

ANTIMICROBIAL ACTIVITY OF LEMON EXTRACT INTENDED AS NATURAL PRESERVATIVE IN FOOD APPLICATIONSA. CONTE^a, B. SPERANZA^a, M. SINIGAGLIA^{ab}, M. A. DEL NOBILE^{ab}Department of Food Science- University of Foggia^aInstitute for research and biotechnological applications to safety and quality of typical food products (BIOAGROMED) - Foggia^{ab}

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In this work a quantitative investigation on the antimicrobial effectiveness of a natural extract agent against some microorganisms, representing spoilage risk in different food matrices, is presented. The investigated microorganisms are represented by three yeasts species (*Candida lusitanae*, *Pichia subpelliculosa* and *Saccharomyces cerevisiae*), three bacillus species (*Bacillus subtilis*, *Bacillus cereus* and *Bacillus licheniformis*) and two acid lactic bacteria (*Lactobacillus curvatus* and *Lactobacillus plantarum*). Due to the sporigen nature of the *Bacillus cereus*, lemon extract activity was also tested on the spores of the bacterium. In order to assess potential biostatic or biocide activity of the natural agent, both the growth kinetics and dose-response profiles were obtained and analyzed. A suitable macro-dilution methodology based on a turbid metric technique was adopted to produce inhibitory data used for characterizing microbial susceptibility against lemon extract. Microbial growth was monitored through absorbance measurements at 420 nm as a function of contact time between microbial suspensions and active compound. Growth and no-growth status of microbial suspension containing natural active extract were expressed by means of two critical concentration of the compound: the Non-Inhibitory Concentration (NIC) representing a threshold value under which any effect on microbial growth can be observed, and the Minimum Inhibitory Concentration (MIC) that indicates a threshold concentration over which the active compound exerts a complete inhibition. The microbial sensibility was related to the NIC values, whereas the microbial resistance to the MIC ones. Results prove that lemon extract is effective in inhibiting the growth of the investigated vegetative cells and spores of microorganisms with similar antimicrobial effectiveness on the investigated groups of bacteria and yeasts. In particular, the NIC levels for all microorganisms are very small, ranged between 8,5 ppm and 20 ppm, with a prevalence of values ranging around 10 ppm. Results on the bacterial resistance showed that as far as the bacillus species is concerned the more resistant bacterium is *Bacillus licheniformis*; as concern the yeasts, *Saccharomyces cerevisiae* seems to be the less resistant with a MIC value equal to 28 ppm; a similar behavior was observed in the case of *Pichia subpelliculosa*, whereas *Candida lusitanae* has a MIC value higher than 100 ppm. Acid lactic bacteria showed similar MIC levels and, within the investigated microorganism groups, both of them represent the more resistant bacteria to lemon, even if the concentrations necessary to provoke a complete inhibition are very small, ranging around 150 ppm.

Keywords: Antimicrobial compound, essential oil, food spoilage

EFFECTS OF EDIBLE COATINGS ON DEEP FAT FRIED CHICKEN NUGGETS

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Deep-fat frying is a widely used operation for preparing foods because of the desirable and unique sensorial characteristics of fried products, such as flavor, appearance and texture. However, oil consumption is related to obesity and health problems like coronary heart disease and hypertension. Edible coatings may become a good alternative to solve this problem. Thus, the aim of this study was to evaluate the effect of edible coatings based on methylcellulose (MC) and cassava starch (CS) to reduce oil uptake and improve water retention of chicken nuggets. The effect of the plasticizer concentration was also evaluated. The edible coatings were prepared with 1 g of MC/100g solution and 4 g of CS/100g solution, both with 25 or 55 g plasticizer/100 g biopolymer. Glycerol was used as plasticizer. These solutions were applied on the nugget samples before battering the products. Pre-fried (180°C/30seg.) and fried (180°C/3min.) nuggets were analyzed to find out oil and water contents. These analyses were made in the crust, with the internal portion and with the whole sample. Also, color and texture were measured only in the fried nuggets. Texture of samples was determined by knife measuring shearing and compression forces. The coatings based on MC with 25% of plasticizer were more effective than CS for reduction of oil content in the internal portion after frying. But, for the crust, all coatings were more effective than the control (without coatings). During the pre-frying, the samples coated using MC with 55% of plasticizer presented the lowest oil content. However, after frying, the whole fried nuggets coated with CS with 25% of plasticizer, had the highest reduction of oil content. Overall, addition of the coatings on the nuggets didn't change the moisture content significantly. The coated samples were darker and presented yellower color when compared with the control. Also, a significant decrease in the peak force of the fried coated samples was verified, indicating a reduced hardness of the fried samples. This could be interesting for improving technological characteristics. Although the results had shown a reduction in the fat uptake, it is difficult to evaluate the possible protection of the coatings, because the highest oil absorption occurred substantially in the crust.

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Keywords: Edible coatings; deep-fat frying; oil-uptake; water retention, chicken nuggets

ISOLATION AND CHARACTERIZATION OF A NOVEL BACTERIOCIN PRODUCED BY LACTOCOCCUS LACTIS RUC 9 WILD STRAIN

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Lactic acid bacteria have been used in the production of fermented foods and feeds for many centuries. The preservative ability of LAB (lactic acid bacteria) in food is attributed to the production of antimicrobial metabolites including bacteriocins, acid organic, and hydrogen peroxide that play an essential role in ensuring the safety and extending the shelf-life of products (O'Sullivan et al., 2002). Bacteriocins generally exert their anti-microbial action by interfering with the cell wall or the membrane of target organisms, either by inhibiting cell wall biosynthesis or causing pore formation, subsequently resulting in death (O'Sullivan et al., 2002). The incorporation of bacteriocins as a biopreservative ingredient into model food systems has been studied extensively and has been shown to be effective in the control of pathogenic and spoilage microorganisms. *Listeria monocytogenes* is an opportunistic psychrotrophic foodborne pathogen that has been used as a model organism to study the efficacy of many different preservation methods (Teixeira de Carvalho et al., 2006).

The aim of the present work was to characterize the antimicrobial compound, produced by *Lactococcus lactis* wild strain RUC 9, previously isolated from MPV (minimally processed vegetables) products. The antimicrobial peptide was screened by the agar spot assay and identified by using different proteases. The bacteriocin exhibits a broad inhibitory spectrum against *Listeria innocua* DSMZ 20649 and several LAB strains used as target microorganisms. Moreover the peptide was stable over a wide range of pH (3-11) and temperatures (100 °C for 5 min). In order to establish the molecular size of the peptide, SDS-PAGE analysis was applied. To investigate the MPV product preservation of the *Lactococcus lactis* strain RUC 9 bacteriocin, further efforts are currently in progress.

Keywords: Minimally Processed Vegetables products, Bacteriocin, Lactic acid bacteria.

USE OF AN INERT GAS DURING THE OLIVE OIL BOTTLING: SHELF LIFE EVALUATION

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An urgent practice of bottling is needed to preserve the precious characteristics of the extra virgin olive oil during the commercialization. Previous results suggest that it is possible to realize a good stability for just produced the extra virgin olive. Moreover it is possible to obtain the inhibition of the oxidation process by storing oil in bottles which should be dark well closed and almost completely filled to ensure the lowest headspace. In this work we have investigated how to improve the olive oil shelf life using an inert gas to fill the headspace up during the bottling. A stability study was performed, monitoring the standard quality indices (free acidity, peroxidase value, specific extinction coefficients K_{232} e K_{270} , sensory score, induction time) and the phenolic compounds. Furthermore, the evolution of volatiles compound, responsible of the pleasant aroma of extra virgin olive oil, was studied by means a PURGE and TRAP system. The present paper reports on the study of extra virgin olive oils bottled in dark glass with nitrogen gas and stored under conditions similar to those found in supermarkets. The results suggested that the oil stability was improved by filling the headspace up with nitrogen and the main characteristics were maintained unchanged.

Keywords: Virgin olive oil, nitrogen gas, olive oil stability

NEW MIXER EQUIPPED WITH CONTROL ATMOSPHERE SYSTEM: INFLUENCE OF MALAXATION ON THE SHELF LIFE OF EXTRA VIRGIN OLIVE OIL

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To study the influence of operative conditions adopted during the malaxation of pastes on the shelf life of resulting oils we compared phenol compounds and the volatile compositions of the oil extracted from homogeneous batches of olive fruits from *Coratina* cultivar by using different malaxation atmosphere compositions. During the malaxation step there are the optimal conditions to the development of various enzymatic reactions simultaneously with a remarkable variations of the reological property of the olive paste. Results reported in this research shown higher concentration of total phenols in extra virgin olive oil using hermetically close mixer with modified atmosphere. But in this condition, the oil obtained was poor in volatile compounds, then less rich in pleasant aroma. It is necessary to arrive to a compromise in the atmosphere composition. A reduced concentration of oxygen in the mixer allowed to obtain an oil with a high total phenol content and with an equilibrium aromatic profile respect to the oil obtained filling up the mixer with nitrogen. These oils were richer in phenols respect to the oils obtained employing a mixer totally exposed to the air, consequently they shown a higher stability.

Keywords: Virgine olive oil, malaxation, olive oil stability

EFFECT OF THE CHEMICAL TREATMENT ON MECHANICAL PROPERTIES, WATER VAPOR PERMEABILITY AND SORPTION ISOTHERMS OF GELATIN-BASED FILMS

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Proteins contain hydrophilic groups which can bond to the water molecule through hydrogen bridges resulting in water adsorption. The high solubility coefficients or water absorption, and an increase on the diffusivity coefficient provoked by the rupture of intermolecular interactions, are responsible for the high water vapor permeability in proteins based films. Also, water molecules acts as plasticizer and affect strongly the mechanical properties of these films. Chemical modification of proteins such as the cross-linking of adjacent polypeptides can be an alternative to improve functional properties of these materials. Thus, the objective of this work was to evaluate the effect of chemical modification of gelatin, with formaldehyde and glyoxal, on the mechanical properties, water vapor permeability and water vapor adsorption of gelatin based films. Native films (NF), formaldehyde modified film (FMF) and glyoxal modified film (GMF) were produced by the casting method with 10% (w/w) of gelatin and 4.5% (w/w) of glycerol. The formaldehyde and the glyoxal were added at concentrations of 8.8mmoles/100 g of filmogenic solution and 26.3mmoles/100 ml of filmogenic solution, respectively. The mechanical properties of films were determined by tensile tests using a texturometer TA.XT2i (TA Instruments) and the water vapor permeability (WVP) was determined gravimetrically, both at 25°C. The water vapor sorption isotherms were determined gravimetrically at temperatures of 15, 25 and 35°C with water activity ranging from 0.11 to 0.85. The models of GAB, BET, HALSEY and OSWIN were fitted to experimental data of equilibrium moisture content versus water activity. Concerning mechanical properties, only treatment with formaldehyde provoked an increase of the tensile strength as compared to the NF and GMF films, however, the elongation of films did not vary significantly. On another side, the water vapor permeability of the films modified with formaldehyde (0.155 ± 0.008 g.mm/h.m².kPa) and glyoxal (0.174 ± 0.007 g.mm/h.m².kPa) were significantly lower when compared to the observed for native films (0.198 ± 0.003 g.mm/h.m² kPa). According to results of non-linear regressions, the GAB and HALSEY models could be used for representing the water vapor sorption isotherms of gelatin-based films, with an average relative deviation lower than 5%. Analyzing the GAB model, it was possible to observe that the chemical modifications provoked a slight reduction on the monolayer moisture content as following: NF (12.57 to 11.70g/100g of dry matter), GMF (12.46 to 11.47 g/100g of dry matter) and FMF (12.20 to 11.14 g/100g of dry matter). Thus, although the effect of chemical modifications on water vapor sorption was not important, the mechanical properties and water vapor permeability of the formaldehyde modified films were improved at low relative humidity condition.

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Keywords: physical properties, protein, cross link, formaldehyde, glyoxal

EFFECT OF SOME ESSENTIAL OILS AS NATURAL FOOD PRESERVATIVES

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This work studied the effects of some essential oils against the growth of some microorganisms potentially danger to the safety of foods. These were *Escherichia coli*, *Staphylococcus aureus* and *Listeria innocua*. The bacteriostatic, bactericidal effect and the minimum concentration of these activities of the essential oils were observed. The essences tested were: lavender (*Lavandula officinalis*), lemon-balm (*Melissa officinalis*), juniper (*Juniperus communis*), lemon verbena (*Lippia citriodora*), rosemary (*Rosmarinus officinalis*) and cypress (*Cupressus sempervirens*).

Considering the well known activity of the essential oils against the contaminating microorganisms in food, these substances could be utilised as an alternative to synthetic antimicrobial agents which are largely widespread. In the same way, these essential oils could be used to extend the shelf-life of several kind of food, due to their biological activity.

All tested essential oils were obtained through vapour distillation and preserved in dark sealed glass containers kept at 4 °C. The trials to evaluate the inhibition of bacterial growth were carried out on solid selective media by sterile dishes imbued with the different concentrations of essences. The results were observed by means of the measure of inhibition area of bacterial growth around the dishes. The biological effect of essential oils was evaluated also in liquid medium at different concentration of them. The over-night bacterial cultures were inoculated in test-tube with Tryptone Soya Broth added with the essential oils at different concentrations. The optical density was evaluated by spectrophotometric apparatus at 600 nm of wavelength.

E. coli was the most sensitive bacterium respect the other tested. The cypress essential oil showed a bacteriostatic activity against *L. innocua* at concentrations of 5 and 10% in the medium. The essential oil of *Lippia c.* was a bactericide at the same concentrations. *S. aureus* was sensitive at essential oils of cypress, lemon verbena and juniper: they showed bactericide activity at 5 and 10% of concentration. The inhibition of *E. coli* by the different essences was tested also in liquid medium. In this way the essential oils effects were measured also at concentration less than 5%. The most effective essential oils, as lemon verbena, cypress and lemon-balm, were tested on two different foods: sticked carrots and minced fresh meat, inoculated with bacteria, and stored at 4-6 °C as they are sold in market.

Keywords: essential oils, shelf life, *Escherichia coli*, *Staphylococcus aureus*, *Listeria innocua*

ANALYSIS OF VOLATILE COMPOUNDS DURING THE STORAGE OF BREAKFAST CEREALS. EFFECTS OF THE EMPLOYMENT OF NATURAL ANTIOXIDANTS

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Cereals, due to their high starch contents, are a particularly suitable raw material for the extrusion-cooking process. This kind of technology involves high thermal and mechanical solicitations, with the purpose of obtaining products with new physical and structural characteristics.

Thanks to its limited duration, extrusion-cooking does not determine an immediate oxidative degradation of fats; nevertheless, it makes the lipid fraction more susceptible to such alteration during the subsequent storage of the product. During the storage period, the lipid fraction undergoes oxidative phenomena that at the same time compromise the nutritional and sensorial quality of the product and reduce its shelf-life. Such rancidity processes lead to the formation of volatile compounds that are responsible of off-flavours.

The aim of this work was to monitor the evolution of these new-formed compounds during the storage and to verify the effectiveness of the employment of natural antioxidants in order to slow down their rise.

The investigation has been carried out on corn flakes produced at a factory sited in Corato (BA). The product without antioxidants (test) has been compared to the same kind of product but containing natural antioxidants. The flakes were packaged into polypropylene and stored at room temperature. At different storage times, the samples were submitted to headspace analysis by means of SPME/GC/MS technique. Such technique foresees the sampling of the headspace of the product by using a special fiber, and the subsequent injection of the compounds adsorbed by the fiber in a gas-chromatograph coupled with a mass spectrometer.

The obtained results underlined a noticeable increase, during the storage, of some secondary oxidation products. The composition and evolution of the pattern of the found compounds revealed, besides, that the lipid degradation also underwent a pathway of further oxidation. The data, in fact, showed a significant increase of pentanoic and hexanoic acids as a result of the oxidation of the pentanal and hexanal aldehydes, respectively.

Concerning the effectiveness of the employment of natural antioxidants, the results showed a significant difference in the oxidative degradation levels among the examined samples. In particular, the levels of volatile oxidation compounds the test showed were significantly higher than the levels found in the samples containing the added antioxidants. The obtained data are encouraging with regard to the employment of such natural products in order to increase the shelf-life of breakfast cereals. The present work furnishes, therefore, original information about the lipid oxidation and the corresponding onset of off-flavours in low-fat extruded products, either in presence or in absence of natural antioxidants.

Keywords: breakfast cereal, shelf-life, natural anti-oxidant, volatile oxidation compounds, SPME/GC/MS analysis

STUDY OF MASS TRANSPORT PROPERTIES OF A MICROPOROUS BIODEGRADABLE FILMG. G. BUONOCORE^a, L. NICOLAIS^a, A. MASSARO^a, M. A. DEL NOBILE^bInstitute for Composite and Biomedical Materials - National Research Council, Portici (Na)^aDepartment of Food Science - University of Foggia^b

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Biodegradable materials obtained from natural or synthetic sources have attracted interest in recent years due to the fact that they can reduce environmental pollution by lowering solid disposal waste. Polycaprolactone is one of the synthetic polymers that meets the ASTM definition of biodegradability. It has attracted attention of researchers due to its lack of toxicity and low cost if compared to other biodegradable polyesters. Due to its high degree of crystallinity (about 50%), it is considered an hydrophobic material. Enhancement of hydrophilicity can be achieved by blending the polymer with suitable additives such as polyethyleneglycol. In this work a microporous PCL membrane was obtained by leaching out water-soluble polymeric additive, PEG, using a solvent-casting leaching method. The structure of the obtained membranes was studied; from SEM micrograph, it was shown that the pores were spherical and had identical size, in particular, the micropores obtained by leaching PEG 600 were larger than those obtained using PEG 4600.

As well known, permeability of gases (oxygen and carbon dioxide) and vapours (water and organic compounds) is a critical property of polymers in their application as food packaging materials. For many food product, the mass exchange must be restricted to guarantee their quality and safety. For many others, gas and vapor permeation is needed to maintain their physiological activities. The knowledge of the correlations between the structure and transport properties of polymer materials is of great relevance in order to be able to properly design new food packaging materials and to improve the packed food quality. In this work we studied the mass transport properties of the obtained microporous membrane and the results were compared with those ones of the non porous PCL film. Water vapour sorption kinetics and water sorption isotherm at 25 °C were investigated using an electronic microbalance (Intelligent Gravimetric Analyser, Hiden). Water vapour transmission rate was measured at 25 °C using a Mocon Permeabilimeter. It was possible to determine the dependence of permeability coefficient from the water activity at downstream side of the film, ranging from 0.35 to 0.90. A significant increase in water sorption and water permeability was revealed. As far as permeation of organic aromatic compounds is concerned, permeation of hexanal, trans-2, eptenal and octanal through the PCL membranes was studied by means of a GC/MS.

Keywords: biodegradable, water sorption, barrier properties

GAS BARRIER PROPERTIES OF POLYMERIC FILMS WITH HYBRID ORGANIC/INORGANIC COATINGS FOR FOOD PACKAGING APPLICATIONS

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The sorption and transport properties of O₂, CO₂, N₂ and water vapor in polymeric films of PET, PP and LDPE coated with a thin layer of an hybrid organic/inorganic (ceramer) material have been determined, at temperatures up to 65 °C. The ceramer layer is obtained via sol-gel technique and is composed of two phases, organic and inorganic. The addition of coating dramatically improves the gas barrier properties, the apparent permeability of the multilayer film being, in some cases, 2-3 orders of magnitude lower than that of the pure polymeric support. The reduction of gas permeation through the membrane depends strongly on the type of coating used and on the procedure followed during the synthesis and coating processes, and, more generally, on the degree of adhesion of the coating on the polymeric support. We have studied the effect of different organic/inorganic mass ratios, as well as the effect of ageing, by comparing the gas transport properties before and after immersion in water of the multilayer films, to study the possible degradation of the hydrophilic organic phase. The permeability data are collected using a closed-volume permeation technique and the diffusivity values have been calculated based on the time-lag method: the coating properties are opportunely calculated by taking into account the effect of the different diffusion resistances in the various layers.

Keywords: Ceramers, barrier properties, nanocomposites, oxygen permeability, barrier materials

USE OF LEMON EXTRACT AS NATURAL PRESERVATIVE FOR DEPRECIATION OF WINE

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In this work the possibility to use lemon extract as natural preservative to inhibit the growth of microorganisms responsible for malolactic fermentation in wine was assessed. Two different wine spoilage microorganisms were separately tested in this investigation: *Oenococcus oeni* and *Lactobacillus plantarum*. Growth tests were run at 30 °C using both specific microorganism medium broths, deprived of glucose and supplied with L-malic acid. The inoculated broths were loaded with different concentrations of active compound (100ppm, 200ppm, 300ppm and 500ppm), and closed aseptically in a vial. As control, samples of media containing each microorganism without active compound were also prepared. The enzymatic activity of the strains was evaluated on the basis of the rate of Carbon dioxide (CO₂) production in the vial headspace. The antimicrobial activity of the investigated natural preservative was expressed in terms of Non Inhibiting Concentration (NIC) and Minimum Inhibition Concentration (MIC). The observed antimicrobial activity at sub-MIC levels suggests that lemon extract is active on each phase of the growth cycle for both tested microorganisms with a comparable effectiveness. In particular, the active compound could be added at concentrations slightly higher than the NIC levels in order to appreciably slow down the microbial growth rate as well as to reduce the maximum microbial growth.

Keywords: Antimicrobial compound, malolactic fermentation, wine

EVALUATION OF INTOLERANCE TO FOOD PRESERVATIVES BY NON-INVASIVE BIOPHYSICAL TECHNOLOGIES

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Food preservation represents one of the main interests of food science, and various synthetic and natural compounds are available nowadays for food industry. A high concern exists among consumers towards the toxicity of food additives and preservatives, in particular, however the role that some of these compounds can have in causing food intolerance to consumers should be underlined.

The last decades have been characterized by an exponential progress of knowledges in the scientific field, which have undisclosed the extremely complex nature of the living systems. Such advances allow to understand that at the basis of the cellular transmission there is not only a biochemical message, but also an electromagnetic one.

Such biophysical principles are at the basis of new technologies which, exploiting the interactions between low-frequency electromagnetic waves and the human organism, can be used for diagnostic tests.

One of the most common, non-invasive technologies is derived from the principles of electro-acupuncture according to Voll (EAV), based on the variations of conductivity or cutaneous electric potential in correspondance with the points of acupuncture meridians and due to the interaction between the electromagnetic waves, spontaneously emitted by certain substances, and the human organism. A first experimental approach of the research staff was addressed to verify this modern diagnostic methodology, which allows to essay the overall energetic pattern of the subject and the presence of hypersensitivity or intolerance of various nature. The experimental protocol involves the use of a new electronic equipment (Bicom) which allows to perform the diagnostic EAV test. The results obtained have been compared with those given by the Prick test and a correspondance >76% was found between the two methods. Moreover, a further validation of data was obtained by the comparison between diagnostic data obtained with EAV and with Patch test in the essay of the intolerance to some basic components used in the preparation of cosmetic products.

Data concerning 18 substances tested on 66 patients (overally 1180 double tests in comparison) gave an average value of 88% of diagnostic concordances, with a variability, for the different substances, ranging between 95% and 78%. A collaboration between the Departments of Physiologic Sciences and DOFATA is aiming to test the incidence of food preservatives in provoking intolerance and/or allergies on a population of young healthy subjects, which is the topic of the present research.

Keywords: Intolerance disease, food preservatives, Non-invasive analytical techniques, EAV

EFFECTS OF TWO DIFFERENT HUMECTANT AGENTS ON QUALITY OF "AMARETTI" COOKIES

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"Amaretti" are typical Sardinian cookies, whose shelf-life is strictly limited by a qualitative decay due to a severe hardening of the internal almond paste; in this work, the "Amaretti" were manufactured in two different recipes in addition to the original one, by using two ingredients with water binding capacity. Glucose syrup has been chosen as conventional sugar frequently used in the confectionery industries. It is characterized by a low cost, availability on the internal market, no-toxicity and, from a legal point of view, it is considered as an ingredient.

Bamboo fibre has been chosen as a new cellulose-based ingredient derived from the fiber-rich parts of bamboo plants. It can contribute beneficial textural properties; in fact, it is considered as a texturizing and water binding agent, improving mouth feel too. To evaluate the effect of these ingredients on the quality of the cookies, a_w and moisture content (wet basis) were measured, sorption isotherms were determined and texture and colour evolution were followed. The results indicate that both the new ingredients are effective on "Amaretti" texture and water distribution; glucose syrup, although allowed to lower the initial a_w of "Amaretti", then reducing the a_w gradient between crust and paste, led to a such severe modification affecting the typicality of this regional product.

In fact, the presence of glucose, as proved by the sorption isotherms determination, promotes the sugars crystallization after about 21 days. Moreover, the syrup seems to promote the water redistribution between the components of the matrix, resulting in a softening of the crust, well perceptible after 3-4 days of storage. The textural analysis, in fact, showed that the crust lost its crispness, probably due to a water migration from the paste to the crust (due to the a_w gradient) and to the hygroscopic sugars in the amorphous form too. Finally, the high amount of sugars caused a more intense Maillard reaction, as seen by the colour analysis. Contrary, the addition of the fibre led to an absolutely positive global effect, promoting the water retention into the matrix for a longer time than the traditional recipe and maintaining the textural difference between crust and paste. Only a small difference in colour was appreciated, that can be easily solved by bringing a small change in the time-temperature conditions during baking.

Keywords: Cookies, hardening, humectant, texture

ANTIOXIDANT ACTIVITY OF α -TOCOPHEROL INCORPORATED INTO BIODEGRADABLE FILMS

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Active packaging concepts change the condition of the packaged food in order to extend its shelf-life or to improve safety or sensory properties while its quality is maintained. Promising active packaging materials are those ones containing an antioxidant which is initially incorporated into the polymer and is then released into the packaged food product. The release is aimed to provide a sufficient concentration of antioxidant in order to prevent lipid oxidation and rancidity of the foodstuff. In the literature the incorporation of α -tocopherol into polyolefins (i.e., PE and PP) has been widely studied. Some works deal with the effectiveness of the antioxidant-impregnated film in prolonging the shelf life of both dry and liquid foodstuff, others deal with the action of α -tocopherol in protecting the polymer against heat during processing and UV-light induced oxidative.

Due to the current interest in biodegradable polymers, in the present study the incorporation of α -tocopherol into polycaprolactone (PCL) and polylactide (PLA) films was investigated. Thin films (150 micron) were obtained using both solvent-casting technique and extrusion process.

In order to study the release kinetic of the chosen antioxidant into the foodstuff, 95% ethanol was chosen as food simulant, as olive oil has a relatively high initial α -tocopherol content. Release kinetics of the active compound from the different films were monitored at 25 °C, a simple model was used to fit the experimental data in order to obtain information about the diffusion mechanism.

Since the addition of high amounts of antioxidant may influence the properties of the obtained polymer films, their water barrier properties were investigated. Water sorption kinetics and isotherm were obtained using an electronic microbalance at 25 °C and water permeability coefficient was determined at 25 °C using a Permatran apparatus. Tests were performed in order to assess the effectiveness of the released α -tocopherol in preventing the oxidation of linoleic acid. The method is based on the spectrophotometric determination of the rate formation of conjugated diene from linoleic acid in the absence and in the presence of the chosen antioxidant.

Keywords: active packaging, antioxidant, biodegradable

Note

Shelf-life testing

Chairmen:

Dario DAINELLI, Cryovac Sealed-Air, Passirana di Rho - Italy

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INNOVATIVE AND INTEGRATIVE APPROACHES APPLIED TO SHELF-LIFE TESTING AND IMPROVEMENT OF PACKAGED FOODS

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Keywords: Quality tracers, transfer, reactions, integrative approach

THE EFFECT OF LIGHT OF DIFFERENT WAVELENGTH ON PHOTO-OXIDATION IN ICE CREAM

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The use of transparent plastic boxes is becoming more and more common also in the ice cream industry. The purpose of this investigation was to reveal the sensitivity/stability of vanilla ice cream towards light of different wavelengths and to elucidate whether packages with different colour could protect the ice cream against light and still be transparent. Ice cream manufacturing consists of incorporation of gas phase into an emulsion pre-mix before freezing, which represent more than 50% (v/v) air, and is therefore very sensitive to light. Vanilla ice cream was commercially packed in specially made 2 L injection moulded PP tubs and injection-moulded black PP Snap-On lids. During exposure by flat-emitting HMI studio lamps, the black lids were replaced by studio-filters with well-defined transmission-curves. All filters were balanced with neutral (grey) filters and transparent PP-lids to transmit the same amount of light energy (W/m²) in the 330-800 nm region. Except for one of the clear lid samples, all filters were supplemented with an UV filter. After 24 hours of light exposure the quality was assessed by fluorescence spectroscopy, by measurement of colour, and by a trained sensory panel.

The fluorescence spectra showed two peaks in the 410 - 500 nm and 500 - 600 nm region. The peak at 530 nm was the highest, and was due to the riboflavin in the cream. It is generally known that riboflavin in dairy products is degraded when exposed to light and it absorbs light in the UV region and up to about 500 nm. There was a clear decrease of the riboflavin peak for violet, blue and white light with and without UV filter. Red, orange and yellow light had little or no influence on riboflavin. There was an increase in fluorescence intensity in the 410 - 500 nm region for all colours, but most for blue and green filters. Peaks in this region could be oxidation products from lipid and protein oxidation. This region was closely related to oxidised flavour. The colour was best protected by red, orange, and yellow light. Red and orange transparent plastic boxes would be best to protect vanilla ice cream from photo-oxidation.

Keywords: light, photo-oxidation, ice cream, fluorescence spectroscopy

PIGMENT STABILITY IN PISTACHIO KERNELS

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Often, the use of suitable packaging is very important to decrease the degradation of food product. Aim of this study was to test pigment stability (chlorophyll *a*, chlorophyll *b* and lutein) of pistachio kernels stored, for 14 months, in the dark, at three different temperatures: 10, 25 and 37 °C. The samples were packaged using two films (nylon and ethylen vinyl alcohol), with or without oxygen scavengers; packages were hermetically closed. Moreover, per each temperature, some samples were packaged in bags without hermetic seal. After 14 months, at the end of storage, pigments underwent a degradation that it was influenced by storage time and temperatures. Lower concentrations were observed at 37 °C with a degradation of about: 62% for chl *a*, 44% for chl *b*, 57.5% for lutein. At 10 and 25 °C, the samples didn't show any differences statistically significative and pigments degradation was: 45% for chl *a*, 35% for chl *b*, 37.5% for lutein. During storage no difference, due to oxygen scavengers presence or films of packaging, was observed among samples stored. At 25 °C, after 11 months, samples, packaged in bags not closed hermetically, showed presence of moulds and bugs (*Plodia interpunctella* - Hubner). About kinetic study, rate of degradation of the three pigments fitted a zero-order kinetic and it was observed an activation energy of: 11,7 KJ/mol, 12,1 KJ/mol and 18,2 KJ/mol, respectively for chl *a*, chl *b* and lutein.

Keywords: Pistachio, Pigments, Storage, Oxygen scavengers, Kinetic

SHELF-LIFE MONITORING OF BROCCOLI PACKED WITH DIFFERENT FILMS

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Broccoli can be a viable source of anticarcinogenic moieties, such as vitamin C and sulfur containing molecules (sulforaphane). However, while a regular daily intake of these compounds can exert a positive action, the sulforaphane and antioxidants content of broccoli can be dependent on the ripening stage, as well as on the storage conditions of the vegetable. Although for marketing reasons the shelf-life of vegetables is often requested to be as long as possible, mainly pointing to preserve the organoleptic properties of the products, in this research the improvement of the shelf-life of the broccoli was investigated taking into account not only easily perceivable characteristics (i.e. color, weight loss), or alterations (i.e. growth of moulds and yeasts) that can be of interest for the consumer or for the shop management, but also the preservation of acceptable levels of nutrients that play a key role for the human health.

The shelf-life of broccoli packed with different films and stored at 6 °C and 95% R.H. was studied up to 35 days, using physical, chemical and microbiological indexes (weight loss, vitamin C, sulforaphane and chlorophyll content, total mesophilics, yeasts, moulds and psychrotrophics level) to assess the product quality throughout the storage time. Immediately after harvesting, broccoli samples (cv. Marathon) were washed in potable water, trimmed, packed in wooden boxes and transported to the laboratory within two hours. For each experimental thesis two broccoli of similar size (total weight 300-350 g) were packed using a polystyrene tray (19.3 cm x 11.5 cm x 9.5 cm) and inserted in pouches (35 cm x 30 cm) made of different polymeric films, perforated (OPTI 330A, OPTI 330 C, OPTI 330 E) and not perforated (MRX; AFG-E 60). When the AFG-E60 film was tested, broccoli were packed without tray and tightly wrapped with the film. The samples were stored at 6 °C and 95 % R.H. using a climatic chamber; the tests lasted 35 days and broccoli were sampled every 7 day for analyses. Unpacked broccoli stored at the same conditions were utilized as control.

The experimental results indicated that the use of packaging could extend the shelf-life of broccoli, with an allowable storing time of about 5 weeks, without any major loss of nutrients and modification of color. In particular, among the different perforated films tested, the OPTI 330A, characterized by a considerable lower percentage of holes, gave the worst results, in comparison with all the other films. The best results were obtained using the AFG-E60 film, while no appreciable differences could be pointed out when OPTI 330 C, OPTI 330 E and MRX films were utilized.

Keywords: broccoli; chilling; packaging; shelf-life; vegetables

SURFACE ANALYSIS BY ATOMIC FORCE MICROSCOPY OF BACTERIOCIN ACTIVATED PLASTIC FILMS FOR FOOD PACKAGING

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Antimicrobial agents can be used to give antimicrobial properties to polymeric materials used to envelope foods for packaging purposes. Different kinds of antimicrobial packaging are described in the current literature and the bacteriocin coating of polyethylene surfaces is reported as an effective method to confer antimicrobial properties to food packages. However, no information is reported about the interactions, between bacteriocins and the surface of plastic films, which are implicated in the adhesion and release mechanisms of antimicrobials. In this study we exploited an Atomic Force Microscopy (AFM) analysis of active and non active surfaces of plastic films. The analysis was carried out by using an easyScan 2 (Nanosurf AG, Switzerland) followed by post-processing of images by the software Scanning Probe Image Processor (SPIP, Image Metrology). In particular, the surface images were obtained by a dynamic AFM analysis and roughness parameters of 3D images were investigated by SPIP. Different polyethylene films were involved in this research and different bacteriocins were used for their activation. Significant differences were found between the bacteriocin activated and control films and the activated surfaces showed lower values of average roughness and higher values of summit density indicating that an homogeneous distribution of the bacteriocin preparation was obtained following the coating procedure. Information on the interactions between plastic film and antimicrobial preparations could dramatically improve the production and implementation of bacteriocin activated food packages.

Keywords: Active packaging, bacteriocin, Atomic Force Microscopy

STUDY ON RELATIONSHIPS BETWEEN FOOD KINETIC STATE AND SOME REACTIONS OF FOOD DEGRADATION

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It is well known that food matrixes may exist as high viscous glass, rubbery or liquid states. Glassy state is defined as an amorphous very high viscosity and kinetically stable system; instead rubbery state is defined as an amorphous low viscosity and kinetically unstable system. In food point of view, moisture content is the most important component that can act as plasticizer modifying the glassy transition temperature (T_g). Traditionally if a food is in glassy state ($T < T_g$) it will be considered stable because the translational mobility of water is considered to be non-existent. Instead if a food is stored at $T > T_g$ it will be in an unstable state and all reaction degradation will occur in short time. Some researchers studied the effect of moisture content on kinetic state of food and on degradation rate, but literature concerning the effect of glassy or rubbery state on the rate of degradation reactions is not exhaustive. We put a question: which would be the rate of reaction of some degradation if a food is at $T > T_g + T_i$ °C? This work will tent to answer, dealing with the eventual connections between food kinetic state and rate of some degradation reactions.

Keywords: degradation reactions, glassy state, mobility water

NEAR INFRARED SPECTROSCOPY AS A TOOL TO STUDY THE SHELF LIFE OF FOODS

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Classical studies on shelf life of foods are based on the parameterisation of the time dependence of some microbiological, chemical and physico-chemical indices. These tests are usually time consuming, and require high manual ability and qualified personnel.

Recently, NIR spectroscopy has been used more and more in the agricultural and food industries. This analytical technique is relatively low-cost and it can be applied in both fundamental research and in factory for on-line control, by using optic probe. NIR technique is easy to use, fast and non destructive method, and in recent years it is becoming a valid tool, alternative to classic wet chemical methods. As known, NIR spectra contain some information related to macro composition of food (water, proteins, lipids and carbohydrates) and to its chemical or structural modifications.

The aim of this research was to investigate the feasibility of applying Near Infrared Spectroscopy (NIR) to study the shelf life of selected foods.

As first study, the shelf life of three different types of dairy products was studied: Crescenza cheese, stored at two different temperatures (8° and 15°C) and analysed at fixed times; Italian Butter, having its shelf life influenced by oxidation reactions, stored at 10°C up to 47 days of storage and Ricotta samples, stored at three different temperatures (3°, 10° and 20°C) up to 48 days of storage. FT-NIR spectral data were collected in the whole NIR range (from 12000 cm⁻¹ to 4000 cm⁻¹) by using a FT-NIR spectrometer equipped with a fibre optic probe working in diffuse reflectance (MPA, Bruker Optics). Measurements were carried out in different replicates directly on the surface of the sample. Results show that NIR spectroscopy reveals the water and fat modifications occurring during the storage.

FT-NIR spectroscopy was also applied to discriminate fresh olive oils from olive oils stored in different conditions: under light for one year and under dark for one and two years. The results showed how this method was able to define olive oils freshness and olive oils oxidation and appeared to be able to describe the different storage conditions.

The freshness of farm raised European sea bass (*Dicentrarchus labrax*) was studied by applying both chemical methods and FT-NIR spectroscopy, using a fibre optic probe operating in diffuse reflectance directly on the fish surface. The results showed the ability of FT-NIR spectroscopy to monitor the shelf life of this types of fishes stored at three different temperatures, underlining a critical day of shelf life for each storage temperature. Results are in agreement with those obtained by classical techniques, allowing to define also a Q₁₀ value in order to describe the time-temperature relationship.

Overall, the results obtained in these case studies, show that FT-NIR spectroscopy, being a non destructive method, could be implemented together with conventional techniques to study the shelf life of foods and to monitor the quality of the products during storage.

Keywords: NIR, shelf life, cheese, fish

RANCIDITY EVALUATION AND SHELF-LIFE MONITORING OF VIRGIN OLIVE OIL BY ELECTRONIC-NOSE

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The main alteration occurring during the shelf-life of bottled virgin olive oil is the auto-oxidation and photooxidation of polyunsaturated fatty acids leading to rancidity, with the loss of chemical and sensory quality. The official measure of the "rancid" off-flavour is usually achieved by chemical and sensory analysis made by a trained panel (Reg. CE 2568/91 and subsequent modifications). Other different analytical methods have been developed in the last few years. The volatile compounds responsible of rancidity are sampled by static head-space (SHS), dynamic head-space (DHS) by solid phase micro-extraction (SPME) and quantified and monitored by gas chromatography (GC) or GC coupled with mass spectrometry (GC/MS). The use of Artificial Olfactive System (AOS) or "electronic nose" (EN) for the evaluation of virgin olive oil odours could be represent a valid and direct alternative to evaluate oil rancidity and monitor its shelf-life.

This study was carried out with the aim to apply the EN for the rapid and objective evaluation of rancidity in different virgin olive oil samples and to monitoring the shelf-life of bottled virgin olive oils. An EN equipped with an array of 10 electronic chemical sensors with partial specificity and an appropriate pattern-recognition system, capable of recognising simple or complex odours was tested. The results obtained by the EN were compared with those obtained by the sensory assessment and those obtained from the GC/MS of volatile markers of oxidation.

EN parameters of electronic nose analysis were optimized: volume of the flask, amount sample, time and temperature analysis, number of response sensors utilized, time interval to collect data, statistical tests to interpret the instrumental data. Principal component analysis (PCA) and Partial Least Squares (PLS) were applied to data sets evaluation.

A rancid standard virgin olive oil of the International Olive Oil Council (Madrid, Spain) was added to a refined olive oil at different percentage and analysed to training the nose and to build a calibration of EN response with respect to rancidity assessed by the sensory analysis and GC/MS. A good correlation was found between sensory and instrumental data. Extra virgin olive oil (without off-flavours), commercial oil with different level of rancid perception (measured by the Panel and by DHS-GC/MS and SPME-GC/MS) and a series of standard samples (refined olive oil spiked with known amount of rancid oil) were analyzed and undergone to statistical analyses.

Usefulness and limits in applying the electronic nose to distinguish among oil of different quality (extravirgin, virgin and lampante) and to monitoring oxidation during the shelf-life of bottled virgin olive oil are discussed.

Keywords: rancidity, electronic nose, virgin olive oil, sensory quality

MEASURING THE QUALITY OF PACKED FOODS

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Degradation of food products may cause off-flavors or loss of nutritional value resulting in inferior product quality and consumer rejection. The mechanism of degradation and the reaction products highly depend on the type of product. Degradation of nutrients, flavor and additives, are mainly determined by light, pH, oxygen, moisture and other product matrix components.

Optimization of the composition and packaging of food products with respect to shelf-life and sensory quality can be done with classical techniques as high performance liquid chromatography (LC) and gas chromatography (GC) and microbial testing.

Application of these techniques allows e.g. the selection of an appropriate UV barrier in packaging materials to avoid unwanted photo degradation of vitamins.

The combination of analytical techniques and food packaging research techniques such as gas permeability and optical oxygen measurements allow the selection of appropriate measures to avoid food degradation processes like lipid oxidation and growth of aerobic microorganisms.

To study complex research issues such as off-flavor research, optimization and control of products and ageing or alteration of food during storage such as oxidation in complex food stuffs, holistic analytical methods are applied. Holistic analysis is the comparative non-biased determination of nearly all components in a broad range of polarity and molecular weight in a sample. This is in contrast to target analytical techniques as mentioned above which aims at measuring a limited number of known parameters and/or compounds. Such comprehensive techniques are based on GC or LC coupled to mass spectrometry (MS). Next, data pre-processing tools and multivariate data analysis (MVA) are applied to find the relevant correlations between the analytical data with certain properties of interest.

This paper provides an overview of advanced analytical approaches in combination with food packaging research techniques to obtain insight in the key processes of food degradation. Also examples are given in which the obtained insight resulted in an appropriate packaging strategy to improve product quality and shelf-life.

Keywords: packaging performance, advanced food analysis

A HEAD SPACE GAS ANALYZING APPROACH FOR EVALUATING THE SHELF LIFE OF MOZZARELLA CHEESE

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A gas evaluating method, based on the analysis of the CO₂ of the head space produced by coliforms, was used to study the shelf life of mozzarella cheese, with or without lysozyme and Na₂-EDTA.

Mozzarella cheeses of about 50g of weight, purchased in cheese factory of Apulian region, were packaged in 200 mL of diluted salt solution, previously added with phosphate buffer 50 mM. This solution was added with lysozyme (0.25 mg/ml) and 50 mM (sample B) or 20 mM (sample C) of Na₂-EDTA. The samples were stored at 4 °C and analyzed after packaging and after 1, 3, 6 and 8 days. Samples without lysozyme and Na₂-EDTA were used as controls (sample A).

10 g of mozzarella cheese were diluted with 90 ml of a sterile saline solution (0.9%) in Stomacher bag and blended for 1 min with a Stomacher Lab Blender 400.

Decimal dilutions of cheese homogenates were performed and microbiological counts of coliforms were carried out on VRBA, incubated at 37 °C for 18-24 hours.

The metabolic activity of coliforms was studied by inoculating 1 mL of cheese homogenates in 20 mL vials, containing 10 mL of a synthetic medium (yeast extract 3.0 g/L; bacteriological peptone 7.0 g/L; NaCl 5.0 g/L and Biliar Salts n. 3 1.5 g/L). The vials were hermetically sealed, incubated at 37 °C and analyzed over 72 h, for monitoring the CO₂ in the head space by a PBI Dansensor.

All the analyses were performed in duplicate and the data of each storage time were modeled according to the modified Gompertz equation, in order to estimate the Minimum Detection Time (MDT), defined as the time necessary to obtain a 3% of CO₂ in the head space.

In the sample A, the maximum value of CO₂ in the head space was about 20-22%; with the passing of the storage time, at the increase of coliforms cell load, as evaluated by the standard plate count, it was observed a decrease of the parameter λ (time necessary to have a significant increase of CO₂ in the head space). The addition of the antimicrobial combination caused a decrease of the maximum value of CO₂ of the head space reached in the stationary phase and a significant prolongation of the lag phase; this effect was more evident at the maximum concentration of Na₂-EDTA (sample B).

During the storage time, the maximum value of CO₂ of the head space decreased in the samples B and C and after 8 days the amount of the gas was of about 4% and 12%, respectively.

The correlation between the MDT and the cell load was good, showing that this method is a good alternative to the traditional standard plate count for monitoring coliforms cell load in a complex food matrix, like mozzarella cheese.

Keywords: head space, mozzarella cheese, shelf-life, MDT, coliforms

APPLICATION OF PCR-DGGE AND CLONING ANALYSIS TO STUDY BACTERIAL POPULATION IN MINIMALLY PROCESSED VEGETABLES

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The bacterial population of minimally processed vegetables (MPV) products is generally detected by classical microbiological methods, which are tedious and time-consuming, and allow only a partial picture of the microbial community. New molecular approaches, as cultivation-independent methods, based on direct analyses of DNA, have been developed and allow more precise descriptions of microbial dynamics in complex ecosystems. In particular, single-strand conformation polymorphism, denaturing gradient gel electrophoresis (DGGE) methods of PCR-amplified rDNA have been applied to a variety of environmental studies for analyzing microbial communities.

The aim of this research was to describe the diversity and dynamics of the microbial communities of MPV products packaged in ordinary and modified atmospheres during storage at 4 °C, combining the PCR-DGGE analysis with the construction of clone libraries. The results of DGGE analysis indicated a great diversity of bacterial communities. Secondly the DGGE results showed no appreciable difference between the microbial population detected in MPV products packaging both in ordinary and modified atmosphere. Cloning and sequencing of rDNA amplicons revealed that microbial population including species of genera *Pseudomonas*, *Enterobacter*, *Pantoea* and *Acinetobacter*. The results obtained indicate that the chosen monitoring strategy combining DGGE analysis of 16S rDNA amplified fragments and the construction of clone libraries can give important new information about the microbial population on MPV products.

Keywords: Minimally Processed Vegetables products, Bacterial communities, denaturing gradient gel electrophoresis and cloning

PHYSICAL-CHEMICAL CHARACTERISTICS OF FROZEN GROUND BEEF AND AGED BEEF FROM *BOS INDICUS* STEERS SUPPLEMENTED WITH α -TOCOPHEROL ACETATE

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The color and lipid stability are important factors, among others, on the quality and acceptability of meat and meat products. The lipid oxidation can produce free radicals which can lead to the oxidation of meat pigments and to off flavors production. Antioxidant agents, such as α -tocopherol may prevent these oxidations in meat during storage, improving meat shelf life. Thus, the objective of this work was to evaluate the effects of diet supplementation with vitamin E of Nellore steers, on physical and chemical characteristics of frozen stored ground *Quadriceps femoris* (QF) or aged *Longissimus dorsi* (LD) muscles. These muscles were obtained of 24 animals, with 30 months of age and 279 kg of mean live weight, confined for 98 days with high proportion of concentrate in diet. Daily, half of animals received 1000 mg of α -tocopherol acetate (VITE) added to 100 g of corn meal. Other half received 100 g of corn meal without the antioxidant. The animals were slaughter when the subcutaneous fat thickness measured between the 12th and 13th ribs reached 6 mm. These determinations were made with an ultrasound equipment. Twenty four hours after slaughter, 7 samples of QF were ground, frozen at -25°C and stored for 0, 1, 2, 3, 4, 5 and 6 months at -18°C. Moreover, 4 beefs with 2.5 cm in thickness were cut from the LD, individually vacuum packed and kept between 0 and 1°C for aging for 1, 7, 14 and 21 days. After that, these samples were frozen at -25°C until analysis. All samples were analyzed to determination of the pH, with a digital pHmeter, of the color, using a HunterLab miniscan colorimeter and L*, a*, b* CIElab system scale, and the water lost (WL) by exudation, by gravimetric measures. The WL of QF was affected by treatment ($P<0.01$) with lower water lost on meat from animals treated with VITE. Also, a quadratic effect ($P<0.01$) with frozen time of QF was observed. The pH presented a linear effect ($P<0.01$) with frozen time, but it was not affected by treatments. The color parameters of QF were not affected by treatments. However L* had cubic effect ($P<0.01$), a* had linear effect ($P<0.05$) and b* had quadratic effect ($P<0.01$) with frozen time of QF. Furthermore, no significant differences were found for the WL or pH values of LD between the VITE and the Control group along the aging time, but the L* and a* color parameters had linear effects ($p<0.01$) and the b* had cubic effect ($P<0.01$) among the days of aging. But, these parameters were not affected by the treatments. Thus, the VITE supplementation improved only the water loss characteristics of frozen ground QF and did not present positive effect over the physical-chemical characteristics of the LD aged for up to 21 days.

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Keywords: antioxidant, color stability, pH, Nellore

PHYSICO-CHEMICAL, SENSORY AND MICROBIOLOGICAL EVALUATIONS OF MINIMALLY PROCESSED BLOOD ORANGES USING A PILOT-PLANT

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The increasing popularity of minimally processed fruits and vegetables has been attributed to the health benefits associated with fresh product, combined with the ongoing consumer trend toward eating out and consuming ready-to-eat foods.

Recently, a study on minimally processed blood oranges demonstrated that Tarocco orange slices, manually processed and stored for 15 days at 4 ° C, had good physico-chemical, sensory, and microbiological characteristics.

The possibility of providing an immediately utilizable new technology for citrus factories, has prompted our interest into the study and the layout of a pilot-plant for fresh-cut blood orange productions. Initially, elements that might affect environmental quality and technological performance were characterized. Then, an environmental dimensional study was conducted to locate the right spaces for equipment and operators.

For this purpose, functional spaces, grouped together in "environmental units" (EU), were defined on the basis of a careful analysis of the work to be carried out and on the needs that derive from this in terms of dimensions, environment, relationships and intensity of use. In particular, a total of 4 EU (processing area, anteroom filter area, entry and dressing-room area, selection and disinfection fruits area) were chosen for the production functional space. In addition, in the production area, UV lamps (lighted before processing) and air-conditioner systems were installed. In this area, also, a machine suitable for peeling orange fruits (PND mod. BABY, Salerno, Italy) and a heat-sealing vacuum/gas machine (TecnoFoodpack mod. Easy Pack, Pavia, Italy) were equipped.

Moreover, the "elementary activities" (EA) necessary to obtain a product that satisfies consumer requirements were planned.

To evaluate that our pilot-plant was suited to produce fresh-cut oranges, fruits of Tarocco 'TDV', Moro 'NL 58-8D-1' and Sanguinello 'NL 49-5-5' were processed and packaged with three films with different permeability to oxygen.

No important physico-chemical changes were observed in orange slices during 15 storage days. In particular, only a reduction in vitamin C content was noted in almost all the examined cultivars, especially in Sanguinello packaged with film C. Concerning the sensory evaluation, only Tarocco 'TDV' showed statistically significant differences ($p < 0,05$) for the 'freshness', 'juiciness' and 'overall' descriptors. From the microbiological results, it could be affirmed that fruits, packaged in the three films, had lower count values than established by microbiological criteria for minimally processed fruits and vegetables up to the 6th storage day. The only exception was Tarocco 'TDV' packaged in film A and B, which exhibited lower microbial counts up to the 12th day.

Keywords: pilot-plant, fresh-cut oranges, shelf-life, packaging

VIRGIN OLIVE OIL SHELF LIFE - STUDY OF OIL STABILITY AT ROOM TEMPERATURE AND UNDER ACCELERATED STORAGE CONDITIONS

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Virgin olive oil (VOO) is an appreciated food product nowadays due to its peculiar sensory characteristics, beneficial fatty acids composition and the content of nutritionally valuable minor compounds such as α -tocopherol (vitamin E), β -carotene (precursor of vitamin A) and phenolic compounds, some of which possess *in vitro* and *in vivo* antioxidant activity, which may play a role in the prevention of certain diseases such as cardiovascular disease and cancer.

Lipid oxidation occurs fairly slowly in VOO at room temperature, since its natural antioxidants act as radical scavengers delaying the rate of oxidation. Nevertheless, it is the main cause of olive oil quality deterioration and its reaction rate determines the shelf life of this product. The stability of virgin olive oil usually ranges from 9 to more than 18 months and hence accelerated shelf life testing (ASLT) is generally employed to estimate the induction period of the oxidation reaction in a relatively short period of time. However, accelerated assays have a drawback in that the oxidation process takes place in drastic conditions, quite unlike those typically occurring in oil mill storage tanks or even during the marketing of the product, in which the autoxidation mechanism may be significantly different. Moreover, the methods selected to determine the end-point of the accelerated stability assays, and therefore the chemical changes observed in the oil, have no satisfactory correlation with the autoxidation process that takes place at room temperature, and hence with VOO shelf life. As a consequence, oxidative stability is not recognized as a VOO legal quality index.

The main goal of this research is, therefore, to study the chemical stability and the autoxidation process of VOO in the attempt to elucidate the role and extent to which each antioxidant contributes to the oxidative stability of the oil and to try to develop an accelerated test for shelf-life prediction. For this purpose, the evolution of major and minor components, as well as of quality and oxidation indexes of several VOO samples, which significantly differ in their initial content in natural antioxidants, were examined during up to 21-month storage at room temperature and under accelerated conditions (40-60°C, and up to 100°C). The relationship between VOO initial composition and the time required to reach the upper limits established by EU Regulations for the quality parameters is also reported.

Keywords: Virgin olive oil; shelf life; accelerated storage conditions; oxidation; antioxidants

DIFFERENTIAL RESPONSES OF ANTIOXIDANTS TO SALT STRESS IN BERRIES OF WILD TYPE AND NON-RIPENING TOMATO MUTANT

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The goal of the present research is to evaluate the possibility of using diluted sea water as alternative irrigation water for tomato (*Lycopersicon esculentum* Mill., cv. Gimar) plant, with the purpose to combine economic needs and environmental sustainability (principle of "cost-recovery") with merceological traits (high berry firmness and long shelf-life) and quality of the fruit (antioxidant contents). To reach this goal we have compared the antioxidant power of NOR, a genotype with long shelf-life, with that of the wild one.

Antioxidants are able to inhibit lipid peroxidation and free radical formation so they contrast oxidative damage correlated to senescence. Therefore berries with a higher content of antioxidants at harvest could have longer qualitative life during storage. Recent studies have demonstrated that tomato plants, grown in a semi-closed hydroponics and subjected to salt stress, improve qualitative characteristics of their berries though the yield is lower. In fact, plants growing in a sub-optimal environment can improve their secondary metabolism and thus produce berries with a higher nutritional and health beneficial value. We studied the tomato plant tolerance to a high salinity level of nutrient solution (EC = 8mS/cm, corresponding to 10% sea water) versus a control (EC = 3mS/cm, representing local irrigation water). We adopted a semi-closed hydroponic system with total weekly-substitution of circulating solution, constant monitoring and adjustment of salinity and pH values. The choice of soilless culture is motivated by our sensibility towards environmental problems, because the use of saline water on soil would cause the loss of chemical-physical fertility.

Plants of two different tomato genotypes were grown: the genotypes consisted of near isogenic line (LIN) of the cv. Gimar, differing in homozygous gene affecting ethylene metabolism (non-ripening, *nor*) in comparison with the wild type. Gimar *wild-type* (WT) plants are characterized by semi-determined growth and bear round, medium-sized fruit. In Gimar *nor/nor* (NOR), *nor*, incompletely recessive gene, determines a reduced ethylene biosynthesis and consequently a slow and reduced synthesis of carotenoids (lycopene and β -carotene), but a better berry shelf-life. The ripening of the NOR berry, alike ripening inhibitor (*rin*), is not influenced by exogenous ethylene. Furthermore, berries of NOR plants exposed to salinity improved their ripening pigmentation, whereas *rin* berries did not. In the berries, picked at well ripe stage, antioxidant activity of both lipophilic and hydrophilic extract, flavonoids and tocopherols have been evaluated.

Results showed a higher concentration of lipophilic antioxidants in WT and of hydrophilic antioxidants in NOR. Higher concentration of tocopherols and quercetin have been observed in NOR. Salt stress induced in WT a reduction of tocopherols and antioxidant activity of lipophilic extract, while in NOR an increase of tocopherols, quercetin and antioxidant activity of hydrophilic extract.

Keywords: antioxidant power; flavonoids; sea water; tocopherol; tomato mutant

MICROBIAL EVOLUTION IN VACUUM-PACKED MIXTURES OF GRATED PROCESSED CHEESES DURING COLD STORAGE

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The objective of this study was to evaluate the survival of *Escherichia coli* and Total Coliforms in 20 similar productions of grated processed cheeses after the introduction of a microbiologically contaminated dairy product in various concentrations. The obtained mixtures were vacuum-packed in thermosealable plastic films and subsequently stored at $2 \pm 2^\circ\text{C}$ for eight weeks. The results were used to obtain four linear expressions for the inactivation of coliforms. In general, the survival of *E.coli* seemed to be extremely difficult in all the contaminated mixtures, while the speed of inactivation decreased when the chemical composition of grated cheeses was theoretically less favorable to coliforms. The results related to Total Coliform count seemed to follow the same pattern, but the inactivation was less evident. These findings seem to imply the crucial role of other competitive microorganisms. In fact, all the packed mixtures showed a number of fungal mycelia after 54 days at most. Consequently, it can be supposed that the higher the presence of spoiling moulds in the above-mentioned dairy products, the lower the temporal survival of *E.coli* and Coliforms. Further research is needed to verify this hypothesis.

Keywords: Total Coliforms; *Escherichia coli*; Grated Processed Cheese; bacterial competition; microbial survival.

MINOR COMPONENTS WITH NUTRITIONAL VALUE, ANTIOXIDANT ACTIVITY AND ORGANOLEPTIC PROPERTIES STATUS THROUGH THE MARKET PERIOD OF COMMERCIAL OLIVE OIL TYPES

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Olive oil is mainly composed by triacylglycerols which are susceptible to oxidation, the prime cause of oil deterioration, which decreases the sensory quality and the nutritional value of the product. However, virgin olive oil also contains minor compounds which greatly improve its stability during its market period.

Virgin olive oil natural antioxidants are important not only for their *in vitro* protection against rancidity, and therefore on the shelf life of the product, but also for their *in vivo* biological activity which participate to the nutritional value of this type of edible oil, together with its beneficial fatty acids composition. Moreover, several minor components, like volatile compounds and some individual phenols, also play an important role in positive sensory attributes, like fruitiness and bitterness, and hence in the consumer preference.

This communication describes and analyses the content of minor components in commercial Spanish extra virgin and olive oils categories, particularly with respect to the individual phenolics compounds, tocopherols (vitamin E), β -carotene (pro-vitamin A) and volatiles compounds, through the market period. The nutritional value of the consumption of virgin olive oil natural bioactive compounds is also discussed, since they are currently under investigation as functional ingredients beneficial to human health.

Keywords: Virgin olive oil; minor components; antioxidant activity; nutritional value

IMPACT OF THE TYPE OF PACKAGING ON THE SCOPE OF FLUCTUATIONS OF THE QUALITY OF THE PACKAGED PRODUCTS

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The purpose of the author's own research was to determine the intensity of the quality fluctuations in a selected product from the group of confectionery bread, offered in various types of packaging. The examined product was packaged in bags with lateral folds, made of laminate OPP/PE, with a mass of 180 g, and moulded plastic recipient with partitions OPP, closed with a cover foil OPP/PE with a mass of 200 g.

The assessment of the scope of fluctuations of the water content in the packaged product and of the index of the overall sensorial quality was made on the basis of the indices of the dynamics of change.

Keywords: packaging, the water content, sensory quality of product, shelf-life.

ACCELERATED TESTS IN ESTIMATION OF THE SHELF-LIFE OF BAKERY PRODUCT

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The purpose of the author's own research was to analyse the changes in the quality of bakery product stored in an environment of accelerated ageing.

The study took into account the impact of the storage temperature on the intensity of variation of the quality parameters in the packaged product.

The scope of specific changes of quality occurring in the accelerated ageing environment was subsequently compared to the dynamics of those changes as occurring in the course of long term storage.

Keywords: shelf-life, accelerated ageing, dynamics of quality changes, packaging

INFLUENCE OF THE STORAGE TEMPERATURE AND THE ETHYL ALCOHOL CONTENT ON THE SHELF LIFE OF THE ASTI SPUMANTE DOCG

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The "Asti" or "Asti Spumante" is the most important aromatic Italian wine. Produced exclusively with the grapes of the "Moscato bianco" cultivar growing in the hilly vineyards of the provinces of Asti, Alessandria and Cuneo (Piedmont, North West Italy), it is commercialized in all the world as sweet dessert wine.

The need to reach new and more distant markets has imposed to the producers a qualitative improvement and an increase of the shelf life extension of the product, currently too limited, in order to allow a more easy marketing.

The application of new techniques of management of the vineyard and of winemaking have allowed improving the dry extract content and the concentration of aromatic compounds in the wines, but a satisfactory improvement of the storability of the wine after the bottling as not achieved yet.

The rapid decadence and transformation in bottle of the terpene compounds, together with to the change of the color, constitute the principal problem of commercial depreciation of the Asti DOCG. The influence of the alcoholic content and of the temperatures of conservation of the bottles on the storability of the product has been evaluate in this work. During twelve months the chromatics characteristics, the concentration of acetaldehyde and, by means of GC-MS analysis, the volatile composition have been monitored in samples of bottled wines. They derived from an only mass of must, and have been produced with three different levels of ethyl alcohol (6.5%, 7.5%, 9.5%) and preserved at three different temperatures (5, 15, 22 °C). At the same time a sensory analysis test was performed on each sample.

The results showed that keeping the wine to 22 °C reduces the content in linalol, the principal monoterpene alcohol, already from the first months of conservation. At 15 °C the process of degradation is very slower. Only with temperatures of storage of 5 °C it as been possible to preserve the chromatics and organoleptic characteristics of the product for more than six months. At more elevated alcoholic levels the degrading enzymatic processes are more slowed down.

Keywords: Asti Spumante, storage temperature, volatile compounds, dessert wine

USE OF POLYMERIC MATERIALS AS PACKAGING FOR WHITE AND RED TABLE WINES PRODUCED IN PUGLIA

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Packaging materials can have a significant effect on the quality of foods, especially with regard to both degradation and scalping. In order to prevent this deterioration in food quality, adequate packaging designs should be used to avoid significant losses or degradations of volatile compounds during product storage and to maintain its quality, safety and sensory properties. The shelf-life of wine is a primary concern of the wine industry and the shelf-life of a young wine is directly related to its resistance to oxidation, in fact, both red and white wines undergo oxidative deteriorations and colour alterations. For this reason, in the packaging wine, glass has been always used like ideal container because it is the only packaging material with a total chemical inertia, barrier to gases and vapours, stability in the time, transparency, good recycle attitude and able to keep wine without altering sensory characteristics. However, glass shows some drawbacks, such as cost, heaviness in relation to the product, brittleness, difficulties in managing void spaces and a shape not allowing the space optimization. Therefore, market requirements and consumer trends direct research towards alternative solutions. In this respect, new materials are continuously investigated in order to evaluate their performances. In this study, a comparison between the performance of plastic containers (Polyethylene Terephthalate (PET); PET with oxygen scavenger; PET with external coating; Polypropylene (PP)) and containers made of glass in preserving the quality of white and red table wines produced in Puglia (Italy) has been addressed. The quality decay of stored wines were determined by monitoring the changes of volatile, the amount of oxygen dissolved, tonality, total phenols, volatile acidity and sulfur dioxide, for a period of about six months. No appreciable differences were detected between the two different types of bottles investigated, suggesting that polymeric materials can be advantageously used to pack table wine to improve its commercial competitiveness.

Keywords: wine, packaging, shelf life

LISTERIA INNOCUA DYNAMIC IN MINIMALLY PROCESSED VEGETABLE PACKAGED IN ORDINARY AND MODIFIED ATMOSPHERES

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This work was carried out to study the microbiological and safety aspects of Minimally Processed Vegetables (MPV) packed under different conditions. The type strain *Listeria innocua* DSMZ 20649 was used to study *in vitro* the growth/survival at different initial parameters. The bacteria was cultured in TSB (Tryptic Soy Broth) using a 3x3x3 complete factorial design to assess the impact of temperature (15, 25, 37 °C), initial pH (5.2, 5.7, 6.0) and oxygen (aerobic, microaerophilic and anaerobic conditions) on the microorganism's growth.

In order to verify the behaviour of *Listeria innocua* DSMZ 20649 in the MPV artificially inoculated, packaged both in ordinary and modified atmospheres, the PCR-DGGE analysis was used. Moreover, the same approach was carried out in order to follow microbial dynamics of MPV products without inoculum. *Listeria innocua* DSMZ 20649 showed the highest growth in microaerophilic conditions, at 25 °C and at highest pH value. In aerobic condition the strain grew better at low temperature (15 and 25 °C) and in anaerobic condition at 37 °C showed at the lowest pH value the highest growth. The *in vivo* results showed that *Listeria innocua* became one of the dominant species at the end of the storage time and that indigenous microbial population highlighted slight shifts during storage.

Keywords: Minimally Processed Vegetables; *Listeria spp.*, Shelf-life, Packaging conditions, PCR-DGGE analysis

TIME-TEMPERATURE EXPOSURE OF FRESH FISH IN THE COMMERCIAL CHAIN

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Fresh fish is a very precious and delicate commodity. Testing/modelling of the shelf life of fresh European Sea bass (*Dicentrarchus labrax*), with reference to its thermal history in the commercial chain, was approached by means of a new kind of small temperature recording devices, directly placed in the fish meat and/or in the storage rooms. Twenty selected "real" thermal histories were so collected and split into the different steps experienced during the commercial life (storage at manufacturer, delivery, storage in retail cells, exposition in retail counters).

The fish remains at temperatures lower than 1 °C during the first steps of the commercialisation and reaches 5 °-8 °C during the exposition at the sale point: this step is also the longest one (3-5 hours). The obtained data were statistically analysed and plotted on time-temperature exposure probability charts (50%, 75%, 90% probability values were considered). Delivery at the sale points resulted as the critical step because of the poor control of product temperature and the influence of ambient conditions. Collected data were also employed to parameterise the shelf life: a suitable hypothesis was applied to work out the integration of the most probable time-temperature exposure history. Our findings (75% of the cases) showed that the expected shelf life (or "freshness") just after the purchase of fresh fish was 60% of the whole one. This condition can be related to an integration average temperature of 4 °C, considering 2 days of commercialisation time. Furthermore, our results showed that freshness can be maintained for 1-2 days more during the storage at home. The average exposure temperature could be then reduced by 1-2 °C with a joined effort (manufacturer, sale point management, consumer advertising) thus extending the fresh fish shelf life up to 4-5 days.

Keywords: Time-temperature exposure, shelf life, commercial chain, fish, European Sea bass