. As a result, furosine content rather than HMF content should be used as a main indicator of MR in SDAs.

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Changes in Anthocyanins in Black Carrot Juice Concentrate Stored at Various Temperatures

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Abstract

Anthocyanins are responsible for the attractive red color of black carrot juice concentrate (BCJC). BCJCs are preferred to other ACN-rich concentrates for coloring of fruit juice and nectars, soft drinks, conserves and jellies because of stable anthocyanins. High stability of BC anthocyanins is attributed to intramolecular copigmentation of their acylated anthocyanins. This reaction is highly dependent on storage temperatures. Therefore, this study was conducted to determine the effects of

temperatures (-23°, 5°, 20° and 30 °C) on anthocyanins of BCJC (68 Bx). Anthocyanin concentration was found in BCJC before storage by pH-differential method (3747 mg/kg) and HPLC (4125 mg/kg). Degradation of anthocyanins and formation of brown color progressed at a faster rate with increasing storage temperature. For example, t_{1/2} for anthocyanin degradation was 603, 137 and 29 days at 5°, 20° and 30 °C, respectively. Zero-order k values for polymeric color formation were 0.0207, 0.1435 and 0.5581% days⁻¹ at the respective temperatures. While color of BCJC stored at -23 °C almost unchanged during 319 days of storage, very fast color deterioration occurred in concentrate samples stored at 30 °C. Over 52% loss in monomeric anthocyanins and 28% increase in polymeric color were observed in concentrates after 33 days of storage at 30 °C. Losses in anthocyanins were only 6% at 5 °C and 22% at 20 °C for the same storage period (33 days). Although anthocyanins from BCJC were much more stable than those from other sources, BCJC should still be kept at sub-freezing temperatures to minimize anthocyanin degradation as well as polymeric color formation.

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Changes in Physicochemical, Biochemical, Microbiological and Sensory Characteristics of Fermented Sausages as Affected by Starter Cultures During Ripening

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Abstract

Lactic acid bacteria (LAB) and coagulase negative cocci (CNC) are the most dominant microflora in fermented meat products. They contribute to faster acidification and denitrification to produce a standard and safe final product, respectively. LAB starter cultures protect the product by forming of some metabolites, such as organic acids and bacteriocins which have antimicrobial properties while CNC cultures responsible for nitrate reduction, lipolysis and proteolysis. In this study, *Pediococcus pentosaceus* and *Staphylococcus xylosum* (S1), *Lactobacillus sakei* and *S. carnosus* (S2), and *S. xylosum* (S3) starter cultures were used to produce the traditional Turkish fermented sausages (TFSs). Physicochemical, microbiological, lipolytic and sensory quality of the sausages were evaluated during 9 days of ripening period. Sausages containing S1 and S2 had lower pH, higher acidity, and higher lactic acid and acetic acid contents. Sausages with *L. sakei* and *S. carnosus* had significantly low a_w than the others. Moreover, fermentative strains, *P. pentosaceus* and *L. sakei* showed a better preserving effect (high LAB numbers) than the *S. xylosum*. At the end of ripening, total saturated fatty acids decreased while total mono unsaturated fatty acids and polyunsaturated fatty acids (PUFA) increased in all sausage samples. Control sausages had the highest PUFA, indicating intensive lipid oxidation. However, sausages contained CNC culture showed similar FFA profile. Sausages prepared with S1 and S2 received the highest odor, color, taste and overall scores compared with S3. Therefore, application of *P. pentosaceus* and *S. xylosum* or *L. sakei* and *S. carnosus* in TFSs contributes to improving microbial and sensory quality.

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Antibacterial Activity of Blueberry Extract on Meatball During Cold Storage

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Abstract

Blueberry is an important source of phenolics which are known as phyto-antimicrobial substances. The antibacterial effect of blueberry extracts (BE) on the growth of Gram-positive (*Bacillus subtilis*, *B. cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Lactobacillus plantarum*, *L. brevis*) and Gram-negative (*E. coli* O157, *E. coli*, *Salmonella enteritidis* and *Pseudomonas fluorescens*) bacteria was determined. Direct addition of concentrated lyophilized water extract of blueberry to meatballs was also investigated during 10 days of refrigerated storage at 4 ± 1 °C. The blueberry extract was incorporated into meatball formulations at 0.5% and 1% concentrations and compared with 0.01% butylated hydroxy toluene (BHT) as a reference and the control without natural or synthetic additives. While BE had antimicrobial activity against *E. coli* O157, *E. coli*, *S. enteritidis* and *P. fluorescens* at concentrations from 0.125 to 1%. However, no inhibitory effect of BE was observed against *S. aureus*, *L. monocytogenes* and some lactic acid bacteria (LAB). During refrigerated storage, 0.5% BE and 1% BE significantly (P<0.01) suppressed viable,

psychrotrophic and *Enterobacteriaceae* counts compared to control and BHT samples. BE resulted in reductions by 1.47 and 1.53 log CFU/g in 0.5 and 1% BE added meatballs, respectively, in the numbers of *Enterobacteriaceae* after 10 days of refrigerated storage. Sensory evaluation results indicated that meatballs containing BE in both levels were acceptable for 8 days with respect to off-flavor and overall acceptability while control was rejected after 3 days. Therefore, results show that BE is a promising additive for keeping the safety of meatballs during refrigerated storage.

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Comparison of Quality Parameters of Sugar Beet Pectin Produced by Classical and Ultrasonic Treatment by using Taguchi Method

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Abstract

Sugar beet pulp is a byproduct of sugar (sugar beet) production that can be said to be a rich source of pectin because of its pectin content (~15-20% in dry matter). In this study, the ultrasonic (45 kHz) method was compared with classical method for pectin production from sugar beet waste by using Taguchi L25 array design. The optimum extraction conditions, rheological properties and esterification level of sugar beet pectin was also determined by Taguchi L25 array design. In both production ways, the array design consists of four factors; temperature (60-95 °C), pH (1, 1.5, 2, 2.5, 3), time (1-5) and solid/liquid ratio (1:4, 1:5, 1:6, 1:7, 1:8) respectively. As a response, degree of esterification (DE) and yield analyses were performed to calculate individual signal-to-noise (S/N) ratios. The optimum DE level and the yield of pectin production was found at 80 °C temperature, 3 h and pH value was 1.5, 1/5 solid/liquid ratio for classical production method, 95 °C temperature, 3 h and pH value was 1, 1/5 solid/liquid ratio for the ultrasonic method. In result, it was seen that the classical method of the production pectin from sugar beet waste is better than ultrasonic treatment. The rheological properties of the sugar beet pectin in classical production was found better than ultrasonic method.

Antimicrobial Activity of Phenolic Compounds Obtained from Spent Coffee Ground

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Abstract

Spent coffee ground (SCG) is an interesting waste generated by the coffee industry. SCG contents phenolic compounds (PC), such as chlorogenic acid (CGA), caffeic acid and quinic acid. The PC have deployed antimicrobial activity against bacteria GRAM+ and GRAM- having high efficiencies similar to conventional antibiotics, such as gentamicin and streptomycin. Also, pure CGA [2-4] is able to inhibit bacteria at concentrations in the range of 20-80 µg·mL⁻¹ by increasing the permeability of the cellular membrane, depletion of the electrostatic potential, homeostatic imbalance, protoplasm release and cell lysis. The aim of this work was to produce an extract from SGC (E-SGC) by solvent extraction (water-ethanol) and to evaluate the inhibitory effect on bacteria GRAM+ and GRAM- using antibiogram. Additionally, the minimum inhibitory concentration (MIC) was determined by microtiter plate assay, whenever inhibition was detected. The bacteria tested were: GRAM+ strains: *Staphylococcus aureus* VQSA68, *Listeria monocytogenes* ATCC 19115; and *Staphylococcus aureus*, *Micrococcus* sp and *Bacillus subtilis* all wild types. GRAM- strains: *Salmonella typhimurium* ATCC140285, *Salmonella enteritidis* SARB16; and *Salmonella typhimurium*, *Proteus* sp. and *Escherichia coli*, all wild types. For the antibiogram, concentrations in the range from 10 to 100 mg·mL⁻¹ were

tested. All strain showed inhibition halo >1 cm diameter at $50 \text{ mg}\cdot\text{mL}^{-1}$. Gram + strains showed a MIC of $<1 \text{ mg}\cdot\text{mL}^{-1}$, while GRAM- strains had a MIC of $<10 \text{ mg}\cdot\text{mL}^{-1}$. Therefore, Gram- strains are less sensitive than Gram+ strains to E-SCG, showing the same behavior against antibiotics.

Determination of Mercury in Fish Sauces Using DGT Technique and TD-AAS

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Abstract

In this work the possibility to use the DGT technique for determination of mercury in fish sauces was studied. The preparation of a new sorption gel has been tested. After optimization of sorption gel preparation, this new gel was inserted into the plastic DGT sampling unit together with agarose diffusive gel and cellulose filter membrane [1] and correct function of DGT technique was tested in model solution containing mercury and NaCl. Mass of mercury accumulated in sorption gel and mercury concentration in model solution was analyzed by TD-AAS [2]. After validation of DGT technique this technique was used for determination of mercury in fish sauces. The content of mercury in the sauce did not exceed the limit set by the valid legislation for fish products. Thanks to the DGT's preconcentration capability, mercury at concentrations below the detection limit of TD-AAS was determined. The unique feature of DGT technique is separation the analyte from the complex matrix of fish sauce, which prevents the corrosion of the metal parts of the TD-AAS instrument and the wear on other parts of the TD-AAS.

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Influence of Filtration and Pasteurization on Content of Vitamins B in Beer

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Abstract

Beer is one of the most popular alcoholic beverages worldwide. Beer technology is essentially based on age-proven practices which are not significantly changing over time. In the present times, improvements of the beer technology are mostly focused on the increase of the stability and shelf life of beer. It is mainly achieved by the development of post-fermentation technologies - mainly Filtration and Pasteurization. These treatments of the beer can significantly prolong durability, but it can also change the chemical composition of the beer which is closely connected with technological, sensory and nutritional properties. This work focused on the influence of the filtration and the pasteurization on the content of the selected vitamins in beer. Specifically, the vitamin B2, B3, B6, B9 and B12. Effects of the post fermentation procedures were studied on 3 groups of Czech lager beers (filtrated unpasteurized, filtrated pasteurized, and no filtrated unpasteurized). The high-performance liquid chromatography with diode array detector was used as a separation method for the vitamin content analysis. The data obtained by analyzing 28 beer samples were evaluated using a statistical method - the analysis of variance. Based on the results, a significant effect of the filtration was observed for the vitamin B2 and B3. The filtration has reduced the amount of the vitamin B2 while it increased the concentration of the vitamin B3. The pasteurization had no significant effect on the vitamin content.

Identification of ACE-I and DPP-IV Inhibitory Peptides from Giant Grouper (*Epinephelus lanceolatus*) Roe Protein Using Combined Proteomics and *in silico* Technique

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Abstract

Bioactive peptides are widely observed due to their potentials as pharmaceutical products to improve human health, such

as the antihypertensive and anti-diabetic peptides. The aims of this study were to identify major proteins contained in giant grouper roe and to predict the release of bioactive peptides from giant grouper roe proteins using combined proteomics and *in silico* techniques. Eight protein bands detected from giant grouper roe were selected for further identification. Vitellogenin (from *Epinephelus coioides*; NCBI accession number: AAW29031.1), apolipoprotein A-1 precursor (from *Epinephelus coioides*; NCBI accession number: ACI01807.1) and apolipoprotein E (from *Epinephelus bruneus*; NCBI accession number: AEB31283.1) were discovered by proteomics. These proteins were further analyzed using *in silico* analysis including BLAST and BIOPEP. Based on BLAST results, the sequence of vitellogenin from *Epinephelus coioides* was aligned to that from *Epinephelus lanceolatus* found in another research. It was reported that identities value was 70% indicating high homologous AA sequences between those species. Furthermore, BIOPEP revealed two major bioactivities possessed by the intact proteins, such as angiotensin-I-converting enzyme (ACE-I) and dipeptidyl peptidase-IV (DPP-IV) inhibition with 247 and 429 fragments, respectively. Pepsin (pH >2) and the combined action of trypsin and chymotrypsin (simulation of gastrointestinal digestion) theoretically released numerous ACE-I and DPP-IV peptides in BIOPEP proteolysis simulation. Overall, this work highlights that giant grouper roe is a potential resource of ACE-I and DPP-IV peptides and it demonstrates the utilization of *in silico* analysis to streamline the identification and generation of those bioactivities.

Effect of Physical and Chemical Pre-Treatments on the Enzymatic Hydrolysis of Keratin Feathers

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Abstract

The effect of physical and chemical pre-treatments on the enzymatic hydrolysis of keratin was studied. Physical pre-treatment consisted on 1 h autoclaving, meanwhile the chemical pre-treatments were made using different concentrations of NaOH (0.01, 0.025, 0.050, 0.075 and 0.10 N) and Na₂SO₃ (0.1, 0.5 and 1% w/v). Released amine-groups were quantified using the OPA method. The results indicated that autoclaved keratins were not hydrolyzed unless a chemical process was coupled. NaOH was more effective than Na₂SO₃ pre-treatment with an increase in hydrolysis ratio of 13 and 3, respectively, compared to the untreated keratin. Enzymatic hydrolysis using subtilisin were carried out after NaOH (0.025 and 0.050 N) and Na₂SO₃ (0.1 and 0.5% w/v) treatments. Hydrolysis experiments were performed at 11.25 mUA/g of reaction mixture, 10% w/w feathers, 50 °C and pH 8.0. Amino groups were quantified through pH-stat technique during hydrolysis. Meanwhile autoclave pre-treatment does not generate hydrolysis, it does produce an increasing susceptibility to enzymatic hydrolysis yielding 26.5 mM of amine groups after 1 h of reaction compared to 10.7 mM obtained with untreated feathers. The Na₂SO₃ and NaOH pre-treatments generated an increase in the enzymatic hydrolysis of keratin. It can be inferred that the biodigestibility of keratin is enhanced by the enzymatic treatment. The combination of physicochemical pre-treatment and enzymatic hydrolysis is a promising alternative to produce a food ingredient from keratin feathers.

Brewing Whey Beverages Using *Kluyveromyces* spp. and Sake Brewing Yeast

Naoki Yamahata* and Mamoru Wakayama

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Abstract

The aim of this research is to develop fermented whey beverages with high functionality. Whey is a main by-product of the cheese production and it contains lactose (73%), protein (13%), ash (8%), fat (1%), and other bioactive substances as dry components. Whey also contains many essential nutrients such as vitamins, β-lactoglobulin and α-lactalbumin, whereas there is lower fat in whey. The production of fermented whey beverages is difficult because the common brewing yeast *Saccharomyces cerevisiae* including sake brewing yeast cannot assimilate lactose. In this study, we used lactose-fermenting yeast, *Kluyveromyces* spp. isolated from food. In addition, lactose hydrolysis process enabled sake yeast to brew whey beverages indirectly. The maximum amount of ethanol produced by the strain *Kluyveromyces marxianus* NBRC 1735 was 10 v/v% for 7 days, whereas *Kluyveromyces lactis* NBRC 433 produced only 6.1 v/v% ethanol for 14 days from the standard medium which contains 15 w/v% lactose. After hydrolysis of whey using β-galactosidase, sake yeast, *S. cerevisiae* Kyokai 7, could assimilate glucose and galactose, however the fermentation rate was very slow because of glucose repression. It is necessary to make sake yeast assimilate glucose and galactose at the same time or ferment lactose directly. The protoplast fusion method might be useful way to make a fused yeast between *S. cerevisiae* and *Kluyveromyces* spp. This fused yeast will be able to ferment lactose and produce much ethanol faster than parental strains. Besides, the fusants between these yeasts are not genetically modified organisms under Cartagena Protocol on Biosafety.

A Study on Brewing Method of a New Liquid Seasoning, Lact-sho

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Abstract

There are various reports about the value of milk because it contains many nutrients such as protein, calcium, amino acids and fats. However, it is difficult to preserve the milk and control the production so that milk is often discarded in large quantities. Here, we introduce an alternative method of milk processing. Lact-sho is a soy sauce like seasoning made from milk protein instead of soy protein. Lact-sho is expected to prevent corruption of milk ingredients because of salt, ethanol and lactic acid, and to create new fermented seasonings. In the previous study, the method of making card koji which is made from card and koji mold was used in brewing process of Lact-sho. However, it takes time and effort at large scale brewing. The aim of this study is to shorten the brewing process and evaluate the brewed Lact-sho. First, we determined the optimum materials of koji and water addition rate depending on four activities: protease, β -galactosidase, α -amylase and glucoamylase. As a result, mixed koji which contained the same ratio of wheat bran and roasted crushed wheat showed the highest enzyme activity at the water addition ratio of 70%. Next, Lact-sho was brewed on the above conditions and aged for 90 days. During maturation, pH and concentration of reducing sugar, lactic acid, ethanol and salt were measured. After completion of Lact-sho, it was filtered and sterilized by heat. Finally, amount of amino acids was measured, and sensory analysis was performed.

A New Type of Liquor Made from Whey - Selections of *Aspergillus* spp. Raw Materials for Mold, and Determination of Brewing Conditions

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Abstract

Whey is the main by-product of cheese production. Most of whey was wasted to river until 1980 and it caused environmental pollution. Then, full-studies on utilization of whey have begun since then. Whey is high protein, low fat and highly nutritious, and interest in its function has been increased. We focused on the utilization of whey and the development of a new alcoholic beverage made from whey. First, we determined the optimal combination of koji mold and raw material, which showed the highest activity of β -galactosidase to hydrolyze lactose in whey. As a result, two strains of *Aspergillus oryzae* that one is high liquefaction and the other is high saccharification were selected as koji molds and wheat bran was selected as a raw material. In addition, this koji prepared with 150% water content showed the highest β -galactosidase activity. We brewed whey beverage from deproteinized whey using two types of koji and *Saccharomyces cerevisiae* Kyokai 7. After the hydrolysis, koji residues were taken out from whey by filtration and yeast was added for ethanol fermentation. Sampling was carried out at every 24 hours, and reducing sugar concentration, ethanol concentration, β -galactosidase, and protease activities were measured. As a result, the ethanol concentration reached 8.5% in the high liquefaction koji preparation and 7.6% in the high saccharification koji preparation. All reducing sugars were consumed in both preparations within 10 days. Based on these analyses, we evaluated the quality of the brewed products as a new type of liquor made from whey.

Screening of Lactic Acid Bacteria Highly Producing L-Asparaginase for Acrylamide Reduction in Food and Characterization of Recombinant L-Asparaginase

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Abstract

L-Asparaginase is an enzyme that catalyzes the hydrolysis of L-asparagine to L-aspartic acid and ammonia. L-asparaginase has been used for treatment of leukemia and lymphomas. Also, it can be used to reduce the formation of acrylamide in heat-processed foods. Acrylamide is a carcinogenic compound and formed from asparagine during the heating of foods at high temperature. Most of lactic acid bacteria are found in foods and used for various foods. However, little study of L-asparaginase from lactic acid bacteria has been reported. In this study, we screened lactic acid bacteria highly producing L-asparaginase and cloned their genes for application of L-asparaginase to acrylamide reduction in foods.

We searched lactic acid bacteria exhibiting high L-asparaginase activity from lactic acid bacteria of cultures collections and isolates from food. The Nessler's method was used for detection of L-asparaginase activity. As a result, highest L-asparaginase activity was detected in *Streptococcus thermophilus* LMG18311. The L-asparaginase gene from *S. thermophilus* was cloned into the pET28a (+) vector, and the recombinant L-asparaginase (StAsn) was highly expressed in *Escherichia coli* BL21 (DE 3) strain. The recombinant StAsn was purified using Ni Sepharose affinity, anion exchange, and gel filtration chromatography, and the purified StAsn showed a specific activity of 46.98 $\mu\text{mol}/\text{min}/\text{mg}$ (30 mM L-asparagine, pH 7.0, 30 °C, 15 minutes reaction). The optimum pH of StAsn was 8.5, the optimum temperature was 50 °C, and StAsn retained the activity over 70% at pH 7.0-9.0 and below 50 °C. Based on SDS-PAGE and gel filtration chromatography analysis, it was suggested that it was a homotetramer.

Cloning and Characterization of Chitinase Derived from *Streptomyces thermodiastaticus* HF3-3 strain

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Abstract

Chitin, a β -(1,4)-linked polymer of N-acetyl-D-glucosamine, is the second most abundant carbohydrate polymer and can be a source of biomass. Chitinases, which catalyze the hydrolysis of chitin, have been used in the bioconversion of chitin, and classified into families 18 and 19 of the glycosyl hydrolase superfamily. Chitin which is a component of the crab's shell can be decomposed by chitinase, N-acetyl-D-glucosamine which is used in food can be produced. Conventionally, the crab's shell was discarded and it could not be used effectively.

The purpose of this study was cloning and elucidation of various properties of chitinase derived from *Streptomyces thermodiastaticus* HF3-3 strain for using in food industry. By elucidating the properties of chitinase, we aim for efficient degradation of chitin and effective utilization of N-acetyl-D-glucosamine.

Chitinase from the thermophilic strain *S. thermodiastaticus* was purified and characterized and some of its properties were investigated to evaluate the potential application of the enzyme in the chitin industry.

Cloning was carried out to prepare a plasmid (Chi19.ssp1), and the enzyme was purified by culturing. We examined various properties.

The optimum pH was 9.0 and the optimum temperature of Chi19.ssp1 was 70 degrees. Because of its heat resistance, it can be expected to be for industrial use.

Study of Enzyme Associate with Antioxidant Stress Derived from Acetic Acid Bacteria (*Komagataeibacter xylinus*)

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Abstract

When aerobic organisms use oxygen, the aerobic organisms produce reactive oxygen species (ROS) as a by-product just a little. ROS brings useful work for organism if little amount, but it oxidize a cell indiscriminately when it formed excessively and have a potential to cause a cancer and mutation in cell. Aerobic organisms have a system to remove ROS, a lot of studies are conducted, but the system and mechanism are still indistinct. Therefore, I focus on SOD which removed O_2^- which was a precursor of the ROS in this study. In present, SOD is expected the application in the medical and food field. I studied using SOD derived from *Komagataeibacter xylinus* NBRC 13773. *K. xylinus* may have two kinds of SOD gene sequence (Cu/Zn SOD and Fe-Mn SOD) unlike the aerobic organisms including other acetic acid bacteria. In this study, elucidation of biochemical mechanism of two kinds of SOD was intended and performed the influence of growth environment gave to SOD, the cloning of the SOD gene. I added ethanol, acetic acid, acetaldehyde in each culture and cultured it. After measuring SOD activity of the cell-free extract, with all additives, SOD activity decreases in comparison with control. I tested the cloning of the SOD gene derived from *K. xylinus* NBRC 13773. SOD activity of *Escherichia coli* which tested cloning of Fe-Mn SOD derived from *K. xylinus* NBRC 13773 did not rise much.

Development of New Fermented Seasonings Using Degreased Rapeseed as Raw Material

Masaki Nose^{*}, Mamoru Wakayama and Junji Hayashi

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Abstract

The pressed lees (meal) after the oil extraction process are widely used. Meal contains rich nutrients such as proteins and amino acids. As a representative example, there are soybean meal and rapeseed meal. Soybean meal is a good quality protein source and is used as raw material for brewing sauce as well as feed. In contrast, rapeseed meal is mainly used as a raw material for feed. From this fact, rapeseed meal has limited use in comparison with soybean meal. Therefore, expansion of use of rapeseed meal is an issue to achieve high added value. In this study, we focused on the high protein content of rapeseed meal and tried to use it as a raw material for brewing.

From previous research, a novel fermented seasoning which has been researched and developed in this laboratory has dairy sauce and we called "Lact-sho". It uses cow's milk protein as a raw material, and it has a cheese taste and a cheese smell. These are the characteristics of Lact-sho. However, it is desirable to expand the functionality in order to enhance the utility value as Lact-sho. Therefore, we aim to further improve functionality by using rapeseed meal as a raw material for Lact-sho.

Effects on the Activity and Stability of the C-Terminal Region of γ -Glutamyl Transpeptidase (GGT) Derived from *Pseudomonas aeruginosa* PAO1

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Abstract

γ -glutamyl transpeptidase (GGT) is a heterodimer type enzyme that catalyze both hydrolytic and transfer reactions. The enzyme is widely distributed in humans, animals and bacteria. It can hydrolyze γ -glutamyl bonds and transfer γ -glutamyl moiety to mostly amino acids and peptides.

GGT is widely used in food and medical field. In this study, we have made improvements to GGT for synthesis useful γ -glutamyl compounds like γ -Glu-Val-Gly as *Kokumi* component and theanine (γ - glutamyl ethyl amide) as umami of tea.

GGT from *Pseudomonas aeruginosa* PAO1 (PaGGT II) is expressed heterologously in *E. coli* using pET-22b(+) vector. However, neither expression nor activity could be confirmed. Therefore, when adding 25 amino acids to the c-terminus of PaGGT II (PaGGT 25 a.a.), the activity increased about 500-fold. For the purpose of further increasing the enzymatic activity, a mutant type GGT was prepared so as to take a random coil (PaGGT II Gly 19), α helix (PaGGT II Ala 19), β sheet (PaGGT II Val 19), whose elongated portion is a characteristic secondary structure. In addition, by alignment comparing with other GGT, it is confirmed that PaGGT II has extra 8 amino acids in c-terminus. So, a mutant GGT whose 8 amino acids in C-terminus was truncated was prepared (PaGGT Δ 8).

When comparing the activity of each mutant GGT in the cell-free extract, it was confirmed that the activity was similarly increased in a manner other than PaGGT II Val 19.

Improvement of Val-Gly Synthesis Method Using L -Amino Acid Esterase

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Abstract

γ -Glu-Val-Gly is a tripeptide useful as a food additive and seasoning, because it has a strong taste of *Kokumi*. *Kokumi* enhances the five basic tastes, particularly sweet, salty and umami, and modifies the thickness and richness of food. However, it is very expensive, because efficient production system of Val-Gly has not been established. Therefore, we have examined enzymatic synthesis of Val-Gly from valine-methyl ester and glycine using L-amino acid esterase (LAE) of *Elizabethkingia* sp. TT1. This

method is useful for industrial application, since it does not need both complicated steps like chemical synthesis and energy materials such as ATP. For further improvement of synthesis efficiency, it is desirable to acquire structural information of LAE by X-ray crystallography. However, the expression level of LAE in the soluble fraction is insufficient and its crystallization is difficult. Moreover, since it aims at the production of γ -Glu-Val-Gly by co-expression with γ -glutamyltransferase in *Escherichia coli* in the future, construction of Val-Gly synthesis system at the culturing stage of LAE-expressing *E. coli* (Fermentation Method) is also desired. Hence, we attempted to construct such expression systems and designed pLAE-TF (LAE co-expressed with Trigger Factor under the control of *cspA* promoter) for crystallization and pUC-LAE (LAE expressed under the control of *lac* promoter) for Fermentation Method. As a result, pLAE-TF showed high expression into the soluble fraction. Also, the optimum culture condition in the Fermentation Method using LAE was to cultivate BL21 (DE3) as a host in LB medium at pH 6.0 for 24 hours at 25 °C.

Breeding of *Kluyveromyces lactis* with Metabolic Engineering

Yo Kato* and Mamoru Wakayama

Ritsumeikan University, Japan

Abstract

This study aims for developing a new brewed beverage from whey. Whey is a byproduct of the cheese production and includes much nutrition such as lactose, protein and ash. It is known that whey has a good nutritive value as source of protein and an excellent composition of amino acids. On the other hand, a part of whey is still disposed because of bad preservation, palatability and processing cost. Disposed whey may cause environmental pollution problem including offensive smell or bad effects to ecosystem, and therefore the effective use method of whey is searching. In this study, we will try to develop a new brewed beverage from whey through ethanol fermentation of lactose, its main component. Brewing can improve whey from the viewpoint of taste and preservation. We decide to use *Kluyveromyces lactis* with lactose utilization for this study because *Saccharomyces cerevisiae*, most popular sake yeast, cannot utilize it. However, *K. lactis* have lower ethanol productivity and slower fermentation rate than *S. cerevisiae*. In recent studies about sake yeast, some factors of high fermentation and detail of metabolic system in sake yeast were revealed through comparison with laboratory yeast. In addition, it has been reported that some genes of stress tolerance influence ethanol production. Therefore, we decide to breed *K. lactis* with metabolic engineering on the basis of knowledge of sake yeast and construct improved *K. lactis* which has high ethanol productivity and rate of fermentation. Using this improved strain will produce high concentration ethanol brewing from whey.

Study of Synthesis of β -Aspartyl Compounds by β -Aspartyl Transpeptidase

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Abstract

Gene cloning, purification, and characterization of β -aspartyl transpeptidase (BAT) from *Escherichia coli* (ybk/spt/iaaA gene product) were performed in *E. coli* cells. BAT was partially purified to 3.60-fold, with a specific activity of 0.52 U/mg. The molecule is presumed to be a heterodimeric consisting of large (27 kDa) and small (13 kDa) subunits. The optimal pH and temperature for hydrolytic activity of L-Asn like L-asparaginase were 8.5 and 50 °C. The hydrolytic activity is down with γ -butyl lactone, Zn^{2+} , Co^{2+} , Fe^{2+} . BAT also has the transfer reaction which synthesizes β -aspartyl compound. This enzyme can synthesize 0.90 mM of β -Asp-hydroxamate, the optimal pH and concentration of hydroxylamine is 10-10.5 and 200 mM. It also can synthesize 0.05 mM of β -Asp-Ala, the optimal pH and concentration of L-Ala is 10.5 and 400 mM.

The products of BAT biosynthesis are expected to have new physiological properties such as umami taste and therapeutic effect, because γ -glutamyl transpeptidase (GGT) synthesizes γ -glutamyl ethylamide which showed not only a strong umami taste (good taste) of tea but also relaxing and blood-pressure lowering effect. GGT is similar enzyme to BAT, both belong to the group of N-terminal nucleophile (Ntn)-hydrolase family. There is no published work on application of L-asparaginase for peptide compounds synthesis whereas the use of synthesized peptides and/or its derivative for commercial products is fascinating.

Oat-Buckwheat Breads – Technological Quality, Staling and Sensory Properties

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Abstract

Due to the more and more available information on buckwheat and oats, they find a variety of applications: in dietetics, medicine, pharmaceutical, cosmetics and chemical industries. They are also have become the object of many scientific researches, which are aimed at confirming their nutritional properties and demonstrating pro-healthy values.

The effects of oats and buckwheat flours, used in different proportions, on the technological properties, staling and sensory properties of model bakery products were studied. Generally, it was found that model bakery products were significantly differ in the content of protein, starch, and mineral compounds, due to significant differences in composition of the buckwheat and oat flours used. With the increased participation of buckwheat flour in the recipe the deterioration of the technological properties of dough and bakery products was demonstrated, also the increasing of crumb hardness was noticed. Bread with a higher amount of oat flour was characterized by a lighter color and smaller pores, which were uniformly distributed in the crumb. In sensory analysis it was found an overall improvement in the attractiveness of bread, in which oat flour was used. It should be noted the proposed bakery products can be attractive to the consumer, who is looking for new food products.

Acknowledgments: This research was supported by statutory found from the Department of Chemistry and Biodynamics of Food of the IAR&FR PAS in Olsztyn, Poland.

Induced Resistance against *Penicillium expansum* in Peach Fruit by Chlorogenic Acid via Activating the Salicylic Acid Signaling Pathway

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Abstract

This study investigated the effect of chlorogenic acid (CGA) treatment on induction of resistance against pathogens in peach fruit. Peach fruit were infiltrated by CGA, followed by *Penicillium expansum* infection and storage for up to eight days at 25 °C. Our results showed that treatment with CGA at 25-150 mg L⁻¹ significantly reduced the lesion diameter and decay index of peach fruit during storage. Meanwhile, CGA treatment had also significant beneficial effects on fruit quality parameters including firmness, soluble solids contents, pH value, and titratable acidity. Moreover, the activities of main defense-related enzymes (including phenylalanine ammonia lyase, polyphenol oxidase, peroxidase, β-1,3-glucanase and chitinase) and expression of key genes involved in the salicylic acid (SA) signaling pathway, such as *PAL*, *ICS*, *WRKY*, *NPR1*, *PR1*, *CHI*, *GLU*, *PR5*, and *POD*, were significantly enhanced by CGA treatment. To the best our knowledge, this is the first report that CGA treatment is a promising approach to controlling postharvest blue mold rot in peach fruit. Its mechanisms may be closely related with CGA-induced plant disease resistance via activating the SA signaling pathway.

Improving Gluten-Free Bread Quality by Enzymatic Modified Egg White

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Abstract

As the incidence of celiac disease increases continuously, an increasing demand for good quality gluten-free products especially bread is rising. Gluten is a key ingredient for bread structure and quality. The aim of this study was to evaluate the effect of enzymatic modified egg white on the quality of gluten-free bread (GFB). Egg white suspension was hydrolysed with a commercial protease from *Bacillus stearothermophilus* (Thermoase PC10F) at enzyme protein ratio (E/S) of 0.03 at 65 °C for 70, 130, and 190 min, then the hydrolysate was added in the GFB formulation at the level of 0.5% calculated from original protein prior to the hydrolysis. The results show that addition of the enzymatic modified egg white at optimum time can significantly ($p \leq 0.05$) improve the quality of GFB by increasing specific volume, decreasing hardness and chewiness, improving crust and crumb color. In addition, sensory scores showed that overall acceptability of GFB containing modified egg white was significantly ($p \leq 0.05$) higher than that of the control. Furthermore, the staling rate of the GFB was significantly ($p \leq 0.05$) retarded by the addition of modified egg white during 7 day storage. These results indicate that enzymatic modified egg white can be used to improve the quality of GFB.

Influence of Succinylation on the Physicochemical and Emulsifying Properties of Mung Bean Protein Isolate

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Abstract

The influence of the weight ratio of succinic anhydride to protein (0-0.10) on the degree of N-acylation, physicochemical properties and protein functionalities of mung bean protein isolates (MPI) including emulsifying properties, were investigated. The extent of N-acylation of MPI increased as the succinic anhydride: protein ratio increased, while the ζ -potential at neutral pH decreased. In addition, succinylation had impact on the average particle size of MPI depending on the succinic anhydride: protein ratio. Succinylation also increased the exposed free sulfhydryl groups but had no impact on the total free sulfhydryl groups. Moreover, the isoelectric point (pI) of the succinylated proteins shifted from pH 5 to lower pH. Succinylation increased the emulsifying activity index (EAI), while the impact on the emulsion stability index depended on the succinic anhydride: protein ratio.

Safety Assessment of *Terminalia ferdinandiana* Fruits Using Four Different Cell Lines

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Abstract

Freeze dried powders of *Terminalia ferdinandiana* are commercially available and known for their high levels of vitamin C and ellagic acid content. The aim of the present research is to assess the application level of solvent (methanol, ethanol) and aqueous extracts (water) of *T. ferdinandiana* fruits and seeds in mammalian intestinal and liver cell lines. Undifferentiated and differentiated Caco-2 (enterocytes), HT29-MTX-E12 and Hep G2 cell lines were used to represent absorptive enterocytes, goblet and liver cells respectively. Nine different dose points ranging from 33 to 200,000 $\mu\text{g/ml}$ were investigated. The Cyquant NF Cell proliferation assay was performed to determine the DNA content of the viable cells. Changes to cell viability produced IC50 values between 4415 and 12,878 $\mu\text{g/ml}$ for all of the extracts and cell lines tested. The IC50 values for standard ellagic acid varied from 1055 to 2244 $\mu\text{g/ml}$ across the different cells. Undifferentiated (IC50 1204 $\mu\text{g/ml}$) and differentiated Caco-2 cells (IC50 1055 $\mu\text{g/ml}$) showed more sensitivity towards standard ellagic acid than Hep G2 (IC50 2244 $\mu\text{g/ml}$) and HT29 (IC50 2139 $\mu\text{g/ml}$) cells. However, when compared to standard Ellagic acid, higher doses were required to inhibit the proliferation of cells by the fruit (IC50 4415 to 9214 $\mu\text{g/ml}$) and seed (IC50 4147 to 12878 $\mu\text{g/ml}$) extracts. Vitamin C, ellagic acid and other phytochemicals present in both extracts may provide antioxidant and other bioactivities contributing to higher dose levels required

to inhibit cell proliferation compared to ellagic acid. Results reported here will inform future *in vitro* digestion and absorption studies that will contribute valuable information regarding safe dosage levels of *T. ferdinandiana* in commercial products.

Effect of Drying Treatments on Carotenoid Composition in Organic Carrots

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Abstract

Introduction: Drying process of fruits and vegetables is known to positively affect their shelf-life and nutritional quality. At the same time, drying can cause damage and severe physicochemical changes in flavor and color, oxidation of fat and degradation of nutritional compounds (vitamins, carotenoids, polyphenols) [1]. Carotenoids, which exhibits important sensory, nutritional and biological properties, could occur extensive oxidation during food processing with the formation of degradation compounds [2].

Objective: The aim of this study is to evaluate the variation of carotenoids content and composition as well as the degradation products in organic carrot roots, blanched and unblanched, during drying.

Methodology: Slices of organic carrots (var. Amsterdam) were subjected to two pretreatments: hotwater blanching pretreatment at 95 °C for 1.5 min and without blanching. Next, samples were subjected to 0, 1, 2, 3, 4 and 5 h hot-air drying at 40 °C followed by 24-h freeze drying. Carotenoids were extracted with 1% BHT (butylated hydroxytoluene) (w/v) in cold acetone. The separation and characterization of the carotenoids were monitored by using High Performance Liquid Chromatography (HPLC).

Results: Qualitative analysis revealed similar carotenoid profiles for all carrot samples. The results showed the predominant presence of α -carotene followed by β -carotene, but *cis*- β -carotene, γ -carotene, phytofluene, phytoene, lutein and lycopene were also identified. β -carotene-5,6-epoxide and β -carotene-5,8-epoxide were formed as degradation compounds. The higher carotenoids content was in blanched carrot at 2-h and unblanched samples at 1-h.

Conclusions: This study showed that there are variations in carotenoid composition which depend on both duration of drying and treatments.

Minerals and Phenolic Compounds in Seeds of Different Buckwheat Cultivars and Species

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Abstract

Buckwheat is considered to be an excellent food with various beneficial effect on human health. The newest trend in food industry is developing new functional foods and perspectives could be focused to buckwheat: high quality protein, dietary fiber, mineral elements and flavonoides (rutin and quercetin). The study was performed to compare the content of minerals and phenolic compounds in seeds of different buckwheat cultivars and species.

Material: *Fagopyrum esculentum* : Panda, Kora cultivars, Green corolla and Red corolla lines; *Fagopyrum tataricum* : LIT 111 98 and 63181. Sowing term – 25.05.2016; Soil – very good rye complex; Mineral Fertilization – 40 kg N; Harvest – 21.08.2016; Plot design: four replications.

Methods: Total phenolic content was determined spectrophotometrically measuring the absorbance at 720 nm against the reference sample. The content of phenolic acids was determined by HPLC method. The same chromatographic set was used to determine the rutin content. Minerals composition Flame Atomic Absorption Spectrometry (FAAS) was used to measure: Cu Mn, Fe, Zn, Mg and Ca; Na and K were analyzed with Atomic Emission Spectrometry (AES); and P by colorimetric methods (610 nm.). There was a significant difference in the amount of bioactive compounds between varieties and types of buckwheat. *Fagopyrum tartaricum* showed a significantly higher content of phenolic compounds, rutin, less of kumaric acid, syringic acid and

vanillic acid compared to *Fagopyrum esculentum*. *Fagopyrum tartaricum* seeds contained smaller amounts of Mg, however, higher Ca compared to *Fagopyrum esculentum*.

Assessment of Fruit Juices Authenticity Using Spectroscopic and Chromatographic Techniques

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Abstract

In this contribution, the utilization of spectroscopic (EPR, UV-VIS) and chromatographic techniques (HPLC) for orange and pineapple juices authentication was explored. Antioxidant properties, color characteristics, concentrations of selected organic acids, sugars and/or amino acids of pineapple and orange juices with well-defined origin from Columbia, Costa Rica, Ecuador, South Africa, Ghana and Mauritius (pineapple), and from Brazil, Mexico and Greece (orange) were evaluated. Moreover, basic characteristics such as pH, titratable acidity, formol number or °Brix were monitored in an effort to obtain complex information on juices. All experimental characteristics were processed by multivariate statistics in order to evaluate correlation between feedstock origin and properties of produced juices. The results confirmed the strong relationships between juice feedstock origin and its properties. The created dataset of measured juice characteristics allowed unambiguous differentiation of orange and pineapple juices according to the country of origin.

Acknowledgement: This contribution is the result of the project APVV-15-0023 “Quality and authenticity of fruit juices – study of relationships between the origin of feedstock, processing technology and quality of fruit juices” and of the project ITMS 26220220175. Improvement of nutritional and sensorial parameters of fruity and vegetable drinks via an inert gases application” implementation, supported by the Research & Development Operational Program funded by the ERDF.

Multi-Experimental Characterization of Costa Rican Pineapple Juice

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Abstract

Sensory perception evoked by fresh hand-squeezed fruit juice is quite different from that of commercial one, because individual stages of industrial processing as well as long-term storage in retail chain result in some alterations in original juice aroma. One potential way how to reduce degradation of these juices can be their production under inert gas atmosphere. Complex profile of volatile fraction of defrosted raw pineapple juice, and immediately “slightly” pasteurized was studied in context of N₂ processing atmosphere application. Its effects to individual volatiles was investigated by HS-SPME with GC-MS, and in parallel, with GC-FID/Olfactometry, subsequently during four months-long shelf-life of juice. Besides this, basic physico-chemical and color characteristics, antioxidant properties, concentration of ascorbic acid, sugars and/or hydroxymethylfurfural were evaluated involving UV-VIS, EPR, HPLC techniques. Gained results showed that used inert atmosphere can preserve the juice aroma profile to a considerable extent during the storage. GC-FID/Olfactometry analyses proved that high odor intensities of principal aroma-active compounds (some: esters, furan derivatives, lactones) were significantly stable during the entire storage period. On the contrary, perceptible changes in odor intensities of present relatively reactive aldehydes were observed already immediately after pasteurization (hexanal, (E)-2-heptenal, nonanal, (E)-2-decenal). However, these changes were not significant sensorially insofar, to lead to the obvious worsening of the overall aroma of the stored juice. In addition, the storage of pineapple juice led to minimal changes in other's previously mentioned parameters.

Acknowledgement: This contribution is the result of the project APVV-15-0023 “Quality and authenticity of fruit juices – study of relationships between the origin of feedstock, processing technology and quality of fruit juices”.

The Research of Green Test for Qualitative Analysis of Preservatives

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Abstract

Benzene is a carcinogenic material classified as group 1 by IARC and class 1 by ICH Q3C (R5). Recently, green test is increasingly developed to replace the solvent for safety of researcher. However, Korea Food Additives Code (KFAC) is still using that for the purposes of extraction, titration and mobile phase on identification, impurities and assay. Among preservatives are also using for identification, although those are widely used as food additives. Therefore, the aim of this study is to develop the green method which are not using poisonous reagent 'benzene', could reduce the harmfulness and guarantee the safety for food researcher. The preservatives including methyl-paraben, ethyl-paraben, propyl-paraben and butyl-paraben were carried out for the qualitative analysis using thin layer chromatography (TLC). The benzene used mobile phase of TLC was replaced by acetone-water solution. The results were confirmed at wavelength of 245 nm.

Acknowledgement: This research was supported by a grant (16162MFDS021) from Ministry of Food and Drug Safety in 2017.

A Research for Improving of Qualitative & Quantitative Analysis of Turmeric Oleoresin in Foods

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Abstract

Pigments used as food additives are divided into natural pigments and artificial pigments. Among turmeric oleoresin is a natural yellow pigment from turmeric extract (*Curcuma longa* Linne) that is called curcuminoids including curcumin (C), demethoxycurcumin (D.C), bisdemethoxycurcumin (B.D.C). In Korea, those are regulated by qualitative analysis using thin layer chromatography (TLC) and quantitative analysis using high-performance liquid chromatography (HPLC). The purpose of this research is improving accuracy and convenience of each analytical method for turmeric oleoresin. The method was developed sample preparation using rapid dispersive-solid phase extraction (d-SPE) which is followed by a cleanup procedure based on dispersive-SPE. The three compounds were determined by TLC and HPLC, validation of the quantitative method using HPLC was carried out linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy and precision. Those results were satisfied with international validation guidelines.

Acknowledgement: This research was supported by a grant (16162MFDS013) from Ministry of Food and Drug Safety in 2017.

Functional CNT/Silica Nanohybrid Spheres from Pickering Emulsion Templating and their Potential as Gas Sensors in Food and Agriculture

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Abstract

Microcapsules have received considerable attention in various fields, like catalysis, microencapsulation or as smart fillers in nanocomposites for gas sensing. Carbon nanotubes (CNT)-reinforced silica nanocomposites combine the extraordinary strength, hardness, and thermo-chemical stability of the ceramic material with the unique optical, mechanical, and electrical properties of the CNTs and facilitate the percolation of electrically insulator ceramics into electrically conductive composites. The potential applications of CNTs, however, are hindered by their poor dispersibility in most common organic solvents and low

interfacial compatibility with ceramic matrices. We describe here a simple, and effective way of preparing electrically conductive CNT/silica nanohybrid spheres based on the self-assembly of the colloidal particles at the interface between two non-miscible liquids (Pickering emulsion templating). The oil-in-water (o/w) emulsions are stable against coalescence and sedimentation. The emulsion volume fraction was highly dependent of the silica and CNT content and the o/w ratio. There is a point of collapse where emulsification fails. CNTs and silica particles both are located in the interface, as evidenced by confocal laser scanning microscopy. The Pickering emulsions can only be generated in the presence of reactive functional groups at the particles surfaces. (3-Aminopropyl) triethoxysilane is crucial for the formation of the shell structure due to *in situ* polycondensation with surface hydroxyl groups. It is serving as a binder to fix the nanoparticles at the o/w interface. After drying, hierarchical, open porous structures have been generated. These new nanocomposites are promising as electrically conductive fillers for polymers or ceramics.

2,5-Diketopiperazines in the Wine: Chemical Synthesis and Identification of Isomers

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Abstract

2,5-diketopiperazines are the simplest class of cyclodipeptides obtained by the cyclisation of linear dipeptides of two α -amino acids. These compounds are found in wines and that they are likely to be formed by the yeast. Six diketopiperazines were identified in most of the twenty-two samples analyzed, namely, the cyclo (Leu-Pro) the cyclo (Phe-Pro), the cyclo (Leu-Phe), the cyclo (Leu-Leu), the cyclo (Ala-Phe) and the cyclo (Val-Phe). Four dipeptides were chemical synthesized. The coupling reagent was HBTU. The BOC-protected dipeptides were treated with an excess of trifluoroacetic acid. For BOC-Leu-Pro-OCH₃ and BOC-Phe-Pro-OCH₃, we received the cyclic final products after the un-protected procedure; however, for BOC-Val-Phe-OCH₃ and BOC-Ala-Phe-OCH₃, 8 hours heating is necessary. The cyclo (Leu-Pro) was analyzed by ¹H NMR. The spectrum obtained from this analysis, was similar to one of the three spectra, obtained from the analysis of cyclo (Leu-Pro) which was determined in the GC-MS analysis of wine samples. Synthetically prepared cyclo (Leu-Pro) has been shown to be the 3 isomeric form of cyclo (Leu-Pro), which is the naturally occurring cyclo (Leu-Pro) of wines.

Characterization of Native Lipases Produced by a Novel Isolated Bacterium Cultivated in Wheat Bran by Solid-State Fermentation

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Abstract

Lipases are biocatalysts known for their hydrolytic role, being capable of acting not only on a variety of natural substrates such as animal fats, vegetable oils and fatty acid esters, but also on artificial substrates such as synthetic triglycerides contained in milk formulas. This broad performance spectrum, when combined with their acknowledged resistance to solvents and capability of action in different reaction mediums, makes lipases ideal biocatalysts for biotechnological applications. Among these, microbial lipases have been extensively used in food industry for synthesis of nutraceuticals, modification of fats and development of new organoleptic properties in dairy products. Hence, this work aimed at characterizing the lipolytic activity produced by CNPAE 99 579, a novel bacterial strain isolated from oil palm fruits. The bacterium was cultivated in wheat bran by solid-state fermentation for five days. Later, the fermented solids were washed with an extraction solution resulting in a crude extract (CE) with lipolytic activity. The CE exhibited preference for long chain substrates (synthetic or natural), indicating the presence of true lipases. Some bivalent cations, such as Ba²⁺, Mg²⁺ and Fe²⁺, behaved as activators of lipase activity. However, EDTA, Fe³⁺, Zn²⁺ and SDS revealed to be major inhibitors of lipolytic activity. CE lipases remained functional in a broad spectrum of solvents analyzed. These characteristics open, therefore, opportunities for further studies and reveal a lipase with potential for applications in food processing and other biotechnological processes.

Physicochemical Properties of Melipona Honey Living in the Indigenous Reservation La Ceiba, Guainia, Colombia

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Abstract

Stingless bees are eusocial insects that live in all tropical and southern subtropical areas throughout the world [1], their honey is distinctive for its unusual degree of sweetness, sourness, acidity, high moisture percentage and this is greatly appreciated due to its quality and its medicinal value [2]. Commercialized honey refers (almost entirely) to that produced by the species *A. mellifera*; therefore, the majority of research is focused on its characterization, with few studies related to the characterization of honeys produced by Meliponinos. In this work, the physicochemical characterization of the honey produced by the species *M. eburnea*, *M. marginata*, *M. compressipes*, *M. crinita* and *M. titania*; located in the Colombian Amazon (La Ceiba, Indigenous Reservation) was carried out.

The properties related to the maturity of the honey (content of sugars and moisture), purity (insoluble solids in water, and ash) and deterioration (acidity, diastase, hydroxymethylfurfural (HMF)) were measured, following the protocols established by the International commission of honey and the Colombian technical standard NTC 1273. A multivariate chemometric treatment was carried out to demonstrate the differentiation between honeys, providing relevant information for the establishment of quality parameters that allow its easy commercialization, strengthening meliponiculture and also promoting the protection and conservation of these insects.

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Multivariate Analysis of Physicochemical Properties of Colombian Stingless Bee Honey

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Abstract

Honey is a complex mixture made by bees from the nectar of flowers or honeydew, the most common and commercialized honey worldwide is produced by *Apis mellifera*; however, in tropical countries, stingless bees, which are called “stingless” because the size of the sting is greatly reduced, produce a specific type of honey called “pot honey” because it is stored in wax containers with a pot-shape. Several studies have shown that “pot honey” and “honey-bee honey” have dissimilar values of physicochemical properties; therefore, further investigations on pot honey properties are required to improve its consumption and to protect the bees and their natural habitat 1.

In this work, the physicochemical properties (by triplicate) of honey produced by seven species of stingless bees and by *Apis mellifera* were determined periodically for one year (August 2014 to August 2015) using the methodology proposed by the official methods of Analysis² and the International Honey Commission³. The honey samples were collected every three months from *Trigona (Frieseomelitta) nigra*, *Scaptotrigona* sp., *Nannotrigona* sp., and *Trigona (Tetragonisca) angustula*, and every two months from *Apis mellifera*, *Melipona fuscipes*, *Melipona favosa* and *Melipona compressipes*. The results showed that physicochemical properties depend on bee species ($p < 0.05$) and not on the time during the year ($p > 0.05$). The samples were 97.2% correctly classified using multivariate analysis, being acidity the most important factor to differentiate honey produced by *Apis mellifera* from pot-honey.

Development of HPLC-MS/MS Method for the Simultaneous Quantitation of Toxic Compounds of 4-Hydroxy-2-Hexenal, 4-Hydroxy-2-Nonenal, 4-Oxo-2-Nonenal and Malondialdehyde in French Fries

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Abstract

A HPLC-MS/MS method was developed for the simultaneous quantitation of toxic compounds of 4-hydroxy-2-hexenal, 4-hydroxy-2-nonenal, 4-oxo-2-nonenal and malondialdehyde in French fries. The MRM conditions including the qualitative and quantitative transitions and collision energies for the toxic components were optimized. The method validation was conducted by studying the linearity of the standard calibration curves, limit of detection (LOD), limit of quantification (LOQ), intra-day and inter-day reproducibility, and accuracy. Matrix effect was also studied by conducting the HPLC-MS/MS for the samples with spiked authentic compounds. The established method showed high linearity of the standard calibration curves in the tested range, low LOD and LOQ, and high precision and accuracy. The extractions of the compounds from French fries were conducted with different solvents (methanol, ethanol and acetonitrile) to compare the recovery of the compounds with different solvents. This method provided the reliable analytical results on the contents of these 4 toxic compounds present in French fries.

Simultaneous Characterization and Quantitation of C8 Volatiles in Shiitake Mushrooms

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Abstract

An automatic headspace (HS)-solid phase microextraction (SPME)-gas chromatography-tandem mass spectrometry (GC-MS/MS) was developed for the simultaneous characterization and quantitation of 10 different C8 volatiles in shiitake mushrooms. The SPME fiber type, sampling temperature and time were optimized. Multiple reaction monitoring mode conditions such as qualitative and quantitative transitions, collision energy, retention times for the C8 compounds were studied. The contents of C8 volatile compounds in two different shiitake mushroom species were determined using the established HS-GC-MS/MS. In this research, it was found that the quantity of C8 volatile compounds in two different mushroom species was not considerably different. However, the contents of C8 volatile compounds in shiitake mushrooms greatly dependent on the culture condition.

Starch Contents and Antioxidant Activities and of 5 Different Sweet Potatoes Cultivars

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Abstract

Starch contents and antioxidant activities of 5 different sweet potato cultivars were determined. The antioxidant activities in sweet potatoes were studied using spectrophotometric analysis of DPPH radical scavenging activity, reducing power, and total phenolic contents after the extraction of antioxidant components with methanol. The chlorogenic acids in sweet potatoes were identified using HPLC-MS, HPLC-MS/MS. The contents of chlorogenic acids were determined by HPLC-DAD. It was found that the antioxidant activities and contents of chlorogenic acids of sweet potato were greatly dependent on the cultivar and cultivating field type. The antioxidant activities obtained by the spectrophotometric analysis showed high positive correlation with the chlorogenic acid contents. The crude starch contents in sweet potatoes were in the range of 8.5 – 17.1%, showing the considerable difference with cultivar. However, the starch content was not greatly dependent on the cultivating field type.

Multiresidue Analysis and Monitoring of 69 Veterinary Drugs in Fishery Products with LC-MS/MS

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Abstract

Veterinary drugs have been widely used to cure or protect diseases in livestock and aqua cultural animals and the increased use of these drugs could be potential risks for human and environment. To manage these potential risks, national monitoring of veterinary drugs and their products should be required. In this study, a simple multiresidue analytical method using liquid chromatography with tandem mass spectrometry (LC-MS/MS) was used to detect and monitor 69 veterinary drugs including 6 metabolites in fishery products. The analytical method was validated using three different matrixes (shrimp, eel and flat fish) according to the Codex guidelines (CAC/GL 71-2009). Monitoring of 69 veterinary drugs was performed on the 399 samples of fishery products including eel, mud loach, flat fish, rockfish, red seabream, rock bream, sea bass, salmon and shrimp purchased from local markets in Korea from 2017 to 2018. The most frequently detected veterinary drug was enrofloxacin, followed by ciprofloxacin, oxytetracycline, trimethoprim, oxolinic acid and amoxicillin. The detection rate of enrofloxacin was 23.06% (92 samples) and the concentration was ranged from 0.0006 ~ 0.194 mg/kg. The detected concentrations of tested veterinary drugs were below MRLs except one mud loach sample in 2017. These monitoring results showed that the use of veterinary drugs in fishery products on the markets was relatively well managed.

Quantitative Real Time PCR-Based Method Validation for GM Cotton GHB119 and GHB614

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Abstract

Cultivation of Genetically modified (GM) plants has constantly increased for cultivation and commercialization. Recently, GM cotton GHB119 and GHB614 were estimated at the Busan Regional Food and Drug Administration in Korea. To make sure of GMO labeling regulations, GMO identification and quantification methods have been developed and verified by Quantitative Real-time PCR (qPCR). We validated the quantitative methods for detection of GM cotton GHB119 and GHB614. To validate the methods, GM or Non-GM samples were ground by an electric mill, then DNA extraction and purification were carried out using the Promega's automated Maxwell16 system. Then DNA samples containing GM at the mixing levels of 0.08, 0.1, 1.0, 3.0, 5.0% were prepared and conducted qPCR (Life Technology, USA). The precision of the method was evaluated as the RSD of reproducibility (RSDr) and repeatability (RSDr), linearity (R²), slope and PCR efficiency. The result from both GM cotton GHB119 and GHB614 showed that the values R² were more than 0.98 and their RSDr and RSDr were within the range between 10.41% and 15.61%. The trueness, precision and LOQ of the method were at the same levels as those of the previously established Ministry of Food and Drug Safety (MFDS) standard methods. In conclusion, we validated qPCR-based quantification method for GM cotton GHB119 and GHB614. The results showed that validated methods would be applicable for the detection and quantification of both GM crops to ensure the appropriateness of food labeling.

Textural Properties of Convective, Vacuum and Freeze Dried Seedless Black Raisins

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Abstract

Black seedless grape is highly valued fruit due to its irresistible taste and large processing possibilities in products such as raisins, juices, wine and other alcoholic drinks. Drying is the most common way of grape preservation since raisins have longer shelf-life and need less storage capacities compared to other products from grape. The quality of a dehydrated food products is strongly affected by drying processes used for preservation. Almost all raisins are produced by natural drying in sunlight and in order to improve quality of dried products, this traditional technique could be replaced by convective drying, vacuum or freeze drying.

External stress during drying process causes solubilization of the cell walls, destruction of cell integrity and loss of firmness. Textural properties are very important quality indicator of dried products which affects a lot consumer acceptability. Thus, the main goal of this study was to investigate textural properties of convective, vacuum and freeze dried seedless black raisins. Texture analyser and light microscope were used for obtaining textural characteristic of dried samples. The differences were successfully obtained by analysing shear forces observed by samples shearing and comparing the images observed by the light microscope.

Color and Anthocyanins Determination of Red Wines from Three Different Romanian Vineyards

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Abstract

The aim of this paper was to investigate the anthocyanins content and color of red wines by using UV-VIS spectrophotometer and CM 700 D spectrophotometer - KONICA MINOLTA Colorimeter (CieLab Method). Nine samples of red wine Feteasca Neagra, Cabernet Sauvignon and Merlot, the harvest of 2012, purchased from three different Romanian vineyards (Murfatlar, Dealu Mare - Tohani and Samburesti) were analyzed. The chromatic parameters such as: L^* , a^* , b^* , C^* and H^0 were investigated. Results showed that L^* parameter which indicates the degree of brightness, is between 0.27 (darkest) for Merlot wine from the Samburesti Vineyard and 15.48 for the Feteasca Neagra wine from the Dealu Mare-Tohani Vineyard, while chromatic parameter a , located on the color coordinate red-green, has values ranging from -0.09 (Feteasca Neagra from Dealu Mare-Tohani) and 1.47 (Cabernet Sauvignon from Dealu Mare-Tohani). The total anthocyanins content of the wines was determined by applying the method proposed by Rabino and Mancinelli (1986). The highest total anthocyanins content of the Feteasca Neagra wine was recorded in the vineyard of Sâmburești (25.12 mg/L), followed by the Murfatlar vineyard, and the lowest content (9.72 mg/L) was recorded for the wine from the Dealu Mare-Tohani vineyard. Feteasca Neagra, Cabernet Sauvignon and Merlot wines have different anthocyanins content from one vineyard to another, demonstrating that the variation of anthocyanins and implicitly variation in wine color is influenced by pedo-climatic conditions in the area, cultivated varieties and vinification technology applied.

Influence of Osmotic Dehydration on the Color and Chemical Characteristics of Some Fruits

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Abstract

Osmotic dehydration is the pre-treatment method of preservation the fruit and vegetable to increase its shelf-life in which these are immersed in concentrated salt or sugar solutions.

The effect of an osmotic dehydration was investigated on the color and chemical characteristics of dehydrated fruits (apple, pear and quince) in fructose osmotic solutions. Difference in CIE-LAB, chroma - C^* and hue angle H^* were performed with a Chroma Meter CR-400/410. Three aqueous solution of fructose (40, 60 and 80%) were used for dehydration, during 3 h of process at temperatures of 25 °C, with fruit/osmotic agent ratio of 2:1. Water loss and solids gain showed significant differences depending on the concentration of the osmotic agent and process time. The use of highly concentrated osmotic solutions induced losses of phenolic content (TPC) and ascorbic acid in sliced apple, pears and quince. Fructose concentration, osmosis time and temperature induce significant increase of a^* and b^* colorimetric parameters but did not affect the lightness (L^*) of pear slices.

Rheological and Textural Characterization of Dough with Added Rosehip and Inulin Powder

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Abstract

The aim of this work was to study the effect of adding the native inulin (IN) and the addition of rosehip powder (RP) in wheat flour type 550 in terms of rheological and textural properties of the dough. Wheat flour has been enriched with IN and increased hip powder in a proportion of 0-10%. Dynamic oscillatory rheology was studied in the linear viscoelastic field (frequency testing and oscillating temperature measurement). The texture properties of wheat dough were determined using a texturometer using texture profile (TPA) analysis of the dough (e.g., elasticity, hardness, consistency, adhesion and bonding). The water content, gluten quality and added bread and inulin powder were the main factors determining the texture properties of the dough.

Fruit Juices as Probiotic Carrier

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Abstract

Lactic acid fermentation of fruit juices represents a modality to obtain probiotic food products, designed for people suffering by lactose intolerance or interested by a nutritional health status and wellbeing too. Different raw materials and various beneficial microorganisms can be used to develop marketable beverages. Managing the technology and the quality of the final products remain a challenge for producers in order to meet the consumers' expectation.

Multi-fruit juices were subjected to fermentation with *Lactobacillus acidophilus* LA-5 and *Bifidobacterium lactis* BB-12. The cultures used have grown in multi-fruit juices, achieving cell counts specific for probiotic-containing products. Carbohydrates content decreased with 35-48% and the volumetric productivity ranged, depending on type of culture, initial cell concentration and substratum used, between 9.4×10^8 cells/L·h and 3.1×10^{10} cells/L·h. The stoichiometric coefficients describing the fermentation process, biomass and product yield coefficients on substrate reached values by 0.05 to 0.07 g/g and 0.4 to 0.55 g/g respectively. In kinetic terms, *Lactobacillus acidophilus* had the highest specific growth rate constant (μ_{max}). Time to reach pH 5.0 in all the experimental guaranteed the microbial stability of beverages and beneficial bacteria survived up to 5 weeks.

The investigated probiotics proved biochemical activities in fruit juices, regardless of the food matrix. Average volumetric productivities and an outstanding production of lactic acid should be exploited in relationship with the technology applied and probiotics survival. Consumption of these beverages is advantageous, in terms of basic hydration and nutritional properties. Encapsulating bacteria and suitable packaging methods are future research area.

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Impact of Vacuum Impregnation on Quality Parameters of Apple Cubes

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Abstract

Vacuum impregnation is an effective method of introducing solutions into porous materials such as apple tissue. Many changes of physical and chemical properties of impregnated material may depend on used solutions. The aim of this study was to evaluate the properties of apple cubes after vacuum impregnation process with solution consisting of water, sugar, ascorbic

acid, citric acid and calcium chloride. The vacuum impregnation was conducted using different pressure 7, 15, 20, 30 and 50 kPa. The apple cubes after impregnation were analyzed for mass increase, pH, soluble solids, color parameters, texture parameters and ascorbic acid content. All samples after vacuum impregnation showed similar pH (about 3.3), soluble solids (about 12.5%) and ascorbic acid content (about 140 mg/100g). In general, the application of the lower pressures resulted in higher weight increase of cubes. At the lowest applied pressures (7 kPa and 15 kPa), the increase of mass was 27% in relation to the mass of apples, while at the highest pressure (50 kPa), only 16%. Regardless the solution saturation, low pressure applied during impregnation adversely affected other tissue parameters, such as hardness and color. At the lowest applied pressure, the reduction in hardness of the tissue expressed as the force of its crushing reached about 20%, while in the other cases the decrease was between 6 and 12%. Moreover, lower pressure caused a greater decrease in the lightness (L^*) value. The possible explanation for the changes in the hardness and color of cubes is that the application of low pressure led to mechanical stress and damage of apple tissue.

Non-Invasive Monitoring of Red Beet Leaf Senescence for Agriculture

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Abstract

The main task of our work is to introduce the optical Raman spectroscopy method into the monitoring of the plant state – red beet. This method requires little or no sample preparation, compared with most other biochemical methods. The Raman measurements can be carried out in the field, without damaging the plant.

Raman spectroscopy has proven itself in determining the chemical composition of substances. This method is sensitive to carotenoid content and is successfully used for structural and complex analysis of various plants. As a result of the measurement, we obtain a spectrum where each peak corresponds to a certain vibration of the molecule, by which we can determine its composition.

In our work, we study red beet using Raman spectroscopy. Sugar beet is actively used as a source of sugar, and red pigment in food, so this study is of great economic importance.

As model plants we selected the beet varieties “Bona”. Seeds were germinated and then placed in pots. The pots were located in cells of climatic chambers – Phytotrons (shown in the Figure 1). Throughout the experiment, all growing cells were kept under the same conditions, but the spectral composition of the radiation was different for two cells.



Figure 1: Intellectual greenhouse layout – the Phytotron.

As a result, we observed the change in the pigment ratio in red beet with age. With time it may become possible to make conclusions about the lack of nutrients or early stage infection to increase the agricultural yield.

Preservation of Margarine by Pomegranate Peel Extract (*Punica granatum* L.)

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Abstract

This study aims to develop in industrial pomegranate byproducts. It was conducted on pomegranate peels (*Punica granatum* L.) called Lahlou, in the region of Bejaia (Algeria). The objective was to substitute the vitamin E used as an antioxidant in the preservation of several foods such as margarine. The ethanol extract obtained from the pomegranate peels was used for all analyzes. The results of the assays showed high level in antioxidants content, 926.74 mg GAE, 625.20 mg QE and 174.79 CE per 100 g DM were recorded for phenolic compounds, flavonoids and tannin respectively. Evaluation of antioxidant and radical scavenging activity by two methods (reducing power and inhibition of DPPH) revealed interesting reducing effects that are close to those of quercetin and BHA. Tests conducted in laboratory scale have shown that with the extract of pomegranate peel, even at 50% lower concentration than vit. E, margarines developed were resistant to oxidation, without modification of the physico-chemical and microbiological properties. Pomegranate peels is a good source of bioactive substances that are proving of great interest and that could be used in different food sectors.

Simultaneous Determination of 16 Macrolide Antibiotics and 4 Metabolites in Milk by Using Quick, Easy, Cheap, Effective, Rugged, and Safe Extraction (QuEChERS) and HPLC-MS/MS

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Abstract

A novel analytical method was developed and validated for simultaneous analysis of 16 macrolide antibiotics and 4 metabolites in milk. A modified quick, easy, cheap, effective, rugged, and safe extraction method (QuEChERS) optimized by response surface methodology (RSM) was used for sample preparation. All the drugs were subsequently separated and detected by high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS), using roxithromycin as internal standard for maximum accuracy and precision. The method was validated following the guidelines specified in Commission Decision 2002/657/EC. The recoveries of all the analytes were in a range of 62.27%-115.28%. Most macrolide antibiotics and metabolites could be detected in the concentration of 1-100 ng/mL with correlation coefficient ($r^2 > 0.998$). The LODs and LOQs of all analytes were respectively in the range of 0.30-0.85 $\mu\text{g}/\text{kg}$ and 1.1-4.0 $\mu\text{g}/\text{kg}$, respectively. The intra-day and inter-day precision were respectively lower than 10% and 15%, respectively. The developed method has been successfully applied to screen these compounds in different milk products.

Characterization of Marine Oils from Fish Transforming Industry By-Products – A Comprehensive Approach

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Abstract

Introduction: Lipids from marine origin have a composition determined by an accurately identification and quantification of the fatty acid (FA) content. Essential long chain fatty polyunsaturated fatty acids (PUFAs) play an important part in human health, concerning the n-3/n-6 ratio used as index for nutritional value. It has been well established that fish oils and marine microalgae are abundant sources of n-3 fatty acids, being predominantly in perivisceral organs, with uptakes exceeding 50% of the wet weight.

This work focuses on discards from fish transforming industry (FTI) and offers a characterization of marine oils and fats from different sources representative of regional fishing sector and methods of extraction. The activities were developed under the project Marine Blue Refine, in a consortium with a local company (Madebiotech).

Results and Discussion: An *in situ* simultaneous lipid extraction and transesterification was performed to avoid manipulations and rapid analysis. Results of characterization by spectroscopic methods in conjunction with PCA multivariable analysis (FTIR-ATR and high-resolution ¹H and ¹³C RMN), provides insight in molecular structure of FA and relative concentration of n-3 PUFAs. This data is in direct correlation with characterization of FAs profiles by GC-FID and show a favorable ratio towards n-3. Another index of the nutritional value of fish oils indicates a good value of PUFA relative to saturated FA.

Acknowledgements

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Sessions - II: Analysis of Food and Nutrition | Food Technology

The Influence of Honey Adulteration with Sugar Syrups on the Honey Bioactive Compounds and Biochemical Properties

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Abstract

The assessment of honey authenticity is a major concern that has gained a lot of interest internationally since honey has always been subjected to various fraudulent practices which become more sophisticated in the present day. Consumers and regulatory bodies are interested to have reliable analytical tools and information to allow the detection of adulterated honey.

The objectives of this study were to develop and validate new comprehensive and cost-effective methods to confirm the authenticity of honey in reference to detection of direct and indirect adulteration of honey by addition of sugar syrups, even for fine adulteration.

Authenticity of different floral honeys in terms of adulteration identification was investigated by fingerprinting low-molecular organic compounds responsible for honey therapeutic effect (phenolic compounds, organic acids and vitamins) and sugars profile by HPLC-MS, UV-VIS and NMR techniques and verified using the isotopes signature ($\delta^{13}\text{C}$ and (D/H)I) by IRMS and SNIF-NMR techniques. In this respect, we have established reference databases, estimating the variation of honey bioactive constituents and isotopic parameters due to botanic and geographic factors, which can be compared with the variation observed by direct or indirect adulteration of honey. Using the proposed methodologies, direct and indirect adulteration experiments with different sugar syrups (from corn, rice and beet) were conducted in order to highlight the minimal amount of incorporated sugar syrup from which the bioactive compounds profile and honey biochemical properties are definitively modified.

Differentiation of Berry Species in Foods by DNA Barcoding and High-Resolution Melting (HRM) Analysis

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Abstract

The consumption of berries has been associated with various health benefits. Thus, numerous berry products are commercially available. However, studies indicate that berry products are frequently adulterated, most commonly by replacing berries of higher value by less expensive berries or even by other fruit species.

Selective methods are required for the identification and differentiation of berry species in food products. In the present study, we investigated the potential of combining DNA barcoding and high-resolution melting (HRM) analysis for the authentication of berry species.

The aim of DNA barcoding is to identify and differentiate organisms by analyzing distinctive regions in the DNA, so-called "DNA barcodes". The first step of DNA barcoding is the selection of an appropriate DNA barcode. The DNA barcode should consist of species-specific bases to allow the discrimination of the target species. In order to be able to amplify the barcode sequence in all species of interest with a universal primer pair, the variable part should be flanked by conserved regions.

In the present study, we designed primers for the differentiation between bilberry (*Vaccinium myrtillus*) and blueberry (*Vaccinium corymbosum*) and for the differentiation between American cranberry (*Vaccinium macrocarpon*), European cranberry (*Vaccinium oxycoccos*) and lingonberry (*Vaccinium vitis-idaea*). After amplification of the barcode region by the polymerase chain reaction (PCR), the amplicons were subjected to HRM analysis and the melting profiles were used to identify the berry species. The methods were validated by performing spiking experiments and applied to a variety of commercial products including jams, juices and supplements.

S-Nitroso-N-Acetylcysteine as a Nitrite Replacement Additive in Meat Products

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Abstract

Nitrite reacts with secondary amines to form N-nitrosamines (N-NA) that lead to gastrointestinal cancers. The aim of this study was to compare nitrite with S-nitrosocysteine (Cys-SNO) and S-nitroso-N-acetylcysteine (NAC-SNO) with respect to N-NA formation, which was evaluated by determining the conversion of N-methylaniline to N-nitrosomethylaniline. Under neutral and acidic pH conditions, N-NA formation rate was nitrite > Cys-SNO > NAC-SNO. In the presence of copper or nucleophiles, NAC-SNO generated much less N-NA than Cys-SNO. Nitrite and Cys-SNO produced higher amounts of N-NA in the presence of oxygen, whereas NAC-SNO was almost oxygen insensitive. In meat in the stomach medium, NAC-SNO produced much lower amounts of N-NA than other additives. In heated meat, Cys-SNO and NAC-SNO generated the nitrosyl-hemochrome pink pigment, better than nitrite. In conclusion, NAC-SNO was much less reactive for N-NA formation than nitrite and Cys-SNO in conditions relevant to meat production and stomach digestion.

Optimization of Enzymatic Protein Hydrolysis Condition of Edible Bird's Nest Using Alcalase® to Obtain Maximum Degree of Hydrolysis

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Abstract

The purpose of this study is to determine the optimum hydrolysis conditions for edible bird's nest by using Alcalase® in terms of temperature (45 °C - 65 °C), pH (7.5-9.5), enzyme to substrate level (1% - 2%) and hydrolysis time (60 minutes-180 minutes) to obtain maximum degree of hydrolysis. The optimization study was employed at equidistant level with three level face-centered central composition design (CCD) by using response surface methodology (RSM). The optimum conditions for enzymatic hydrolysis of edible bird's nest were found at temperature of 64.99 °C, enzyme concentration of Alcalase® at 2%, hydrolysis time at 179.55 min and pH at 9.46. The enzymatic hydrolysis of edible bird's nest gave a two-factor interaction (2FI) model for the experimental data. Under these optimum conditions, the predicted value for degree of hydrolysis was 37.90%, while the experimental data gave 37.92%. Proximate analysis (dry basis) revealed that edible bird nest contained 70.55 ± 1.33% protein, 11.59 ± 0.33% ash, 5.43 ± 1.02% fat, and 12.43 ± 0.31% carbohydrate, while lyophilized edible bird's nest hydrolysate prepared under optimum condition consisted of 67.63 ± 0.78% protein, 2.62 ± 0.37% ash, 0.61 ± 0.30% fat and 29.14 ± 0.11 carbohydrate. The edible bird's nest hydrolysate prepared under optimum condition contained all essential amino acids and their levels were higher than suggested profile of essential amino acid requirements for adults (FAO/WHO, 2007), except for methionine. As compared with previous study on other types of protein hydrolysate, edible bird's nest gave the highest composition of valine and threonine in terms of essential amino acid.

Inactivation of *Listeria monocytogenes* on Paperboard Using 410 nm Light Emitting Diodes

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Abstract

Light emitting diodes of wavelength 410 nm were used to inactivate *Listeria monocytogenes* stains in Luria Bertani broth on paperboard, an increasingly popular food packaging material. The integrity of the cell membranes was examined using differential fluorescent staining. Scanning electron microscopy (SEM) was used to obtain a deeper understanding of *L. monocytogenes* stain formation on paperboard and the damage caused to the cells by the LEDs. While the planktonic *L. monocytogenes* population could be completely inactivated following a brief lag phase that lasted about 20 min, the illumination of the sessile population left some persisters despite immediate commencement of the inactivation. Planktonic populations of inocula sized 3, 5 and 7 log CFU/mL were reduced below the detection limit in 54, 80 and 84 min respectively, whereas it took 120 and 390 min to reach constancy in the sessile populations of inocula sized 5 and 7 log CFU/cm². The number of membrane-damaged cells was seen to increase with the illumination time. SEM images provided evidence of the protection conferred by the stain on the underlying cells. This study demonstrates that blue LEDs have the potential to reduce the risk of contamination from paperboard cartons under refrigeration conditions.

Novel Sorbitol Ester of Norbixin Disperses Bixin in Aqueous Solution Through a Nanostructure Complex that Shows Higher Storage Stability than Norbixin at Moderate Temperature

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Abstract

Bixin is one of the most used yellow-orange food colorants in the food industry. The polyene chain of bixin makes it highly hydrophobic and less suitable for aqueous foods. Upon saponification, bixin is converted into the water-soluble derivative, norbixin. However, norbixin also has its limitation towards aqueous food matrix and is only completely soluble at alkaline condition.

To synthesize a new derivative of bixin with potential hydrophilic properties, it was enzymatically modified with sorbitol. A lipase-catalyzed reaction of bixin lead to a transesterification reaction and formation of a sorbitol ester of norbixin (SEN). A combination of 2-methyl-2-butanol and tetrahydrofuran (80:20 v/v%) was optimal for reaction with immobilized *Candida Antarctica* lipase B (Novozyme 435) resulting in a 50% yield of the SEN-product.

In combination with reverse phase HPLC separation, dynamic light scattering and a shift in absorption spectra suggested a complexation of SEN with bixin (SEN-complex) in a well-defined nanostructure with a radius around 40 nm in aqueous solution. We proposed that a self-association of SEN in aqueous medium might develop a hydrophilic structure with a hydrophobic core, which can uptake bixin and disperse it into the aqueous medium. The SEN-complex showed slightly higher solubility at pH 8 than at 7 and 6, but at pH 5, precipitation and loss in color were observed.

The SEN-complex showed higher stability than norbixin when exposed to moderate temperatures 4-40 °C, but in light, norbixin showed higher stability up to 28 h, whereas no difference was observed after 72 h.

Synthesis of New Inorganic Janus Particles: Self-Assembly, Nanostructuring and Applications

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Abstract

Janus particles (JPs) have two or more distinct surfaces with different physical properties, allowing different types of chemistry to occur on the same particle. Due to their ability to self-assemble at the interface between two immiscible fluids, JPs have great potential as a new class of colloidal materials with advanced functionalities. These JPs are usually fabricated by immobilizing isotropic particles on a given surface and region selectively modifying their exposed hemispheres. However, this approach is complex in various aspects, and therefore of lower interest for potential industrial applications. Spontaneous, template-directed strategies or self-assembly of the particles are thus highly preferred.

This research presents *in-situ* functionalization of silica nanoparticles in oil-in-water (o/w) biphasic system with two equimolar organosilanes of antagonistic polarity to generate JPs with a desired distribution of hydrophilic and hydrophobic surface regions (amphiphilic particles). Homogeneous and stable Pickering emulsions have been obtained, where droplet size can be tuned according to the silica particle concentration and the o/w ratio used. The micro- and nano-structure was analyzed by optical and fluorescence microscopy and cryo-SEM. Such formulations enabled the successful individual encapsulation of fungal spores of *Metarhizium anisopliae*, which is implemented in efficient formulations for biopesticides in agricultural applications.

In addition, we present new, rapid and facile approach for the synthesis of JPs via polymer precipitation process, resulting in core-shell structure that allows selective chemical modification of the exposed hemispheres of the particles. This opens up novel and emerging applications of JPs, such as chemical sensors, superhydrophobic surfaces and encapsulation techniques.

Ethnic Fermented Food Products of Nagaland, India

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Abstract

Fermentation process is traditional knowledge-based food preparation and preservation process from the locally available raw materials and has been passed down to next generation. Ethnic fermented foods are tagged to identity of a community and cultural heritage. Nagaland, a hilly state of India inhabited by different tribal communities and each tribe has its own ethnic fermented foods. Here, we present the different ethnic fermented foods of the state, nutritional assessment and microbiology of certain food products. Some of the prominent fermented foods and beverages of Nagaland are *zutho*, *axone/akhuni*, *anishi*, *Jang kap*, *hungrii*, *rhujuk/bastanga*, *jangpangngatsu*, *tsutuocie*, fermented pork fat and fermented fruit beverages.

Microbiological studies of *axone/akhuni*, *anishi*, *hungrii*, *rhujuk/bastanga* and *tsutuocie* was conducted. Based on phenotypic and genotypic characterization, *Bacillus* species was found as dominant microorganism all the foods. Products like *axone/akhuni*, *anishi*, *hungrii*, *rhujuk/bastanga* and *tsutuocie* have been analyzed for nutritional values (protein, reducing sugars, crude fiber, TPC, TFC and antioxidant activity). Most of the foods were acidic except *Axone* and *tsutuocie*. Four products (*axone/akhuni*, *anishi*, *hungrii* and *rhujuk/bastanga*) were highly protein rich (38.9-43%) and crude fiber content was high. Of the 5 products, *rhujuk/bastanga* had the highest phenolic content, followed by *hungrii*, *anishi*, *axone/akhuni* and *tsutuocie*. While *anishi* was flavonoid richest followed by *hungrii*, *axone/akhuni*, *rhujuk/bastanga* and *tsutuocie*. *Anishi* was antioxidant richest while, *tsutuocie* was poorest.

Fermented foods in Nagaland are still prepared at the household level by indigenous unscientific methods and food security is one a major challenge and need scientific awareness for commercial exploitation.

In vivo Exposure Spectrum Analyses of Acrylamide and Related Chemoprotective Sites of Action Based on Metabolomics

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Abstract

The acrylamide related food safety issues have raised wide concern all over the world since a large amount of acrylamide in thermally processing foods was discovered in 2002. In recent years, the research focus on acrylamide has gradually shifted from *in vitro* studies to *in vivo* studies. The *in vivo* exposure levels, metabolic analyses, biomarkers and metabolic protective ways of acrylamide have become the focus of scientific attention in related fields. Here we investigated the changes in the levels of metabolic exposure biomarkers in the rat urine and the difference of exposure spectrum with and without the intervention of

catechins. After oral gavage with $^{13}\text{C}_3$ -acrylamide, the metabolic process *in vivo* was tracked and the exposure spectrum was profiled. The differential metabolites of acrylamide *in vivo* were collected through the intervention of catechins. The urine samples of rats within 16 h were collected and the exposure spectrum analysis was conducted using UHPLC-Q-Exactive-HRMS. The trace level of N-acetyl-S-(2-methylethyl)-L-cysteinyl sulfoxide (AAMA-sul) was discovered in rat urine. The mass spectra of rat urine samples were processed by metabolic and statistical software's, and the orthogonal partial least squares discriminant analysis model (OPLS-DA) was established. A total of 27 kinds of differential metabolites of $^{13}\text{C}_3$ -acrylamide in rat urine were found using this model, while 13 different metabolites were identified by the database for the comparison and qualitative analysis. Similarly, 75 kinds of $^{13}\text{C}_3$ -acrylamide were found under the intervention, while the chemical structures of 21 metabolites were identified.

Advantages of Model Predictive Control in Food Industry-Case study on Pasteurization Temperature Control

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Abstract

Artificial intelligence (AI) is imposing itself as the new trend for decision making, classification, modeling, and much more. The most representative example of AI tools used in the World Wide Web is deep Artificial Neural Networks (ANN). However, AI is not only present in IT and communication technologies; it is conquering other engineering fields, such as control, modeling, medical applications, etc. Food industry and food technologies, even if being behind the above-mentioned fields in terms of AI use, the last decades witnessed an increase in ANN applications. Indeed, interest in using Artificial Neural networks as a modeling tool in food technology is increasing due to their ability in solving many complex real-world problems. Food technology systems are often complex, mixing mechanical, thermodynamics, chemical and biological concepts, making the development of first principles mathematical models very difficult to obtain, opening the way to data driven AI approaches, mainly ANN. Artificial neural networks are dynamic systems which are composed of a simplified version of biological neurons in the brain. Organized in a networked topology, they have the ability to approximate any nonlinear function providing enough comprehensive explanatory data, and sufficient training time as well as a validation phase. Once obtained, such models may help reaching the main objectives of the food industry which focus on food safety, food quality control, increasing yield, minimizing production cost and preserving environment. The present talk presents an overview of ANN usage in food industry is given. Finally, a real application of ANN, and more precisely Multi Layered Perceptron (MLP), in dairy industry is detailed.

Phytoestrogen Content in Organically- and Conventionally Produced Beer and Hops

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Abstract

Prenylflavonoids are unique secondary metabolites in hops. They are phytoestrogens because of their estrogenic effects. Besides having an estrogenic effect, which can be positive (reduce osteoporosis, antineoplastic in prostate cancer) or negative (menstrual disturbances, feminization, carcinogenic in estrogen-induced cancers), they also have antioxidant effects. In the absence of pesticides and mineral fertilizers, organically produced crops have been shown to produce more secondary metabolites that can serve to protect the plant. The aim of this study was to investigate if there is a significant difference between the content of the selected prenylflavonoids in organically produced hops and beer compared to that in conventionally produced ones.

An UHPLC-MS/MS method for the analysis of three selected prenylflavonoids: xanthohumol, isoxanthohumol, and 8-prenylnaringenin in hops and beer was developed and validated. The instrumentation used was a Shimadzu Nexera x2 UHPLC coupled to Sciex 4500 QTRAP system. The analyzed samples were commercially available hops and beer produced from the sampled hops. Thirteen matched pairs of organically- and conventionally produced hops of different varieties from four producing countries were collected in 2016 or 2017. Contrary to the theoretical assumptions, no significant difference between the content of the selected phytoestrogens neither in hops, nor in beer was observed. Also, there was no significant influence of production year within a region. In conclusion, levels of prenylflavonoids most likely depend on hop variety and these compounds seem to have a minor role in plant protection mechanisms. In addition, contents of the selected prenylflavonoids cannot serve as indicators for organic production frauds.

Young Researchers Forum

Cross-linking of Caseins with Microbial Transglutaminase: A Comprehensive Analysis Using Asymmetrical Flow Field Flow Fractionation

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Abstract

Enzymatic cross-linking of caseins with microbial transglutaminase (mTGase; EC 2.3.2.13) has been extensively studied as a promising tool for enhancing the firmness and physical properties of dairy products [1]. The size of casein oligomers and polymers produced by mTGase has been reported to play a major role in the formation of structured acid gels [2].

The suitability of size exclusion chromatography (SEC) for the size determination of larger casein polymers is limited, because of its restrictions concerning separation range and resolution that mainly depend on the column material. In contrast, asymmetrical flow field flow fractionation (AF4) enables a high-resolution separation of a wide range of molar masses, from approx. 103 up to 1010 Da, because of the lack of a stationary phase and reduced shear forces [3].

In this study, AF4 coupled to static and dynamic light scattering detection (AF4-MALS-DLS) is performed to evaluate casein particles concerning molar mass, size, and scaling behavior prior to and after enzymatic cross-linking with mTGase. Additionally, *in situ* Atomic Force Microscopy (AFM) is subsequently applied for verification of their conformational properties. The first results revealed increasing molar masses with proceeding of cross-linking time, whereas size distributions are marginally affected by enzymatic treatment. This would suggest that mTGase mainly cross-links casein molecules within distinct particles, which get more compact after extensive polymerization.

A New Biodegradable Active Food Packaging Film with Seaweed Extracts for Frozen Salmon

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Abstract

In the framework of the European project AMALIA – Algae-to-Market Lab IdeAs there has been an effort to develop new strategies for the valorization of invasive seaweeds with the objective of provide the industry with high added value compounds, transforming its harvesting from the ocean in an effective management practice to sustain good environmental status, or even to restore impacted areas.

In this study, the potential incorporation of bioactive seaweed extracts in alginate films to develop a biodegradable active packaging was addressed. Ethanolic extracts (EE) of invasive seaweeds, *Sargassum muticum* and *Grateloupia turuturu* were incorporated in alginate, and its effects on the maintenance of salmon was assessed after 6 months of storage at freezing temperature. A traditional plastic film used by the industry to preserve frozen fish and alginate films without EE were used as controls. The effect of films on the maintenance of fish was evaluated in terms of color, texture, moisture, pH, lipid peroxidation, fatty acid profile, water holding capacity, drip loss, and cooking loss.

The incorporation of EE of *S. muticum* and *G. turuturu* resulted in an effective maintenance of fish quality after the 6-month storage period. After thawing, samples with EE of *S. muticum* and *G. turuturu* showed lower drip and cooking loss values. However, due to the incorporation of colored extracts, in some cases, interferences can be detected in samples packaged with these films.

The results showed that EE incorporation in alginate films can lead to a new type of biodegradable active packaging to replace plastic formulations actually used in food industry, which also has the potential to be used in ready to cook meals.

DNA Metabarcoding of 28 Meat Species to Detect Food Adulteration

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Abstract

One aspect of meat adulteration is the substitution of expensive meat species by cheaper ones. Meat species differentiation can be performed with either protein- or DNA-based methods. DNA based methods are advantageous due to the higher stability of DNA compared to proteins. In routine analysis, multiplex real-time polymerase chain reaction (PCR) methods currently play the most important role. By combining several species-specific primers and probes, they enable the identification of more than one meat species per tube. The maximal number of species that can be differentiated is, however, limited by the number of optical channels of the real-time PCR instrument. This limitation can be overcome by DNA metabarcoding, a combination of DNA barcoding and next generation sequencing (NGS). DNA barcoding is the analysis of DNA barcode regions, short DNA sequences containing conserved regions but also species-specific differences. NGS is a powerful technology to sequence a huge number of amplicons in parallel.

Here, we present a DNA metabarcoding method for the simultaneous amplification of a mitochondrial 16S ribosomal DNA target region of 17 mammalian and 11 poultry species. Indexed and pooled DNA libraries were sequenced on the benchtop sequencer MiSeq[®] (Illumina). NGS-reads were analyzed in a workflow implemented in Galaxy to identify and discriminate the respective animal species. The method was validated by analyzing individual DNA extracts from muscle meat, DNA extract mixtures and model sausages. Our results indicate that indexed massively parallel amplicon sequencing is a reliable method to detect target species down to a concentration of 0.1%.

Effect of Steam Treatment on the Nutrient Stability of Chloroplast-Rich Fraction Derived from Post-Harvest, Pea Vine Field Residue

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Abstract

Green waste materials are rich sources of chloroplasts, an organelle in plants and other photosynthetic organisms that are responsible for the conversion of light energy into chemical energy. The removal of intact chloroplasts from their cell-wall confinement offers a novel way to enrich nutrients from green biomass. In this work, the impact of heat treatment to stabilize green biomass on nutrient retention was studied. Peroxidase enzyme activity was used as a measure of heat-induced enzyme inactivation. After various heat treatments of postharvest pea vine field residue (haulm), a chloroplast-rich fraction (CRF) was recovered and freeze-dried and the following nutrients were measured: β -carotene; lutein; and α -tocopherol. The CRF material was also stored under a range of conditions: temperature (-20 °C, 4 °C, 25 °C and 40 °C); vacuum packed and non-vacuum packed; and foil and transparent pouches for 12 weeks. This result showed steam-treated pea vine juice could reduce peroxidase activity by 66% compared with fresh pea vine juice. The nutrient stability has been observed at low temperatures, with and without air exposure, nutrient contents, including β -carotene, lutein and α -tocopherol were stable over the duration of the 12 weeks. However, a decrease is observed at week 12 in non-vacuum sealed pouch stored at 25 °C and the higher temperature at 40 °C. They showed the nutrient degradations over time, especially in the presence of air and light. In addition, β -carotene content degraded over time more than the other two micronutrients. However, this degradation was particularly seen at high temperature or with exposure to air and light.

Effect of Temperature, Oxygen, and Light on the Degradation of Micronutrients in Spray-Dried Spinach Juice Powder During Storage

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Abstract

Vitamin A deficiency is a public health concern especially in developing countries. Generally, the active materials such as β -carotene, lutein and α -tocopherol in spinach are concentrated in the chloroplasts. Therefore, encouraging dietary carotenoids as precursors is a promising strategy. With the growing public awareness of healthy eating and sustainable nutrition, opportunities exist for the vegetable sector if dried spinach powder was stable and bioaccessible.

This study evaluated these two parameters during *in-vitro* digestion of carotenoids from fresh raw spinach, fresh spinach juice, freeze-dried spinach juice, spray-dried spinach juice and freeze-dried chloroplast-rich-fraction (CRF) from spinach juice.

Both β -carotene and lutein in CRF and fresh spinach juice had lower stability (10-45%) than the rest of the samples that retained more than 50% stability. All samples had bioaccessibility greater than 30% for β -carotene and greater than 40% for lutein. Heat-treatment from spray-drying positively influences the digestive stability and bioaccessibility of spinach carotenoids. Spray-dried juice provided the greatest amount of bioaccessible β -carotene with 800 $\mu\text{g/g}$ d.m., followed by freeze-dried juice, fresh juice, raw spinach and CRF from spinach juice (508, 301, 248 and 136 $\mu\text{g/g}$ d.m. respectively). However, fresh juice and CRF from spinach juice had the most bioaccessible lutein with 1743 and 1490 $\mu\text{g/g}$ d.m., followed by raw spinach, spray-dried juice and freeze-dried juice (837, 777 and 622 $\mu\text{g/g}$ d.m. respectively).

Antioxidants and Their Behavior After Processing and Digestion

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Abstract

Antioxidants, known as health-promoting agents, are responsible to slow down or prevent reactive oxygen reactions which are responsible for cancer, atherosclerosis, several inflammation and similar disorders in the body. Food sources especially fruits and vegetables are rich in antioxidant compounds such as polyphenols, carotenoids and vitamins. Various processing procedures for instance heat treatment, high pressure applications, fermentation etc. are applied to extend the shelf life of the food and obtain new products. However, these compounds are generally sensitive to high temperature, pH, light, oxygen etc.; moreover, bioaccessibility of them is very low due to pH changes in the gastrointestinal track. Encapsulation which is an entrapment process to cover desired compounds within an appropriate material can be solution for proper protection of sensitive compounds. Encapsulated compounds for functional foods have recently become important due to latest demands and tendencies of consumers towards high quality, convenient and healthy food. Encapsulation technology offers protection of valuable compounds against chemical, physical and biological degradation, enhancement in handling, improvement of bioaccessibility and controlled release at a certain time and point. Several techniques such as spray and freeze drying, extrusion, electrospinning etc. can be applied for the encapsulation of antioxidants. Each method has own advantages and disadvantages; therefore, suitable method has to be selected for every single substance to obtain desired final product.

Importance of Molecular Nutrition on Functional Food Designing: Physiological Functionality of Iron Mineral for the Development of Edible Natural Iron Supplements Against Anemia

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Abstract

Iron deficiency anemia (IDA) is the most common global nutritional deficiency especially in women, children and infants.

Consuming of low dietary iron and inadequate intestinal iron intake cause IDA. Intestine plays a vital role to maintain iron homeostasis since mammals do not have active physiological iron excretion mechanism. Efficient intestinal iron absorption is essential to control iron level in the body. There are two iron supplies for enterocyte cells of intestinal including blood and diet. Thus, we aimed to investigate the effects of dietary and blood iron minerals on the molecular and genetic regulation of intestinal iron metabolism during IDA. The results will help us to design iron containing functional foods. The Caco-2 cells used in the study were grown on special bicameral cell culture insert systems to mimic the human small intestine. The apical and basolateral polarization of cells were analyzed by TEER measurement. IDA was induced by DFO and effect of iron on IDA was investigated at the molecular and genetic levels by RT-PCR. Our main result showed when iron was given into basolateral side of cells, iron deficient phenotype was disappeared compared to apical side iron treatment by analyzing marker genes (Dmt1 and TfR) mRNA expression levels. Our results suggest that blood iron is essential to maintain intestinal iron metabolism. The blood iron level is regulated through dietary iron. Therefore, it is important to develop edible natural iron supplements that can be used as an additive to food products to make them “functional” via enhancing their iron bioavailability.

Effect of Sublethal Temperature and UV-LED Irradiation on the Inactivation of *E. coli* K12 in Cloudy Apple Juice

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Abstract

Ultraviolet Light Emitting Diodes (UV-LED's) offer many superiorities compare to conventional mercury lamps due to their non-toxicity, compact size, low-energy consumption rate and long-life span. UV-LEDs use UV light emitted either at multiple and individual wavelengths or in combination with different wavelengths. However, their lethal effect on microorganisms in fruit juices is limited because of low transmittance of juices; therefore, it is necessary to design a process which uses hurdle concept for gentle and effective preservation of fruit juice products.

The aim of this study is to investigate the effect of sublethal temperature (mild heat (MH)) and UV-LED processing on the inactivation of *E. coli* K12 and natural microflora (total aerobic mesophilic bacteria (TAM) and yeast & molds (YM)) of cloudy apple juice (CAJ). For this purpose, pre-pasteurized CAJ inoculated with *E. coli* K12 (6-7 log CFU/mL) and freshly-squeezed CAJ (FSCAJ) were treated with UV-LED (280/365 nm) irradiation at 55 °C with different exposure times ranging from 5 to 15 min.

Individual processes of UV-LED and MH treatment applied to CAJ samples for 5 to 15 min were not sufficient to inactivate neither *E. coli* K12 nor TAM and YM of juice samples. The combination of these two processes with 5 to 15 min treatment times resulted in complete inactivation of the target microorganism in pre-pasteurized CAJ. TAM count of FSCAJ subjected to UV-LED+MH for 5, 10 and 15 min were reduced by 1.49 ± 0.06 , 1.65 ± 0.15 and 2.31 ± 0.18 log CFU/mL; YM count of FSCAJ was significantly reduced by 3.98 ± 0.03 , 4.01 ± 0.03 , 4.05 ± 0.02 log CFU/mL, respectively. This investigation demonstrated that UV-LED+MH treatments would be an alternative to heat pasteurization, enabling the inactivation of pathogenic bacteria and reducing the natural microflora of CAJ, obtaining a safe and stable product at sublethal temperatures.

Effect of Blueberry Extract on Oxidative, Microbiological and Sensory Quality of Meatballs during Frozen Storage

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Abstract

Research on the role of plant-derived antioxidants in food safety has been gaining interest. The antioxidant and antimicrobial capacity of freeze-dried phenolic extracts obtained from blueberry were studied in beef meatballs during frozen storage for 6 months. Concentrated extract was used in 0.5% and 1% concentrations and they were compared with BHT (0.01%), synthetic antioxidant. Antioxidant sources were added to freshly prepared meatballs before freezing at -18 °C. The addition of blueberry extracts and BHT efficiently protected meatballs from pigment and lipid oxidation. The a* values of BE-added meatballs changed slightly, in comparison with significant changes in the control and BHT containing samples. Peroxide value (PV) and TBARS significantly (P<0.01) reduced by BE and BHT addition while no significant differences were observed between

antioxidant sources. Total viable, psychrophilic and Enterobacteriaceae counts of meatballs were lower in BE and BHT added meatballs, except lactic acid bacteria (LAB). Blueberry extract added meatballs in both concentrations had higher LAB than that of BHT and control samples. However, microbial counts which decreased during storage were significantly ($P < 0.01$) affected by storage time. Color and rancid odor scores were correlated with a^* value, PV and TBARS results in BE and BHT added meatballs. Meatballs with 0.5% BE had higher sensory scores than the others. Considering its antioxidative effectiveness, blueberry freeze-dried extracts have the potential to be used as a natural antioxidant to reduce lipid and pigment oxidation and enhance the microbial and sensory quality of raw meatballs during frozen storage.

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Quality Properties of Freeze-Dried Verjuice Powder as Affected by Maltodextrin Concentration

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Abstract

Verjuice is produced from unripe grapes and used in foods to give flavor. Freeze drying is the best drying technique to obtain high nutritional and sensorial quality powders. The aim of this study is to evaluate the effect of different maltodextrin concentrations used as carriers on the quality properties of verjuice powder produced by freeze drying.

Freshly squeezed verjuice was prepared and freeze-dried for 48 h after addition of Maltodextrin (DE20) at three different concentrations (MD10%, 15% and 20% [w/w]) to avoid stickiness of the powder and increase the yield. Product yield and some quality parameters of verjuice powders such as the moisture content, water activity, bulk and tapped density, flowability, cohesiveness, solubility, Tg and color parameters were measured and compared. Drying yield of FD was very high at all conditions (>96%). Moisture contents and water activity of powders were significantly reduced, i.e. from 6.24 ± 0.05 to $3.98 \pm 0.11\%$ and from 0.40 ± 0.01 to 0.18 ± 0.02 , when increasing MD concentration from 10% to 20% [w/w]. Maltodextrin addition to juice at different levels did not influence the bulk density of powders. Powders containing MD15% and MD20% had nearly good flowability (21.46–20–48) and intermediate level of cohesiveness (1.27–1.26) ($p > 0.05$), however powder containing MD10% was in poor powder quality. Highest solubility (95.88%) was obtained from freeze-dried powders having the highest MD concentration. Similarly, higher L^* , lower a^* and b^* values of powders were reported when MD concentration was increased. Thus, browning was reduced. Yellow powders were obtained with around 90° hue angle. Larger size of particles was detected in powders having higher MD concentration.

Novel Mixing Approach for Enhancing Chemiluminescence Intensity Using Nanodroplets Mixing in a Microfluidic Platform for Estimating the Total Phenolic Content in Honey and Pomegranate Samples

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Abstract

In a precedent approach, nano-droplet microfluidic mixer system using acidic potassium permanganate and colloidal manganese (IV) chemiluminescence reactions has been conducted and developed to estimate total phenolic content (TPC) in honey samples and pomegranate juices. The advantage of applying very low flow rates in nano-droplet mixer microfluidic system was chosen to enhance reaction mixing and minimize reagents consumption and waste generation. A comparison between the two chemiluminescence systems was conducted by investigating 21 of phenolic compounds that are commonly found in food samples. Many parameters have been optimized including the concentration of reagents, flow rates and the chip design. The LOD obtained using colloidal Mn (IV) for standard solutions of gallic acid, caffeic acid, ferulic acid, catechin and rutin were 1.32, 2.40, 11.5, 7.60 and 16.5 ng mL⁻¹ respectively while the LOD ranged from 6.2 ppb for caffeic acid to 11.0 ng mL⁻¹ for quercetin using acidic potassium permanganate.

Finally, the *acidic potassium permanganate* method was applied for the determination of TPC in 29 local and commercial honey samples while colloidal Mn (IV) was used with 9 local and commercial *pomegranate juices*. The conducted reactivity and interference tests revealed that the methods were selective, rapid and sensitive in estimating the total phenolic content and the results were in good agreement with those reported for honey and *pomegranate juices*. The comparative study showed that colloidal Mn (IV) might be a favourable system in detecting polyphenolic compounds and thus in estimating TPC in food.

Antioxidant and Antihypertensive Activities of Enzymatic Hydrolysate from Commercial Gelatins

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Abstract

Gelatin is a good example of utilizing by-products from hydrolyzed collagen of animal connective tissues. It has been applied widely in the food industry to improve the texture and quality of foods. Apart from the functionalities of gelatin, a large number of reports have focused on the biological activities of enzymatic gelatin hydrolysates from various sources. Currently, the antioxidant and antihypertensive characterization of hydrolysates has gained increasing attention. The objectives of this study were to investigate the antioxidant and antihypertensive activities of commercial gelatin hydrolysates including bovine, porcine, and tilapia sources prepared by bromelain, collagenase, papain, and thermolysin, respectively.

In vitro results showed that hydrolysates generated with bromelain had the lowest degree of hydrolysis (DH), <25%. While there were higher DH in bovine hydrolysate with collagenase (BC, 45.7% at 2.5 h) and thermolysin (BT, 53.6% at 3.5 h). Moreover, the hydrolysate BC also had the highest antioxidant activity in DPPH radical scavenging activity ($22.53 \pm 2.81\%$), ferric reducing power test ($0.0056 \pm 0.0002 \text{ mM Fe}^{2+}/\text{mg}$), and superoxide radical scavenging activity ($41.64 \pm 0.64\%$). On the other hand, porcine gelatin hydrolyzed by papain (PP) had the best angiotensin converting enzyme (ACE) inhibitory ($60.94 \pm 2.94\%$). The results of this study suggest that BC and PP may be served as nutraceutical ingredients for the production of functional food to alleviate the oxidative stress and hypertension.

Carcinogenic Risk Assessment of Heavy Metals in Fruits and Vegetables Consumed in Syunik, Armenia

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Informational Analytical Center for Risk Assessment of Food Chain of the Center for Ecological Noosphere Studies (CENS) of National Academy of Sciences, Republic of Armenia

Abstract

Food safety is the leading issue all over the world, particularly in Armenia, where mining industry is developed. Thereby population is exposed to many toxic elements, particularly heavy metals. Since non-carcinogenic health risk of heavy metals was assessed in previous researches, the present study was conducted to assess carcinogenic risk of heavy metals (Ni, Cr, As, Pb and Cd) in fruits and vegetables, grown in some mining areas of Syunik region, Armenia. Overall 16 fruits and vegetables were investigated.

Carcinogenic risk was calculated using the estimated daily intake (EDI) and oral slope factor (SF). Risk of less than 10^{-6} was considered as the acceptable limit. The obtained results showed that each of studied heavy metal poses significant level of carcinogenic risk through consumption of the majority of studied food items. Cumulative cancer risk values for investigated heavy metals showed a decreasing order of $\text{Ni} > \text{Cr} > \text{As} > \text{Pb} > \text{Cd} > 10^{-6}$. Among investigated fruits and vegetables potato, bean and maize were considered the most hazardous. Moreover, cumulative cancer risk for each of studied fruits and vegetables exceeded US-EPA acceptable limit (10^{-6}), which should warrant concern.

Quantification of Mercapturic Acid Metabolites from Dietary 3-MCPD and Glycidol for Evaluation of Toxicokinetics in Rats and Daily Internal Exposure in Humans

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Abstract

The 3-monochloropropane-1,2-diol (3-MCPD), glycidol and their esters are main chlorine source risk factors during food processing. The mercapturic acid metabolites from dietary 3-MCPD are important short-term exposure biomarkers for evaluating the *in vivo* toxicity of 3-MCPD. Here we developed an isotope dilution ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) method for the quantitative determination of 2,3-dihydroxypropyl mercapturic acid (DHPMA), which derived from the metabolism of 3-MCPD, glycidol and their esters under the electrospray ionization negative

(ESI⁻) mode in urine of rats and humans. Iso-DHPMA, a novel urinary metabolite, was discovered and successfully separated on an Acquity UHPLC HSS T3 column (2.1×150 mm i.d., 1.8 μm), and detected in urine of rats, which were orally administered with glycidol but not 3-MCPD. Using the high-resolution mass spectrometry technique, we demonstrated that iso-DHPMA appears a specific biomarker which derived from glycidol. The limit of quantification (LOQ) of the analytes in urine of rats and humans were 1.33 ng/mL and 1.56 ng/mL, respectively. Acceptable within-laboratory reproducibility (RSD<9.0%) substantially supported the use of current method for robust analysis. Our proposed UHPLC-MS/MS method was successfully applied to the toxicokinetic study of DHPMA in rats and short-term internal exposure to 3-MCPD and glycidol in humans. The ENV+ solid-phase extraction (SPE) cartridges were additionally employed for the clean-up treatment in urine of humans. Based on the robust quantitative method established in current study, both DHPMA and iso-DHPMA could be successfully measured in urine of humans during daily internal exposure to 3-MCPD and glycidol and their esters in food.

Effects of Wall Materials Composition on Physical Structure of Pumpkin Seed Oil-in-Water Emulsions

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Abstract

Formation of stable emulsion is the crucial step for oil encapsulation. The aim of this study was to investigate the influence of wall material composition on emulsion viscosity, emulsion stability and emulsion droplet size of pumpkin seed oil-in-water emulsions. Maltodextrin (MD), whey protein (WP) and gum Arabic (GA) were used as wall materials, whereas pumpkin seed oil was core material. Eighteen trials were performed according to a 3-factor D-optimal mixture design. Independent variables were WP concentration (10-19%), MD concentration (0.5-5%) and GA concentration (0.5-5%) with the constraint of WP + MD + GA = 20% (total solid content of wall materials). Pumpkin seed oil: wall materials ratio was fixed to 1:20. The response of the study was encapsulation efficiency.

All emulsions showed pseudo plastic (shear-thinning) behavior. As the concentration of GA increased, the viscosity of emulsions increased. The droplet size of emulsions (D_{3,2}) ranged between 1.11–3.00 μm. The composition of emulsion had statistically significant (p<0.05) effect on droplet size of emulsions. It is thought that possible interactions between proteins and carbohydrates such as steric or electrostatic forces may influence the size of droplets. The emulsion stability was expressed as phase separation. Phase separation of emulsions ranged between 13.65–35.28%. As the concentration of components having emulsifying activity (WP and GA) increased, the stability of emulsions also increased. The results of this research suggest that physical structure of pumpkin seed oil-in-water emulsions were greatly affected by the composition of emulsion.

Comparison of Quality Parameters of Sugar Beet Pectin Produced by Classical and Ultrasonic Treatment by Using Taguchi Method

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Abstract

Sugar beet pulp is a byproduct of sugar (sugar beet) production that can be said to be a rich source of pectin because of its pectin content (~15-20% in dry matter). In this study, the ultrasonic (45 kHz) method was compared with classical method for pectin production from sugar beet waste by using Taguchi L25 array design. The optimum extraction conditions, rheological properties and esterification level of sugar beet pectin was also determined by Taguchi L25 array design. In both production ways, the array design consists of four factors; temperature (60-95 °C), pH (1,1.5,2,2.5,3), time (1-5) and solid/liquid ratio (1:4,1:5,1:6,1:7,1:8) respectively. As a response, degree of esterification (DE) and yield analyses were performed to calculate individual signal-to-noise (S/N) ratios. The optimum DE level and the yield of pectin production was found at 80 °C temperature, 3 h and pH value was 1.5, 1/5 solid/liquid ratio for classical production method, 95 °C temperature, 3 h and pH value was 1, 1/5 solid/liquid ratio for the ultrasonic method. In result, it was seen that the classical method of the production pectin from sugar beet waste is better than ultrasonic treatment. The rheological properties of the sugar beet pectin in classical production was found better than ultrasonic method.

Evaluation of Polyphenols as Milk Plasmin Inhibitors

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Abstract

Functional dairy products, enriched with polyphenolic substances, are thought to be the one of the most effective candidates as being natural delivery systems of bioactive compounds. In this regard, the effects of polyphenol-milk protein interactions on the final characteristics of dairy products have been extensively studied. However, the effects of polyphenol binding on minor constituents of milk were rarely studied. The aim of this study is to evaluate the potential inhibitory effects of several flavonoids on plasmin activity. The effects of selected flavonoids (catechin, epicatechin gallate, epigallocatechin gallate, quercetin, myricetin, resveratrol and curcumin) on plasmin activity in model systems and milk were evaluated via spectrophotometric tools. The IC₅₀ values of each flavonoid were determined for plasmin inhibition. The obtained results revealed that, catechin and resveratrol did not influence plasmin activity. However, epicatechin gallate (ECG), epigallocatechin gallate (EGCG), quercetin, myricetin and curcumin inhibited plasmin by 57%, 86%, 65%, 90% and 54%, respectively. IC₅₀ values for plasmin inhibition were lower for myricetin (10 µM) and EGCG (17 µM) compared to quercetin (32 µM) and ECG (30 µM). For milk matrices, higher concentrations of flavonoids are required for indicated inhibition levels. The interactions between flavonoids and plasmin were characterized via ITC measurements and found to be dominated by noncovalent attractions. This study shows that phenolic compounds might interact with plasmin in milk via weak interactions and the consequences of these interactions can cause several alterations of milk and dairy products especially caused by proteolytic activity of plasmin, which are needed to be investigated in detail with future studies.

Comparison of a Conventional Plate Heat Exchanger Pasteurizer and a Tunnel Pasteurizer for Beer Product in a Certain Capacity

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Abstract

Pasteurization is a heat – treatment process that destroys pathogenic microorganisms in certain foods and beverages. The process is found by French Scientist Louis Pasteur in 1864 to improve the keeping qualities of wine. According, to structure of the machine, there are four pasteurizers; plate, tubular, autoclave and tunnel type. With regard to method, there are two type of pasteurization process; batch and continuous. In continuous pasteurization, flow-through heat exchangers, e.g. tubular, plate and frame types can be used. The Pasteurization process of Beer is an important operation in production that inactivates the spoilage microorganisms. Therefore, the pasteurization process should be good at reach desired flavor and shelf – life. The process enables a can or bottle of beer to be stored at room temperature for periods of time up to 120 days and beyond while draft beer has a life 45 to 60 days. The paper focuses on comparisons of the beer tunnel pasteurizer and the conventional plate heat exchanger pasteurizer for the same product and capacity. The pros and cons of the systems are defined in order to use in the pasteurization process for beer. The conventional high capacity pasteurizer consumes high electricity and also quantity of water, while, in the tunnel pasteurizer, energy and water consumption is decreased 50% and 80%, respectively. Moreover, the PU control unit and automation system bring about the high precision in the calculation of the Pasteurization Unit measurement as desired level at the instance of consumer.

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