Report of the 2nd RCM on
Application of Radiation Technology in the Development of Advanced Packaging Materials for Food Products

8 to 12 September 2014
Bejaia, Algeria
The prepared convenience food sector has become a significant part of the economy and/or is evolving in many developed and developing countries. Packaging technology underpins the development of this sector and ensures food quality and safety. Radiation processing could provide attractive options for the food packaging industry worldwide. Radiation technology enables the development of new polymeric materials (modified natural polymers and nanocomposites), new packaging technologies (surface irradiation) and new dye printing methods (e.g. high gloss, abrasion and chemical resistant finish produced without volatile chemicals), which can be both sustainable and environmentally friendly. In addition, the irradiation of pre-packaged food requires radiation tolerant packaging that retains the necessary properties to prevent post-treatment contamination. There is a need and an opportunity for collaboration between radiation chemists, material scientists, food irradiation specialists and food safety specialists to examine the complete life-cycle of food packaging and encourage the development of novel and environmentally friendly food packaging materials and technologies.

The CRP proposal was formulated based on the conclusions and recommendations of the Consultants Meeting on “Applications of Radiation Techniques in Development of Advanced Packaging Materials for Food Products” held at IAEA Headquarters, Vienna, Austria, 14-18 May 2012; organized jointly with the Food and Environmental Protection (FEP) Subprogramme of the Joint FAO/IAEA Programme for Nuclear Techniques in Food and Agriculture (NAFA). The objective of this CRP is twofold: on one hand it is to assess the effects of ionizing radiations (gamma, electrons, and X-rays) on commercial and emerging food packaging materials, while on the other hand, to develop new functional packaging materials based on natural polymers with improved stability, sealability, biodegradability, and recyclability by using radiation techniques. The focus of the CRP is on pre-packaged foods intended for irradiation with the aim of improving food safety, enhancing shelf life and promoting international trade.

The CRP was constituted with 14 participants, and the 1st RCM was organized on 22-26 April 2013, in Vienna, Austria, attended by all chief scientific investigators and one observer from Brazil. The Meeting Report is available at http://www-naweb.iaea.org/napc/iachem/working_materials/F2-22063-CR-1-report.pdf. Following the recommendations of the 1st RCM, the second RCM was organized on 08-12 September 2014 in Bejaia, Algeria, attended by 11 chief scientific investigators and one observer from Algeria. The participants have reported their research results, and agreed on the way forward. The achievements of the RCM are presented in this meeting report, giving first the background information and the short summaries of the presentations, followed by individual full reports.

The IAEA wishes to thank all participants of the Meeting for their valuable contributions. The IAEA officer responsible for this Research Coordination Meeting was Agnes Safrany of the Division of Physical and Chemical Sciences.
INTRODUCTION

1. BACKGROUND

The world’s food supplies need to be increased and protected to meet the demands of the growing global population. The World Summit on Food Security in 2009 reported that by 2050, food production needs would have to increase by as much as 70% in order to feed the anticipated 9 billion people (FAO 2009). However, improving crop yield is only just one aspect of meeting this increased food supply needs. It is equally important to expand arable lands and protect what is produced. A significant quantity of harvests around the world does not make it to the consumer. It is estimated that the loss between the point of production and consumption constitutes as much as 30-40% of the total amount of food that is produced. Where the food is wasted differs in different parts of the world. In the developing countries majority of the food is wasted at the farm and during shipping and transportation, while in the developed parts of the world such as the US and UK, majority of food waste occurs at the home (Science, 2010).

Plant and animal products are also at risk of microbial pathogen contamination during their journey from the producer to the consumer. Also, processing and prolonged storage can potentially increase food safety risks especially when the food is not adequately packaged and protected from fungal infestation and toxin contamination. The US Center for Disease Control reported 48 million cases of food borne illnesses and 3000 deaths in 2011, while Agriculture Canada reported 13 million cases of food borne illnesses per year (Agriculture Canada, 2006). The Foodborne Diseases Active Surveillance Network (Food Net) of the USA states that in 2007 incidence of infections caused by Campylobacter, Listeria monocytogenes, Salmonella, Shigella, Vibrio, and Yersinia did not decline significantly compared to 2004-2006 data and estimated incidence of Cryptosporidium infections increased by 44% (Imran et al., 2010). The most recent FoodNet data point out that Salmonella and Campylobacter contamination of foods continue to be the leading cause of bacterial food borne illnesses. Foodborne illnesses cause immense economic burden due to food recalls and medical treatment costs. According to recent estimates, foodborne illnesses linked to domestic and imported fresh fruits and vegetables cost the US economy over $150 billion per year (Scharf, 2010). The post-process contamination caused by product mishandling and faulty packaging is reported to be responsible for approximately two thirds of all microbiologically related recalls in the USA (Gounadaki et al., 2007). Post-processing protection and active packaging and coatings has been proposed as an innovative approach that can be applied to ready-to-eat products to minimize or prevent the growth of pathogenic microorganisms (Min et al., 2008).

Considerable research and development is being conducted in different Member States to improve and develop new packaging materials and coatings for use in the food sector. These research activities include developing recyclable, biodegradable, bioactive and smart packaging material and food coatings. Ionizing irradiation plays a major role in the development and improvement of packaging polymers as well as in sterilizing packaging material used in aseptic packaging. However, the use of ionizing radiation for pre-packaged foods is a major technology used worldwide to combat foodborne pathogens. However, the behavior of these materials (especially those directly in contact with foods) under ionizing radiation doses needs to be evaluated and quantified in order to obtain regulatory approval prior to their commercial use. Innovative packaging based on natural polymers in conjunction with other technological advanced material modifications (e.g. in nanotechnology) have considerable promise for the future in this field.

Radiation induced degradation of various newly developed polymers and their components including adjuvants, antioxidants, plasticizers, coatings, release agents, stabilizers, and their emerging technologies (for example incorporating nanoparticles, sensing agents, and “intelligent”/bioactive components) also need to be evaluated at the radiation doses recommended for phytosanitary treatment and food safety applications.
The effect of ionizing radiation doses around 10 kGy on packaging materials, and the packaged food in contact with these materials, needs to be evaluated for ensuring the wholesomeness (safety and quality) of food. Likewise, the suitability of packaging materials when used in conjunction with lower doses (≤ 1000 Gy) when used for extending the shelf life, reducing post-harvest losses, and for quarantine applications for trade in agricultural commodities, also needs to be evaluated.

The successful commercialization of new materials for food packaging has to overcome many multidisciplinary barriers (science and technology, safety regulation, standardization, training and technology transfer). In order to ensure that economic and social benefits can occur, research and development in this area should be encouraged and involve a high degree of multidisciplinary collaboration. The issue of developing and recommending packaging materials for radiation processing of pre-packaged foods therefore needs to be addressed through research networks and coordinated interactions of multidisciplinary expertise. This can be best addressed by means of a coordinated research effort through a CRP. In a similar, but much broader approach to developing sustainable food packaging, a network of 35 Countries and more than 80 research and industrial Institutions is already in place under the EU 7th Framework Project Cost Action FA0904 entitled “Eco-sustainable food packaging based on polymer nanomaterials” (www.costfa0904.eu), with an aim to develop new packaging materials. Collaboration and joint events with this Cost Action have already been taken place, while others are planned.

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2. CRP OVERALL OBJECTIVE

The objective of this CRP is to develop new packaging materials based on natural and synthetic polymers using radiation techniques, and to assess the effects of ionizing radiations (gamma, electrons, and X-rays) on commercial and emerging food packaging materials, in particular for their use in pre-packaged foods intended for radiation processing.

2.1. SPECIFIC RESEARCH OBJECTIVES

- to assess the effects of ionizing radiations on selected commercially relevant polymers used in contact with food (polyolefin and derivatives, polyamides, polyesters, ethylene vinyl alcohol, ethylene vinyl acetate, and polystyrenes);
- to assess the relationship between radiation processing and the structural and functional properties of emerging advanced food packaging materials and coatings in contact with food, including nano-filled polymers, polymer films and coatings with advanced/active/smart functions, natural and synthetic biodegradable polymer-based materials;
- to develop new functional packaging materials based on natural polymers with improved stability, sealability, biodegradability, and recyclability by using radiation techniques;
- to harmonize protocols and methodologies for measurements and testing of packaging materials.

2.2. EXPECTED RESEARCH OUTPUTS

- Advanced and improved packaging materials and their components using radiation technology.
- Recommendation for packaging materials for pre-packaged irradiated foods suitable for extending shelf-life and providing environment sustainability advantages like recyclability, disposability, degradability, and life cycle improvement.
• Internationally harmonized protocols and methodologies for measurement and testing of packaging materials.
• Data on the influence of radiation processing on functionality of packaging materials in relation to bioactive, antioxidant, antimicrobial, insect repellent functions, and effect on overall food quality.
• Data on the effects of irradiation on physicochemical, structural and mechanical, and functional properties of materials leading to their commercial exploitation with respect to irradiation of pre-packaged foods.
• Data in the detection limits of at least 15 ppb in polymers on migrating chemical species using food simulating solvents for irradiated pre-packaged foods targeted for regulatory approvals.

2.3. RCMS

The 1st RCM was organized on 22-26 April 2013, in Vienna, Austria, and was attended by all 14 chief scientific investigators and one observer from Brazil. The Meeting Report is available at http://www-naweb.iaea.org/napc/iachem/working_materials/F2-22063-CR-1-report.pdf.

Following the recommendations of the 1st RCM, the second RCM was organized on 08-12 September 2014 in Bejaia, Algeria, attended by 11 chief scientific investigators and one observer from Algeria. Three chief scientific investigators could not attend the meeting (Italy, Romania and Thailand), but they have sent their reports, thus their contribution could also be included in this Meeting Report. The meeting was opened by Prof Boualem Saidani, rector of the University of Bejaia, and Prof Nacer Bezzi, vice rector, external relations, University of Bejaia. The participants elected Mr Mustapha Kaci (Algeria, host) as the chairperson of the meeting, while Ms Lucille Abad (Philippines) moderated the preparation of the meeting report.

The participants have reported their research results and achievements, which were discussed in great detail. The reported progress towards expected outputs of this CRP is summarized in Table 1.

<table>
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<tr>
<th>Expected Outputs</th>
<th>Actual Accomplishments</th>
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| Advanced and improved packaging materials and their components using radiation technology | • Blends of natural biopolymers and synthetic treated by irradiation in the solid state (UK)  
• Biodegradable films from polycaprolactone (PCL) and cellulose acetate using clay, chitin whiskers and chitosan (Egypt)  
• Sodium alginate-polyethylene oxide (SA-PEO) blend film and methacrylate (MA) treated SA-PEO (Bangladesh)  
• MMA treated Gelatin-PVA blend films and MMA treated gelatin films (Bangladesh)  
• Antimicrobial (sorbic acid)) grafted polyethylene (PE) active packaging film (Malaysia)  
• Functionalized alginate and chitosan active beads and films (Canada)  
• PE-g-PAAc/Natamycin antifungal films (Turkey)  
• EVA, EVOH, PBAT/Starch and PBAT/PLA reinforced with micro and nanofiller from smectic clay, Bio-CaCO₃, green silica and rice husk (Brazil)  
• PVA, starch-PVA, starch-PVA-NCC (nanocrystalline cellulose) films (Poland)  
• PLA and polypropylene reinforced with montmorillonite clay 7-9 microns (Italy) |

| Recommendation for packaging materials for | For extended shelf-life, nanocrystal cellulose in films and beads based on alginate; radiation grafted LDPE film with |
| pre-packaged irradiated foods suitable for extending shelf-life and providing environmental sustainability, as well as advantages like recyclability, disposability, degradability, and lifecycle improvement. | antimicrobial (AM) additives (sorbic acid)  
- For enhanced biodegradability, aliphatic-aromatic copolyester: polyactic acid reinforced with nanofiller containing pre-irradiated PLA; PLA and polypropylene reinforced with montmorillonite clay |
| Internationally harmonized protocols and methodologies for measurement and testing of packaging materials. | GPC-MALLS of biopolymers and solution properties (UK) |
| Data on the influence of radiation processing on functionality of packaging materials in relation to bioactive, antioxidant, antimicrobial, insect repellent functions, and effect on overall food quality. | Food quality studies with clay amended and other biopolymers (USA)  
- Gamma irradiation in air and at RT to control MW and MW distribution of cellulose acetate and chitosan (Egypt)  
- Relationship between irradiation and the functional properties of active beads based on nanocrystal cellulose (Canada) |
| Data on the effects of irradiation on physicochemical, structural and mechanical, and functional properties of materials leading to their commercial exploitation with respect to irradiation of pre-packaged foods. | Mechanical and thermal properties of some commercial packaging materials and their detectable leachates by GPC (Philippines)  
- eBeam dosing studies on PLA + clay and PPR + clay and functionality and structural studies completed (USA)  
- Effects of irradiation on gum arabic, alginate, and xanthan including the effects of dose rate and irradiation condition (UK)  
- Effect of gamma radiation at higher doses on morphology and functional properties of blends based on PHBV and PLA biomaterials (Algeria)  
- Mechanical properties and functionality (emulsification and encapsulation) following irradiation (UK)  
- Gamma and e-beam effect on starch, PVA, starch-PVA, starch-PVA-nanocellulose films (Poland) |
| Data in the detection limits of at least 15 ppb in polymers on migrating chemical species using food simulating solvents/for irradiated pre-packaged foods targeted for regulatory approvals. | Preliminary data using stable isotope technique (Philippines) |

While most of the work done in the participating institutions in this CRP, there are several products that are already in a stage in which commercialization could follow, as shown in Table 2.
### TABLE 2. Products that have past the research stage

<table>
<thead>
<tr>
<th>Products that have been developed</th>
<th>Remaining Issues / Challenges</th>
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<tbody>
<tr>
<td>Encapsulation of antimicrobial compounds involving nanocrystal cellulose in films and beads based on alginate for the protection of the bioactivity during irradiation treatment and storage to assure food safety.</td>
<td>Funding, industrial investments and regulations</td>
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<tr>
<td>Radiation grafted LDPE film with antimicrobial (AM) additives (sorbic acid) with inhibition of mould and fungi growth for bread.</td>
<td>Upscaling to evaluate continuous grafting of AM onto polymer film e.g. using CVD line and inline irradiation process of the film</td>
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<tr>
<td>Biodegradable flexible film based on aliphatic-aromatic copolyester: polyactic acid reinforced with nanofiller containing pre-irradiated PLA for dried food packaging</td>
<td>Funding, industrial investments and regulations</td>
</tr>
<tr>
<td>Biodegradable PLA and polypropylene reinforced with montmorillonite clay for fruits and vegetable packaging for food quality and safety</td>
<td>Commercialization</td>
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### 2.3.1. Collaborations:

The above results could not all be achieved without the extensive collaboration among the participants. Most of these collaborations were on bilateral basis, and are listed as follows:
- Brazil and UK on modification of new material for packaging
- UK and Turkey has been successful in obtaining funding from the Turkish research council to support the PhD students to investigate the effects of irradiation on biopolymers
- UK and Bangladesh on the irradiation of biopolymers for a successful link with Saudi Arabia for PhD funding through UK
- Italy and USA on choosing e-beam doses and performing e-beam dosing studies
- Philippines sent a fellow to Canada for training
- Bangladesh and Canada for PhD students
- Italy and Algeria on the preparation and characterization of PHBV zinc oxide nanocomposites through exchange of PhD students
- Canada and Italy – approved project on irradiation of PLA
- Italy and Egypt – preparation and modification of PLA through PhD students
- Malaysia and Turkey in the characterization of grafted polymers
- Poland and Turkey for funds on ERASMUS project for cooperation in studying radiation effect and safety food packaging through exchange of PhD students and lecturers
- CRP facilitated US and Mexico to initiate collaboration of biopolymer development and testing
- Synergy with other IAEA Projects, for example: Philippines collaborated with Pakistan on Stable Isotope Project.

### 2.3.2. Joint activities of IAEA and EU COST Actions

In continuation of the collaboration between IAEA and EU COST Action FA0904 Eco-sustainable Food Packaging Based on Polymer Nanomaterials, the project officer of this CRP was invited to attend the final meeting of this action held 26-28 February 2014 in Rome, Italy, and deliver a talk entitled “Preparation of New Food Packaging Materials by Radiation-Initiated Reactions and Relevant IAEA Support to Member States Institutions”. A group of participants, with Italy as the originator and leader, have prepared and submitted a proposal entitled “Worldwide preservation of food through advanced packaging materials obtained by synergetic exploitation of new technologies”, for funding by the EU. The proposal was invited for submission as full proposal with excellent review, but has not received
funding. On the other hand, Poland participates in COST project FP1205 entitled “Development and innovative application of cellulose”.

3. PROGRESS OF THE RELEVANT WORK IN PARTICIPATING INSTITUTIONS

3.1. ALGERIA

Summary:
A study of morphological, thermal, rheological and barrier properties of poly(3-hydroxybutyrate-co-3-hydroxyvalerate)/polylactide blends prepared by melt mixing and their durability under gamma irradiation exposure

Biodegradable polymers derived from renewable resources have attracted much attention due to the great demand, in particular for food packaging applications. Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and polylactide (PLA) have received even more attention in view of sustainability. They offer the potential for an attractive combination of mechanical performances and biocompatibility. The interest in polymer blends is also growing because this is an opportunity to finely adjust the functional properties of these materials. Therefore, the objective of the work was to study blend properties of biodegradable polymers of PHBV and PLA prepared by melt mixing. Blend compositions based on PHBV/PLA were investigated according to the following weight ratios, i.e. 100/0, 75/25, 50/50, 25/75 and 0/100 wt. %. The study showed through scanning electron microscopy (SEM) that blends of PHBV/PLA are not miscible. This is consistent with differential scanning calorimetry (DSC) data which indicates the presence of two distinct glass transition temperatures (Tg) attributed to the neat polymers, over all the range of blend compositions. Water and oxygen barrier properties of PHBV/PLA blends are significantly improved with increasing the PHBV content in the blend. Further, structural analyzes indicated that increasing the PHBV content in the polymer blends results in increasing the PLA crystallinity due to the finely dispersed PHBV crystals acting as a filler and a nucleating agent for PLA. Moreover, the addition of PLA to the blend results in a large increase in the complex viscosity, storage modulus and loss modulus of PHBV matrix.

To improve the miscibility between the two blend components, low amount of compatibilizing agent (5 wt. %), obtained by grafting maleic anhydride onto PHBV (PHBV-g-MA), was used. When compared with the uncompatibilized blends, the presence of the compatibilizer induces a greater interfacial adhesion. The effect of organo-modified montmorillonite (OMMT) on the blend morphology and properties was also investigated. A synergistic effect of compatibilizer and OMMT was highlighted leading to an improved miscibility of the two blend components.

The effects of γ-irradiation under ambient conditions on properties of PHBV/PLA blend with 50/50 composition (wt.%) have been also examined in the presence of both PHBV-g-MA and OMMT, up to 100 kGy. Several techniques were used to investigate the chemical structure, the morphology and the property changes using FT-IR spectroscopy, SEC, TGA, DSC, SEM, and nanoindentation measurements. The results showed that at higher doses, the oxidative degradation under gamma irradiation of neat PHBV, neat PLA and PHBV/PLA blends causes main chain scissions responsible for a significant decrease in the average molecular weight of the materials. However, the thermal stability as well as the nanomechanical properties of PHBV/PLA blends remain almost unchanged even after 100 kGy of exposure.

Work plan:
- Study of the effects of e-beam and γ-irradiation at lower doses (1 – 15 kGy) on the physico-chemical properties of PHBV/PLA blends with 50/50 composition in the absence and the presence of PHBV-g-MA and organo-modified montmorillonite (OMMT).
- Study of the gas permeability of PHBV/PLA blends subjected to e-beam and γ-irradiation to evaluate their applicability as food packaging materials.
- Toxicity studies (microtox and/or salmonella Ames tests) on irradiated PHBV/PLA blends.
3.2. BANGLADESH

Summary:
Sodium alginate (SA)-Polyethylene oxide (PEO) blend films with glycerol as plasticizer were prepared by solution casting method. For the preparation of SA-PEO blend film, the concentration of PEO and glycerol were optimized. The SA-PEO blend films in presence and absence of methacrylate (MA) were irradiated by gamma radiation from Co-60 gamma source at room temperature (27ºC). The parameters like effect of radiation dose and concentration of MA as monomer were investigated. The properties such as tensile properties (Tensile strength and elongation at break) and thermal stability of SA-PEO blend films were examined. The tensile strength of SA-PEO blend films increased with increase in radiation dose and it attained a maximum value at 12.2 kGy absorbed dose. The tensile strength of irradiated MA treated SA-PEO blend film was obtained 44.58% higher than that of irradiated SA-PEO blend films at 12.2 kGy absorbed dose. The thermal stability of SA-PEO blend films were investigated using thermo gravimetric analysis (TGA) and dynamic mechanical analyzer (DMA). The thermal stability of irradiated MA treated SA-PEO blend film was obtained higher than that of irradiated SA-PEO blend films.

Gelatin films and gelatin-polyvinyl alcohol (gelatin-PVA) blend films with glycerol were prepared by casting method and their mechanical properties were studied. The tensile strength of gelatin films and gelatin-PVA blend films was decreased by addition of glycerol but elongation at break was increased. Glycerol incorporated gelatin films and gelatin-PVA blend films were further modified with methyl methacrylate (MMA) as monomer by the application of gamma radiation from Co-60 gamma source at room temperature (27ºC). The mechanical properties of gelatin film and gelatin-PVA blend films attained a maximum value at 3.1 kGy absorbed dose. The MMA treated gelatin films and MMA treated gelatin-PVA blend films shows improvement of tensile properties than gelatin films and gelatin-PVA blend films. The tensile strength was found 35.45 MPa for gelatin-PVA blend film with 3% MMA and 24.93 MPa for gelatin film with 3% MMA, which is 42.20% higher than that of gelatin film with 3% MMA treated. Thermo gravimetric analysis (TGA) and dynamic mechanical analysis (DMA) shows that the MMA treated gelatin film and MMA treated gelatin-PVA blend film shows less thermal degradation than that of gelatin film and gelatin-PVA blend film.

The objective of the proposed research project (CRP) is to prepare biodegradable material for packaging purpose from natural polymers or natural polymer/synthetic polymer blend by radiation processing for the benefits of the end users.

Work Plan
The work will be continued:
1. Study on morphological and structural properties of alginate-polyethylene (SA-PEO) blend films and gelatin-polyvinyl alcohol (gelatin-PVA) blends films.
2. Study on the degradation of SA-PEO blend films and gelatin-PVA blend film by natural weathering or artificial weathering.
3. Application of developed films on fruits or vegetables to investigate the shelf life.

3.3. BRAZIL

Summary:
During this CRP, studies have been made for the modification of conventional food packaging materials (polymer petroleum-derived) by addition of natural clay and ionizing radiation treatment, for pre-packaged irradiated foods and for the modification of biobased and compostable materials, by addition of micro and nanofiller from natural resources (renewable resources) and also ionizing radiation treatment. Composite based on EVOH, EVA, PBAT/Starch (aliphatic-aromatic copolyester/starch blend) and PBAT/PLA (aliphatic-aromatic copolyester/polyactic acid blend) reinforced with micro and nanofiller from renewable resources were prepared by melt extrusion, using a twin-screw extruder machine and blown extrusion process and treated by electron-beam radiation. It
was observed that the modified Brazilian smectitic clay addition (1-3 % wt) in conventional polymer, consisting of EVA followed by irradiation led to a composite with greater tensile strength at break and with improved elongation at break at the same time. The results of tensile strength at break test show that the use of reinforcement in EVA can increase this property, but results also indicate that greater increases are due to the use of ionizing radiation. The mechanical results of composites based on PBAT/Starch and PBAT/PLA blend showed that the addition of micro or nanofiller led to obtaining flexible films with improved properties when compared with neat blends. On the other hand, when these composites were irradiated at the highest radiation dose (200 kGy) the mechanical behavior of composite was worse than neat blend. Therefore, chain scission, as induced by e-beam irradiation, led to associated alterations in surface polymer morphology. SEM micrographs of the irradiated samples showed evidence of mechanical degradation (cracking) whereas irradiated PBAT/PLA and PBAT/Starch blend composite reinforced with micro and nanofiller and neat blend. However, when 10-20 % (wt %) of pre-irradiated PLA was added to the composite blend, before extrusion process, changes in surface morphology and significant gain in mechanical properties were observed indicating that surface adhesion between filler and polymeric blend matrix enhanced, resulting in better property gains.

**Work Plan**

- Preparation and characterization of graphene oxide from natural graphite powder;
- Preparation and characterization of flexible film based on EVOH and graphene oxide;
- Characterization of flexible film based on EVOH and EVA reinforced with modified Brazilian smectitic clay;
- Preparation and characterization of PBAT/Starch and PBAT/PLA blend reinforced with green silica and metal nanoparticles;
- Preliminary tests for choosing the better composition for dry food packaging based on PBAT/Starch and PBAT/PLA blend reinforced with filler from renewable resources;
- Production and characterization of multilayer food packaging structure prototypes based on EVA/EVOH/EVA with micro and nanofiller from renewable resources.
- Production and characterization of flexible dry food packaging based structure prototypes based on PBAT/Starch and PBAT/PLA blend with micro and nanofiller from renewable resources.
- Support for other composite studies involving reinforcements from fibers or waste of piassava, sugarcane bagasse, Brazil nut shell, coffee husk and peels into PBAT/Starch Blend and PBAT/PLA Blend.

### 3.4. CANADA

**Summary:**

This study focused on the antimicrobial efficiency of micropencapsulated nisin/essential oil (EO) nanocomposite bead systems against *Listeria monocytogenes* in ready-to-eat (RTE) meat for in situ food applications. For the evaluation of nisin/EO microbeads, a preliminary study in vitro was undertaken to develop nisin/EO-microencapsulated edible beads in order to inhibit the growth of *L. monocytogenes* RTE ham. Different concentrations of nisin (16, 31 and 63 μg/mL) were encapsulated into alginate-cellulose nanocrystals (CNC) beads. Microencapsulation kept the available nisin (63μg/mL) content 20 times more than free nisin during 28 days of storage at 4°C, by exhibiting 31 μg/ml of availability. Cooked ham slices were coated by nisin beads, inoculated with *L. monocytogenes* (3 log CFU/g) and stored at 4°C under vacuum packaging for 28 days. The beads containing 16, 31 and 63 μg/ml of nisin significantly \((P \leq 0.05)\) reduced the bacterial counts by 2.6, 1.5 and 3.0 log CFU/g after storage compared to free nisin, without changing the physicochemical properties (pH and colour) of RTE ham. A second microencapsulation study was to compare oregano essential EO (*origanum compactum*; 0.025% w/v), cinnamon EO (*cinnamomum cassia*; 0.025%) and nisin (0.25% or 16 μg/mL), used alone or in combination with γ-irradiation, to evaluate their in situ inhibiting capacity against the growth of *L. monocytogenes* in RTE ham. Microencapsulation of these formulations allowed verifying the potential of the polymer to protect their antimicrobial efficiency.
during storage time. Combined treatments of antimicrobial formulations with $\gamma$-irradiation were also carried out to determine synergistic antimicrobial effects. Microencapsulated cinnamon EO/nisin combined with $\gamma$-irradiation (1.5 kGy) showed 0.03 log CFU/g/day growth rate of bacteria compared to 0.17 log CFU/g/day for non-encapsulated counterpart. Microencapsulation also showed a significant improvement of bacterial radiosensitivity ($P \leq 0.05$). Microencapsulated oregano EO/nisin and cinnamon EO/nisin combinations showed the highest bacterial radiosensitization with relative sensitivities of 3.4 and 6.9 respectively. Finally, microencapsulation can protect the bioactivity of the antimicrobial compounds during irradiation and the both process act in synergy to eliminate pathogens in food.

**Work Plan:**
- Modeling to develop optimized antimicrobial formulation to inhibit *L. monocytogenes, E.coli* but also *C. botulinum* in sausage using the microencapsulation method developed in combination with irradiation
- Develop a method of crosslinking and immobilization of nisin on film surface based on chitosan and evaluate their antimicrobial properties in combination with irradiation
- Develop formulation and active packaging to control pest during storage of rice and evaluate the synergistic effect of the active packaging with irradiation on desinfestation and on mold control.
- Evaluate the combined treatment of irradiation and active packaging using supramolecular PLA on RTE vegetables and meat

### 3.5. EGYPT

**Summary:**
Preparation of active biodegradable films suitable for radiation sterilization with good mechanical and barrier properties from polycaprolactone (PCL) and cellulose acetate (CA) using low amount of clay, chitin and chitosan was developed. Cellulose acetate (CA) matrix reinforced with bentonite (in nano scale) was obtained by film casting technique. The resulting composite materials were characterized by means of differential scanning calorimetry, thermo-gravimetric analysis, X-ray diffraction, FTIR spectroscopy, water vapor permeability (WVP), oxygen transmission rate (OTR) and mechanical tests. The addition of low amount of bentonite significantly improved both strength and barrier properties of cellulose acetate (CA)-based films. The oxygen transmission rate (OTR) and water vapor permeability (WVP) decreased significantly. Biodegradability of the Cellulose acetate (CA) was not affected by adding bentonite clay.

On the other hand, a method based on melt processing for incorporating Chitin whiskers and Chitosan into PCL was developed for homogenous active food packaging without using a compatibilizing agent. Chitin whiskers were prepared and characterized using different techniques. Also, CS of high viscosity was incorporated after irradiation at 10-20 kGy and 1-5 wt % in order to maintain the mechanical properties of PCL and reduce the yellow coloration of CS caused by irradiation. Nevertheless, the preparation method should be modified, e.g. by changing the technique of mixing, to obtain more homogenous films with better mechanical properties. Characterization and evaluation of the antioxidant and antimicrobial capacities of the developed PCL/CS or Chitin whiskers films were carried out.

**Work plan:**
1- Active and smart packaging from biodegradable polymer nano-composite films will be prepared and characterized.

A- Nanoscale antioxidant and antimicrobial agents will be used to expand packaging material functional properties (such as anti-microbial activities and antioxidant properties).

B- Polymer nano-composite film is prepared via polymer melt processing, or casting techniques according to the nature of selected polymer.

C- To obtain homogeneously dispersing nano-composite materials, radiation modification of nanoparticles filler surface will be done in the presence of different chemical reagents. Also, some plasticizers will be added for the composites homogeneity.
2- Characterization and properties of the obtained green nano-composite films will be investigated using different tools.

A- Characterization of nano-composite films will be investigated using different tools
B -The moisture permeation, gas barrier, electric properties, etc. of the films will be determined.
C- Biodegradability and antimicrobial properties as well as toxicity of the prepared nano-composite films will be tested.
D- Effect of ionizing radiation on the properties and oxidation stability of the prepared nano-composites films will be studied.

3.6. ITALY

Summary:
The principal aim of the 3-year agreement research project in the framework of the CRP F22063 is to assess through simulation modelling and experimental approaches the relationship between radiation processing and properties of emerging advanced polymer nanomaterials for food packaging. Three research groups are involved: 1. Italian National Research Council (CNR) –Institute of Chemistry and Technology of Polymers (ICTP)- Italy; 2. The Department of Physics and Astronomy is part of the Faculty of Sciences of Ghent University; 3. The food irradiation Group of the National Reference Center for the Detection of Radioactivity in Veterinary and Husbandry - at the Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata Italy; 4. Two systems are under evaluation: 1 Polypropylene/Oligocyclopentadiene/clay, 2 Polylactic Acid/clay;

For both systems the studies on the properties in dependence of preparation conditions indicates that depending on processing conditions nanocomposites with different phase structures and properties (morphological, rheological and mechanical properties) are obtained. In particular the methodology using a master batch is to be preferred as more homogeneous morphologies along with improved mechanical properties are obtained. Both systems were then irradiated by using X-ray and e-beam radiation. The results for the system Polyactic Acid/clay show that the obtained nanocomposite is suitable for sterilization by e-beam irradiation at the doses studied (1-10 kGy). Moreover it was observed that the clay addition decreased the oxygen permeability and that after e-beam irradiation there was a further decrease of the permeability values due to matrix stiffening caused by crosslink formation.


Work plan:
• Study of the effect of X-ray radiation on the structural, thermal and mechanical and barrier properties of Polylactic Acid/Montmorillonite systems used for pre-packaged foods intended for radiation;
• Study of the effect of X-ray radiation/ebeam on the structural, thermal and mechanical and barrier properties of Polypropylene/Oligocyclopentadiene/clay systems used for pre-packaged foods intended for radiation;
• Comparison of the properties of the systems reported above in dependence on the kind of irradiation source;
• Dissemination and Exploitation of the results.

3.7. MALAYSIA

Summary:
The reports highlighted the progress of work related to a novel non-migrating antimicrobial (AM) coating through co-polymerization grafting of selected AM to produce active packaging film. A characterization of polyolefin film with antimicrobial additive (AM), sorbic acid (SA) was reported. Radiation induced grafting of sorbic acid on low density polyethylene (LDPE) film has been performed with the aim to developed antimicrobial active packaging film. The covalent attachment of sorbic acid onto LDPE film surface was performed by pre-irradiation method. The grafted samples were characterized with respect to their oxygen permeability, water contact angle and mechanical properties, FTIR, XPS, SEM, AFM evaluation. The report summaries that optimum grafting parameters and the grafting efficiency. The grafting yield of SA onto PE film was observed to be low with maximum around 2.8% only. However, evidences for FTIR, XPS, SEM and AFM analysis confirmed that the grafting does occurred on the surface of polymer film. Oxygen permeability and water contact angle of the grafted film slightly increased compared with raw LDPE film (control). Tensile strength increased from 20.48MPa to 32.61MPa, while elongation at break increased from 193.25 % to 254.47% when 10% of SA was incorporated. The grafted film were then use to wrap slice of freshly baked bread of which were prepared without addition of AM additive as a normal bread. The visual inspection was performed against fungal growth and compared with LDPE control film at 0, 5, 7 and 10 days of its wrapping. It was found that SA grafted film could delay the appearance of mould and yeast on the slice of freshly baked bread for up to 10 days with respect to control film. Total plate count & yeast & mold testing carried using standard of FDA-BAM Chapter 3 & Chapter 18 respectively at accredited laboratory validated that SA grafted film could delay the appearance of mould and yeast on the slice of freshly baked bread for up to 10 days with respect to control film.

These results reveals that grafting of sorbic acid onto polyethylene is a very promising strategy to generate novel non-migrated sorbic acid based antimicrobial materials with potential advantages for active antimicrobial packaging applications.

**Commercialization Potential**
The results obtained so far has indicated the commercial potential of active packaging film based on non-migration grafting of specific antimicrobial additive onto the commercially used packaging film such as polyethylene film. However, further development is necessary to be done at pre-commercialization proof of concept stage. The need to carry out this work is in order to ascertain the commercial viability of product and overall process. This will involve evaluating continuous grafting process. Effort will be made to follow through pre-commercialization work that includes continuous vapor deposition (CDV) of additive (AM) onto polymer film and subsequent irradiation by electron beam accelerator, preferably inline process. A proposal for pre-commercialization project will be submitted for funding with relevant authority in collaboration with company involved with manufacturing packaging material.

**Work plan:**
The work will continue;
- To evaluate effectiveness of antimicrobial grafted polyethylene developed with respect of fungi and mould inhibition i.e. to wrap food (bread or pastries) & analyzed the growth.
- To conduct preliminary evaluation of continuous grafting using chemical vapour decomposition (CVD) methodology
- To undertake a study of using other antimicrobial (AM) such as niacin, Habatus Sauda (black seed oil, ‘nigella sativa’) and others to produce bioactive packaging film by means of radiation grafting and evaluate its potential and type of food suitable for different AM for example meat and fruit.
- To evaluate possibility of using low energy electron beam accelerator to achieve grafting
- To continue characterization of AM-PE grafted film
- To study antimicrobial properties of AM-PE grafted film (toxicity, minimum inhibition concentration, shelf life study & migration test)

3.8. PHILIPPINES
Summary:
Commercial packaging films made up of Plain PET 12 / Foil 7 / PE 100, VMPET 12 / PE 70, OPP 20 / Foil 6.5 / PE 40, PET 12 / CPS 40, PET 12 / PE 50, laminated PET / PE, Nylon / PE, and Nylon 15 / PE 50 were investigated for its effect with gamma radiation at an absorbed dose of 10 kGy. Mechanical properties indicated no changes in the tensile strength but increased in elongation at break for all films. Thermal properties likewise did not show any changes after irradiation except for 20 OPP 20 / Foil 6.5 / PE 40 which slightly decreased in stability. Gel Permeation Chromatography of leachates from water samples detected the presence of high molecular weight radiolytic products especially from laminated PET/PE films. Radiation effects were minimal for VMPET12 /PE70, Nylon/PE, and Nylon 15/PE 50 films. Preliminary results on the use of stable isotope technique to study the water leachates from the packaging materials revealed an indicative increase in $\delta^{18}$O‰ and $\delta^{2}D$‰. This can be a promising sensitive technique to determine possible contamination of leachates from packaging materials.

Work Plan:
- Refinement of Stable isotope technique for $d^{18}$O‰ and $d^{2}D$‰ of leachates from packaging materials
- Na Alginate films with Ca++ and nanoclays
- Chitosan grafted PE films

3.9. POLAND

Summary
The trials were initiated for elaboration of the method for preparation of the composite films based on starch-PVA system by modification of the composition and introducing of the potential reinforcing agents (cellulose, nanocellulose), as well as evaluation of the effect of irradiation. Accordingly, the selection of the appropriate PVA and starch preparations was done basing the results of the experiments carried out in the frame of the project, as well as search of nanocellulose preparations and adaptation of the experimental conditions that enables to obtain the a good quality films. Afterwards, the conditions were adopted for preparation of the films containing additives of fibrinal, microcrystalline, and nano-sized (nanocrystalline nanofibrinal, bionanocellulose) celluloses. Following elaboration of the synthesis methods, the starch:PVA films characterized by various starch:PVA ratios were prepared and examined. The compositions were then selected for preparation of the films containing various addition of nanocellulose (NCC). Furthermore, the effect of ionising radiation on the films properties was studied. The experiments have been also carried out dealing with the influence of gamma radiation on particular substrates by EPR and gas chromatography.

In the latest stage of the project the systematic studies were conducted dealing with optimization of the methodology of synthesis of starch-PVA and starch-PVA-nanocellulose films, in that cases when it is supported or followed by ionising radiation. This concerns verification of the selection of the basic components, initiation of the studies dealing with application of the various nanocelluloses, as well as introduction of the natural antioxidants into composition of these films predicted for irradiation.

Irradiations were carried out using Co$^{60}$ gamma rays and E-beam applying the doses in the range of 5 – 75 kGy. Mechanical properties, hydrophilicity (wetting angle, water uptake, swelling), gel content in the non-irradiated and the irradiated films, and microstructure (SEM) were examined. The studies by means of diffuse reflectance spectroscopy (DRS), TGA, and DSC were also conducted.

It was stated that appropriate addition of NCC enable to obtain films with the improved functional properties. The effect of irradiation depends on the sample composition and on the applied condition. Irradiation causes decrease in hydrophilicity of the selected compositions prepared basing starch-PVA-glycerol or starch-PVA-glycerol-NCC systems. None particular effect after irradiation on mechanical properties, or even improvement, was found in the cases of some composition, although deterioration of these properties was noticed for the majority of starch-PVA films. Irradiation improves homogeneity and compatibility of the films’ components. Degradation was found to be the prevailing process taking
place under irradiation. However, the evidence of crosslinking was also observed. Strong interaction between the particular films components were also discovered. Addition of the natural antioxidants into the films’ composition has an protective effect against oxidation processes induced by irradiation. It can be considered that reactivity of nanocellulose under gamma irradiation is generally higher as compared to micro-sized cellulos.

**Work Plan**

- Continuation of the systematic studies on optimization of the methodology of synthesis of starch-PVA films and those compositions containing nanocellulose additive in the case when it supported or followed by ionising radiation. This concerns:
  - Systematic studies of the effect of application of the various starches on the irradiated films’ properties.
  - Studies of the effect of various nanocellulose preparations on the irradiated films properties.
  - Studies of the effect of the introduction of the natural antioxidants into the films composition.
- Continuation of the studies dealing with valuation of the effect of ionising radiation carried out under standard technological condition applied during food irradiation on the functional properties of the ready films.
- Studies dealing with evaluation of the safety of potential packaging for food during storage and processing:
  - Evaluation of the possibility for formation of the low-molecular products due to irradiation.
  - Adaptation of the methods for evaluation of the migration of packaging components into the films stimulant and accomplishment of experiments for the selected films.
- Initiation of the studies concerning preparation of the active films with the antimicrobial properties.

3.10. ROMANIA

**Summary:**

Two biodegradable (polylactic acid (PLA) and cellulosic materials (CC)) and one non-biodegradable substrates (polyethylene (PE)) exposed to cold plasma (cp) and γ-irradiation (irradiation doses: 5, 10, 20, 30, 50 kGy) have been modified with different antimicrobial/antioxidant agents (e.g. chitosan (CHT), lactoferrin (LF), phenolic compounds such as eugenol (Eu), vitamins E (VE) and C (VC), essential natural oils from grape seeds (GO) (*Vitis Vinifera*), Tea Tree (*Melaleuca alternifolia aetheroleum*) – Fares Comp, rosehip seeds (RO) (*Rosa rubiginosa* or *Rosa moschata*) – Herbavita Comp) by a coupling reaction in order to obtain active/bioactive food packaging materials. The characterization was made both by examination of bulk and surface properties using specific methods such as chemiluminescence, thermal analysis, ATR-FTIR, XPS, SEM, AFM, and contact angle measurements. Antimicrobial and antioxidant properties were also evaluated by standard methods and also the interaction of some of them with apple and juice was tested.

For all modified substrates by plasma activation or gamma irradiation the following orders were established:

**PLA packaging materials:** for antioxidant activity: PLA/N2/EDC+NHS/LF < PLA/N2/EDC+NHS/CHT < PLA/20kGy/EDC+NHS/CHT ≈ PLA/20kGy/EDC+NHS/TT, while in respect with antibacterial activity: PLA/N2/EDC+NHS/LF < PLA/20kGy/EDC+NHS/CHT ≈ PLA/20kGy/EDC+NHS/LF ≈ PLA/20kGy/EDC+NHS/CHT ≈ PLA/N2/EDC+NHS/CHT.

**CC packaging materials**, in case of plasma or gamma irradiation activation the following order of the antimicrobial properties was established: CC/RO/cp air > CC/GO/20kGy > CC/Eu/cp air > CC/RO/20kGy > CC/Eu/20kGy > CC/GO/ cp air. All functionalized CC samples showed 100% antioxidant activity.

**PE packaging materials:** antibacterial character: PE/20kGy/EDC+NHS/TT < PE/30kGy/EDC+NHS/RO ≈ PE/20kGy/EDC+NHS/CHT ≈ PE/air/EDC+NHS/CHT and for antioxidant
activity: PE/20kGy/EDC+NHS/CHT < PE/30kGy/EDC+NHS/RO < PE/air/EDC+NHS/CHT ≈ PE/20kGy/EDC+NHS/TT

It is seems that in most cases rosehip seeds oil imparts the best antioxidant and antimicrobial properties. As gamma irradiation affects also the bulk properties of the selected polymers and for a proper functionalization longer time is needed, it has been concluded that the most efficient procedure is plasma treatment. Choosing two biodegradable polymers as functionalizable substrates, the environmental concerns are avoided.

Work Plan:
Detailed characterization of different vegetable oils with antimicrobial and antioxidant activity and various benefits for consumers health and also new materials obtained based on complex stratified composites assembled by covalent bonding and also effects and interactions with food products as meat, fresh fruits and juices is underway in the next 18 months. This objective will include the following activities

- Determination of the composition by GC-MS, GC-FID and HPLC of vegetable oils with antimicrobial, antioxidant activity and various benefits for consumers health – we need collaboration for last method;
- Elaboration of new types of bioactive formulations for food packaging (different techniques, substrates and bioactive agents);
- (Bio)active compounds release and migration tests;
- Investigation of (bio)active compounds release from modified polymeric materials in a simulant (distillated water) media;
- Testing of the other presented formulations mainly those containing vegetable oils for fresh fruits preservation;
- Monitoring of (bio)active compounds release from modified polymeric packages in foods;
- Testing of laboratory packaging technology to micropilot scale;
- Results dissemination.

3.11. THAILAND

Summary:
The beginning part of this research aims to study the effects of gamma irradiation on commercially available polymers currently being used for food products sterilized by radiation processing at Thailand Irradiation Center (TIC). The chosen product is fermented pork sausages. The effects of gamma irradiation on packaging material of fermented pork sausages were investigated using FTIR, DSC and universal testing machine. FTIR and DSC revealed changes in chemical and thermal properties of the packaging material, respectively, where the universal testing machine showed changed in mechanical properties. The results showed that the high fat content, the direct contact between acidic fermented pork sausages and the packaging material as well as irradiation led to changes in chemical and mechanical properties of the packaging material. Two types of biodegradable polymers poly(lactic acid) (PLA) and poly(butylene succinate) (PBS) were chosen as candidates for biodegradable food packaging materials suitable for food irradiation. The effects of gamma and electron beam irradiation on properties of these two polymers were investigated. Results showed that PLA can undergo radiation-induced crosslinking, in a presence of a suitable crosslinking agent. PLA crosslinked by radiation is able to maintain its physical shape and transparency at high temperature. Results also showed that, upon irradiation, PBS can crosslink, without a crosslinking agent.

Work plan:
Future work plan is to find suitable food-grade crosslinking agent for PLA as well as for PBS to make them more suitable for food-packaging application.

3.12. TURKEY
Summary:
The incorporation, immobilization, surface modification of antimicrobial agents directly into polymeric packaging is an exciting development, which allows industry to combine the preservative functions of antimicrobials with the protective functions of preexisting packaging concepts. There are other synthetic and naturally occurring compounds that may be exploited by the packaging industry. These include organic acids, bacteriocins, spice extracts, chelating agents, antibiotics and enzymes, etc. The use of bacteriocins and other biologically derived antimicrobials in packaging materials is attracting increasing interest in recent times. In this study, The objective of the present work was to graft some food additives onto commercial packaging film polyethylene (PE) by gamma-irradiation under O₂ and N₂ atmosphere. With this aim, We tried to graft some food additives (FA) with antimicrobial properties such as fumaric acid (FAc), grafting acrylic acid (AAc) and then loaded natamycin (Nat) onto PE film. We investigated the irradiation dose and concentration of FA on grafting yield. The grafting yield of FAc onto PE film was very low. The antimicrobial activity of FAc grafted PE films was investigated. There was no grafting of natamycin onto PE film. In addition to this study we have been working on the grafting of acrylic acid (AAc) onto PE films and AAc grafted PE film can be then used to bind antifungal agents, such as natamycin, and the antifungal properties of the films were investigated. It was found that this process results in highly conformal and uniform PAAc grafts on the surface PE films. The synthesized PE-g-PAAc copolymers were characterized by Grazing FTIR spectroscopy, X-ray photoelectron spectroscopy, elemental analysis, scanning electron microscopy. The results of various techniques confirmed the existence of well-defined PAAc chains in copolymer composition.

Work plan
- The different processes will test and compared in terms of loading yield and release behavior of natamycin. Processed and non-processed samples will continue to characterize by
  - FTIR spectroscopy,
  - thickness,
  - drop contact angle,
  - water vapor permeability,
  - water uptake,
  - mechanical resistance,
  - thermal properties,
  - surface roughness,
  - color and micro-morphology,
  - antimicrobial activity,
  - MA will try to graft to PE.

3.13. UNITED KINGDOM

Summary:
Hydrocolloids, including polysaccharides and protein, applications in packaging materials have been mainly focused on improving their mechanical properties and water resistance to match those offered by synthetic (non biodegradable) counterparts. Method such as radiation treatment, chemical cross-linking, radiation grafting, thermal or UV curing have been proposed for various systems. Another approach to extend the application of natural hydrocolloids and utilise their biocompatibility, low toxicity is the production of biocomposite through blending with synthetic polymers in order to obtain the desired functionality as well as cost reduction. This is an area which requires great deal of understanding which mainly includes (i) accurate characterisation to account for the natural built in variability widely acknowledged in hydrocolloids, (ii) compatibility of mixed polymers in a given solvent and (iii) subsequent processing conditions. Our research group has been active in studying the emulsification of natural food emulsifiers such as gum Arabic, sugar beet pectin etc (see 1st CRP report). Our research has continued to focus on studying the interactions between two macromolecules. In particular to develop a method for physical fractionation by using phase separation. Polymer phase separation could potentially offer an alternative method to enhance the functionality of a given
polymer without using complex blends. The technology could be utilised in the formulation of food packaging as well as formulation for food coating.

Work Plan
- To investigate the role of Mw on the phase separation. (Application of irradiation of biopolymers in various conditions to produce a series of molecular weight materials from the same biopolymer)
- To investigate the effect of irradiation on the properties of polymer blends. (Blends will be prepared by designing conditions specific for biopolymer such controlling pH etc.)

3.14. UNITED STATES OF AMERICA

Summary:
The trans-boundary shipment of fruits and vegetables treated by ionizing irradiation (to meet phytosanitary treatment requirements) from overseas into the United States has increased over 6000% since 2007 and as much as 14% since 2012. All indications suggest that these volumes are expected to increase substantially over the next years. Plastic polymers are extensively used in the packaging of these high value products. Biopolymers, if they are to be of commercial value to this market segment would have to meet a variety of mechanical and functional properties. The original focus was to evaluate the behavior of commercially available materials Cryovac RD-45® and RD-106® shrink films and Dri-Loc® absorbent pads (that are recommended for pre-washed fruits and vegetables). However, these studies have not commenced yet. Instead, we developed collaborative linkages as part of a COST program with the research group in Italy(Institute of Chemistry and Technology of Polymers, Naples, Italy) under the direction of Drs. D. Duraccio and C.Silvestre. We also developed collaborative linkages with Dr. Tomas Madera Santana of CIAD, Sonora, Mexico. The focus of these collaborations is to evaluate the structural and functional properties of biopolymers. For the collaboration with the Italian group, our contribution was focused on providing scientific consulting regarding choosing the appropriate eBeam doses and delivering the eBeam doses. This collaboration was successful in that the outputs included multiple conference presentations as well as publication of meeting proceedings. With respect to our collaboration with Mexico, we have initiated laboratory studies to understand the shelf-life and quality attributes of fresh produce packaged using a variety of biopolymers and eBeam processed. We are currently pursuing funding to provide additional support to these studies.

Work Plan
- Evaluate toxicity tests when commercial polymers are eBeam treated at 1 kGy and 10 kGy using off-the-shelf biological toxicity assays
- Perform shelf-life and microbial pathogen reduction studies when using biopolymers in combination with MAP and other packaging approaches

CONCLUSIONS

The current RCM provided an excellent opportunity for participants to discuss the recent trends, developments and demands in the area of food coating and packaging as well as the regulatory aspects in different countries. Specifically,
- The participants reiterated the importance of using of irradiation technology in the development of the polymers as well as in studying the resistance of these biopolymers in food packaging applications;
- Advances in biopolymer development have occurred and some of these may have commercial value;
- New approaches to detect and quantify leachates from polymeric packaging materials are being developed;
- Emerging technologies e.g. encapsulation, immobilization of active compounds in films, beads and coating can be synergistically enhanced by radiation technology;
The participants acknowledged that majority of their current activities are categorized under R & D stage and they are actively engaged in further development. Some of the products developed have reached level of maturity that the next step would be on upscaling;

- Outreach activities performed under this CRP has further highlighted the importance of this project to society as a whole;
- At the onset of this CRP Meeting, collaboration has been established among participants and has matured to provide more opportunities for exchanges of capabilities;
- Exchange of researchers, fellowships, PhD students have been accomplished within the framework of this CRP.

**RECOMMENDATIONS**

- The participants recommend that the irradiation resistance of packaging materials be tested at doses appropriate for use in each country;
- Additional research is needed to exploit the stable isotopic technique for determining leachates from packaging materials;
- Research on bacterial radio-sensitization (to reduce absorbed dose) is needed to address removal of pathogens and insects in food;
- Further research on food-borne viruses and bacteria is needed as related to food coating and packaging materials;
- Focus groups on specific topics related to food packaging materials need to be formed to synergize efforts;
  - Collaborative efforts can be expanded further through sharing of resources, equipment, and expertise:
    - USA – e-beam processing at different conditions and microbiological studies on the materials;
    - Poland – gamma or e-beam irradiation, diffuse reflection spectroscopy (DRS);
    - Canada – gamma irradiation, microbiological analyses and compression molding;
    - Malaysia – gamma and e-beam irradiation;
    - Brazil – e-beam irradiation and plastics film preparation by blown extrusion process;
    - Bangladesh – gamma irradiation;
    - Italy – Permeability testing;
    - UK: Biopolymer characterization (GPC-MALLS);
- The CRP participants are invited to consider submitting manuscripts for a special issue on the area of food packaging in the International Journal of Biological Macromolecules and the Journal of Applied Polymer Science.
- Participants recommended that the third RCM will be organized either at Texas A & M University (USA), Italy (Naples) or Malaysia (Kuala Lumpur).

**PUBLICATIONS BY THE PARTICIPANTS**


Presentations during the Conferences and Meetings (published abstracts).


Oral Presentations

15. PILLAI S, SHAYNAFAR S (2014). A holistic approach of ensuring public health, animal health, and environmental health using electron beam as a bioprocessing technology platform, IFIBOP, 7-10 Sep, Lille, France.

1. LACROIX, M., The use of Poly(lactic acid)-Nanocrystalline Cellulose supramolecular composite films to improve the quality of food products. Fibre meeting, Cornwall, 15 Mai. 2013

16. LACROIX, M., Encapsulation of natural antimicrobial agents into Poly(lactic acid)-Nanocrystalline Cellulose (PLA-NCC) supramolecular composite films to improve the shelf life of food products. ArboraNano annual meeting, 18 April 2013, Edmonton


**Poster presentations**


**Accepted presentations for the nearest future (abstracts to be published):**

1. CIEŚLA K., ABRAMOWSKA A., BUCZKOWSKI M., GŁUSZEWSKI W; “Ionising radiation influence on the films formed in the starch-PVA- nanocellulose system”. 11th International Meeting on Ionising Radiation and Polymers IRAP 2014, to be held in Jeju, Korea, 5-9.10.2014 (accepted oral presentation D-0S1).

2. CIEŚLA K., SARTOWSKA B.: “Modification of the microstructure of the films formed by gamma irradiated starch examined by SEM”. 11th International Meeting on Ionising Radiation and Polymers IRAP 2014, to be held in Jeju, Korea, 5-9.10.2014 (accepted poster presentation 5P-4).

3. LACROIX, M., Overview of using gamma irradiation for grafting and crosslinking for the fabrication of active beads and packaging films. Int. meeting radiation applied on polymers (IRAP). Jeju, Korea. 5-9 October 2014.

4. LACROIX, M., Bioactive packaging, capsules and edible coating. Benefiq 2014, 24 September, Québec, Canada.


A study of morphological, thermal, rheological and barrier properties of poly(3-hydroxybutyrate-co-3-hydroxyvalerate)/ polylactide blends prepared by melt mixing and their durability under gamma irradiation exposure.

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1. INTRODUCTION

The design of biodegradable plastics is an appropriately eco-efficiency approach to enhance the environmental quality for many products, as well as to minimize the waste disposed in landfills. Different markets are found in the fields of biodegradable polymers, including packaging (trash bags, wrappings, loose-fill foam, food containers and laminated papers), disposable non-woven (engineered fabrics), hygiene products (diaper back sheets and cotton swabs), consumer goods (fast-food tableware, containers, egg cartons, razor handles and toys) and agricultural tools (mulch films and planters) [¹]. Unfortunately, the use of biodegradable polymers as bulk commodity materials is still restricted to few applications because of the strong cost-competition with cheaper petroleum-based polymers, and their limited thermo-mechanical properties [²].

Blends were explored as an alternative way of acquiring novel materials with desired properties. Polymer blending quite often is a very convenient industrial process since it provides tailored made materials excluding any synthetic stage [³]. For instance, by blending two polymers, an easily processable material may be obtained preserving the major properties of the moieties [⁴].

Individually PHBV and PLA polymers have serious disadvantages when compared to thermoplastics that are currently used. To address high costs and thermal instability, PLA and PHBV have poor processing properties and are brittle at room temperature [⁵]. Blends of PHBV with PLA were explored as an alternative way of achieving novel materials with desired properties. The blends of PHBV and PLA are environmental and ecologically sound polymers and give economical benefits and combined properties [⁶]. Recently, some publications on PHBV/PLA blends are reported in literature [⁷] and they are devoted mainly to characterization studies of morphology and properties. However, research is still needed in this direction.

Therefore, the first objective of this work is to report some experimental data on PHBV and PLA blends prepared by melt mixing through a comprehensive study of the structure, the morphology and the thermal, mechanical, rheological and barriers properties. The influence of each component on the other is evaluated and the structure/property relationships are studied at various PHBV/PLA blend compositions involving 75/25, 50/50 and 25/75 wt%, respectively.
To improve the miscibility between PHBV and PLA, low amount of compatibilizing agent (5 wt. %), obtained by grafting maleic anhydride onto PHBV in combination with organo-modified montmorillonite (OMMT) (3 wt. %) were added to the blend components. The resulting properties were correlated with the morphology observed for the different blends. The effects of gamma irradiation on PHBV/PLA blend properties were also investigated up to 100 kGy using FT-IR spectroscopy, TGA, DSC, SEM, nanoindentation measurements and size exclusion chromatography (SEC).

2. EXPERIMENTAL

2.1. MATERIALS USED

PLA was supplied in pellets form by NatureWorks under the trade name 7001D. The polymer has the following main properties: density = 1.25 g.cm\(^{-1}\), MFI = 6 g/10 min (210°C, 2.16 kg), \(T_g = 60^\circ\)C and \(T_m = 160^\circ\)C.

PHBV was manufactured by Tianan Biological Materials Co. Ltd. (China) and commercialized in pellets form under the trade name ENMAT Y1000P. According to the manufacturer, PHBV has the following properties: density = 1.25 g.cm\(^{-1}\), \(T_g = 8^\circ\)C and \(T_m = 165^\circ\)C. This grade has been comprehensively characterized in a recent paper [8].

Cloisite 30B (C30B) is an organically modified montmorillonite which is commercially available and was supplied by Southern Clay Products (Texas, USA). C30B is a montmorillonite modified with bis-(2-hydroxyethyl) methyl tallow alkyl ammonium cations. C30B was dried under vacuum at 60°C for at least 24 h before use.

Maleic anhydride (MA) and dicumyl peroxide were purchased from Sigma-Aldrich and used as received.

2.2. GRAFTING OF MA ONTO PHBV

The grafting of MA onto PHBV was carried out according to the process described by Salim et al. [9]. Maleated PHBV was prepared by mixing 48.5 g of PHBV with 1.5 g of MA and 0.75 g of dicumyl peroxide, at 180°C, in a Brabender Plasticorder mixer (model W 50 EHT) which have the following characteristics: chamber volume of 55 cm\(^3\), sample weight of 40-70 g, maximum torque of 200 N and maximum temperature equal to 500°C. The rotor speed was set at 30 rpm for 3 min in the early stage of the blending, and was increased progressively to 40 rpm for 5 min when MA and dicumyl peroxide were added. Finally, the blended samples were collected and dried under vacuum at 100°C to remove the non-reacted MA.

2.3. PREPARATION OF PHBV/PLA BLENDS AND PHBV/PLA/C30B NANOCOMPOSITES

Prior to use, all materials were dried under vacuum at 60°C for 24 h. Drying is necessary to minimize the hydrolytic degradation of the polymers during the melt processing in the extruder. The different samples were prepared by melt mixing in the mixer previously described. Different formulations based on PHBV/PLA blend with 3 and 5 wt. % of C30B contents were prepared. The compatibilized PHBV/PLA/C30B nanocomposites were prepared by adding 5 wt. % of PHBVMA. The major processing parameters were mixing temperature, screw speed and residence time; they were set at 180°C, 50 rpm and 8 min, respectively.
2.4. TECHNICAL CHARACTERIZATION

2.4.1. Fourier Transform Infrared Spectroscopy (FT-IR)
FT-IR spectra of various film samples based on PHBV and PHBV-g-MA were recorded using a FT-IR spectrometer (Shimadzu 8400 M) using 4 cm⁻¹ resolution and 40 scans. All spectra were recorded in the transmittance mode in the 4000-400 cm⁻¹ region.

2.4.2. Differential Scanning Calorimetry (DSC)
DSC analyzes were performed on weighted samples of about 10 mg, using a calorimeter (Mettler-Toledo DSC-882). The samples were first heated from -40 to 200°C at a heating rate of 10°C.min⁻¹ under nitrogen atmosphere and maintained at this temperature for 2 min to eliminate thermal history. The samples were then cooled to -40°C at a cooling rate of 10°C.min⁻¹. Then the samples were reheated to 200°C at 10°C.min⁻¹ so that the melting could be studied.

2.4.3. Scanning Electron Microscopy (SEM)
Morphologies were observed with a scanning electron microscope (Jeol JSM-6031) to examine the fracture surface of the samples. The neck region for the broken specimens fractured in liquid nitrogen is parallel to the draw direction in order to reveal the internal morphology. Prior to observation, the fracture surfaces were coated with a thin gold layer by means of a polaron sputtering apparatus (Polaron E5100).

2.4.4. Wide Angle X-Ray Scattering (WAXS)
The WAXS measurements were performed at room temperature using advance diffractometer equipment (Bruker AXS D8), operating at the CuKα radiation (wave-length λ = 0.154 nm) for 40 kV and 40 mA. The 2θ scan range was used from 2° to 10° at a scan speed of 40 step/s.

2.4.5. Rheological measurements
Oscillatory shear measurements were performed using a controlled stress rheometer (Anton Paar Rheometer MCR 301) equipped with parallel disks of 25 mm diameter using a gap of 1.5 mm. Sample disks were vacuum dried at 60°C for 24 hours prior to testing. Strain sweep viscoelastic tests were first performed at a fixed angular frequency of 1 HZ in order to determine the extent of the linear regime; then, frequency sweep experiments were carried out at a fixed strain in the linear regime in order to determine the linear viscoelastic moduli, G' and G'', as well as the complex viscosity η*. The angular frequencies were swept from 100 to 0.01 HZ with five points per decade at temperatures of 175. All rheometrical data obtained were shown to be reproducible within ±5%.

2.4.6. Thermogravimetric analysis (TGA)
TGA experiments were carried out in a thermal analyzer (Setaram TGDTA 92-10) using a scanning rate of 10°C.min⁻¹ under nitrogen in the temperature range starting from 20°C up to 600°C.

2.4.7. Tensile testing
The static tensile tests were carried out in a laboratory where the temperature was 23°C and the humidity was 48% according to ISO 527 using a testing apparatus (MTS Synergie RT1000). The loading speed was 1 mm.min⁻¹. An extensometer was used with a nominal gauge length of 25 mm. The dumbbell-shaped samples with a dimension of 75×4×1 (mm)³ were stamped from the compression molded sheets, using a hydraulic press equipped with two heated plates at 180°C with a pressure of 30 bars for 3 min. The tests were carried out at least five times for each material and the results were averaged arithmetically.
2.4.8. Dynamic mechanical analysis (DMA)
The thermo-mechanical behavior of polymer samples has been investigated using a dynamical mechanical analyzer (TA Instruments DMA 2980). The specimen was a thin rectangular strip with dimensions around 30×6×2 (mm)³ prepared from the compression molded sheets, using a hydraulic press equipped with two heated plates at 180°C with a pressure of 30 bars for 3 min. A temperature scan from -40°C up to 150°C was performed at the rate of 3°C.min⁻¹ while a dynamic tensile test was performed at a frequency of 10 Hz with amplitude of 10 μm.

2.4.9. Oxygen permeability test
The oxygen permeability tests were performed using an Oxysense 4000B device equipped with a film permeation chamber. All experiments were performed at 23°C and 50% relative humidity. The permeation chamber consists of a cylinder divided in two parts by the investigated film. One of these is instrumented with an UV sensor sensitive to the oxygen ratio. This last chamber is purged with nitrogen while the other is kept open to air. The experimental procedure consists of monitoring the oxygen uptake with time. Oxysense OTR software uses this oxygen evolution to determine the oxygen transmission rate (OTR). As the OTR value is relative to both thickness and partial pressure between rich and poor oxygen atmosphere, results were normalized using Equation (1):[8]

\[ OP = \frac{OTR \times e}{\Delta P} \]  

Where OP is the oxygen permeability coefficient (mL.m⁻².s⁻¹.Pa⁻¹), OTR is the oxygen transmission rate (mL.m⁻².s⁻¹), e is the film thickness (m) and ΔP is the oxygen partial pressure difference (Pa). In our experimental setting, the oxygen ΔP has the value of 0.21 atm, i.e. 2.127 × 10⁴ Pa.

2.4.10. Water vapor permeability test
The water vapor permeability of the film samples was studied using the “Cups methods” referring to ISO 7783. The experimental setting consists of a cylindrical vessel filled with a desiccant powder and sealed with the investigated film. For our test, 10g of CaCl₂ were used as the desiccant powder, while the temperature was set to 23°C with a relative humidity of 50%. This method consists of monitoring the water mass uptake of the desiccant powder with time. The water vapor transmission rate (WVTR) is then calculated from the slope of the mass uptake profile versus time as soon as the steady state is reached using Equation (2):[8]

\[ WVP = \frac{WVTR \times e}{\Delta P} \]  

Where WVP is the water vapor permeability coefficient expressed in g.m⁻².s⁻¹.Pa⁻¹, WVTR is the water vapor transmission rate in g.m⁻².s⁻¹, e is the film thickness in m and ΔP is the water vapor partial pressure difference in Pa. From the experimental conditions, the water vapor ΔP is 1.4 ×10³ Pa calculated at temperature of 23°C and 50% as relative humidity.

2.4.11. Molecular weight measurements by SEC
Size exclusion chromatography (SEC) was used to determine the evolution of molecular weight. The apparatus is equipped with a set of three columns: two ResiPore and one PL gel Mixed C (Polymer Labs.). The detection system is composed of a refractometer and a UV detector. Chloroform was used as eluant with a flow rate of 0.8 mL/min. The elution profiles were analyzed by the Empower SEC module software (Waters). Calculations are based on calibration curves obtained from polystyrene standards ranging from 200 g/mol up to 6 ×10⁶. The weight-average molecular weight $\bar{M}_w$ and number-average molecular weight $\bar{M}_n$ are
obtained from the SEC analysis. The polydispersity index (PDI) was calculated as \( \frac{M_w}{M_n} \).

Moreover, the average number of random chain scissions per unit mass \( (n_t) \) is calculated according to the following Eq. (3):

\[
n_t = \frac{1}{M_n} - \frac{1}{M_n(0)}
\]

in which \( n_t \) represents the number of chain scission at a given processing cycle, while \( M_n(0) \) and \( M_n(t) \) are the number average molecular weight of samples after the initial and a given processing cycles, respectively.

3. RESULTS AND DISCUSSION

3.1. Study of properties of PHBV/PLA blends prepared by melt compounding

3.1.1. Morphological analysis by SEM

Figure 1 shows the SEM micrographs of the fracture surface of neat PHBV, neat PLA and various PHBV/PLA blends. Neat PHBV presented in Figure 1(a) shows an irregular fracture surface due to its crystalline structure, whereas neat PLA in Figure 1(b) exhibits a smooth and uniform surface of an amorphous polymer containing some holes resulting probably from processing. Figures 1(c), (d) and (e) show the fracture surface of 75/25, 50/50 and 25/75 wt% of PHBV/PLA blends, respectively. The higher the PHBV content, the rougher is the surface of fracture. By comparison of the micrographs of all blends with those of neat biopolymers, it seems that this surface roughness could be due to the phase separation between PHBV and PLA.[10] In Figure 1(c), the PHBV phase is easily seen, and PLA domains appear as beads, whose size and distribution are very irregular. This is an indication that PHBV and PLA form mainly a two phase-system, while in 25/75 blend, shown in Figure 1(e), the phase is formed by PHBV and the inclusions (bead-shaped), are PLA domains. Blends with phases being in more or less equal proportions should make a co-continuous morphology. This seems to be the case for 50/50 blend as illustrated in Figure 1(d).
3.1.2. Thermogravimetric analysis (TGA)

The thermal stability of neat polymers and different blend compositions was evaluated by TGA under nitrogen atmosphere. From Figure 2, it is observed that the degradation of each polymer occurred separately. As expected, PLA is more thermally stable than PHBV. In general, the thermal weight-losses of PHBV and PLA were quite sensitive to temperature, with narrow decomposition temperature ranges [11]. The temperatures corresponding to the different stage of decomposition (5%, 10% and 50% for instance) for a polymer are essential for evaluating its thermal stability and are summarized in Table 1. According to Table 1, the degradation temperatures at 5%, 10% and 50% loss shift to higher values, i.e. 332, 341 and 362 °C for the neat PLA in comparison with those of the neat PHBV which are 268, 272 and 284°C, respectively. All the decomposition temperatures of the blends are between those of PLA and PHBV. The thermal stability of the blends is regularly improved by the addition of PLA into the blend. This is due to the higher thermal stability of PLA. Further, all samples have less than 1 wt. % residue at 600°C suggesting that no additive is present in both polymers. TGA thermograms show that PLA and PHB degrade in only one step.

<table>
<thead>
<tr>
<th>Samples</th>
<th>T5% (°C)</th>
<th>T10% (°C)</th>
<th>T50% (°C)</th>
<th>Char yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHBV</td>
<td>268</td>
<td>272</td>
<td>284</td>
<td>1.2</td>
</tr>
<tr>
<td>PHBV/PLA 75/25</td>
<td>273</td>
<td>278</td>
<td>290</td>
<td>1.2</td>
</tr>
<tr>
<td>PHBV/PLA 50/50</td>
<td>275</td>
<td>279</td>
<td>305</td>
<td>1.1</td>
</tr>
<tr>
<td>PHBV/PLA 25/75</td>
<td>287</td>
<td>291</td>
<td>353</td>
<td>0.9</td>
</tr>
<tr>
<td>PLA</td>
<td>332</td>
<td>341</td>
<td>362</td>
<td>0.3</td>
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</table>
Table 2 reports in details the values of $T_g$ for both neat polymers and various PHBV/PLA blends. The data given in Table 2 indicate that $T_g$ of PHBV shifts slightly to higher values as the PLA content increases in the blend. On the contrary, it is observed a decrease in the values of $T_g$ for PLA in the blends with increased PHBV content ratio. This decrease is most likely due to interactions between the amorphous and crystalline regions in the blend and probably to an increase in mobility resulting from the increase in PHBV content.[12]

**Table 2.** $T_g$ values of PHBV/PLA blends determined by DSC

<table>
<thead>
<tr>
<th>Samples</th>
<th>$T_g,_{PLA}$ by DSC [°C]</th>
<th>$T_g,_{PHBV}$ by DSC [°C]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHBV</td>
<td>-</td>
<td>2.8</td>
</tr>
<tr>
<td>PHBV/PLA 75/25</td>
<td>57.1</td>
<td>3.0</td>
</tr>
<tr>
<td>PHBV/PLA 50/50</td>
<td>57.9</td>
<td>3.3</td>
</tr>
<tr>
<td>PHBV/PLA 25/75</td>
<td>58.4</td>
<td>4.5</td>
</tr>
<tr>
<td>PLA</td>
<td>59.8</td>
<td>-</td>
</tr>
</tbody>
</table>

**3.1.3. Thermal properties**

The values of the melting characteristics are presented in Table 3. It is observed that the crystallization of PHBV is restricted by the presence of PLA phase. The number of heterogeneous primary nuclei of PHBV may decrease because of their migration from PHBV to PLA. Another possible reason may be due to a decrease in the crystallization growth rate, which can result from the dilution effect of PLA melt, thus reducing the amount of PHBV chain segments toward the growing crystals.[13] It is therefore reasonable to speculate that PHBV forms small-dispersed crystals during the cooling process, which may act as nucleation sites for PLA.

**Table 3.** Thermal characteristics of PHBV/PLA blends determined by DSC.

<table>
<thead>
<tr>
<th>Samples</th>
<th>$T_c$ [°C]</th>
<th>$\Delta H_c$ [J/g]</th>
<th>$T_{m,PLA}$ [°C]</th>
<th>$T_{m,PHBV}$ [°C]</th>
<th>$\Delta H_{m,PLA}$ [J/g]</th>
<th>$\Delta H_{m,PHBV}$ [J/g]</th>
<th>$X_c,_{Blend}$ [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHBV</td>
<td>109.3</td>
<td>-22.8</td>
<td>-</td>
<td>147.0</td>
<td>-</td>
<td>51.6</td>
<td>47.3</td>
</tr>
<tr>
<td>PHBV/PLA 75/25</td>
<td>107.1</td>
<td>-20.0</td>
<td>151.0</td>
<td>173.1</td>
<td>16.3</td>
<td>45.6</td>
<td>35.1</td>
</tr>
<tr>
<td>PHBV/PLA 50/50</td>
<td>105.6</td>
<td>-17.2</td>
<td>153.2</td>
<td>172.8</td>
<td>17.8</td>
<td>39.3</td>
<td>26.2</td>
</tr>
<tr>
<td>PHBV/PLA 25/75</td>
<td>101.3</td>
<td>-12.3</td>
<td>152.6</td>
<td>170.1</td>
<td>10.0</td>
<td>4.1</td>
<td>7.8</td>
</tr>
<tr>
<td>PLA</td>
<td>-</td>
<td>-</td>
<td>154.0</td>
<td>-</td>
<td>17.8</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**3.1.4. Oxygen permeability**

Gas barrier properties of PHBV/PLA blends were investigated using oxygen and water vapor permeation tests. Evaluations were performed on extruded films with thicknesses between 100 and 150 mm. Samples were stored at 23°C and 50% RH for at least 2 weeks before test. The resulting data are depicted in Figure 3(a). PHBV features an oxygen barrier capacity up to more than PLA; the semi-crystalline nature of PHBV should be the main factor explaining this difference. PHBV is the most crystalline and seems to be quite pure considering the DSC analysis. In this way, it logically features the higher oxygen barrier properties. PHBV show
good oxygen barrier properties, the property barrier of PLA is improved with increased PHBV ratio in the blends. In contrast, the value of oxygen permeability coefficient of PLA decreases by about 35.3, 43.2 and 81.5% with the addition of 25, 50 and 75 wt% of PHBV in the blend, respectively. This shows clearly the role of PHBV as an efficient barrier promoter for PLA, even at low content.

3.1.5. Water vapor permeability
Crystals in a polymer reduce the water transmission due to their small cross-sectional and low permeability restricting chain mobility, thus lowering water permeability. The resulting WVP data are presented in Figure 3(b). From Figure 3(b), it is observed that increasing the PHBV content has a positive effect on water vapor barrier properties. PHBV/PLA blends show intermediate water vapor barrier properties. On the other hand, the value of WVP permeability of PLA decreases by about 22.7, 36.6 and 58.9% by addition of 25, 50 and 75 wt% of PHBV, respectively. It means that the incorporation of PHBV is more effective to improve the barrier properties of PLA, even at quite low ratio. The decrease of the gas permeability with crystallinity is generally explained in terms of two factors. The first one is the inclusion of impermeable crystallites which decreases the amount of amorphous phase through which the gas molecules can permeate. The second one is that impermeable crystals increase the tortuosity of the transport path.

![Figure 3. Effect of PHBV content on oxygen permeability (OP): (a) and water vapor permeability (WVP): (b) of PHBV/PLA blends.](image)

3.1.6. Rheological measurements
Figure 4 shows the variation of complex viscosity vs. frequency for neat PHBV, neat PLA and their blends at various compositions at 175°C. The plots in Figure 4 indicate a decrease in complex viscosity with increasing rotational frequency due to the duration of experiment. The neat PHBV and PLA samples exhibit a non-Newtonian flow profile with a plateau at low frequencies and shear thinning behavior when the frequency increases. It is observed in Figure 4 that the complex viscosity is significantly higher for pure PLA than PHBV. However, PHBV shows a very impressive increase of the complex viscosity with increased the PLA content in the blend. An increase in viscosity is largely due to an increase in molecular weight of the polymer. Another factor which may contribute to the increased viscosity of PHBV is an increase in its elasticity. For the blend compositions of 75/25 and 50/50, one can notice clearly
an increase in complex viscosity at low frequencies which can be explained by the fact that PHBV forms small-dispersed crystals and very small droplets, well dispersed in PLA.

Figure 4. Complex viscosity of PHBV, PLA and their blends at 175°C under 2% of dynamical shear strain.

3.2. SYNERGESTIC EFFECTS OF PHBV-G-AM AND ORGANOMODIFIED MONTMORILLONITE (OMMT) ON COMPATIBILITY OF PHBV/PLA BLENDS

3.2.1. Morphology
Figures 5(a), (b) and (c) show SEM micrographs of the fracture surface of PHBV/PLA 25/75, PHBV/PLA 25/75 at clay loading of 3 wt%, without and with PHBV-g-MA compatibilizer, respectively.

Figure 5. SEM micrographs of fracture surface of PHBV/PLA 25/75 (a), PHBV/PLA 25/75 with OMMT (3%) (b), PHBV/PLA 25/75 with OMMT (3%) and PHBV-g-MA (5%) (c).
By adding only 3 wt. % of OMMT, the dispersed phase becomes more deformed and less discernible and the morphology is rather co-continuous. It is not easy to distinguish the phases and the nearly phase-separated domains are not visible. The morphology can be considered as relatively homogeneous at this magnification, suggesting a change in the miscibility between the two blend components in the presence of OMMT. The SEM image of the fracture surface of PHBV/PLA: 25/75 at a clay loading of 3 wt. % with PHBV-g-MA reveals that the compatibilizer incorporation into the PHBV/PLA blend nanocomposites changes the co-continuous to dispersed-type morphology. This can be attributed to the reduction of interfacial tension of the system.

The droplet sizes are noticeably reduced and this reduction in droplet size may be attributed to the formation of an interphase resulting from the migration of PHBV-g-MA to the interfacial area. This induces a relatively homogeneous morphology. These results indicate the efficiency of OMMT and PHBV-g-MA in improving the interfacial adhesion and the miscibility between PHBV and PLA.

3.2.2. Wide angle X-ray diffraction (WAXS)
WAXS patterns of OMMT powder and PHBV/PLA blend nanocomposites with PHBV-g-MA compatibilizer are presented in Figure 6.

![Figure 6. WAXS patterns of OMMT and PHBV/PLA blends with of OMMT (3%) and PHBV-g-MA (5%).](image)

The WAXS pattern of OMMT shows a diffraction peak at 4.8°, corresponding to a basal spacing. The d-spacing values (basal distance between clay layers) were calculated using Bragg's law ($\lambda = 2dsin\theta$; $d$ is the interlayer d-spacing and $\lambda$ is the wave length). The $d_{001}$ peak of OMMT at $2\theta = 4.8^\circ$, corresponds to an interlayer spacing of 1.8 nm. No peak is observed in the WAXS patterns between 2 and 10° for the obtained PHBV/PLA blend nanocomposites with PHBV-g-MA compatibilizer, probably suggesting the formation of nanocomposite structures more or less exfoliated.

3.2.3. Thermal characteristics by DSC
The thermal characteristics of the nanocomposites PHBV/PLA/OMMT can be significantly affected by the crystallization characteristics of PHBV and PLA. Indeed, Table 4 summarizes the thermal data obtained for all the samples through DSC experiments during a cooling scan and a second heating cycle. The main thermal parameters are as follows: the crystallization
temperature ($T_c$), the crystallization enthalpy ($\Delta H_c$), the glass transition temperature ($T_g$) and the cold crystallization temperature ($T_{cc}$). The melting process is characterized by the melting temperature ($T_m$). Regarding the glass transition, two $T_g$ are observed for the whole samples. This indicates phase immiscibility between PLA and PHBV. Moreover, it is noticeable that a $T_g$ depression occurs for the nanocomposites compared to the non reinforced ones. This may be due to the fact that OMMT has a significant influence on the chain flexibility and intermolecular attraction of PHBV/PLA blend [14]. However, the determination of $T_g$ of PHBV in the nanocomposite blends compatibilized or not, was difficult according to the chosen heating rate. $T_{cc}$ shifts to lower temperature for the uncompatibilized and compatibilized nanocomposites. This indicates that the clay particle acts as nucleating agent increasing the crystallization rate of PHBV/PLA blend. The clay particles seem to promote the mobility of PHBV/PLA chains and the rearrangements of the macromolecular chains during crystallization. The melting temperatures relative to PHBV and PLA slightly decrease for all the samples with the addition of OMMT and PHBV-g-MA compatibilizer indicating that the thermal behaviors of the blends are influenced by the presence of clay. Furthermore, $\Delta H_{cc}$ and $\Delta H_m$ values (not reported in Table 4) significantly increase with the addition of OMMT and PHBV-g-MA compatibilizer. It suggests that the clay favors the nucleation process considering that blend/clay interactions would induce a slight delay either into the melting or into the crystallization process.

### TABLE 4. Thermal characteristics of neat PHBV, neat PLA, and various PHBV/PLA blends.

<table>
<thead>
<tr>
<th>Samples</th>
<th>OMMT (wt. %)</th>
<th>PHBVM (wt. %)</th>
<th>$T_{c,PHBV}$ ($^\circ$C)</th>
<th>$T_{cc,PLA}$ ($^\circ$C)</th>
<th>$T_{g,PHBV}$ ($^\circ$C)</th>
<th>$T_{g,PLA}$ ($^\circ$C)</th>
<th>$T_{m,PHBV}$ ($^\circ$C)</th>
<th>$T_{m,PLA}$ ($^\circ$C)</th>
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</thead>
<tbody>
<tr>
<td>PHBV</td>
<td>-</td>
<td>-</td>
<td>118.3</td>
<td>-</td>
<td>2.8</td>
<td>-</td>
<td>174.0</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>119.8</td>
<td>-</td>
<td>1.1</td>
<td>-</td>
<td>172.0</td>
<td>-</td>
</tr>
<tr>
<td>PHBV/PLA 75/25</td>
<td>-</td>
<td>-</td>
<td>116.7</td>
<td>119.9</td>
<td>3.0</td>
<td>57.1</td>
<td>173.1</td>
<td>151.2</td>
</tr>
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<td>-</td>
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<td>55.8</td>
<td>172.0</td>
<td>150.1</td>
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<td>115.6</td>
<td>125.1</td>
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<td>172.8</td>
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<td>-</td>
<td>57.0</td>
<td>-</td>
<td>151.2</td>
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</tr>
</tbody>
</table>

### 3.2.4. Thermogravimetric analysis (TGA)

TGA was performed on PHBV/PLA blends and PHBV/PLA/OMMT nanocomposites to determine the influence of the addition of clay on the thermal stability. The thermal stability of neat PHBV, neat PLA and PHBV/PLA blends systematically increases with the addition of 3 wt. % of OMMT. For all nanocomposite samples, an increase by 6-12°C of the temperature at 5% weight loss and by 5-14°C of the temperature at 10% weight loss can be noticed when comparing with the corresponding blends. Furthermore, a significant increase of the temperature at 50% weight loss is observed in the presence of OMMT. This increase can be
associated to the presence of the nanodispersed clay phase which appears as effective barriers to heat and volatile compound diffusion and thus can counterbalance the degradation effect.
TABLE 5. TGA results of neat PHBV, neat PLA, PHBV/PLA blends and PHBV/PLA/C30B

<table>
<thead>
<tr>
<th>Samples</th>
<th>OMMT (wt.%)</th>
<th>T_{degrad} 5% (°C)</th>
<th>T_{degrad} 10% (°C)</th>
<th>T_{degrad} 50% (°C)</th>
<th>Char at 600°C (%)</th>
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<td>PHBV</td>
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<td>290</td>
<td>1.2</td>
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<td>301</td>
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<tr>
<td>PLA</td>
<td>-</td>
<td>332</td>
<td>341</td>
<td>362</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>344</td>
<td>355</td>
<td>378</td>
<td>2.2</td>
</tr>
</tbody>
</table>

3.2.5. Tensile measurements

The main mechanical results are reported in Table 6. It is obvious that the incorporation of 3 wt. % of OMMT induces significant change in the mechanical properties of the PHBV/PLA blend. The addition of 3 wt.% of OMMT leads to an improvement in the Young’s modulus of PHBV/PLA blends equal to 16, 11 and 14 % for PHBV/PLA 75/25, 50/50 and 25/75, respectively. It is believed that the enhancement in the Young’s moduli of blends may be due to the formation of supramolecular assemblies obtained by the presence of dispersed anisotropic nanoplatelets [15]. Furthermore, the Young’s moduli of nanocomposite blends with 5 wt. % of PHBV-g-MA compatibilizer increase up to 27, 20 and 23 % in PHBV/PLA 75/25, 50/50 and 25/75 wt. %, respectively. The intensities of these polymer/clay interactions are higher in the case of compatibilized nanocomposites comparatively to uncompatibilized ones. The stronger interfacial interaction between the blend and the silicate layers induces a more pronounced improvement of the modulus value in the presence of 5 wt. % of PHBV-g-MA which favors the expansion of the gallery space of the reinforcing nanoclay by inclusion of polar groups intercalating between the clay layers through hydrogen bonding with the oxygen groups of clay [16-18].

The elongation at break of the nanocomposite blends is less as compared to their non reinforced blends, independently of the compatibilizer presence. The filler presence may increase the stress concentration, which causes the composite to fail in a brittle manner resulting in the decrease of the elongation at break of the nanocomposite as compared to their pristine counterpart [16].

Concerning the evolution of the maximal stress at break which expresses the ultimate strength that the material can bear before break, the differences observed are sufficiently notable to draw some comments. The relationships between tensile stress, compatibility of blend components, filler/matrix interaction and dispersion are more complex than for the modulus, so no attempt is made at this time to justify the result with quantitative models [19].
3.2.6. Rheological properties

The embedding of nano-size clay particles into polymer matrices changes their rheological properties. The filler-polymer and the filler-filler interactions lead to an increase in the complex viscosity, particularly at low frequencies, and to a more pronounced shear-thinning behavior. Moreover, the matrix molecular weight and the degree of clay dispersion strongly affect the rheological properties of the nanocomposite samples [20]. The region of linear viscoelastic behavior of polymers is greatly modified in the presence of the intercalated clay [21]. To study the OMMT effect with and without PHBV-g-MA on the rheological properties of PHBV/PLA blends, the complex viscosity, the storage modulus (G') and the loss modulus (G'') were investigated as a function of frequency.

Figure 7 summarizes the results obtained for neat PHBV, PHBV/PLA 25/75 blend, PHBV/PLA 25/75 nanocomposite with 3 wt. % of OMMT and PHBV/PLA 25/75 nanocomposite with 3 wt. % of OMMT and previously compatibilized. The incorporation of OMMT in PHBV/PLA blend leads to a very impressive increase of the complex viscosity (Figure 7(a)), the storage modulus (Figure 7(b)) and the loss modulus (Figure 7(c)). The comparison of the viscoelastic response of the materials shows the significant effect of the organoclay, particularly at low frequencies and this effect becomes even more substantial when 5 wt.% of PHBV-g-MA compatibilizer is used.

It is observed that the melts of PHBV/PLA/OMMT nanocomposite exhibit higher G' compared than those of both neat PHBV and PHBV/PLA blend. This increase becomes more intense when the PHBV-g-MA compatibilizer is added to the PHBV/PLA/OMMT nanocomposite. At high frequencies, however, the G' of all the sample melts come close together, except for the neat PHBV. The reason for the increase in G' may arise from the confinement of polymer chains within the OMMT layers. Indeed, the large increase of G' observed for PHBV/PLA/OMMT nanocomposite compatibilized with 5 wt.% of PHBV-g-MA melts at low frequencies with the formation of a quasi-plateau is generally interpreted as the result of both the confinement effect and the interparticle interactions [21]. The interparticle interactions come from frictional interactions between the tactoids which are predominant at low frequencies. This suggests a pseudo-solid behavior for the compatibilized
nanocomposite. This is pictured as a well structured nanocomposite revealing the good dispersion of the platelets in the matrix [22].

3.3. GAMMA IRRADIATION EFFECTS ON THE MORPHOLOGY AND THE FUNCTIONAL PROPERTIES OF PHBV/PLA BLENDS

3.3.1. FT-IR spectra analysis

The material usefulness depends on their durability in a particular environment in which materials are used and their interaction with environmental factors. In this connection, the changes in the chemical structure induced by gamma irradiation exposure of neat biopolymers and various PHBV/PLA/OMMT nanocomposites with PHBV-g-MA were determined using FT-IR spectroscopy. Representative FT-IR spectra of non-irradiated and irradiated PHBV/PLA/OMMT samples with compatibilizer at 25 and 100 kGy, recorded in the hydroxyl and carbonyl regions are shown in Fig. 8(a) and (b), respectively. From Figure 8(a), it is observed that the FT-IR spectrum of the non-irradiated sample exhibits large absorption bands
Figure 8. FT-IR spectra of PHBV/PLA/C30B/PHBV-g-MA before and after 25 and 100 kGy of exposure recorded in the domains: (a): 3600 – 3400 cm\(^{-1}\) and (b): 1900 – 1500 cm\(^{-1}\).

centered at 3505 and 3438 cm\(^{-1}\), which correspond to OH groups in alcohols and hydroperoxides. After exposure to gamma irradiation, these bands become stronger in intensity with absorbed doses. In the region 1900 – 1500 cm\(^{-1}\) as shown in Figure 8(b), significant changes in the chemical structure have occurred on exposure the sample to gamma irradiation up to 100 kGy. At this absorbed dose, it is observed the disappearance of the main absorption bands localized at 1750 and 1700 cm\(^{-1}\) and the formation of a large absorption band involving various carbonyl products at 1770, 1745 and 1698 cm\(^{-1}\) corresponding to perester, ester and carboxylic groups, respectively. This indicates that gamma irradiation of PHBV/PLA/OMMT with the compatibilizer leads to oxidation reactions of ester groups inducing the formation of hydroxyl groups which can be present in hydroperoxides or alcohols. [23] Similar conclusions are deduced for PHBV/PLA blend samples in comparison with neat polymers, i.e. PHBV and PLA.

3.3.2. Molecular weight changes by SEC

The changes in weight average molecular weight (\(\overline{M}_w\)), number average molecular weight (\(\overline{M}_n\)) and polydispersity index (\(\overline{M}_w/\overline{M}_n\)) taking place during gamma irradiation exposure of neat PHBV, neat PLA and various PHBV/PLA blends are shown in Table 7, which provides the values of \(\overline{M}_w\) and \(\overline{M}_n\) for 50 and 100 kGy. It is observed that the average molecular weight of all irradiated samples decreases with increased absorbed doses, which is synonymous of the occurrence of polymer degradation. Indeed, after 50 and 100 kGy, \(\overline{M}_w\) of PHBV decreases by almost 55 and 73%, respectively. Further, the significant decrease in both \(\overline{M}_w\) and \(\overline{M}_n\) of irradiated PHBV, samples associated to an increased polydispersity index, confirms the predominance of chain scission mechanism as a result of degradation. Similar trend is also observed for \(\overline{M}_w\) and \(\overline{M}_n\) of PLA which shifts toward smaller values. For PHBV/PLA blend, it is obvious that all samples exhibit also a significant reduction in both molecular weights \(\overline{M}_w\) and \(\overline{M}_n\) with irradiation dose, except PHBV/PLA blend which appears to be almost stable up to 50 kGy.
3.3.3. Thermogravimetric analysis (TGA)
The effects of gamma irradiation on the thermal stability of neat PHBV, neat PLA and various PHBV/PLA blends in the presence of both OMMT and compatibilizer are shown in Table 8 where the values of $T_{5\%}$, $T_{10\%}$, $T_{50\%}$ and percent residue are given after absorbed dose of 100 kGy.

**TABLE 8.** Values of $T_{5\%}$, $T_{10\%}$, $T_{50\%}$ and residue at 600°C

<table>
<thead>
<tr>
<th>Samples</th>
<th>Doses (kGy)</th>
<th>$T_{5%}$ (°C)</th>
<th>$T_{10%}$ (°C)</th>
<th>$T_{50%}$ (°C)</th>
<th>Résidu (600°C) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHBV</td>
<td>0</td>
<td>265</td>
<td>270</td>
<td>283</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>267</td>
<td>272</td>
<td>285</td>
<td>1.2</td>
</tr>
<tr>
<td>PLA</td>
<td>0</td>
<td>332</td>
<td>341</td>
<td>362</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>311</td>
<td>329</td>
<td>361</td>
<td>0.3</td>
</tr>
<tr>
<td>PHBV/PLA</td>
<td>0</td>
<td>276</td>
<td>280</td>
<td>302</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>275</td>
<td>280</td>
<td>302</td>
<td>0.5</td>
</tr>
<tr>
<td>PHBV/PLA/OMMT</td>
<td>0</td>
<td>286</td>
<td>291</td>
<td>317</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>284</td>
<td>289</td>
<td>310</td>
<td>2.6</td>
</tr>
<tr>
<td>PHBV/PLA/OMMT/PHB-V-g-MA</td>
<td>0</td>
<td>286</td>
<td>290</td>
<td>315</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>282</td>
<td>287</td>
<td>310</td>
<td>2.6</td>
</tr>
</tbody>
</table>

From Table 8, it appears clearly the effect of gamma irradiation up to 100 kGy is almost negligible for neat PHBV and various PHBV/PLA blends. Only, PLA is significantly affected since $T_{5\%}$ and $T_{10\%}$ are reduced by – 21 and -12°C, respectively.
3.3.4. Nanoindentation measurements

<table>
<thead>
<tr>
<th>Samples</th>
<th>Dose (kGy)</th>
<th>Modulus (GPa)</th>
<th>Hardness (GPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHBV</td>
<td>0</td>
<td>6.31 ± 0.5</td>
<td>0.220 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.10 ± 0.8</td>
<td>0.052 ± 0.01</td>
</tr>
<tr>
<td>PLA</td>
<td>0</td>
<td>4.32 ± 1.1</td>
<td>0.108 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.36 ± 0.9</td>
<td>0.082 ± 0.05</td>
</tr>
<tr>
<td>PHBV/PLA</td>
<td>0</td>
<td>5.53 ± 0.4</td>
<td>0.24 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>5.10 ± 0.5</td>
<td>0.21 ± 0.02</td>
</tr>
<tr>
<td>PHBV/PLA/OMMT</td>
<td>0</td>
<td>5.13 ± 0.7</td>
<td>0.13 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>5.86 ± 0.8</td>
<td>0.21 ± 0.04</td>
</tr>
<tr>
<td>PHBV/PLA/OMMT/PHBV-(g)-MA</td>
<td>0</td>
<td>5.51 ± 0.9</td>
<td>0.20 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>5.56 ± 0.5</td>
<td>0.19 ± 0.03</td>
</tr>
</tbody>
</table>

4. CONCLUSION

Biodegradable PHBV and PLA blends have been prepared by melt mixing. This compounding technique is easy and potentially commercially viable. It can be concluded that PHBV/PLA blends form a biphasic system over the whole composition range. This immiscibility is displayed by SEM and DSC analyzes. Furthermore, DSC data show an improvement in crystallization of PLA due to the positive effect played by PHBV crystals on the nucleation of PLA. PHBV provides better water and oxygen barrier properties to PHBV/PLA blends acting as an efficient barrier promoter for PLA, even at quite low ratio. The rheology of PHBV/PLA blends investigated in the dynamic mode reveals that PHBV exhibits a very impressive increase in complex viscosity with increasing the PLA content. It can be concluded that the production of blends based on PHBV and PLA can be an efficient and promising route to extend the applications as biodegradable materials, PHBV for instance, considering the possibility to finely tune their functional properties playing with the respective proportions of each component.

The incorporation of OMMT contributes to enhance the thermal stability of PHBV, PLA and PHBV/PLA blends, as usually observed when clay is homogeneously dispersed. Besides, PHBV/PLA/OMMT nanocomposites exhibit improved mechanical properties in terms of Young’s modulus, comparatively to PHBV/PLA blend, especially when PHBV is previously compatibilized. Last, the rheological study reveals a significant increase of the complex viscosity, the storage and the loss modulus by the addition of OMMT in the PHBV/PLA blend. This is particularly noticeable when samples are submitted to low shear frequency. Moreover, this increase in the rheological response is much more pronounced in the presence of a compatibilizer as PHBV-\(g\)-MA.

Biopolymers like PHBV or PLA often have inferior properties compared to commodity polymers. This paper highlights that biopolymer blending is an efficient and promising way to
improve properties and achieve some property combinations required for specific applications. The use of nanofillers and/or compatibilizers can constitute a supplementary approach to more finely tuned functional properties of biopolymer blends. The effects of oxidative degradation under gamma irradiation of neat PHBV, neat PLA and various PHBV/PLA: 50/50 blends result in drastic modifications in the chemical structure, and the average molecular weight of the materials after 100 kGy of exposure while thermal and nanomechanical properties were remained almost unchanged.

REFERENCES

PREPARATION OF BIODEGRADABLE FILM FROM NATURAL AND SYNTHETIC POLYMERS BY THE APPLICATION OF GAMMA RADIATION

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Nuclear and Radiation Chemistry Division, Institute of Nuclear Science and Technology, Atomic Energy Research Establishment, Savar, Dhaka, Bangladesh

1. Sodium alginate-polyethylene oxide (SA-PEO) blend films

Sodium alginate (SA)-Polyethylene oxide (PEO) blend films with glycerol as plasticizer were prepared by solution casting method. For the preparation of SA-PEO blend film, the concentration of PEO and glycerol were optimized. The SA-PEO blend films in presence and absence of methacrylate (MA) were irradiated by gamma radiation from Co-60 gamma source at room temperature (27ºC). The parameters like effect of radiation dose and concentration of MA as monomer were investigated. The properties such as tensile properties (Tensile strength and elongation at break) and thermal stability of SA-PEO blend films were examined. The tensile strength of SA-PEO blend films increased with increase in radiation dose and it attained a maximum value at 12.2 kGy absorbed dose. The maximum value of tensile strength of SA-PEO blend film was obtained from 3% MA treated film. The tensile strength of irradiated MA treated SA-PEO blend film was obtained 44.58% higher than that of irradiated SA-PEO blend films at 12.2 kGy absorbed dose. The thermal stability of SA-PEO blend films were investigated using thermo gravimetric analysis (TGA) and dynamic mechanical analyzer (DMA). The thermal stability of irradiated MA treated SA-PEO blend film was obtained higher than that of irradiated SA-PEO blend films.

Introduction

Present worldwide environmental consciousness strongly motivates the introduction of biodegradable materials instead of petroleum based synthetic polymers used for packaging materials. The petroleum based synthetic polymer possesses excellent mechanical and thermal properties and it is cost effectiveness. But most of these synthetic polymers are not biodegradable and environmental friendly. Due to worldwide environmental awareness, versatile efforts are in progress to develop biodegradable packaging materials that simultaneously will be cheap and possesses good mechanical and thermal properties. Such challenged materials can be fabricated from natural polymers. But major drawbacks of the materials are low mechanical properties. That is why, many studies are in progress to overcome these limitation1-4.
Sodium alginates are the salt of alginic acids. Alginic acid is a polysaccharide obtained from the various species of brown seaweeds (phaeophyceae). It is a natural polysaccharide. Alginic acid is a linear copolymer containing mainly of residues of 1, 4-linked β-D-mannuronic acid and α-1, 4-linked L-glucuronic acid. Sodium alginate is an abundant polysaccharide, which can be obtained from marine algae. It has been widely used in food, fabric, and medical fields because of its remarkable gelation properties, application in hemostatic materials. Moreover, blending of sodium alginate with other polymers creates a new application field for it.

Sodium alginate, a polyelectrolyte having rigid molecular chain, and good film forming ability, has been extensively exploited and studied in detail on biomedical applications as a drug carrier. Sodium alginate/carrageenan blend films were prepared and characterized and found that it could be worthy of using as membrane. Sodium alginate films were cured and grafted by silane with photo initiator using ultraviolet (UV) radiation. The results indicated that the sodium alginate films could be used as shopping bags.

Radiation processing is being applied in national economic of developed and developing countries. Sterilization of food products, medical products, cross-linking and grafting of polymeric materials etc, irradiation is the well-established technology in this field. In radiation processing technology initiator, catalyst and cross-linker are not required because ionizing radiation is high energetic. In last few years, considerable success has been achieved in modifying the natural polymers through radiation processing to meet specific applications. In the present study, modification of sodium alginate/polyethylene oxide blend films with methacrylate (MA) is carried out using gamma radiation to improve the mechanical and thermal properties of blend films.

**Experimental**

**Materials**

Sodium alginate (SA) was supplied by Uni-Chem, China. Methylacrylate (MA), glycerol and methanol were purchased from Merck, Germany. Polyethylene oxide (PEO) was obtained from Sigma Aldrich (Iceland).

**Preparation of sodium alginate/Polyethylene oxide blend films**

A 2.5% (w/v) solution of sodium alginate (SA) was prepared in distilled water at room temperature. A 2.5% (w/v) polyethylene oxide (PEO) solution was prepared in distilled water.
using an autoclave. SA/PEO blend solutions were prepared by mixing SA and PEO solutions in different composition. SA/PEO blend films were prepared from SA/PEO blend solutions by casting. To improve the properties of SA/PEO blend films, glycerol was added to SA/PEO blend solution in different composition.

Modification of SA/PEO blend films with methacrylate (MA)
SA/PEO blend films incorporated with glycerol were modified with MA by the application of gamma radiation from Co-60 at the dose rate of 3 kGy/h in presence of oxygen. The total dose was determined with the help of the Fricke dosimeter. 3, 5, and 7% (v/v) MA solutions were prepared in methanol (Table-1). The blend film was immersed in methanolic solution of MA in test tubes and then irradiated by gamma radiation. Radiation dose was varied from 3 to 15 kGy.

Table-1: Composition of different monomer formulation (v/v).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>MA (%)</th>
<th>Methanol (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M\textsubscript{1}</td>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>M\textsubscript{2}</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>M\textsubscript{3}</td>
<td>7</td>
<td>93</td>
</tr>
</tbody>
</table>

Tensile properties
Tensile properties of the films were measured by using Universal Testing Machine (Testometric, model M 500-100CT, UK) with a cross-head speed of 5 mm/min. The load range of 500N and the gauze length 20 mm were used throughout the experiment.

Thermogravimetric analysis
The thermal gravimetric analysis (TGA) was conducted on a Perkin-Elmer TGA 7-thermal analyzer from 30 to 600ºC with a heating rate of 10ºC/min. under nitrogen atmosphere with a flow rate of 20 mL/min.

Dynamic mechanical analysis
The thermal properties of films were studied from 27 to 160ºC at a rate 4ºC/min. and oscillating frequency of 1 Hz using dynamic mechanical analyzer (DMA), Triton technology TTDMA, UK.
Results and discussion

Preparation of SA-PEO blend films

SA-PEO blend films (thickness ~0.3 mm) were prepared by mixing SA and PEO solution in different composition by casting. Table-2 shows tensile strength and elongation at break of SA-PEO blend films. The tensile strength of blend films increases with increased amount of PEO in SA-PEO films and it attains a maximum value for 10% (v/v) PEO in SA-PEO films. After 10% PEO in SA-PEO blend film, the tensile strength decreases with increased amount of PEO in blend films. The SA-PEO blend film is obtained smooth with 10% PEO and more than 10% PEO, blend film becomes rough. The elongation at break of SA-PEO blend films decreases with increased amount of PEO in blend films. At 10% (v/v) PEO in blend film the elongation at break is 4.25%. In respect to tensile strength the blend films with 10% PEO can be considered as required amount of PEO for good SA-PEO blend film. Therefore, the rest of the work were performed the blend film with 10% (v/v) PEO.

Table-2: Tensile strength and elongation at break of SA-PEO blend film.

<table>
<thead>
<tr>
<th>SA/PEO composition</th>
<th>Tensile strength (MPa)</th>
<th>Elongation at break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100/0</td>
<td>9.96</td>
<td>8.01</td>
</tr>
<tr>
<td>0/100</td>
<td>9.07</td>
<td>4.36</td>
</tr>
<tr>
<td>95/5</td>
<td>19.75</td>
<td>3.98</td>
</tr>
<tr>
<td>90/10</td>
<td>22.39</td>
<td>4.25</td>
</tr>
<tr>
<td>80/20</td>
<td>16.82</td>
<td>3.26</td>
</tr>
<tr>
<td>70/30</td>
<td>14.66</td>
<td>3.19</td>
</tr>
<tr>
<td>60/40</td>
<td>12.61</td>
<td>4.23</td>
</tr>
</tbody>
</table>

For the improvement of mechanical properties of SA-PEO blend films, glycerol was added to SA-PEO solution in different composition. The concentration of glycerol added was varied from 10 to 30% of total polymers (SA-PEO) weight. After addition of glycerol elongation property of the blend was improved but tensile property was decreased. The good mechanical properties were obtained for 15% glycerol in SA-PEO blend film. The tensile strength and elongation at break of SA-PEO blend film with different composition of glycerol is shown in Table-3.

Table-3: The effect of glycerol on tensile strength and elongation at break of SA-PEO blend film.

<table>
<thead>
<tr>
<th>SA/PEO composition</th>
<th>Glycerol (%)</th>
<th>Tensile strength</th>
<th>Elongation at break</th>
</tr>
</thead>
</table>

45
The results of elongation at break are plotted in Fig 2 against radiation dose as a function of monomer concentration. The elongation is related to elastic and brittle character of the film. It
is observed that SA-PEO blend film treated with monomer, elongation at break is higher than that of SA-PEO blend film without monomer treated after irradiation. The elongation at break of all irradiated blend films is decreased with increased radiation dose. The elongation at breaks are 3.51%, 6.10%, 6.55% and 7.27% for 0.0%, 3%, 5% and 7% monomer treated films at 12.2 kGy absorbed dose respectively. It is also found that treated with monomer, films become flexible and gain higher elongation than that of film without monomer treatment may be due to the interaction between MA and SA-PEO blend film.

**Thermogravimetric analysis of SA-PEO blend film**

The TGA thermograms for SA-PEO, SA-PEO irradiated and irradiated MA treated SA-PEO films are shown in Fig. 3. It is observed that the thermal stability of SA-PEO blend film improved by irradiation. This may be due to cross-linking of polymer chain by the action of gamma radiation. The thermal stability of SA-PEO blend film treated with MA shows more thermal stable than irradiated film. The total weight loss of no-irradiated SA-PEO blend film at 250°C is found to be 47.53% while the weight losses for irradiated SA-PEO blend film and MA treated SA-PEO blend film are found to be 45.05% and 40.45% at 250°C respectively. It can be explained as by the action of gamma radiation MA may be interacted with SA-PEO blend film. The weight loss of all films increases with increased temperature. The initial weight loss may be due to the cause of moisture up to 100°C. After 100°C the weight loss may be break down of polymer chains.

The TGA values clearly indicate that the thermal degradation of SA-PEO blend films reduces after irradiation and monomer treatment which is important for different packaging applications.
Fig. 4 shows the change in the dynamic modulus of SA-PEO blend films, irradiated SA-PEO blend films and irradiated MA treated SA-PEO blend films with temperature. Curves show two distinct peaks, one at lower temperature and the other at higher temperature (a peak corresponding to SA-PEO blend film is ambiguous due to very low intensity); moreover, drop in the modulus is observed in all the cases. The initial moduli also do not exhibit similar trends. It was observed that radiation modification of SA-PEO blend films demonstrates higher modulus than non-radiated one. The maximum value was obtained for the blend films after incorporation of monomer MA with increase in temperature. The storage modulus of the irradiated MA treated SA-PEO blend films was increased up to ~65°C and then showed downhill trend. This transition is called α-transition ($T_\alpha$), and its value around 38°C to 102°C in this case, was defined as glassy region. After the sharp drop of the storage modulus in the glass transition region, the behavior of alginate film moves to leathery state plateau region caused by the micro-Brownian motion of non-crystalline region. Alginate film showed two step transitions. First transition of the film at lower temperature is related to its behavior changes due to water evaporation. The second transition (the higher temperature) is called the Tg at which alginate film changes its behavior from being ‘glassy’ to ‘rubbery’.

**Conclusion**

Sodium alginate (SA)-polyethylene oxide (PEO) blend films were prepared by the application of gamma radiation. The blend films were modified by addition of glycerol as plasticizer. Glycerol incorporated SA-PEO blend films were irradiated in presence and absence of
methacrylate (MA) as monomer. The maximum values of tensile strength of irradiated blend films were obtained at 12.2 kGy absorbed radiation dose. The tensile strength of MA treated irradiated blend film is 44.58% higher than that of irradiated blend film. The maximum value of tensile strength of 3% MA treated irradiated blend film and irradiated blend film are 31.88 MPa and 22.05 MPa at 12.2 kGy absorbed dose respectively. The thermal stability of MA treated blend film is higher than that irradiated blend film and non-irradiated blend film.

References


2. Gelatin and gelatin-polyvinyl alcohol (PVA) blend films

Gelatin films and gelatin-polyvinyl alcohol (gelatin-PVA) blend films with different composition were prepared by casting method and their mechanical properties were studied. The tensile strength of gelatin films and gelatin-PVA blend films was decreased by addition of glycerol but elongation at break was increased. The effect of PVA concentration in gelatin-PVA blend films was examined. Glycerol incorporated gelatin films and gelatin-PVA blend films were further modified with methyl methacrylate (MMA) as monomer by the application of gamma radiation from Co-60 gamma source at room temperature (27°C). The mechanical properties of gelatin film and gelatin-PVA blend films attained a maximum value at 3.1 kGy absorbed dose. The MMA treated gelatin films and MMA treated gelatin-PVA blend films shows improvement of tensile properties than that gelatin films and gelatin-PVA blend films. The tensile strength was found 35.45 MPa for gelatin-PVA blend film with 3% MMA and 24.93 MPa for gelatin film with 3% MMA, which is 42.20% higher than that of gelatin film with 3% MMA. The mechanical properties of gelatin-PVA blend film are found to be higher than those of gelatin film. Thermo gravimetric analysis (TGA) and dynamic mechanical analysis (DMA) shows that the MMA treated gelatin film and MMA treated gelatin-PVA blend film shows less thermal degradation than that of gelatin film and gelatin-PVA blend film.

**Introduction**

Gelatin is a polymer, which is produced by partial hydrolysis of collagen derived from the skin, white connective tissues and bones of animals. Being a protein, it is used in food, cosmetics, pharmaceuticals and photographic industries for its gel forming abilities, non-toxicity and cheap production cost. In pharmaceuticals, gelatin is used as capsule shell manufacturing raw material for controlled drug release. Gelatin has excellent film forming properties. Because of that it is extensively explored in edible and biodegradable film productions and characterization studies, pure\textsuperscript{1-6} and blended with other biopolymers\textsuperscript{7}.

It is reported that gamma radiation improves the tensile properties (tensile strength and elongation at break) of pectin and gelatin based films\textsuperscript{8}. Gamma radiation also increased the
cross-linking between protein chains which increased the mechanical properties of gelatin film\(^9\). The treatment of PEO-gelatin blend film by gamma radiation improved crystallinity, thermal stability and mechanical properties\(^10\). It is reported that when methyl methacrylate (MMA) is polymerized in aqueous medium in presence of gelatin, gelatin graft copolymer macromolecules are formed\(^11\). The thermo mechanical properties of 2-hydroxyethyl methacrylate (HEMA) blended gelatin films were improved by the action of gamma radiation\(^12\).

In the present study, gelatin and gelatin-PVA blend films were prepared by the solution casting method. The gelatin and gelatin-PVA blend films were treated with gamma radiation in presence of methyl methacrylate (MMA). The mechanical and thermo mechanical properties of the films were analyzed.

**Experimental**

**Materials**

Gelatin, methanol, methyl methacrylate (MMA) and glycerol were purchased from Merck, Germany. Poly(vinyl alcohol) (PVA) was supplied by Sigma Aldrich, Iceland.

**Preparation of gelatin and gelatin-PVA blend films**

A 10\% (w/v) solution of granules of gelatin was prepared in hot water and cooled at room temperature (27\°C) and various concentration of glycerol was added to cooled gelatin solution. A 10\% (w/v) solution of PVA was prepared in distilled water using an autoclave. Then gelatin-PVA blend solutions were prepared by mixing gelatin and PVA solution in different compositions with 10\% of glycerol of total polymers (gelatin + PVA). Gelatin and gelatin-PVA blend films were prepared from gelatin with glycerol solution and gelatin-PVA blend solution with glycerol by casting respectively.

**Modification of gelatin and gelatin-PVA blend films with MMA**

Gelatin and gelatin-PVA blend films incorporated with glycerol was modified with MMA using gamma radiation from Co-60 gamma source at the dose rate 3 kGy/h in presence of oxygen. The dose rate was determined with the help of the Fricke dosimeter. Three different monomer (MMA) formulations in methanol was prepared as shown in Table-1 and taken in different test tubes and films were immersed in monomer solution and sealed in polyethylene bags. Then they were irradiated by gamma radiation. The total radiation dose was varied from 1 to 7 kGy.
Table-1: Composition of different monomer formulation (v/v)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>MMA (%)</th>
<th>Methanol (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M₁</td>
<td>1</td>
<td>99</td>
</tr>
<tr>
<td>M₂</td>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>M₃</td>
<td>5</td>
<td>95</td>
</tr>
</tbody>
</table>

Results and discussion

Preparation of gelatin and gelatin-PVA blend films

Gelatin films (thickness ~0.3 mm) were prepared by casting. Tensile strength and elongation at break of gelatin film are 12.12 MPa and 4.54% respectively. To improve the properties of gelatin films, glycerol was added to gelatin solution in different composition. The amount of glycerol added was varied from 5% to 20% of total polymer (gelatin) weight. After addition of glycerol elongation of gelatin films was improved but reduced tensile strength. Gelatin-PVA blend films with different composition by addition of 10% glycerol were also prepared by casting. Good mechanical properties were obtained for gelatin: PVA = 97: 3 (w/w) films. Tensile strength and elongation at break of gelatin: PVA = 97: 3 (w/w) blend film are 17.41 MPa and 12.18% respectively. Therefore, the rest work of gelatin-PVA blend films was done using 3% PVA and 10% glycerol. Tensile strength and elongation at break of gelatin and gelatin-PVA blend films are shown in Table 2.

Table-2: Tensile strength and elongation at break of gelatin and gelatin-PVA films with and without glycerol.

<table>
<thead>
<tr>
<th>Gelatin/PVA Composition</th>
<th>Glycerol (%)</th>
<th>Tensile strength (MPa)</th>
<th>Elongation at Break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100/0</td>
<td>0</td>
<td>12.12</td>
<td>4.54</td>
</tr>
<tr>
<td>100/0</td>
<td>5</td>
<td>8.40</td>
<td>21.05</td>
</tr>
<tr>
<td>100/0</td>
<td>10</td>
<td>7.89</td>
<td>32.25</td>
</tr>
<tr>
<td>100/0</td>
<td>15</td>
<td>4.28</td>
<td>49.65</td>
</tr>
<tr>
<td>100/0</td>
<td>20</td>
<td>1.15</td>
<td>65.52</td>
</tr>
<tr>
<td>99/1</td>
<td>10</td>
<td>10.76</td>
<td>25.35</td>
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<tr>
<td>98/2</td>
<td>10</td>
<td>14.99</td>
<td>22.50</td>
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<td>97/3</td>
<td>10</td>
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<td>20.45</td>
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<tr>
<td>95/5</td>
<td>10</td>
<td>12.50</td>
<td>19.05</td>
</tr>
<tr>
<td>93/7</td>
<td>10</td>
<td>8.95</td>
<td>17.50</td>
</tr>
</tbody>
</table>
Modification of gelatin and gelatin-PVA blend films with MMA

The gelatin and gelatin-PVA blend films incorporated with glycerol were modified with monomer, methyl methacrylate (MMA) using gamma radiation. The films were immersed in different formulation of MMA (1 to 5%) in methanol. MMA solution was taken in test tubes and films were put in test tubes containing MMA solution. Then test tubes were sealed in polyethylene bags and irradiated by gamma radiation. The radiation dose was varied from 1 to 7 kGy. Monomer concentration and radiation dose were optimized on the basis of mechanical properties.

Effect of MMA and radiation dose on mechanical properties of films

Glycerol incorporated gelatin and gelatin-PVA blend films were cut into 9 mm x 50 mm size, put in glass test tubes containing monomer solution and irradiated by gamma radiation. After irradiation, the films were taken out from the test tubes and washed with methanol and dried in an oven for 20 minutes at 50ºC temperature. Then tensile properties of the films were measured. The effect of radiation dose and concentration of MMA on tensile strength of gelatin film is shown in Fig. 1. The tensile strength of gelatin films increases with increased radiation dose. The maximum value of tensile strength is obtained at 3.1 kGy absorbed dose. After 3.1 kGy absorbed dose variation of tensile strength is not so significant. The tensile strength of irradiated films without monomer is lower than that of irradiated film in presence of monomer. The highest value of tensile strength is obtained from irradiated gelatin film with 3% MMA. The maximum value of tensile strength of gelatin film without MMA is found to be 17.25 MPa, while the maximum value of tensile strength of 3% MMA treated gelatin films 24.93 MPa, at 3.1 kGy absorbed dose which is 44.52% higher than irradiated gelatin film without MMA treatment. It is expected that by the action of radiation, free radicals are obtained on polymer chain by abstracting of hydrogen. Interaction of these free radicals on polymer chain is obtained cross-linking and improves tensile strength of films. Another case for improvement of tensile strength in presence of monomer may cause the free radicals of monomer is also obtained by the action of gamma radiation. These free radicals of monomer may react with the free radicals of polymer and can form graft copolymer of gelatin and MMA.

The effect of radiation dose and monomer concentration on elongation at break is shown in Fig. 2. It is found that elongation at break of gelatin film with and without MMA treatment is decreased with increased radiation dose. The elongation at break of gelatin films without
MMA treatment is higher than that of gelatin films with MMA treatment. The elongation at break of gelatin films with MMA also decreases with increased concentration of MMA.

The effect of radiation dose and concentration of MMA on tensile strength of gelatin-PVA blend films is presented in Fig. 3. The tensile strength of gelatin-PVA blend films is increased with increased radiation dose. The maximum value of tensile strength is also obtained at the absorbed radiation dose of 3.1 kGy. After 3.1 kGy absorbed dose, the value of tensile strength is found slight decreasing trend with increased radiation dose. But it is not so significant. The tensile strength of gelatin-PVA blend film without monomer is lower than that of gelatin-PVA blend films with monomer. Tensile strength of gelatin-PVA is 24.75 MPa while tensile strength of 3% MMA treated gelatin-PVA film is obtained 35.45 MPa which is 43.23% higher than that of irradiated gelatin-PVA blend film at 3.1 kGy absorbed dose.
Fig. 4 shows the effect of radiation dose and monomer concentration on elongation at break of gelatin-PVA blend films. The elongation at break of gelatin-PVA blend film without monomer is higher than that of gelatin-PVA blend film with monomer. The elongation at break decreases with increased radiation dose.

The TGA thermograph for non-irradiated gelatin-PVA blend film, irradiated gelatin-PVA blend film and irradiated MMA treated gelatin-PVA blend film are shown in Fig. 5. It is found that the thermal stability of gelatin-PVA blend films is improved by the application of gamma radiation. The thermal stability of MMA treated gelatin-PVA blend films is higher than that of irradiated gelatin-PVA blend films. The total weight loss of gelatin-PVA blend film without irradiation at 250°C is found to be 19.31 wt%, while the weight losses of irradiated gelatin-PVA blend film and MMA treated gelatin-PVA blend film at 250°C are found to be 18.64 wt% and 15.73 wt% respectively. As the temperature increased the weight loss increases. In the temperature ranges from 30°C to 100°C the cause of weight loss may be due moisture in film. The major weight loss of gelatin-PVA blend film after 250°C may be related the char formation of polymers.

Fig. 5. TGA thermograms of gelatin-PVA blend films

Fig. 6 reveals the temperature dependency of storage modulus of non-irradiated gelatin-PVA blend film, irradiated gelatin-PVA blend film and irradiated MMA treated gelatin-PVA blend film. The storage modulus of irradiated MMA treated gelatin-PVA blend film is higher than that of irradiated gelatin-PVA blend film and non-irradiated gelatin-PVA blend film. It is also found that storage modulus of irradiated gelatin-PVA blend film is higher than that of non-irradiated gelatin-PVA blend film. This result indicates that cross-linking of polymer chains and copolymerization with MMA may be the cause of improvement of storage modulus of blend films.

Fig. 6. Storage modulus of gelatin-PVA blend films.
Conclusion

Mechanical properties of the MMA treated gelatin-PVA blend films and MMA treated gelatin films were studied and found that maximum mechanical properties were obtained at 3.1 kGy absorbed radiation dose for 3% MMA treated films. The tensile strength was found 35.45 MPa for gelatin-PVA blend film with 3% MMA and 24.93 MPa for gelatin film with 3% MMA, which is 42.20% higher than that of gelatin film with 3% MMA treatment. The elongation at break was found ~7% and ~8% for gelatin-PVA blend film and gelatin film with 3% MMA treated film respectively. Thermal properties of gelatin-PVA blend film, irradiated gelatin-PVA blend film and MMA treated gelatin-PVA blend film were characterized by thermo gravimetric analysis (TGA) and dynamic mechanical analysis (DMA). It was observed that the thermal stability of MMA treated gelatin-PVA blend films was improved.

References

Summary

During this CRP, studies have been made for the modification of conventional food packaging materials (polymer petroleum-derived) by addition of natural clay and ionizing radiation treatment, for pre-packaged irradiated foods and for the modification of biobased and compostable materials, by addition of micro and nanofiller from natural resources (renewable resources) and also ionizing radiation treatment. Composite based on EVOH, EVA, PBAT/Starch (aliphatic-aromatic copolyester/starch blend) and PBAT/PLA (aliphatic-aromatic copolyester/polyactic acid blend) reinforced with micro and nanofiller from renewable resources were prepared by melt extrusion, using a twin-screw extruder machine and blown extrusion process and treated by electron-beam radiation. It was observed that the modified Brazilian smectitic clay addition (1-3 % wt) in conventional polymer, consisting of EVA followed by irradiation led to a composite with greater tensile strength at break and with improved elongation at break at the same time. The results of tensile strength at break test show that the use of reinforcement in EVA can increase this property, but results also indicate that greater increases are due to the use of ionizing radiation. The mechanical results of composites based on PBAT/Starch and PBAT/PLA blend showed that the addition of micro or nanofiller led to obtaining flexible films with improved properties when compared with neat blends. On the other hand, when these composites were irradiated at the highest radiation dose (200 kGy) the mechanical behavior of composite was worse than neat blend. Therefore, chain scission, as induced by e-beam irradiation, led to associated alterations in surface polymer morphology. SEM micrographs of the irradiated samples showed evidence of mechanical degradation (cracking) whereas irradiated PBAT/PLA and PBAT/Starch blend composite reinforced with micro and nanofiller and neat blend. However, when 10-20 % (wt %) of pre-irradiated PLA was added to the composite blend, before extrusion process, changes in surface morphology and significant gain in mechanical properties were observed indicating that surface adhesion between filler and polymeric blend matrix enhanced, resulting in better property gains.

Work Plan Next 18 Months

- Preparation and characterization of graphene oxide from natural graphite powder;
- Preparation and characterization of flexible film based on EVOH and graphene oxide;
- Characterization of flexible film based on EVOH and EVA reinforced with modified Brazilian smectitic clay;
- Preparation and characterization of PBAT/Starch and PBAT/PLA blend reinforced with green silica and metal nanoparticles;
- Preliminary tests for choosing the better composition for dry food packaging based on PBAT/Starch and PBAT/PLA blend reinforced with filler from renewable resources;
- Preliminary tests for choosing the better composition for EVOH/modified Brazilian clay and EVOH/graphene oxide;
- Production and characterization of multilayer food packaging structure prototypes based on EVA/EVOH/EVA with micro and nanofiller from renewable resources.
- Production and characterization of flexible dry food packaging based structure prototypes based on PBAT/Starch and PBAT/PLA blend with micro and nanofiller from renewable resources.
- Support for other composite studies involving reinforcements from fibers or waste of piassava, sugarcane bagasse, Brazil nut shell, coffee husk and peels into PBAT/Starch Blend and PBAT/PLA Blend.
1. INTRODUCTION

Since the advent of the food can in the 19th century, protection, hygiene, product quality and convenience have been major drivers of food technology and packaging innovation. In recent years, there has been a rising demand for packaging that offers both ease of use and high quality food to consumers with busy lifestyles. From the 1980s, the field of food packaging has grown into a domain that includes high polymer research and discovery. Notably, the materials of food packaging fulfil a very important task as absence of packaging or insufficient packaging would result in fast deterioration of quality and safety giving way to massive commercial losses of valuable foodstuffs [1-4]. The issue of sustainability has been worldwide high, encouraging the development of sustainable alternatives thus aiming to preserve resources for future generations. Moreover, recent developments are raising the prospects that naturally derived resources, the biobased materials, will be a major contributor to the production of industrial products, and a potential new market for these materials is food packaging, a highly competitive area with great demands for performance and cost [5-7].

Because of the recent search for eco-friendly materials and thanks to the use of technological advances, they were reinvented and had, both at nanoscale and microscale level, natural resources reinforcements added to them [6-8]. Food irradiation is a new sterilization method which is effectively used for many packed food items. This process is wholesome as it is a safe process and does not produce a significant change in the nutritional and sensory quality of the food product. Irradiation process helps in extending the shelf life of the product under the recommended storage condition and is also possible to preserve the food product in fresh state. The source used for the irradiation purpose is ionizing radiation [9-12]. Based on the previous important questions the present proposal focuses on the development of advanced food packaging materials in two different fronts:

- modification of conventional food packaging materials (polymer petroleum-derived), based on EVA/EVOH/EVA, by addition of Brazilian smectitic clay, graphene and also ionizing radiation treatment, for pre-packaged irradiated foods suitable for extending shelf-life and providing environment sustainability advantages;

- modification of biobased and compostable materials (aliphatic-aromatic copolyester/corn starch blend – “PBAT/Starch”; aliphatic-aromatic copolyester/polyactic acid blend – “PBAT/PLA”), by addition of filler from natural resource (renewable resources), metal nanoparticles, graphene and also ionizing radiation treatment for application as dry food packaging materials.

Here we report the preparation and characterization of flexible film based on conventional polymer consisting of EVOH and EVA, biodegradable polymer consisting of PBAT/Starch and PBAT/PLA blend reinforced with modified Brazilian smectitic clay followed by electron-beam irradiation. Preparation, electron-beam irradiation and characterization of flexible film based PBAT/Starch and PBAT/PLA blend reinforced with bio-CaCO$_3$ from eggshell and green silica are also reported.

2. EXPERIMENTAL

2.1. Preparation and characterization of micro and nanofillers from renewable resources

The as-received Brazilian smectitic clay consisting of chocolate clay, light green clay, light cream clay and bofe white clay were modified by quaternary salt and sodium carbonate. Brazilian clay is a smectitic clay polycationic, hence the metallic ions occupying the interlayer space (Ca$^{2+}$) had to be changed by sodium ions (Na$^+$): for that the clay was dispersed in deionized water and Na$_2$CO$_3$, at a concentration of 100 meq/100 g of clay, was slowly added to the suspension. The suspension was stirred for about 30 min at 97 °C. Then, an aqueous
solution of quaternary ammonium salt was added to the suspension containing sodium smectite clay, at a concentration equivalent to 1.1 CEC (cation exchange capacity) of the sodium clay. After stirring for 30 min at room temperature, the suspension was filtered and washed with deionized water. The organophilic clays were then dried at 60 °C for 48 h, ground, stored at room temperature, and finally characterized by X-rays diffraction (XRD), wavelength dispersive X-ray fluorescence (WDXRF) and cation exchange capacity by ammonium acetate method [13].

The bio-CaCO$_3$ used in this study is from farm fresh white chicken eggshells. White chicken eggshells were subjected to the following cleaning and size reduction processes: Washing with water, soaking in sodium hypochlorite Solution, 2.5 % (w/w) for 20 min, washing with water, soaking in acetone for 2 hours, drying at 100 ± 2 °C for 2 h in an air-circulating oven. Eggshells were ball milled in (PPG) for 10 h using SPEX SamplePrep 8000D Mixer/Mill. The resulting materials were washed repeatedly with ethanol then dried by heating at 100 ± 2 °C for 24 hours in an air-circulating oven to reduce its moisture content to less than 2 %. The particles were then separated using a set of sieves (125 μm) and a Retsch sieve shaker for 6 hours. Bio-CaCO$_3$ microparticles were irradiated with high intensity ultrasonic horn (Ti-horn, 20 kHz, 100 W/cm$^2$) and nanoparticle were obtained. The micro and nanoparticles of bio-CaCO$_3$ were characterized by XRD, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and surface areas using the Brunauer- Emmett-Teller (BET) surface area analyzer.

Green silica was obtained by keeping sugarcane bagasse ashes in an air insufflating oven at 450 ± 2º C for two days, then ball-milled and classified granulometrically with the aid of sieves so that particle sizes were ≤ 125 μm. Green silica nanoparticles were synthesized using sonochemical methods, by irradiation with high intensity ultrasonic horn (Ti-horn, 20 kHz, 100 W/cm$^2$). The micro and nanoparticles of green silica were characterized by XRD, SEM, TEM and WDXRF.

Rice husk ashes were dried at 150 ± 2 °C for 24 h in an air-circulating oven. The dry fiber was reduced to fine powder, with particle sizes ≤ 125 μm by using ball mills and then it was dried again at 150 ± 2 °C for 24 h to reduce its moisture content to less than 2 %. The rice husk ashes were characterized by SEM, XRD and WDXRF.

2.2. Preparation, irradiation and characterization of flexible film based on conventional polymeric materials and modified Brazilian smectitic clay

EVA reinforced with chocolate and light green clay (97 % EVA; 3 % clay; based on wt %) were obtained with a twin screw extrusion machine “extruder AX 16LD40” made by AX Plásticos Máquinas Técnicas Ltda. EVOH reinforced with light cream and bofe white clay (97 % EVOH; 3 % clay, based on wt %) were prepared using a Haake Rheomex P332 extruder equipped with twin screw operating in the L/D 3:1.33 rate and compression rate X/Y 19/33. The extrudate nanocomposites coming out of the extruder were cooled down by using cold water for a better dimensional stability and wound up manually. Finally, the nanocomposites were pelletized by a pelletizer and fed into an extrusion blow molding (laboratory line), and films samples were obtained. EVOH/Clay nanocomposite pelletized was dried at 110 ± 2 °C for 24 h in an air-circulating oven to reduce its moisture content to less than 2 %, before fed into an extrusion blow molding to obtained flexible films samples. Samples of flexible films obtained from EVA/Clay were irradiated at 100 and 200 Kgy, using a 1.5 MeV electrostatic accelerator (Dynamitron II, Radiation Dynamics Inc, 1.5 MeV energy, 25 mA current and 37.5 KW power). The irradiation was carried out at room temperature, in air, dose rate 28.02 Kgy/s. Irradiation doses were measured using cellulose triacetates film dosimeters “CTA -
FTR -125” from Fuji Photo Film Co. Ltd. Irradiated flexible films were characterized by XRD; DSC, TG, and tensile tests, and melt flow index (MFI) measurement.

2.3. Preparation and characterization of flexible film based on biobased and compostable materials with micro and nanofillers from renewable resources

The PBAT/Starch and PBAT/PLA blends were prepared using a Haake Rheomex P332 extruder equipped with twin screw operating in the L/D 3:1.33 rate. The extrudates coming out of the extruder were cooled down by using air for a better dimensional stability and wound up manually, pelletized and fed into an extrusion blow molding (laboratory line), and films samples were obtained. The formulation of blends are presented in Table I. Flexible film obtained from blends were characterized by XRD, SEM, DSC, TG, and tensile tests.

Table I - Formulation of PBAT/Starch and PBAT/PLA blend

<table>
<thead>
<tr>
<th>Blend</th>
<th>PBAT (wt %)</th>
<th>Starch (wt %)</th>
<th>Neat PLA (wt %)</th>
<th>iPLA(*) (wt %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBAT/Starch</td>
<td>50</td>
<td>50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PBAT/PLA</td>
<td>80-90</td>
<td>-</td>
<td>10-20</td>
<td>-</td>
</tr>
<tr>
<td>PBAT/PLA/iPLA</td>
<td>80-90</td>
<td>-</td>
<td>5-15</td>
<td>5</td>
</tr>
</tbody>
</table>

( *) electron-beam irradiated PLA (200 kGy)

The flexible films based on PBAT/Starch and PBAT/PLA blends reinforced with micro and nanofiller were obtained with a twin screw extrusion machine. Some composite were prepared using an extruder AX 16LD40, equipped with twin screw, made by AX Plásticos Máquinas Técnicas Ltda, other composites were prepared using a Haake Rheomex P332 extruder equipped with twin screw operating in the L/D 3:1.33 rate. The extruded coming out of the extruder were cooled down by using air for a better dimensional stability and wound up manually, pelletized and fed into an extrusion blow molding (laboratory line), and films samples were obtained. The formulation of composite films prepared are presented in Table II.

Table II – Formulation of composite flexible films

<table>
<thead>
<tr>
<th>Blend</th>
<th>Extrusion Machine</th>
<th>Modified Brazilian Smectitic Clay</th>
<th>Micro and Nanofiller</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Chocolat e (%)</td>
<td>Light green (%)</td>
</tr>
<tr>
<td>PBAT/Starch</td>
<td>AX 16LD40</td>
<td>1-3</td>
<td>1-3</td>
</tr>
<tr>
<td>PBAT/PLA</td>
<td>Haake Rheomex P332</td>
<td>3</td>
<td>1-3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Blend</th>
<th>Extrusion Machine</th>
<th>Bio-CaCO₃ nano (%)</th>
<th>Bio-CaCO₃ ≤ 125 µm (%)</th>
<th>Green Silica ≤ 125 µm (%)</th>
<th>Rice husk ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBAT/Starch</td>
<td>AX 16LD40</td>
<td>2</td>
<td>10</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>PBAT/PLA</td>
<td>Haake Rheomex P332</td>
<td>1-3</td>
<td>10</td>
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<td>-</td>
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<tr>
<td>PBAT/PLA/iPL</td>
<td>Haake Rheomex</td>
<td>1-3</td>
<td>10</td>
<td>10</td>
<td>-</td>
</tr>
</tbody>
</table>
The composite film based on PBAT/Starch and PBAT/PLA were irradiated with radiation dose range of 0-300 KGY using a 1.5 MeV electrostatic accelerator (Dynamitron II, Radiation Dynamics Inc, 1.5 MeV energy, 25 mA current and 37.5 KW power). The irradiation was carried out at room temperature, in air, dose rate 28.02 KGY/s. Irradiation doses were measured using cellulose triacetates film dosimeters “CTA - FTR -125” from Fuji Photo Film Co. Ltd. The composite film were characterized by XRD, DSC, TG, SEM, and tensile tests.

3. RESULTS AND DISCUSSION

3.1. Micro and nanofillers from renewable resources

WDXRF results of Brazilian smectitic clay and ash showed that silica (SiO$_2$) is the major component. XRD results of natural and modified Brazilian bentonite clays showed the intercalation of the quaternary ammonium cation in the interlamellar spacings of the clays took place. As example, Figure 1 shows the X-rays diffraction patterns of natural bentonite chocolate clay and after modification with quaternary ammonium salt. The results show that the natural bentonite chocolate presented an interlayer distance (d001) of 1.34 nm and after modification, the interlayer distance increased to 1.93 nm. This increase confirms the intercalation of the quaternary ammonium cation in the interlamellar spacings of the chocolate clay took place.

![Figure 1. X-rays diffraction patterns of natural bentonite chocolate clay and after modification with quaternary ammonium salt.](image)

The surface area determined for the bio-CaCO$_3$ nanoparticle was $\sim 44 m^2/g$, which was much higher than that of microparticles (average particles size 125 μm) that was $\sim 16 m^2/g$. The morphologies of bio-CaCO$_3$ nanoparticles revealed from TEM are showed in Figure 2.
Figure 2. TEM micrographs of bio-CaCO3 nanoparticles after 5 hours of sonication in Decalin.

XRD results of green silica shows the presence of crystalline silica, identified by peaks at $2\theta = 20.8^\circ$ and $2\theta = 26.6^\circ$. WDXRF results shows that silica is the ash major component, corresponding to 57% of its total before burning and to 97.1% after burning (Table III). SEM micrographs for surfaces of the green silica shows that green silica was irregular in shape with a size that varied from the nano to the micron order.

### Table III - Inorganic components of the ash and green silica by WDXRF

<table>
<thead>
<tr>
<th>Component</th>
<th>Ashes (*) (%)</th>
<th>Green Silica (%)</th>
<th>Component</th>
<th>Ashes (*) (%)</th>
<th>Green Silica (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiO$_2$</td>
<td>57±1</td>
<td>97.1±1</td>
<td>TiO$_2$</td>
<td>3.1±0.1</td>
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<tr>
<td>Al$_2$O$_3$</td>
<td>14±1</td>
<td>1.1±0.01</td>
<td>P$_2$O$_5$</td>
<td>2.0±0.1</td>
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<tr>
<td>Fe$_2$O$_3$</td>
<td>13±1</td>
<td>0.7±0.01</td>
<td>MgO</td>
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<tr>
<td>K$_2$O</td>
<td>5.2±0.1</td>
<td>0.6±0.01</td>
<td>Others</td>
<td>0.5±0.01</td>
<td>0.5±0.01</td>
</tr>
<tr>
<td>CaO</td>
<td>3.5±0.1</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(*) sugarcane bagasse ashes

3.2. Flexible film based on biobased and compostable materials with micro and nanofillers from renewable resources

Results showed that the addiction of clay in the PBAT/Starch and PBAT/PLA blend improved the thermal degradation temperature increasing the thermal resistance of material as shown in TG results. The peaks of DSC analysis indicated that blending PBAT with PLA, followed by organophilic clay addition caused structural changes in the polymeric chains of blend component and leads to obtaining a composite material with high melting enthalpy and, consequently, high crystallinity percentage. This can be attributed to the nucleation effects of clay and the improvement in the crystal perfection of PBA/PLA blend. A similar observation is reported by Chow, S. W. for the PBT/Montmorillonite [14]. The superior mechanical properties of PBAT/PLA/Clay nanocomposite observed in this study can be attributed to the stiffness of Brazilian bentonite clay, reinforcing effects, to the degree of the intercalation and good dispersion of the clay layers in the PBAT/PLA matrix. From the results of XRD is possible suggest that the morphology at PBAT/PLA/Clay presents a mixture of intercalated and partially exfoliated structures. As example, the figure 3 shows XRD patterns of PBAT/PLA blend and PBAT/PLA/Clay nanocomposite prepared with 3% (wt%) addition of modified light green clay. It can be seen in Fig. 3 that the XRD pattern for PBAT/PLA/Clay nanocomposite show no characteristic modified light green clay peaks in the range of $2\theta = 4.05^\circ$; that is, the peak corresponding to the basal spacing modified bentonite light green clay ($d_{001}$) of 2.17 nm has disappeared. This indicates that PBAT/PLA chains have diffused into the gallery of the clay.
and that the clay has been successfully intercalated in the PBAT/PLA matrix leading to the formation of exfoliated structures. Subsequently TEM analysis will be performed in order to confirm such suggestions. This result indicates that PLA and PBAT chains have diffused into the gallery of the clay and the clay has been intercalated in the PBAT/PLA matrix leading to obtain flexible films with improved tensile strength and elongation at break properties when compared with the flexible film prepared from PBAT/PLA blend.

![Figure 3. X-rays diffraction patterns of PBAT/PLA Blend and PBAT/PLA/Clay nanocomposite.](image)

However, based on this preliminary study, the better mechanical behavior was presented for flexible film prepared from PBAT/Starch and PBAT/PLA blends reinforced with bio-CaCO₃ nanoparticle. Better results were observed for PBAT/PLA blend, containing irradiated PLA (PBAT/PLA/iPLA blend) reinforced with bio-CaCO₃ nanoparticle. This flexible film presented increases of around 70% in tensile strength; and 90% in Young’s modulus, and a slight reduction of around 10% in elongation at break, when compared with neat PBAT/PLA blend film. This results suggest that PBAT/PLA blend reinforced with bio-CaCO₃ nanoparticles is a good candidate to develop flexible film with high strength and high modulus, for application as mechanical resistance layer, in multilayer packaging for dry food.

3.3. Flexible film based on conventional polymeric materials

3.3.1 EVA and modified Brazilian smectitic clay

It was observed that the combination of EVA with modified clay followed by irradiation led to a composite with greater tensile strength at break and with improved elongation at break at the same time. As example the results of addition of modified Brazilian chocolate clay in EVA are presented. Table IV shows the results of the mechanical tests of the average values calculated from the data obtained in the tests. For each test, 5 specimens were tested. The standard deviations for results were less than 10% for all tests. The results of tensile strength at break test show that the use of reinforcement in the polymer can increase this property, but results also indicate that greater increases are due to the use of ionizing radiation. As expected for elongation at break, composites tend to show significant losses regarding this property. The association of reinforcement and irradiation may change this trend for some specific cases. It was observed in this study that the EVA/clay nanocomposite when irradiated at 100
kGy presents an increase of around 50 % in elongation when compared to the original non-irradiated EVA.

### Table IV - Mechanical test results

<table>
<thead>
<tr>
<th>Materials</th>
<th>Irradiation dose (kGy)</th>
<th>Tensile strength at break (MPa)</th>
<th>Elongation at break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neat EVA</td>
<td>0</td>
<td>5.60</td>
<td>200</td>
</tr>
<tr>
<td>EVA/Clay</td>
<td>0</td>
<td>14.21</td>
<td>47</td>
</tr>
<tr>
<td>EVA/Clay</td>
<td>100</td>
<td>15.85</td>
<td>305</td>
</tr>
<tr>
<td>EVA/Clay</td>
<td>200</td>
<td>16.10</td>
<td>96</td>
</tr>
</tbody>
</table>

The addition of reinforcement in the EVA matrix leads to a decrease in the melt flow index of the material. When compared with neat EVA, a great decrease of around 75 % in this property was observed for the EVA/clay composite. These results apply only to the non-irradiated material. Due to cross-linking in the irradiated materials the MFI cannot be measured.

#### 3.3.2 EVOH reinforced with clay

Neat EVOH and EVOH reinforced with clay were extruded and both tensile strength at break and elongation at break were evaluated. Table V shows the results of the mechanical tests. The standard deviations for these results were much higher than 10 % for all tests. Due to these important variations a study on possible causes was conducted and it was concluded that the EVOH was not dry enough for extrusion. A more meticulous visual evaluation indicated that water bubbles were present in both neat and reinforced EVOH and led to important variation in results of specimens and it was not possible to determine if reinforcement leads to an increase or a decrease in mechanical properties. Because of such initial results with the EVOH, no tests with irradiated material were carried out.

### Table V - Mechanical test results

<table>
<thead>
<tr>
<th>Materials</th>
<th>Tensile strength at break (MPa)</th>
<th>Elongation at break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neat EVOH</td>
<td>13.67±5.51</td>
<td>66.67±28.87</td>
</tr>
<tr>
<td>EVOH/clay</td>
<td>11.30±3.27</td>
<td>42.00±19.37</td>
</tr>
</tbody>
</table>

#### 4. CONCLUSION

**In conclusion:** it may be claimed that the incorporation of modified Brazilian clay in the EVA followed by electron-beam irradiation effectively improved the mechanical properties of neat EVA and led to films materials with superior properties suitable for food packaging development, concerning mechanical properties. EVOH is a more complex material to process and the drying conditions selected for this study were not enough to generate films that would allow a proper assessment of mechanical properties. New drying conditions need to be established to produce the films and re-assess EVOH mechanical properties. Better mechanical behavior were observed for PBAT/PLA blend, containing irradiated PLA (PBAT/PLA/iPLA blend) reinforced with bio-CaCO₃ nanoparticle. This results suggest that PBAT/PLA blend reinforced with bio-CaCO₃ nanoparticles is a good candidate to develop flexible film with high strength and high modulus, for application as mechanical resistance layer, in multilayer packaging for dry food.
REFERENCES


Synergistic effects of gamma-irradiation and antimicrobial nanocomposite microbeads/films against foodborne pathogens in fresh meat product –
Immobilization of nisin and essential oils in alginate and chitosan matrices

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ABSTRACT
This study focused on the antimicrobial efficiency of micropencapsulated nisin/essential oil (EO) nanocomposite bead systems against Listeria monocytogenes in ready-to-eat (RTE) meat for in situ food applications. For the evaluation of nisin/EO microbeads, a preliminary study in vitro was undertaken to develop nisin/EO-microencapsulated edible beads in order to inhibit the growth of L. monocytogenes RTE ham. Different concentrations of nisin (16, 31 and 63 μg/mL) were encapsulated into alginate-cellulose nanocrystals (CNC) beads. Microencapsulation kept the available nisin (63μg/mL) content 20 times more than free nisin during 28 days of storage at 4°C, by exhibiting 31 μg/ml of availability. Cooked ham slices were coated by nisin beads, inoculated with L. monocytogenes (3 log CFU/g) and stored at 4°C under vacuum packaging for 28 days. The beads containing 16, 31 and 63 μg/ml of nisin significantly (P ≤ 0.05) reduced the bacterial counts by 2.6, 1.5 and 3.0 log CFU/g after storage compared to free nisin, without changing the physicochemical properties (pH and colour) of RTE ham. A second microencapsulation study was to compare oregano essential EO (origanum compactum; 0.025% w/v), cinnamon EO (cinnamomum cassia; 0.025%) and nisin (0.25% or 16 μg/mL), used alone or in combination with γ-irradiation, to evaluate their in situ inhibiting capacity against the growth of L. monocytogenes in RTE ham. Microencapsulation of these formulations allowed verifying the potential of the polymer to protect their antimicrobial efficiency during storage.
time. Combined treatments of antimicrobial formulations with γ-irradiation were also carried out to determine synergistic antimicrobial effects. Microencapsulated cinnamon EO/niacin combined with γ-irradiation (1.5 kGy) showed 0.03 log CFU/g/day growth rate of bacteria compared to 0.17 log CFU/g/day for non-encapsulated counterpart. Microencapsulation also showed a significant improvement of bacterial radiosentivity ($P < 0.05$). Microencapsulated oregano EO/niacin and cinnamon EO/niacin combinations showed the highest bacterial radiosensitization with relative sensitivities of 3.4 and 6.9 respectively. Finally, microencapsulation can protect the bioactivity of the antimicrobial compounds during irradiation and the both process act in synergy to eliminate pathogens in food.

1. INTRODUCTION

The safety of ready-to-eat (RTE) meat products is of high concern due to the likelihood of contamination by dangerous food-borne pathogens such as *L. monocytogenes*. This contamination mainly occurs during the post processing stage. The RTE meat products such as cooked ham are completely processed prior to final packaging and are consumed without further cooking which makes them susceptible to contamination by pathogenic bacteria. *L. monocytogenes*, a gram positive, non-spore rod shaped bacteria, is responsible for the food borne disease called listeriosis (1). Scallan *et al.* (2) reported that *L. monocytogenes* causes 94% hospitalization which results 15.6% death rate in the US each year. FDA (US Food and Drug Administration) maintains a policy of “zero-tolerance” for *L. monocytogenes* in RTE meat products (3).

Various methods have been proposed to control the post-processing contamination on RTE meat products and it is found that antimicrobial coating or packaging are the most innovative techniques to control the growth of pathogens during storage condition on RTE meat. The choice of biopolymers is an important step for the success of the microencapsulation processes. Alginate-Cellulose nanocrystals (CNC) matrix was used in current investigation for the development of antimicrobial microbeads, due to its good physicochemical properties which was demonstrated by our previous study (4). The use of CNC improved the physicochemical characteristics of alginate and it has also been demonstrated that CNC has the property to stabilize the emulsion (5) as EOs make emulsion with alginate-CNC matrix.

Direct incorporation of antimicrobials into food may lead to drastic loss of antimicrobial efficacy due to the inactivation of the antimicrobials by food components or dilution below active concentration due to migration into the bulk food matrix. Antimicrobial films provide an innovative alternative and can reduce the addition of larger quantities of antimicrobials that
are usually incorporated directly into the bulk of the food (6). These films can also allow a better efficiency, stability and controlled release of the antimicrobials to the food surface (7). Nisin is an antimicrobial polypeptide or bacteriocin of 34 amino acids, produced by several strains of \textit{Lactococcus lactis} and recognized as GRAS (generally recognized as safe) by the United States. It is a cationic, hydrophobic and amphiphilic peptide, with antibacterial activity against many Gram-positive bacteria such as \textit{L. monocytogenes}. Nisin is a ribosomally synthesized and post-translationally modified antimicrobial lantibiotic. The N-terminal part of nisin contains a large number of hydrophobic residues and the C-terminal part is considered to be hydrophilic due to the presence of positively charged lysine and histidine residues (8).

Essential oils (EOs), one of the most widely used natural antimicrobial compounds, are volatile aromatic oily liquids extracted from plants or spices used in food and beverages to improve its preservation and sensorial quality (9). Oregano (\textit{Origanum compactum}) and cinnamon (\textit{Cinnamomum cassia}) EOs were used in the present study as antimicrobial compounds against \textit{L. monocytogenes}. The antimicrobial activity of these EOs is assigned for the main active compounds such as carvacrol (oregano) and trans-cinnamaldehyde (cinnamon) (10,11). EOs have a GRAS (generally recognised as safe) status but the acceptable concentration is limited due to their organoleptical criteria (12, 13).

Gamma irradiation, one of the post-packaging decontamination technologies, is an effective process for reducing or eliminating the growth of \textit{L. monocytogenes} and ensure food safety (14). This type of ionizing radiation destroys the microorganisms by direct breakdown of chemical bonds in bacterial DNA or by the indirect effects of reactive oxygen species produced by the radiolysis of water on cell membranes and chromosomes (15). These changes may facilitate the contact between antimicrobial molecules and cell membranes and thus increase the inhibitory effects of these molecules against \textit{L. monocytogenes}. The potential implementation of \textgamma-irradiation on RTE cooked food processing is mainly based on the fact that it can effectively be inactivated the DNA of the pathogenic microorganisms (16). In 1981, FAO/IAEA/WHO joint committee was accepted \textgamma-irradiation for stored food products. The approval of meat irradiation by the Food and Drug Administration (FDA) has made consumers more confident and attracted the interest of industries concerned with food quality and safety. It was stated that, irradiation of food at doses up to 10 kGy introduced no special nutritional problem. Currently, more than 26 countries are using this process on a commercial scale (17, 18). Bacterial radiosensitization is a recognized biological phenomenon which has been found to be useful on meat application (17). When \textgamma-irradiation is used in combination
with antimicrobials, the global efficiency is strengthened through synergistic action and it could be possible to reduce the irradiation doses without affecting the food quality.

Bionanocomposites can be defined as a family of materials consisting of a biopolymeric matrix and reinforced with nano-sized fiber, which is obtained from renewable sources (19). Over the years, CNC based bionanocomposites have attracted significant attention due to their renewable nature as well as potential application in various fields (20, 21). Recently, CNC has been used to enhance the mechanical properties of biopolymers such as chitosan (22, 23). A high-pressure homogenization technique such as microfluidization can be also adopted to achieve proper dispersion of the CNC within the polymer matrix. Microfluidization provides an innovative approach to develop processing paths that break down aggregates and maximizes CNC distribution within the polymeric matrix. The applications of a microfluidizer include development of highly stable nanoemulsion or nanodispersions, disruption of cells, micro/nano encapsulation of bioactive compounds in polymer, etc. (24, 25). Microfluidization has also been used effectively for the preparation of cellulose microfibrils from wood (26) and non-wood pulp (27).

The objectives of this study were to characterize and to evaluate in vitro and in situ the potential of alginate-CNC microbeads containing nisin and essential oils to inhibit the growth of *Listeria monocytogenes* during time and to verify the synergistic effects of γ-irradiation combined with microencapsulation of antimicrobials compounds on RTE cooked ham for the determination of the radiosensitivity of *L. monocytogenes* and their level during storage time.

## 2. EXPERIMENTAL

### Materials

**Chemicals.** Sodium alginate (guluronic content 65-70%), calcium chloride (granules) and lactic acid were purchased from Sigma-Aldrich Canada Ltd (Oakville, ON, Canada). CNC was supplied by FPInnovations in their pilot plant (Pointe-Claire, QC, Canada). Nisin (Niprosin™, purity 2.5%, 77.5% salt and 20% vegetable protein) was purchased from Profood International Inc. (Naperville, IL, USA). Oregano (*Origanum compactum*) and cinnamon (*Cinnamomum cassia*) EOs were purchased from Robert et Fils (Montreal, QC, Canada). ALCOLEC® PC 75 lecithin was obtained from American Lecithin Company (Oxford, CT, USA). Brain heart infusion (BHI) broth and Palcam agar were purchased from Alpha Biosciences Inc. (Baltimore, MD, USA). Preservative salts such as sodium chloride, triphosphate, erythorbate and nitrite salts were delivered from BSA Food Ingredients (St-
Leonard, QC, Canada). Ground lean pork meat was purchased from a local grocery store (IGA, Laval, QC, Canada).

**Bacterial strains.** *E. coli* 0157:H7 EDL933 was obtained from INRS-Institut Armand-Frappier (Laval, QC, Canada). *L. monocytogenes* strains HPB 2569 1/2a, 2558 1/2b, 2371 1/2b, 2812 1/2ba and 1043 1/2a were obtained from Health products and Foods Branch of Health Canada (Ottawa, ON, Canada). All the microorganisms were kept frozen at -80°C in BHI containing glycerol (10% v/v). Before use, the stock cultures were resuscitated through 2 consecutive 24 h-growth in BHI at 37°C to obtain working cultures containing approximately $10^9$ CFU/mL. The *L. monocytogenes* strains were mixed together (2 mL each) to obtain a cocktail mixture.

**Preparation of nisin solution**

Different concentrations of nisin (0.25, 0.5 and 1% w/v) were prepared by dispersing Niprosin™ powder in 0.01M CaCl$_2$ solution and the pH was adjusted to 3.0-3.5 with diluted lactic acid. Nisin-CaCl$_2$ solution was stirred overnight and then centrifuged for 15 min at 3500 g at 4°C to remove the undissolved particles and collect the nisin-CaCl$_2$ supernatant.

**Microencapsulation of nisin and EOs for alginate-based microbead application**

For the preparation of nisin formulations, an aqueous suspension containing 3% (w/v) alginate and 5% CNC was homogenized using an Ultra-Turrax T25 disperser (IKA Works Inc., Wilmington, MC, USA) at 23°C and 25,000 rpm for 1 min. The different concentrations of nisin-CaCl$_2$ solutions (16, 31 and 63 μg/mL) were mixed with alginate-CNC suspension (alginate-CNC: nisin-CaCl$_2$ 75:25) to form the antimicrobial microbeads according to Rajaonarivony et al. (28). Free nisin solution was prepared in distilled water without CaCl$_2$ following the same process. The formulations are presented as free nisin-formulations N1-16 μg/mL, N2-31 μg/mL, N3-63 μg/mL and microencapsulated nisin-formulations N1-E 16 μg/mL, N2-E 31 μg/mL, N3-E 63 μg/mL.

For the preparation of EOs/nisin formulations, alginate-CNC suspension was emulsified with oregano or cinnamon EOs (0.025 %) using lecithin (0.25 %). Nisin-CaCl$_2$ solution was also mixed with EOs containing emulsified alginate-CNC suspension according to alginate-CNC-EOs: nisin-CaCl$_2$ 75:25. The final concentration of EOs and nisin in alginate-CNC microbeads were 250 and 16 μg/mL respectively. The free antimicrobials were also verified in order to evaluate the effectiveness of microencapsulation. The formulations were denoted as C: ham; C(E): ham + alginate-CNC microbeads; OR: ham + free oregano; OR(E): ham +
encapsulated oregano; CN: ham + free cinnamon; CN(E): ham + encapsulated cinnamon; N: ham + free nisin; N(E): ham + encapsulated nisin; OR+N: ham + free oregano-nisin; OR+N(E): ham + encapsulated oregano-nisin; CN+N: ham + free cinnamon-nisin; CN+N(E): ham + encapsulated cinnamon-nisin.

**Irradiation treatment of ham samples**

Gamma irradiation was conducted at the Canadian Irradiation Center (CIC, Laval, QC, Canada) with γ-rays generated from $^{60}$Co source at room temperature, at a dose rate of 17.9 kGy/h in a Underwater Calibrator UC-15A Research Irradiator (Nordion Inc., Laval, QC, Canada). Inoculated cooked ham samples were irradiated under refrigerated conditions at 1.5 kGy. Samples were then stored at 4°C and analyzed at different storage interval (1, 7, 14, 21, 28 and 35 days).

**Fourier transform infrared (FTIR) spectroscopy analysis of antimicrobial matrices**

FTIR spectra of microencapsulated nisin in alginate microbeads and chitosan films were recorded using a Spectrum One spectrophotometer (Perkin-Elmer, Woodbridge, ON, Canada) equipped with an attenuated total reflectance device for solids analysis and a high linearity lithium tantalate detector. Spectra were analyzed using Spectrum 10.3.9 software. Samples were dried onto a zinc selenide crystal for 15-20 min and the analysis was performed within the spectral region of 650-4000 cm$^{-1}$ with 64 scans recorded at a 4 cm$^{-1}$ resolution. After attenuation of total reflectance and baseline correction, spectra were normalized with a limit ordinate of 1.5 absorbance units.

**In vitro study: BHI-agar deep-well model to evaluate depletion activity of nisin**

BHI-agar deep-well model for nisin depletion test was adapted from Bi et al. (29). A solution containing BHI (3.7%) and agar (1.0%) was autoclaved for 20 min at 121°C. The solution (225 mL) was then poured into a 600-mL beaker to a height of 40 mm. After gel solidification, 4 wells (from gel surface to bottom) were made in each beaker using a 7-mm pipet tip. Subsequently, 1 mL of each concentration of free and microencapsulated nisin preparation was added into wells. At days 0, 1, 7, 14, 21 and 28 at 4°C, a 100-μL aliquot of each nisin preparation was transferred from the well to a bioassay plate to determine the available nisin concen.

**Microbiological analysis**

Ham was prepared following a model developed in our laboratories (13). Cooked ham was sliced and 8 mL of free or microencapsulated nisin was spread onto each ham slice. The
coated cooked ham slice was then inoculated with ~3 log CFU/g *L. monocytogenes* and vacuum packaged within 24 h of production. Ham samples were stored at 4° C for 28 days and periodically analyzed. Each sample was homogenized for 1 min in sterile peptone water (0.1% w/v; Difco, Becton Dickinson) in a lab-blender 400 stomacher (Seward Medical, London, UK). Serial dilutions from homogenate were prepared in 0.1% peptone and 100 µL of each dilution was spread onto Palcam plate (supplemented with 5 mg/mL acriflavin, 10 mg/mL polymyxin B and 8 mg/mL ceftazidime for the selective detection of *L. monocytogenes*). Plates were incubated at 37°C for 48 h and CFU were counted with a detection limit of 50 CFU/g meat. The pH value of ham coated with nisin was also determined during storage at 1, 14 and 28 days. Ham samples (10 g) were blended with 90 mL of distilled water for 1 min using an Ultra-Turrax T25 blender (IKA Works Inc.) before pH measurements.

**Bacterial growth rate**

The growth rate (μ) of *L. monocytogenes* in the meat samples can be described according to Eq. 1 over duration of 35 days:

\[
\mu = \frac{\log(X_2/X_1)}{T_2 - T_1}
\]

(Equation 1)

where X_2 and X_1 are population of *L. monocytogenes* at day T_2 and T_1, respectively. The growth rate constant is presented as μ (log CFU/day/g).

**Radiosensitization analysis**

Inoculated cooked ham samples were exposed to 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75 and 2 kGy. D_{10} values were calculated (defined as the radiation dose required to reduce 1 log CFU) from the following linear regression of the bacterial destruction kinetics,

\[
\log \frac{N_t}{N_0} = -kt
\]

(Equation 2)

where, N_0 is the initial bacterial count (CFU/g), N_t the bacterial count (CFU/g) at different radiation doses and k the bacterial destruction rate. Bacterial counts (CFU/g) were plotted against different radiation doses and the slope reciprocal of the trend line was deduced to calculate D_{10} value. The degree of radiation sensitivity (RS) was determined by the following equation:

\[
RS = \frac{D_{10} (\text{control})}{D_{10} (\text{treatment})}
\]

(Equation 3)

where D_{10} (control) is the D_{10}-value without antimicrobial and D_{10} (treatment) is the D_{10}-value of samples treated with antimicrobials.

**Measurement of the mechanical properties of the nanocomposite films**
Mechanical properties of the composite films were measured using a Universal Testing Machine (UTM) H5KT (Tinius Olsen Testing Machine Co., Inc., Horsham, PA, USA), equipped with a 100 N-load cell (type FBB) and 1.5 kN-specimen grips. Film thickness was measured using a Mitutoyo Digimatic Indicator (Type ID-110E; resolution: 1 µm; Mitutoyo MFG Co. Ltd, Tokyo, Japan), at five random positions around the film. Film width was measured using a Traceable® Carbon Fiber Digital Caliper (resolution: 0.1 mm; accuracy: ± 0.2 mm; Fisher Scientific). Specimen of films conformed to a thickness of 128.7 ± 2.9 µm, a length of 60 mm and a width of 12 mm before testing. Measurements were carried out following an ASTM D638-99 method adapted by Salmieri et al. (30). Tensile strength (TS; maximum stress, MPa), tensile modulus (TM; elastic modulus, MPa) and elongation at break (Eb, %) were automatically collected after film break due to elongation, using Test Navigator® 7.02.11 software.

Statistical analyses

Each analysis was carried out in triplicate (n = 3) in a randomized experimental design. Analysis of variance (ANOVA) and Duncan’s multiple comparison tests were used to compare all the results. Differences between means were considered significant when the confidence interval was smaller than 5% (P ≤ 0.05). The analysis was performed by the PASW Statistics 18 software (IBM Corporation, Somers, NY, USA).

3. RESULTS AND DISCUSSION

FTIR analysis of alginate-CNC microbeads containing nisin

FTIR spectra of Ca-alginate-CNC microbead containing 16, 31 or 63 μg/mL of nisin are presented in Fig. 1.
Figure 1 - FTIR spectra in the 1801-1192 cm\(^{-1}\) region of alginate-CNC microbeads without nisin (control), microbeads containing 16 μg/mL of nisin (N1-E 16 μg/mL), 31μg/mL of nisin (N1-E 31μg/mL) and 63 μg/mL of nisin (N1-E 63 μg/mL).

For alginate-CNC microbead without nisin (control), the characteristic absorption bands were observed at 3600-3200 cm\(^{-1}\) (O-H stretching), 2934 cm\(^{-1}\) (C-H stretching), 1599 cm\(^{-1}\) (asymmetric and symmetric COO\(^{-}\) stretching) and 1401 cm\(^{-1}\) (symmetric COO\(^{-}\) stretching). A slight increase of typical sharpen peak was observed at 3335 cm\(^{-1}\) related to O-H vibration of CNC which suggesting an increase of hydrogen bonding between alginate and CNC, as previously reported (4). Besides, microbeads containing nisin showed a narrowing of O-H stretching band. Typical characteristic peaks for nisin were also found at 1599 cm\(^{-1}\) due to CO-NH bending (peptide bonds) and 1401 cm\(^{-1}\) due to C-H bending, with sharper peaks shifted to lower frequencies. In addition, the area in the region 1718-1497 cm\(^{-1}\) was reduced (from 100 to 89 units) by addition of nisin (from 16 to 63 μg/mL). This decrease could be attributed to electrostatic interactions between positively charged nisin and negatively charged alginate at neutral pH (pKa of alginate: 3.5; pH of nisin: 8.8), in accordance with other authors who characterized nisin-polyanions interactions (31).

Microencapsulated and free nisin availability during storage: in vitro test

The release activity of free and encapsulated nisin was evaluated by BHI-agar deep-well model (in vitro) during storage at 4°C and the diffusion test was done by evaluating the growth inhibition against \(L.\ monocytogenes\). The peptide diffuses from the free and encapsulated antimicrobial nisin solution into the gel (causing diffusion-based depletion) and BHI components diffuse from the agar gel into the solution (causing irreversible antimicrobial active compounds adsorption or degradation). A 2\(^{nd}\) order-polynomial standard curve for free and microencapsulated nisin was used to correlate the area of inhibitory zones with the amount of available nisin. The available nisin content from free (N) and microencapsulated (N-E) nisin during storage are presented in Fig. 2-A and 2-B. After 1 day, the available nisin of N1-16μg/mL, N2-31μg/mL and N3-63μg/mL (free nisin) was 2.1, 6 and 11 μg/mL, respectively. After 14 days, formulations N1-16μg/mL and N2-31μg/mL drastically lost their antimicrobial activity with a similar available content of 0.12 μg/mL. Formulation N3-63μg/mL showed only 2.6 μg/mL of availability after 14 days and lost its antimicrobial activity after 28 days of storage.

Microencapsulated nisin formulations show after 1 day, a nisin availability of 38, 63 and 89 μg/mL was found for N1-E16μg/mL, N2-E31μg/mL and N3-E63μg/mL, respectively. After 14 days, their availability was respectively 21, 37 and 61 μg/mL. Thus, N3-E63μg/mL
exhibited 20 times more available nisin as compared to free nisin (N3-63μg/mL). After 28 days, all encapsulated nisin formulations still showed efficient available nisin, with respective values of 18.4, 20.6 and 31.3 μg/mL. The present study allowed increasing the available nisin in microencapsulated formulations during storage compared to both Bi et al. (29) due to the novel alginate-CNC microbead carrier formulation. Wan et al. (32) reported that nisin incorporated in alginate microparticles showed a good activity compared to free nisin during storage for the reduction of Lactobacillus curvatus in skim milk. This technique is interesting due to convenient application on the target system which can be manipulated for desirable loading and retention of antimicrobial peptide.

**Figure 2** - Available nisin concentration from A) free nisin and B) microencapsulated nisin against L. monocytogenes during storage at 4°C in in vitro BHI-agar deep well model.

**Antimicrobial activity of microencapsulated nisin against L. monocytogenes: in situ test**

The antimicrobial effect of free and microencapsulated nisin against L. monocytogenes was evaluated on cooked ham as a RTE meat model (Fig. 3-A and 3-B). The effectiveness of nisin to prevent the growth of L. monocytogenes was dependent on nisin concentration. After 28 days
of storage, no significant \((P > 0.05)\) difference in bacterial growth was observed between uncoated control ham (8.2 log CFU/g) and control ham coated with microbeads without nisin (8.25 log CFU/g). The microencapsulated nisin showed better antimicrobial effect as compared to free nisin during storage. Indeed, ham slices coated with N1-16μg/mL, N2-31μg/mL and N3-63μg/mL free nisin exhibited respective bacterial counts of 7.2, 5.2 and 4.7 log CFU/g, after 4 weeks of storage. Similarly, ham slices coated with N1-E16μg/mL, N2-E31μg/mL and N3-E63μg/mL microencapsulated nisin showed respective counts of 4.5, 3.7 and 1.7 log CFU/g, after 4 weeks, corresponding to a reduction of 2.7, 1.5 and 3.0 log CFU/g, as compared to the free nisin. The level of \(L.\ monocytogenes\) was also below detection limit (50 CFU/g) for microencapsulated beads N3-E63μg/mL during all storage.

**Figure 3** - \(L.\ monocytogenes\) counts on vacuum packaged cooked ham slices coated with A) Free nisin and B) microencapsulated nisin during storage at 4°C.

Microencapsulation of nisin also increased the lag phase of bacterial growth during storage. Lag phase is defined as the initial growth phase during which cell number remains constant prior to rapid growth. Indeed, formulations N2-31μg/mL and N3-63μg/mL (free nisin) presented a lag phase of 7 days and after, bacteria started growing exponentially. In comparison, microencapsulated formulation N2-E31μg/mL showed a lag phase of 14 days and during 28 days of storage, formulation N3-E63μg/mL showed bacterial counts below detection limit. \(L.\ monocytogenes\) is a psychrotrophic pathogen that is able to grow on cooked ham at 4°C (33). It is surmised that antimicrobials such as nisin, when directly applied on the surface, may diffuse much faster throughout the product lowering the local surface concentration to sub-active levels. On the contrary, edible antimicrobial coatings maintain the necessary preservative concentration at the product surface for a relatively longer period of time (34). It could be hypothesized that positively charged hydrophobic N-terminal of nisin can electrostatically interact with negatively charged phosphate groups on target cell wall precursor lipid II by forming pores (35). Juck \textit{et al.} (36) reported that alginate coating with nisin (500 IU/g) and sodium diacetate (0.25%) on RTE turkey meat products reduced by 1.1 log CFU/g the growth of \(L.\ monocytogenes\) during 21 days. Comparing these results, the present study revealed that
alginate microbead with nisin (N3-E63μg/mL) can completely inhibit the bacterial growth of *L. monocytogenes* compared to control ham coated with microbeads with 6.6 log reduction after 28 days of storage.

**Effect of γ-irradiation on available antimicrobial content (free or microencapsulated) during storage: *in vitro* test**

The effect of γ-irradiation on available free or microencapsulated antimicrobials, mg/mL chloramphenicol (CAM) (oregano and nisin) is presented in Fig. 4. A 2nd order polynomial CAM standard curve was used to correlate the inhibition zone (mm) of the available antimicrobials. Initially at day 0, the available content of OR was 0.6 mg/mL CAM, but it decreased to 0.05 mg/mL CAM within first 7 days storage and continued to decrease in the later stages. Whereas, microencapsulated OR(E) showed more available content after 7 days of storage with 0.2 mg/mL CAM. N and N(E) exhibited a similar decrease in available content.

Results revealed that microencapsulated combined antimicrobial OR+N(E) showed better activity compared to free combined antimicrobials OR+N during storage. Indeed, after 7 days, the availability for OR+N(E) was 0.4 mg/mL CAM whereas the available content for OR+N was 0.2 mg/mL CAM.

The microencapsulated OR+N(E) showed an availability up to 21 days but free OR+N almost lost its activity after 21 days. Microencapsulation also showed a protection for antimicrobials after γ-irradiation. Indeed, after 7 days, the available content for OR+N (E)-γ was 0.3 mg/mL CAM whereas that for OR+N-γ was 0.03 mg/mL CAM.
Figure 4 - Effect of γ-irradiation on available free or microencapsulated antimicrobials, mg/mL CAM (Origanum compactum and nisin) against L. monocytogenes during storage at 4°C in in vitro BHI-agar deep well model. (A) without irradiation and (B) with irradiation.

The effect of γ-irradiation on available free or microencapsulated antimicrobials, mg/ml CAM (cinnamon and nisin) is presented in Fig. 5. After 7 days of storage, microencapsulated CN(E) had more available content than the non-encapsulated CN. Results showed that the content for CN(E) and CN was respectively 0.3 and 0.03 mg/mL CAM. CN+N(E) showed better protection of antimimicrobials than the CN+N. After 14 days, the content for CN+N (E) was 0.2 mg/mL CAM whereas CN+N lost its activity. In this formulation, γ-irradiation showed a similar activity as oregano-nisin formulation. Thus, after 7 days, the available content for CN+N(E)-γ was 0.3 mg/mL CAM but CN+N-γ lost its activity. Therefore, this study demonstrated that free antimicrobials lost their activity within a very short time after γ-irradiation that microencapsulation could protect initially. But γ-irradiated microencapsulated formulations could not exhibit any activity after 14 days. Huq et al. (4) reported that alginate could be degraded during gamma irradiation due to the formation of hydroxyl radicals by radiolysis of water. These free radicals can reduce the activity of antimicrobials. As a result, free antimicrobials might be directly exposed to γ-irradiation and lose their antimicrobial activity.
activity more than microencapsulated formulations. Later on, *in situ* study was done to verify these results more evidently.

![Graph](image)

**Figure 5** - Effect of $\gamma$-irradiation on available free or microencapsulated antimicrobials, mg/mL CAM (*Cinnamomum cassia* and nisin) against *L. monocytogenes* during storage at 4°C in *in vitro* BHI-agar deep well model. (A) without irradiation and (B) with irradiation.

**Synergistic effect of microencapsulated combined antimicrobials and $\gamma$-irradiation (at 1.5 kGy) during storage: *in situ* test**

*OR+N(E) and $\gamma$-irradiation (at 1.5 kGy) during storage.* The synergistic effect of microencapsulated antimicrobial with $\gamma$-irradiation is presented in Fig. 6. The bacterial counts for C-$\gamma$ and C(E)-$\gamma$ were found to be 2.8 and 2.9 log CFU/g, respectively at 1 day of storage, which represents an immediate effect of irradiation. After 35 days, the bacterial counts for C-$\gamma$ and C (E)-$\gamma$ reached a level of 6.7 and 6.5 log CFU/g, respectively. Both OR+N-$\gamma$ and OR+N(E)-$\gamma$ showed a lag phase of bacterial growth up to 7 days of storage. OR+N-$\gamma$ and OR+N (E)-$\gamma$ showed lower bacterial counts and respective levels of 4.3 and 3.0 log CFU/g were found after 35 days. However, both OR+N-$\gamma$ and OR+N(E)-$\gamma$ showed a bacterial growth rate 0.14 and 0.13 log CFU/g/day. Thus, microencapsulated combined antimicrobials and $\gamma$-
irradiation [OR+N(E)-γ] allowed reducing the bacterial growth rate by 32% compared to microencapsulated combined antimicrobials without irradiation [OR+N (E)].

**Figure 6** - Synergistic effect of microencapsulated oregano + nisin and γ-irradiation on counts of *L. monocytogenes* in RTE cooked ham during storage at 4˚C. A) without microencapsulation and B) with microencapsulation. “γ” indicates formulations irradiated at 1.5 kGy.

**CN+N(E) and γ-irradiation (at 1.5 kGy) during storage.** After γ-irradiation treatment (Fig. 7-A and 7-B), both CN+N-γ and CN+N(E)-γ showed bacterial counts below detection limit (<50 CFU/g) at day 1. Therefore, after 35 days of storage, CN+N-γ and CN+N(E)-γ exhibited the counts 4.0 and 1.8 log CFU/g, respectively. Gamma irradiation increased the lag phase of CN+N (E)-γ up to 28 days but CN+N-γ showed only a lag phase of 7 days. The bacterial growth rate for CN+N-γ and CN+N (E)-γ was 0.2 and 0.03 log CFU/g/day whereas C-γ (control ham) and C (E)-γ (control with microbeads without antimicrobials) showed bacterial growth rates of 0.3 log CFU/g/day. Hence, microencapsulation combined with irradiation allowed a synergistic effect on antimicrobial activity during storage.

Abdollahzadeh et al. (37) reported that combination of thyme EO and nisin reduced the *L. monocytogenes* counts by 1.9 log CFU/g after 4 days of cold storage and then remained unchanged up to 12 days of storage in minced fish meat which was supported by our present findings. The phenolic content of EOs is predominately responsible for their antimicrobial
activity. Turgis et al. (38) also demonstrated the antimicrobial activity of oregano and cinnamon EOs in combination with nisin against *L. monocytogenes* by *in vitro* study. According to specification of manufacturer (Robert et Fils), oregano (OR) and cinnamon (CN) EOs are a mixture of carvacrol (46%), thymol (14%), γ-terpinene (12%), p-cymene (13%) and cinnamaldehyde (65%), methoxy-cinnamaldehyde (21%), respectively. According to Ayari *et al.* (39), the addition of carvacrol in broth and irradiation treatment initiated the disintegration of the outer membrane and disruption of the cytoplasmic membrane permeability of the cell and helped to reduce intracellular ATP (energy), making it nearly impossible for the cell to repair the damage. Nisin, consisting of 34 amino acids, mainly exhibited its antimicrobial properties against gram positive bacteria such as *L. monocytogenes*, involving the formation of pores in the cytoplasmic membrane of target cells and leading to the efflux of essential small cytoplasmic components, such as amino acids, potassium ions and ATP (40). Combination of EOs and nisin was found to be most effective antimicrobial which is believed to be the multiple attack of the cell membrane by individual active components like carvacrol, thymol, trans-cinnamaldehyde and mixture of amino acids, as reported by numerous studies (41, 42). A synergistic effect was found by microencapsulated EOs/nisin system in combination with γ-irradiation, which would generate a high technological impact to improve the RTE meat market in concern with pathogenic contamination. Further studies need to be done to understand the mechanism of microencapsulated antimicrobials with γ-irradiation.
Figure 7 - Synergistic effect of microencapsulated oregano EO + nisin and γ-irradiation on counts of *L. monocytogenes* in RTE cooked ham during storage at 4°C. (A) before microencapsulation and (B) after microencapsulation. “γ” indicates formulation irradiated at 1.5 kGy.

**Effect of microencapsulated antimicrobials on radiosensitization of *L. monocytogenes***

The influence of microencapsulated antimicrobials on radiosensitization of *L. monocytogenes* is presented in Table 1. Results demonstrated that combination of antimicrobial (EOs and nisin) coating enhanced the RS of *L. monocytogenes* on RTE cooked ham compared to individual antimicrobial coating. Microencapsulation of combined antimicrobials was the most effective formulation against *L. monocytogenes*. In current study, D<sub>10</sub> values of 0.54 and 0.55 kGy were observed for control ham (C) and control ham with microbeads C (E). Both free and microencapsulated antimicrobial formulations significantly reduced (P < 0.05) the D<sub>10</sub> values compared to control ham without and with microbeads. Hence, these results revealed that free oregano (OR) and cinnamon (CN) coating on RTE ham increased the RS by 1.2 and by 1.8. Turgis *et al.* (38) showed that addition of nisin enhanced the RS of *L. monocytogenes* by 1.2 fold in sausage meat. Similarly, our result using nisin (N) coating also improved by 1.9 fold the RS of *L. monocytogenes*. Previous studies also showed that combined antimicrobials and γ-irradiation treatments had a synergistic effect on radiosensitization of *L. monocytogenes* (43,44). Microencapsulation of combined antimicrobials (EOs/nisin) increased significantly (P < 0.05) the RS of *L. monocytogenes*. Combined microencapsulated antimicrobials OR+N (E) and CN+N (E) coated ham showed the lowest D<sub>10</sub> values (0.2 and 0.1 kGy respectively) compared to free combined antimicrobials OR+N and CN+N (0.3 and 0.2 kGy respectively). Thus, OR+N(E) and CN+N(E) formulations exhibited the higher RS, representing an increase by 2.9 and 5.0 fold compared to control ham. In addition, results revealed that OR+N(E) and CN+N(E) increased significantly (P < 0.05) by 39 and 113% the RS compared to OR+N and CN+N. Similar data were also obtained in previous studies using EOS mixed in ground beef before irradiation treatment (45). From these results, it can be assessed that ionizing radiation can have its activity enhanced in presence of antimicrobial compounds such as EOs and nisin.

<table>
<thead>
<tr>
<th>Sample</th>
<th>D&lt;sub&gt;10&lt;/sub&gt; (kGy)</th>
<th>RS</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.54 (R&lt;sup&gt;2&lt;/sup&gt;=0.99)</td>
<td>1.00</td>
</tr>
<tr>
<td>Combination</td>
<td>R²</td>
<td>K, ppm</td>
</tr>
<tr>
<td>-------------</td>
<td>----</td>
<td>--------</td>
</tr>
<tr>
<td>OR</td>
<td>0.45 ($R^2=0.90$)</td>
<td>1.20</td>
</tr>
<tr>
<td>CN</td>
<td>0.29 ($R^2=0.67$)</td>
<td>1.86</td>
</tr>
<tr>
<td>N</td>
<td>0.28 ($R^2=0.96$)</td>
<td>1.93</td>
</tr>
<tr>
<td>OR+N</td>
<td>0.26 ($R^2=0.82$)</td>
<td>2.08</td>
</tr>
<tr>
<td>CN+N</td>
<td>0.23 ($R^2=0.99$)</td>
<td>2.35</td>
</tr>
<tr>
<td>C(E)</td>
<td>0.55 ($R^2=0.99$)</td>
<td>1.00</td>
</tr>
<tr>
<td>OR(E)</td>
<td>0.43 ($R^2=0.91$)</td>
<td>1.28</td>
</tr>
<tr>
<td>CN(E)</td>
<td>0.33 ($R^2=0.92$)</td>
<td>1.67</td>
</tr>
<tr>
<td>N (E)</td>
<td>0.30 ($R^2=0.68$)</td>
<td>1.83</td>
</tr>
<tr>
<td>OR+N(E)</td>
<td>0.19 ($R^2=0.87$)</td>
<td>2.89</td>
</tr>
<tr>
<td>CN+N (E)</td>
<td>0.11 ($R^2=1.00$)</td>
<td>5.00</td>
</tr>
</tbody>
</table>

4. CONCLUSIONS

One major finding of the present study is that microencapsulation of nisin in alginate-CNC microbeads can be used as an edible coating for RTE cooked ham to inhibit the growth of *L. monocytogenes* during storage. Microencapsulation of nisin (63 μg/mL) increased the lag phase of bacterial growth up to 28 days. Molecular characterization revealed the interactions between alginate-CNC matrix and nisin and also demonstrated the higher retention activity of microencapsulated nisin during storage. These findings further established the importance of microencapsulation of antimicrobial agents compared to the conventional direct addition method. Furthermore, microencapsulation of EOs and nisin showed a synergistic anti-Listeria effect with γ-irradiation on RTE meat products. Our findings confirmed that CN+N (E)-γ showed a strong inhibitory effect up to 28 days and the bacterial count was below detection threshold. Hence, microencapsulation technology combined with irradiation could be an advanced process to improve the food safety for RTE meat market.

5. ACKNOWLEDGEMENTS

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Development of biodegradable based food packaging materials using gamma radiation

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ABSTRACT

A method based on melt processing for incorporating Chitin whiskers and Chitosan into PCL was developed for active food packaging without using a compatibilizing agent. Chitin whiskers were prepared and characterized using different techniques. Also, CS of high viscosity was incorporated after irradiation at 10-20 kGy and 1-5 wt % in order to maintain the mechanical properties of PCL and reduce the yellow coloration of CS caused by irradiation. Nevertheless, the preparation method should be modified, e.g. by changing the technique of mixing, to obtain more homogenous films with better mechanical properties. Characterization and evaluation of the antioxidant and antimicrobial capacities of the developed PCL/CS or Chitin whiskers films were carried out.

Introduction

Packaging technology is essential to present life styles in developed societies. With the general use of packaging and the development of modern techniques for food safety and commercialization, the universalized consumption of all food has become possible without distance or seasonal limitations, and at an adequate cost. Efficient packaging is necessary for the commercialization of every food type, from fresh produce to ready-to-eat meals. Nowadays, a wide and diversified supply of packaging materials and designs are available to suit the specific requirements of industry and consumers.

Packaging materials provide a means to preserve, protect, merchandise, market and distribute foods. They play a significant role in how these products reach the consumers in a safe and wholesome form without compromising quality. The relationship between the food and contact with the packaging material continuously interact and contribute to changes that can occur over time in these products. It is therefore important that several factors are considered when choosing the right package for a particular food product. Generally, the packaging material may either be rigid or flexible [1].
Development of materials for food packages occurs in response to the needs, and the needs have always been defined by the shortcomings of the materials in use at a given time. Packaging materials available prior to industrial revolution such as plant fibres, pottery and glass had the advantage of ready availability, but fell short of needs. Plant fibres were damaged by moisture and harboured pests. Pottery was heavy and brittle. Paper and paperboards which are made from plant fibres have low strength and high permeability to moisture and gases, and not ideally suited for food packaging. Glass has the advantages of transparency, imperviousness and inertness, but its fragility and high weight-to-strength ratio are limiting factors. Metal containers that came into use during 1700ís for food packaging, have excellent strength besides being retortable, the main disadvantage being their shape limitations.

Plant based fibres, paper and paperboard, glass and metal dominated packaging applications till the middle of the last century. Though they continue to have significant share in present day packaging, the advent of plastics has revolutionized the whole area of food packaging especially during the last quarter of the 20th century [2].

The use of plastics in food packaging has gone up several folds during the last two to three decades owing to the several advantages offered by them as compared to other materials. The most important advantages of plastics are their formabilities into practically unlimited range of shapes and forms and the broad range of their properties that enable design of packages with tailor-made functionalities.

Plastics being synthetic organic polymers, their properties can be adjusted, modified or enhanced by formulation, by adaptation in manufacture and by deploying such processing steps as orientation. One of plastics largest contributions to the packaging industry is its ability to be made into very thin films and containers. In fact, packagers are increasingly substituting plastics for alternative packaging materials because they can achieve significant reductions in packaging weight, volume and cost for the same amount of product delivered.

A major advantage in using plastics for packaging purpose is that most polymers possess excellent physical properties such as strength and toughness, combined with low weight and flexibility, as well as resistance to cracking [1].

The stability of packages in ambient and even hostile environments is reflected in their ability to retain their properties and functionality during the life of the package. Plastics are finite barriers to permeation and this property manifests in the form of oxygen transfer, gain or loss of moisture and sorption of flavour or aroma bodies. This property can often be used to advantage to control gas and moisture transfer as in the case of modified atmosphere
packaging. Most fresh foods need to ‘breathe’ and hence the packaging material used must allow ingress of oxygen and expiration of carbon dioxide. Where the material chosen does not permit sufficient gas transfer, the problem can often be solved by incorporation of a few holes punched onto the film. Fresh meat also requires ingress of oxygen to maintain a satisfactory surface colour. On the other hand, foods with a high fat content (dairy products, bacon, crisps, etc.) become rancid on exposure to oxygen and are often vacuum packed, or packaged in an inert atmosphere, using a material of very low permeability. Low permeability materials are also useful for the packaging of fish or coffee, where the odour must be contained strictly within the package [2, 3].

A number of bio-based materials and their innovative applications in food-related packaging have gained much attention over the past several years. These new materials include starch, cellulose, and those derived from processes involving microbial fermentation.

**PCL/Chitin Whiskers Nanocomposite Films**

**Preparation of Chitin whiskers**

Chitin whiskers were prepared from chitin powder according to the method described by Paillet and Dufresne (2001) with some modification [4]. Briefly, chitin was hydrolyzed with 3 N hydrochloric acid (HCl) under stirring and refluxing for 6 h. The ratio of HCl aqueous solution (3 N) to chitin was 30 ml/g. After hydrolysis, the suspension was diluted with distilled water, followed by centrifugation (10,000 rpm for 10 min) to separate the obtained chitin solid fraction from the aqueous medium. This process was repeated three times to remove residual HCl in the suspension. Finally, the residue was dried at 50 ºC for 24 h to obtain the product in a powder form. The yield was about 50%.

**FTIR analysis**

Characterization of the prepared Chitin whiskers was investigated using FTIR. As we can see from Figure (1), the treatments of chitin using different reagents to obtain Chitin whiskers have no significant effect on the chemical structure of chitin. The chemical structure of Chitin whiskers is the same as chemical structure of chitin [5].
Dynamic Light Scattering Study

The prepared Chitin whisker was investigated using DLS technique. The size of the Chitin whisker was measured using intensity, volume and number Gaussian size distributions for Chitin whiskers. As shown in Figure (2), the mean diameter according to the different methods was; Intensity Weighting (125.8 nm), Volume Weighting (74.7 nm), Number Weighting (39.6 nm).

AFM Study

The prepared Chitin whiskers were investigated using AFM. Figure 3 shows AFM images for Chitin whiskers. The length of Chitin whiskers is 2µm and the Pt is 17.5 nm.
Figure (2) Intensity, volume and number Gaussian size distributions for Chitin whiskers

- **Intensity Weighting (—):** Mean Diameter = 125.8 nm Stnd Deviation = 55.7 nm (44.3 %)
- **Volume Weighting (—):** Mean Diameter = 74.7 nm Stnd Deviation = 33.1 nm (44.3 %)
- **Number Weighting (—):** Mean Diameter = 39.6 nm Stnd Deviation = 17.5 nm (44.3 %)

**Preparation of PCL/Chitin whiskers nanocomposite film**

A required amount of Chitin whiskers was redispersed in glacial acetic acid by sonication for 30 min to obtain a colloidal suspension. PCL granules were dissolved in the prepared colloidal suspension with continuous stirring at 60 °C for 5 min. The mixture was dried at 50 °C for 24 h to obtain a dry blend. Afterwards, the obtained blend was soaked in 2N sodium carbonate solution for 2 h followed by washing with distilled water and drying at 50 °C for 24 h. Finally, PCL/Chitin whiskers nanocomposite film was prepared from the obtained blend by hot pressing in a compression molding machine operated at 80 °C for 1 min. FTIR spectra for PCL, Chitin whiskers and PCL/Chitin whiskers blend was perform. From the figure (4) of PCL/Chitin whiskers blend, it is clear the characteristic peaks for both PCL and Chitin [6].
Figure (3): AFM images for Chitin whiskers
* The mass ratio of Chitin whiskers to PCL is 5/95

**Figure (4):** FTIR Spectra for PCL, Chitin whiskers and PCL/Chitin whiskers blend

Effect of chitin on mechanical properties of PCL

Effect of chitin in the mechanical property of PCL was investigated. It is clear that the mechanical property of PCL has a slightly effect by adding chitin Figure (5).

**Water Vapor permeability of Chitin / PCL composite Films**

Water Vapor permeability of Un-irradiated and 10 kGy irradiated Chitin / PCL composite films was investigated. It was found that water vapor permeability significantly decreases for irradiated and un irradiated films as the amount of chitin increases.
The mass ratio of Chitin whiskers to PCL is 5/95

* The mass ratio of Chitin whiskers to PCL is 5/95

Figure (5): Effect of chitin on mechanical properties of PCL

PCL/chitosan nanocomposite films

In order to obtain CS powders with different molecular weights, CS (high viscosity, Fluka) solution was prepared at concentration of 5 w/v % in 1 w/v % acetic acid solution and exposed to gamma radiation with different doses (10, 20, 30, and 40 kGy). Thereafter, the irradiated chitosan solutions were dried at 50 ºC overnight and grinded to obtain CS powders.

The PCL/CS films were processed through two steps. In the first step, a master batch of CS was prepared by premixing of CS powders with PCL pellets (average Mn=80,000, Sigma-Aldrich, USA) in a beaker in the presence of a little of glacial acetic acid at 60 ºC for 5-10 min with good stirring to obtain a homogenous PCL/CS viscous mixture of composition 75/25 (w/w). Afterwards, this mixture was dried at 50 ºC for 24 h and then soaked in 2N sodium carbonate solution for 2 h followed by washing with distilled water and drying at 50 ºC for 24 h. In the second step, PCL/CS films with different CS contents were prepared by melt blending of the CS master batch (from the first step) with the required amounts of pre-dried PCL pellets at 80 ºC and then by hot pressing in a compression molding machine operated at 80 ºC for 1 min [7].

The present method involves the preparation of CS master batch and then blending with PCL. Photos in Fig. 6 show that the developed two step process can improve the dispersion of CS in PCL matrix by reducing the macroscopic domains of the former if compared with the single
step process where CS is melt blended with PCL directly. The FTIR spectra for PCL, CS, and PCL/CS are shown in Fig. 7. It is clear that there is no interaction between CS and PCL. In addition, the use of acetic acid at these conditions does not alter the structure of PCL.

![Figure 6](image)

**Figure (6): Photos of PCL/CS films prepared by a single step process without acetic acid (A) and by a two step process (B); CS content: 5%, irr. dose of used CS: 20 kGy.**

Incorporation of CS into PCL decreases generally both the tensile strength and elongation of PCL at break as shown in Fig. 8. However, the mechanical properties of the PCL/CS films that prepared from irradiated CS at 5 wt% were better if compared with those prepared from non-irradiated CS. As a result, the decrease of CS molecular weight caused by radiation help reduce its macroscopic domains in the blend. It is worth noting also that there was no a significant difference in the mechanical properties of the PCL/CS films that prepared from CS irradiated with different doses (10-40 kGy).

Fig. 9 shows the effect of CS content (from 0-10 wt %) on the mechanical properties of the prepared PCL/CS films. As the CS content increases, both the tensile strength and elongation at break decrease. The decrease in tensile strength and elongation can be attributed to the thermodynamic immiscibility and inherent incompatibility between CS and PCL which may lead to the formation of pores due to the de-bonding of the polymers upon the application of stress [8].
Figure (7): FTIR spectra of PCL, CS, and PCL/CS 5% films.

It was noted also that the yellow coloration of the prepared PCL/CS films increases with the irradiation dose and CS content (Fig. 10)

Figure (8): Effect of irradiation dose of CS on the tensile strength and elongation at break for the prepared PCL/CS films; CS content: 5%
Antimicrobial and antifungal activities of the prepared PCL/Chitosan films were investigated. It was found that the prepared PCL/Ch films possessed antimicrobial properties against some gram-ve and gram+Ve bacteria and also some fungi. As the amount of Chitosan increases the activity of the film towards the microorganisms increases. Also it is clear that the film activity against the microorganism depends on the Mwt of incorporated chitosan.

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Abstract

The aim of this study is dual: 1) to develop new films based on Polylactic Acid/Montmorillonite (PLA/MMT) with improved properties for applications in the food packaging industry and 2) to examine the effect of electron-beam (e-beam) radiation on the structural, morphological, mechanical and barrier properties of these nanocomposites. PLA with 1, 3 and 5 wt% of MMT were prepared in a twin screw extruder and then filmed by a calendar. All nanocomposites were irradiated with electron beam; one set at radiation doses of 1 kGy and another at 10 kGy. The effect of MMT addition and of e-beam radiation on the properties of polylactic acid were assessed. For all compositions good distribution and dispersion of MMT in the PLA and also orientation and intercalation of MMT were obtained. All nanocomposites showed an increase of the mechanical and oxygen-barrier properties compared to neat PLA. The e-beam radiation caused increase of the crystallinity, formation of crosslink, increase of the glass-transition temperatures and enhancement of the yield values in the stress-strain curves for all nanocomposites. This study demonstrates that PLA/MMT films are materials for irradiation of pre-packed food at the doses analysed in this work.

1. INTRODUCTION

In the last decades, the use of polymers as food packaging materials has increased enormously due to their advantages over other traditional materials. Polymers packaging provides many properties including strength and stiffness, and barrier to oxygen and moisture[1, 2]. Most plastics used in this field are polymers derived from petroleum because they possess many functional advantages united to the low cost and high productivity. However, they have some drawbacks such as the difficulty of disposal and recycling. For these reasons the research in recent years has turned with particular interest to "biopolymers": They are polymers made from renewable natural sources, often biodegradable and non-toxic to produce. Among these, of particular interest for the food packaging industry is the polylactic acid (PLA), a thermoplastic material produced from renewable sources. To improve the properties of PLA a modified montmorillonite (MMT) was used as nanofiller[3]. Radiation technologies are always more often applied by the food industry, in order to ensure food safety and reduce the risk of food-borne illnesses[4]. The World Health Organisation (WHO) and the European Food Safety Authority (EFSA) consider the decontamination of food by ionizing radiation a safe, efficient, environmentally clean and energy efficient process[5, 6, 7]. The aim of this study was to evaluate the changes on the morphology, the structure and the thermal, mechanical and barrier properties of PLA/MMT films due to electron beam irradiation with two doses: 1 kGy and 10 kGy.

2. EXPERIMENTAL PART

2.1 Materials

- Poly(L,L-lactide), PLA 4032 D, provided by NatureWorks, has the following characteristics: $M_n = 1.3 \cdot 10^5$ (g/mol), $M_w = 2.1 \cdot 10^5$ (g/mol), density = 1.24 (g/cm$^3$)
- Montmorillonite, Dellite 67G, provided by Laviosa, has the following characteristics: particle size (dry) 7-9 μm, modifier = dimethyl dehydrogenated tallow ammonium
2.2 Processing Conditions

Before mixing, the PLA was dried in an oven for 24h at 80 °C under vacuum. The blends were prepared by mixing of the components from the melt using a twin-screw extruder; for comparison also the PLA was extruded so that all samples have the same thermal history. The extruder used is a tool Collin ZK 25 (D = 25 mm and L / D = 24). The temperature setting of the extruder from the hopper to the die was 150/170/170/170/160 °C, and the screw speed was 25 r.p.m. The composition used is reported in Table 1.

* partially presented at international conference "Ecosustainable food packaging based on polymer nanomaterials" 26-28 February 2014, Roma

<table>
<thead>
<tr>
<th>Sample</th>
<th>PLA (wt%)</th>
<th>Dellite 67G (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>PLA/D67G 1%</td>
<td>99</td>
<td>1</td>
</tr>
<tr>
<td>PLA/D67G 3%</td>
<td>97</td>
<td>3</td>
</tr>
<tr>
<td>PLA/D67G 5%</td>
<td>95</td>
<td>5</td>
</tr>
</tbody>
</table>

In order to obtain films, the material obtained from the extruder in pellets was again dried in an oven for 24 h at 80 °C under vacuum. The films were prepared using a single screw extruder with a terminal a calender 3-cylinder, two counterrotating including passing the film still in the plastic state and the third useful to direct the output material until the collecting cylinder. The extruder and the terminal are used, respectively, instruments Colin E 20T and 72T Colin CR. the extrusion was conducted using the following temperatures: 160/170/180/170/180 °C; the speed of the extruder screw is 40 rpm. The samples were irradiated with electron beam radiation at 1 kGy and 10 kGy

3. RESULTS AND DISCUSSION

3.1 Morphological analysis

The surface analysis was performed using a SEM Fei Quanta 200 Feg.

Fig. 1a: SEM micrograph of PPR/D67G 3% irradiated 1 kGy
Fig. 1b: SEM micrograph of PPR/D67G 3% irradiated 10 kGy
SEM images of the samples irradiated with electron beam show that the irregularities on the surface increase with radiation doses. This effect can be ascribed to crosslink formation and/or matrix degradation. (Fig. 1a,b)

3.2 Mechanical properties

Stress–strain curves (Fig. 2) were obtained using an Instron machine (Model 4505) at room temperature (25 °C) at a crosshead speed of 2 mmmin⁻¹.

After irradiation all the samples show an increase of yielding-point and Young modulus, increasing e-beam doses [8]. This behaviour is probably due to the crosslink formation.

3.2 Permeability test

The O₂ permeability was tested on films by ExtraSolution Multiperm apparatus(Table 2). Measurements were carried out in triplicate at 23°C and 0% RH. Oxygen permeability was calculated from OTR data by the Eq(1):

\[
\text{Permeability} = \frac{(\text{OTR} \times \text{thickness})}{\Delta P} \quad \text{(Eq.1)}
\]
TABLE 2: Comparison between the values of the OTR and oxygen permeability of the samples treated with electron beam at 1 and 10 kGy with the untreated samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>OTR (cm³ / (24h*m²))</th>
<th>Permeabilità (cc<em>cm/m²</em>24h*bar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA/D67G</td>
<td>573</td>
<td>2,23 ± 0,22</td>
</tr>
<tr>
<td>PLA/D67G_1kGy</td>
<td>519</td>
<td>1,61 ± 0,01</td>
</tr>
<tr>
<td>PLA/D67G_10kGy</td>
<td>539</td>
<td>1,78 ± 0,04</td>
</tr>
<tr>
<td>PLA/D67G 1%</td>
<td>458</td>
<td>1,76 ± 0,01</td>
</tr>
<tr>
<td>PLA/D67G 1%_1kGy</td>
<td>441</td>
<td>1,52 ± 0,01</td>
</tr>
<tr>
<td>PLA/D67G 1%_10kGy</td>
<td>455</td>
<td>1,64 ± 0,08</td>
</tr>
<tr>
<td>PLA/D67G 3%</td>
<td>385</td>
<td>1,52 ± 0,03</td>
</tr>
<tr>
<td>PLA/D67G 3%_1kGy</td>
<td>358</td>
<td>1,43 ± 0,09</td>
</tr>
<tr>
<td>PLA/D67G 3%_10kGy</td>
<td>390</td>
<td>1,58 ± 0,05</td>
</tr>
<tr>
<td>PLA/D67G 5%</td>
<td>380</td>
<td>1,52 ± 0,07</td>
</tr>
<tr>
<td>PLA/D67G 5%_1kGy</td>
<td>374</td>
<td>1,46 ± 0,01</td>
</tr>
<tr>
<td>PLA/D67G 5%_10kGy</td>
<td>403</td>
<td>1,63 ± 0,08</td>
</tr>
</tbody>
</table>

The clay addition decreased the oxygen permeability. After e-beam irradiation there was a further decrease of the permeability values due to matrix stiffening caused by crosslink formation.

4. CONCLUSIONS

This study was aimed to finding the adaptability of PLA/MMT nanocomposites films to the food sterilization by radiation. After e-beam irradiation all samples showed some irregularities on the surface, an increase of $T_g$ and Young modulus and finally a decrease of permeability: these results were related to the effect of the clay on the matrix and to crosslink formation following the irradiation. The results showed that these materials are suitable for sterilization by e-beam irradiation at the doses studied.

REFERENCES


APPLICATION OF RADIATION TECHNIQUES IN DEVELOPMENT OF ADVANCES PACKAGING MATERIALS FOR FOOD PRODUCTS

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ABSTRACT
This paper reports a characterization of polyolefin film with antimicrobial additive (AM), sorbic acid. Radiation induced grafting of sorbic acid on low density polyethylene (LDPE) film has been performed with the aim to developed antimicrobial active packaging film. The covalent attachment of sorbic acid onto LDPE surface was performed by pre-irradiation method. The grafted samples were characterized with respect to their oxygen permeability, water contact angle and mechanical properties. Oxygen permeability and water contact angle of the grafted film slightly increased compared with raw LDPE film. Tensile strength increased from 20.48 MPa to 32.61Mpa, while elongation at break increased from 193.25 % to 254.47% when 10% of SA was incorporated. The grafted film were then use to wrap slice of freshly baked bread of which AM additive was not used. The visual inspection was performed against fungal growth and compared with LDPE control film at 0, 5, 7 and 10 days of its wrapping. It was found that SA grafted film could delay the appearance of mould and yeast on the slice of freshly baked bread for up to 10 days with respect to control film. The results reveals that grafting of sorbic acid onto polyethylene is a very promising strategy to generate novel sorbic acid based antimicrobial materials with potential advantages for active antimicrobial packaging applications.

1. INTRODUCTION
Petrochemical-based plastics such as LDPE have gained much attention in packaging technology because of their physical properties, prominent strength and barrier properties to protect food form spoilage by microbial contaminant [1]. In general, plastic packaging provides several advantages when used in food packaging such as, lightweight and low cost with notable strength compared to other packaging material, good oil and chemical resistance, heat sealing, excellent gas and water vapour barrier properties, thermally stable, easily reused and recycled [2]. However, this traditional packaging only meant for mechanical supporting and protecting food from external contaminant and was considered as passive protection due
to the its key safety when in contact with foods is to be as inert as possible[3]. Thus, new polymer based packaging for food technology was developed during past decade to overcome this problem. At the point of safety, industrial food production had moved towards fresh, less chemical food products with maximum quality and prolonged shelf-life. In addition, trends in market globalization resulting in longer distribution of food and consumer’s way of lifestyle act as driving forces for the development of new and improved packaging to expand the function of traditional packaging. In fact, polymer packagings are now shifting from passive protection to an active role in improving food quality. There have been remarkable developments in recent years in the polymeric packaging films incorporated with antimicrobial agents for improving the preservation of packaged foods. These films possess the potential for improving microbial stability of foods by acting on the food surface [4, 5].

Modification in polymeric structure of plastic material can be achieved either by conventional chemical process or by ionization radiation from radioactive sources or highly accelerated electrons. Among these, radiation grafting is well known as a versatile and a clean method because of its large penetration in polymer matrix, rapid and uniform formation of active sites for initiating grafting throughout the matrix which is useful for the development of new materials that feature unique properties. Radiation-induced grafting in its simplest form involves heterogeneous systems, with the substrate being film, fibre, or even powder and the monomer to be grafted onto the substrate can be liquid, vapour, or solution. Polymers are quite often irradiated for modification of the chemical and physical properties, and are of particular interest for achieving specifically desired properties. Under appropriate experimental conditions, modification of polymer properties can be accomplished not only at the surface but also throughout the internal phase of polymer [6]. Grafting of polyethylene with polar functional groups is a very good method for obtaining new materials of special physical-chemical properties. The incorporation of polar functional groups like carbonyls (C=O) into polymeric chain provides specific sites for interactions such as hydrogen and covalent bonding which improves the compatibility of polymer with other materials [7]. Moreover, the attachment of covalent bond provides long lifetime of chemical stability by means of introduced chains, compared to physically coated polymer chain [8].

Sorbic acid (2,4-hexadienoic acid) is considered a GRAS additive used in beverages, processed fruits and vegetables commonly used as model additive in release studies [9, 10]. Furthermore, the effective concentrations normally do not alter the taste or odor of a food
product and are considered harmless [11]. Recently, the radiation-induced grafting of antimicrobial agent on polymer films with electron beam, x-rays, gamma or ultra-violet has been reported [12-15]. Therefore, the research aimed at evaluating the properties, including oxygen permeability, tensile strength, elongation at break, water contact angle and visual inspection of antimicrobial LDPE film grafted with sorbic acid.

2. MATERIALS AND METHOD

2.1 Materials
Low Density Polyethylene film (thickness ~ 50µm) was extruded using blown film extrusion. Sorbic acid was purchased from Sigma-Aldrich and used as received. Solvent and chemical reagents were of laboratory grade and were used without further purification.

2.2 Irradiation of LDPE Film
The LDPE film was cut into 10cm x 10cm square pieces of known weight and ultrasonically washed in methanol solution for 1 hour to remove surface contaminants. Subsequently the film was dried in a vacuum oven at 60°C until it reached constant weight and thereafter was put in a sealed bag and flashed with nitrogen for 2 minutes to remove the air. The film samples were placed on dry ice and irradiated using electron beam accelerator operated at voltage of 2MeV to a different dose ranging from 10kGy to 100kGy. The irradiated film was directly used for grafting reaction after irradiation.

2.3 Grafting Process
Pre-irradiation grafting method was employed to graft sorbic acid (SA) onto low density polyethylene (LDPE) film. 10cm x 10cm square pieces of LDPE films were placed in a zippered bag, which was sealed after nitrogen flushing and irradiated at dry ice temperature using electron beam accelerator. The irradiated film was directly used for grafting reaction after irradiation. The grafting reaction was carried out in an ampoule, where the irradiated sample was placed inside the ampoule and immersed with sorbic acid solution of 2%, 5% and 10% of SA concentration for 3 hours at 60°C. The samples were then removed from the solution and cleaned by sonicating for 1 hour in distilled water and washed again repeatedly with distilled water until no subsequent weight decrease.

2.4 Grafting Yield
The grafting parameter used to characterize the nature of copolymer is defined with the weight basis expression. The grafting percentage was calculated by the percentage weight gain of LDPE film after grafting process.
\[ Grafting \ Yield \ (GY) = \frac{Final \ Weight \ (W_g) - Initial \ Weight \ (W_o)}{Initial \ Weight \ (W_o)} \times 100 \quad (3.1) \]

where \( W_o \) and \( W_g \) are the weights of the ungrafted and grafted films, respectively.

2.5 Characterization of film

Oxygen permeability rates were measured using a constant-pressure system and a soap bubble flow meter. Permeation tests were carried out at 25°C with feed gas pressure of 5 bar gauge. The measurement was repeated three times for each sample. Pure gas permeability was calculated using Eq. (1):

\[ P = \frac{l}{A \Delta p} \frac{dV}{dt} \quad (1) \]

where \( P \) is the permeability, \( \Delta p \) is the pressure difference across films (Pa), \( A \) is the effective surface area \((12.5 \times 10^{-4} \text{ m}^2)\), \( l \) is the thickness of film (m), \( t \) is the permeation time (s), \( V \) is the volume of the gas permeated through the film \((\text{m}^3_{\text{STP}})\).

Tensile properties were measured on SHIDMADZU Autograph Tensile Test Machine Model AGS-G according to ASTM D1822L. The gauge length was set at 30 mm and the cross head speed of 1 mm/min was used and the test was performed at 25 ± 3°C. The grafted film morphology was analyzed as a function of grafting yield using AFM (Shimadzu SPM-9500J2). The water contact angles of the surface of the samples were measured with Static Contact Angle Measurement using the drop drop method (each sample was analyzed three times).

3. RESULT AND DISCUSSIONS

As shown in Table 3.1, the oxygen permeability \( (OP) \) values of the control film and of the films with sorbic acid (SA) grafted at different concentration were compared. The effect of sorbic acid grafting onto LDPE films plays an important roles in improvement of OP. From the results, lowest OP was observed at LDPE+ 10% SA with \( 3.07 \times 10^{-16} \text{ m}^3\text{m}^2\text{sPa} \), which is significantly different from other film. LDPE grafted with 2% and 5% of SA presented values of \( OP \) of \( 3.34 \times 10^{-16} \) and \( 3.21 \times 10^{-16} \text{ m}^3\text{m}^2\text{sPa} \), respectively, with LDPE+2% being the highest OP value indicates that the film is not qualified for good oxygen prevention as compared with other films. \( OP \) of the films decreased with the incorporation SA probably due to limited inter molecular chain mobility and decreased its free volume resulting to the decrease of OP. This result indicates the potential of LDPE-g-SA films to be used as
antimicrobial packaging to protect food from deterioration. The films with low oxygen permeability are suitable for confectionery products, baked foods, nuts and other products that are susceptible to oxidation [16].

Tensile strength (TS) and elongation at break (EAB) of LDPE film grafted with different concentration of SA are shown in Table 3.1. The incorporation of SA significantly improved both TS and EAB. The control film showed lower TS (20.48 MPa) and lower EAB (193.25%) than those grafted films. When SA concentration increased from 2% to 10%, TS also increased from 31.25 MPa to 32.61 MPa. On the other hand, EAB of antimicrobial film significantly increased when compared with control film from 201.10% up to 254.47% with increasing concentration of SA from 2% to 10%. However, the TS and EAB of film with 2% and 5% SA was lower than those films with 10% SA indicating the film with 10% sorbic acid exhibit superior TS and EAB properties. An improvement of mechanical properties of grafted film was probably due to the grafting of SA onto backbone of LDPE film and therefore increases the flexibility of polymer chains. The tensile results suggested that incorporation of sorbic acid via radiation grafting could improve the tensile properties of LDPE-g-SA film.

**TABLE 3.1**: Oxygen permeability (OP), tensile strength and elongation at break of LDPE and LDPE-g-SA film.

<table>
<thead>
<tr>
<th>Samples</th>
<th>OP × 10⁻¹⁶ (m³/m²sPa)</th>
<th>Tensile strength (MPa)</th>
<th>Elongation at break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDPE (control)</td>
<td>2.93</td>
<td>20.48 ± 0.70</td>
<td>193.25 ± 0.61</td>
</tr>
<tr>
<td>LDPE + 2% SA</td>
<td>3.85</td>
<td>31.25 ± 0.29</td>
<td>201.10 ± 1.31</td>
</tr>
<tr>
<td>LDPE + 5% SA</td>
<td>3.21</td>
<td>31.84 ± 1.12</td>
<td>213.17 ± 0.73</td>
</tr>
<tr>
<td>LDPE + 10% SA</td>
<td>3.07</td>
<td>32.61 ± 0.32</td>
<td>254.47 ± 1.36</td>
</tr>
</tbody>
</table>

Contact angle measurements were performed to provide information regarding the contact angle of water on the surface of LDPE and LDPE-g-SA. As shown in Figure 3.1, generally, all the contact angle for grafted of sorbic acid onto LDPE film are above 90° which means that the films have a hydrophobic surfaces. Before grafting, the contact angle of control LDPE film is about 86.42° which mean that the surface of the material is hydrophilic. As to the grafted film, the water contact angle increases up to 105.25°, indicating that grafting of sorbic acid led to a decreased of surface wettability of the grafted film in comparison to the raw LDPE. The enhanced of LDPE-g-SA wettability properties are determined by the chemical composition and surface morphological structure of material surface, which associated to its
surface roughness. These results correlate well with AFM micrograph observations that indicated attachment of sorbic acid at film surface.

<table>
<thead>
<tr>
<th>LDPE</th>
<th>LDPE-g-SA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left: 86.42°</td>
<td>Left: 105.06°</td>
</tr>
<tr>
<td>Right: 86.06°</td>
<td>Right: 105.25°</td>
</tr>
</tbody>
</table>

**Figure 3.1:** Contact angle images of deionized water droplet over the film surface for LDPE and LDPE-g-SA.

The behaviour of the slice fresh baked bread was visually evaluated to study the effectiveness of the develop film and their ability to act as active packaging to increase the food shelf-life. The antimicrobial activity was assessed against food spoilage and pathogenic bacteria between the bread packed with LDPE control film and grafted film. Figure 3.2 shows the appearance of sliced bread samples at the beginning of the experiment (day 0) and until the observation of microbial growth for a maximum period of 10 days. As the bread formulation did not include any preservative, fungal growth appeared very early. However, satisfactory results were obtained for samples in contact with the active film, since no fungi growth was observed until seven days of storage. On day 5, deterioration signs were observed with the appearance of mould and yeast in the slice bread packed with LDPE control film. These sign were not observed in AM grafted film. Active packaging of slice bread was highly effective in delaying fungal growth as an initial of contamination on the slice bread was observed after 10 days of storage at room temperature. After 10 days, rapid growth of microorganism was more marked for both films.
**Figure 3.2:** Visual inspection of slice fresh baked bread behaviour packaged with LDPE control film and LDPE-g-sorbic acid film.

<table>
<thead>
<tr>
<th>Day</th>
<th>LDPE control film</th>
<th>LDPE-g-sorbic film</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>Day 5</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>Day 7</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
</tbody>
</table>
4. CONCLUSION
This study showed that grafting of sorbic acid (SA) onto LDPE film has significant effect on its film properties. LDPE-g-SA film exhibits an excellent improvement in both tensile strength and elongation at break, a consequence of increasing flexibility of polymer chains. Contact angle of the grafted film revealed a decreased of surface wettability which means the films have a hydrophobic surfaces. It was also found that SA grafted film could delay the appearance of mould and yeast on the slice of freshly baked bread for up to 10 days with respect to control film. Overall, the use of SA as active agent offer high potential for the development of antimicrobial active film for food packaging. Thus, future research in this area should focus on the stability of the grafted film and microbial analysis to evaluate the effectiveness of its antimicrobial properties.

5. REFERENCE


Effects of Gamma Irradiation on Commercial Food Packaging Films

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Abstract

Commercial packaging films made up of Plain PET 12 / Foil 7 / PE 100, VMPET 12 / PE 70, OPP 20 / Foil 6.5 / PE 40, PET 12 / CPS 40, PET 12 / PE 50, laminated PET / PE, Nylon / PE, and Nylon 15 / PE 50 were investigated for its effect with gamma radiation at 10 kGy. Their mechanical and thermal properties generally did not show any changes after irradiation except for 20 OPP 20 / Foil 6.5 / PE 40. Gel Permeation Chromatography of leachates from water samples detected the presence of high molecular weight radiolytic products especially from laminated PET/PE films. Radiation effects were minimal for VMPET12 /PE70, Nylon/PE, and Nylon 15/PE 50 films. Preliminary results, using the stable isotope technique, to study the water leachates from the packaging materials reveal an indicative increase in δ¹⁸O ‰ and δ D ‰.

1. INTRODUCTION

Gamma radiation is a well known technology to inactivate bacterial pathogens in food products. Currently, there is a growing interest in this technology considering its advantages of being a non-thermal process and the convenience of food being pre-packaged in its final form before treatment that prevents possible recontamination. The process of irradiating pre-packaged food requires that appropriate packaging materials are chosen as this would play a vital role in the quality assessment and safety evaluation of the irradiated products. Irradiation can cause changes to the packaging materials that might affect its integrity and functionality as a barrier e.g. to chemical or microbial contamination. Likewise, components of packaging materials that have been irradiated may migrate to food as a result of irradiation. Hence, the type of packaging materials required for irradiated food must also satisfy additional requirements such as resistance to radiation that may produce breakage, change in sensory properties, entrance of microorganisms or insects and migration of toxic substance from the packaging materials to food.

The Philippine Nuclear Research Institute (PNRI) is currently participating in a CRP on “The Development of Irradiated Foods for Immuno-Compromised Patients and other Potential Target Groups” with its specific project entitled “Development of Safe, Quality and Shelf-Stable Filipino Ethnic Foods for Immuno-compromised Patients and Calamity Victims”. This research is aimed towards developing radiation processed ready-to-eat foods for immuno-compromised patients and shelf-stable foods for calamity victims. While the main interest of this research is to evaluate the microbiological, sensory and nutritional contents of the products after irradiation, this current study would complement on-going research on irradiated shelf-stable food by investigating on the packaging materials used for the irradiated food.

2. METHODOLOGY

2.1. Materials

Nine (9) commercial food packaging plastics and laminates were collected from various companies as listed below:
Table 1. List of Commercial Packaging Materials used for Analyses

<table>
<thead>
<tr>
<th>Packaging Material</th>
<th>Thickness (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PET/Foil/PE</td>
<td>119</td>
</tr>
<tr>
<td>Plain PET 12 / Foil 7 / PE 100</td>
<td>125</td>
</tr>
<tr>
<td>VMPET 12 / PE 70</td>
<td>86</td>
</tr>
<tr>
<td>OPP 20 / Foil 6.5 / PE 40</td>
<td>65</td>
</tr>
<tr>
<td>PET 12 / CPS 40</td>
<td>52</td>
</tr>
<tr>
<td>PET 12 / PE 50</td>
<td>62</td>
</tr>
<tr>
<td>Laminated PET / PE</td>
<td>82</td>
</tr>
<tr>
<td>Nylon / PE</td>
<td>115</td>
</tr>
<tr>
<td>Nylon 15 / PE 50</td>
<td>65</td>
</tr>
</tbody>
</table>

2.2. Sample Irradiation
Packaging materials were irradiated at an absorbed dose of 10 kGy using the Co-60 facility of the Philippine Nuclear Research Institute at a dose rate of 1 kGy/hr.

2.3. Mechanical Properties
Tensile strength for both non-irradiated and irradiated films was measured using a Zwick/Roell Universal Testing Machine. Packaging samples were cut using a dumbbell cutter with dimensions of 100 mm x 15 mm. The specimens with known thickness were mounted between the grips of the machine, which were then separated at a constant speed of 500 mm/min. Elongation at break was also measured using the same machine at a speed of 30 mm/min. A 10 mm distance was marked at the center of the film and measured for elongation at break.

2.4. TG-DTA Analyses
Thermogravimetric measurement of the irradiated and non-irradiated packaging materials was carried out using Netzsch Simultaneous TG-DTA apparatus with Al₂O₃ crucible. The samples were heated from 30 to 950°C at a rate of 10°C min⁻¹ and maintained under nitrogen atmosphere at a flow rate of 50 ml/min.

2.5. Over-all Migration Test
Three replicates each of sample pouches with a dimension of 9.5 cm x 9.5 cm were prepared and filled with 50 mL of ultrapure water. Non-irradiated and irradiated samples were heated at 60°C for 30 minutes and cooled down to room temperature. Ten (10) mL of water was collected and contained in pre-weighed aluminium foil pouches. The water was then evaporated and weighed to constant weight. The amount of leachates were calculated gravimetrically.

2.6. Gel Permeation Chromatography
Gel Permeation Chromatography (GPC) analyses of the leachates were performed on a Shimadzu Prominence LC-20AD equipped with CTO-20A column oven, SPD-20A Prominence UV-Vis detector at wavelength of 285nm and three TSK gel PWXL columns in series (G4000 PWXL, G3000 PWXL and G2500 PWXL). Elution was carried out using water as the mobile phase at a flow rate of 0.5 ml/min. The temperatures of the column and detector were both maintained at 40°C. A calibration curve was constructed using polyethylene oxide as standards. All molecular masses reported in this work are based on PEO standards and are not absolute.

2.7. Gas Chromatography – Mass Spectrometry (GC-MS)
Non-irradiated and irradiated packaging material pouches with a dimension of 9.5 cm x 9.5 cm were prepared. Three mL of air was collected from the pouch using a syringe and was dissolved in 0.5 mL of dichloromethane. GC/MS was performed using a Gas Chromatograph, Perkin Elmer Mass Spectrometer. The carrier gas was Helium at 2 Bars column pressure. The temperature of the injector was 50°C to 220°C at 16°C/sec. Separation was carried out on a 5MS silica column, 30 m, 0.25 mm internal diameter and 0.25 µm film thickness with a flow rate of 0.7 ml/min. Mass Spectrometry was
done using a 200°C ion source temperature with an energy of 70 eV. Volatile products were identified by comparing the mass spectra of the recorded chromatographic peaks with those from the GC/MS system using NIST and user-created libraries.

2.8. Stable Isotope Analysis

Packaging material pouches with dimension of 9.5 cm by 9.5 cm were prepared and filled with 50 mL of ultrapure water. Filling of water was done fast enough to reduce probability of isotopic exchanges with O and H in air. All air bubbles were manually removed from the packaging materials before sealing. Non-irradiated and irradiated samples of water were transferred to vials which were filled to the brim before tightening. The IR-MS analysis for the determination of 2H/H (δD) and 18O/16O (δ18O) was done at the Isotope Application Division of Pakistan Institute of Nuclear Science and Technology, Islamabad, Pakistan.

3. RESULTS AND DISCUSSION

3.1. Mechanical Properties of Packaging Films

The results of the tensile strength test for the non-irradiated and irradiated samples are shown in Fig. 1. Tensile strength of all the packaging materials was not significantly altered at 10 kGy. Similar studies have also indicated that no changes are observed at an absorbed dose of 10 kGy. Studies conducted by Goulas et al in 2003 indicate that mechanical properties of packaging materials from PP, EVOH, LDPE, LLDPE and PA are not affected at an absorbed dose of 10 kGy [1]. Changes are only observed at 30 kGy. Mizani et al. also reported that the tensile strength of multilayer films, namely biaxially oriented polypropylene/castpolypropylene (BOPP/CPP) and poly-ethylene terephthalate / polyethylene terephthalate/ linear low density polyethylene (PET/PP/LLDPE) are not significantly changed up to an absorbed dose of 15 kGy [2]. PP-based multi-layer films also have no significant differences at an absorbed dose of 10 kGy [3]. Tensile strength at break of PET/PP irradiated up to an absorbed dose of 45 kGy is not altered [4].

Figure 1. Tensile Strength of the Different Packaging Materials Before and After Irradiation

The effect of percent Elongation at Break of irradiated packaging films is shown in Table 2. All of the packaging materials had an increase in % elongation after irradiation, VMPET 12 / PE 70 being the highest with an increase of 21%. Similar studies also indicate that elongation at break of PET/PP [4],
PET [5] and LDPE [6] films improved after irradiation at absorbed doses of up to 30 kGy. Increase in elongation at break of irradiated PET/PP films has been attributed to a possible dominion of crosslinking over degradation in the structure [4].

Table 2. Elongation at Break of Non-irradiated and Irradiated Packaging Films

<table>
<thead>
<tr>
<th>Packaging Material</th>
<th>% Elongation at Break</th>
<th>% Increase after irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-irradiated</td>
<td>Irradiated</td>
</tr>
<tr>
<td>PET/Foil/PE</td>
<td>51</td>
<td>57</td>
</tr>
<tr>
<td>Plain PET 12 / Foil 7 / PE 100</td>
<td>56</td>
<td>75</td>
</tr>
<tr>
<td>VMPET 12 / PE 70</td>
<td>46</td>
<td>67</td>
</tr>
<tr>
<td>OPP 20 / Foil 6.5 / PE 40</td>
<td>77</td>
<td>94</td>
</tr>
<tr>
<td>PET 12 / CPS 40</td>
<td>29</td>
<td>39</td>
</tr>
<tr>
<td>PET 12 / PE 50</td>
<td>39</td>
<td>53</td>
</tr>
<tr>
<td>Laminated PET / PE</td>
<td>55</td>
<td>63</td>
</tr>
<tr>
<td>Nylon / PE</td>
<td>48</td>
<td>56</td>
</tr>
<tr>
<td>Nylon 15 / PE 50</td>
<td>56</td>
<td>76</td>
</tr>
</tbody>
</table>

3.2. TG-DTA

The TG-DTA thermograms of samples of packaging materials (plain PET 12 / Foil 7 / PE 100 and OPP 20 / Foil 6.5 / PE 40) are shown in Figures 2 - 3. Most packaging materials did not have any significant change in its thermal degradation pattern. Degradation peaks were both the same for the non-irradiated and irradiated samples as shown in Fig. 3. Among the films tested, only the OPP 20 / Foil 6.5 / PE 40 packaging material had a slight decrease from 472.1°C to 469.2 °C. This could denote degradation of the packaging material with irradiation. The film contains poly propylene which is known to easily degrade with radiation. Data on BOPP/CPP indicate that thermal property of this packaging film is decreased with irradiation which has been attributed to some chain scissions especially in the amorphous layer of the BOPP/CPP film structure [2].

Figure 2. TG-DTA thermogram of non-irradiated (N-PA2) and irradiated (PA2) plain PET 12 / Foil 7 / PE 100 packaging material
Figure 3. TG-DTA thermogram of non-irradiated (N-PA2) and irradiated (PA2) OPP 20 / Foil 6.5 / PE 40 packaging material

3.3. Over-all Migration Test

Irradiation of packaging materials results in the release of volatile radiation products which can affect the sensory properties of the packaging materials. Overall migration test was performed to determine the residual concentration of possible leachates in the water samples collected from the packaging material. Results of analyses indicated no detection of residues from both non-irradiated and irradiated films. Welle, et. al mentioned that using the test procedure for overall migration given by EU regulations, volatile substances could not be detected. Therefore, the overall migration is unchanged with increasing absorbed dose [7]. Other alternative methods have to be done to identify specific radiolytic volatile products.

3.4. Gel Permeation Chromatography of Water Leachates

Studies on the radiolytic products from irradiated packaging materials through the determination of volatile components have been done by GC-MS [8,9]. However, no studies have been done so far on the leachates using water as the simulated food stimulant. This study shows the results of GPC analyses of water leachates from non-irradiated and irradiated packaging films (Figures 4-12). In general, leachates were detected even for non-irradiated films. These could be due to some additives such as plasticizers which have been incorporated into the packaging materials. The MW detected consisted of low molecular weight (<1 kDa) to high molecular weight (> 1,000 kDa) with the majority of the leachates within the 500 – 1,000 kDa range. The intensities of the detected peaks were much higher after irradiating the films. In most cases, irradiation at 10 kGy resulted in the appearance of new peaks. These results clearly indicate that radiolytic products are being formed and leached out in water after irradiation.

PET/Foil/PE

Figure 4 shows the GPC chromatogram of non-irradiated and irradiated PET/Foil/PE films. For the non-irradiated film, a large peak had been detected at an MW of 490 kDa. With irradiation, the intensity of this peak had been substantially reduced. This compound may have been degraded into lower MW fragments as indicated by the appearance of a new peak at an MW of 79 kDa with medium intensity. Slight increase in the peak at MW = 19 kDa was also observed. A new peak with very high MW of 2,110 kDa had been detected for the irradiated film. Considering that it is a high molecular weight polymer which is originally absent in the non-irradiated film, this compound may be a result of the degradation of PET or PE.
Plain PET12/Foil/PE100

Figure 5 shows the GPC chromatogram of non-irradiated and irradiated Plain PET12/Foil/PE100 films. There are two distinguishable peaks with an MW of 759 kDa and 33k kDa for the non-irradiated films. With irradiation, these peaks were greatly reduced. Appearance of two (2) new intense peaks at an MW of 306 kDa and a low molecular weight polymer at 4 kDa were observed. Since these two peaks have strong signals, these are not mere degradation products from those detected from non-irradiated films. These are radiolytic products from the degradation of PET12/Foil/PE100 films. Comparing the chromatogram of this formulated PET12/Foil/PE100 with the former (PET/Foil/PE100), the non-irradiated films had peaks within the range of MW = 500 – 1000 kDa. The effect of irradiation produced also similar radiolytic products with an MW range of 100-500 kDa. However, intensity of peaks were higher for the PET12/Foil/PE100 films.

VMPET12/PE70

Figure 6 shows the GPC chromatogram of non-irradiated and irradiated VMPET12/PE70. Four (4) peaks ranging from an MW of 1,002 kDa to 127 kDa were detected. Unlike the previously discussed
films, no new peaks have been detected with the irradiated VMPET12 /PE70. Areas of peaks, however, were approximately twice higher than the non-irradiated film.

![Figure 6. GPC of Non-irradiated and Irradiated VMPET12 /PE70 Films](image)

**OPP 20/Foil6.5/PE40**

Figure 7 shows the GPC chromatogram of non-irradiated and irradiated OPP 20/Foil6.5/PE40. The non-irradiated films had two sets of peak. The first peaks are within the range of MW = 100-1000 kDa with three overlapping peaks and the major peak having an MW of 524 kDa. The second set of peaks had two overlapping peaks of equal intensities within the range of MW 50-100 kDa. With irradiation, the higher MW peaks had been retained with no significant changes in intensities. The lower MW peaks, however, had substantially increased in intensity and had a much broader peak which may signify a wider molecular weight distribution of the leached polymers.

![Figure 7. GPC of Non-irradiated and Irradiated OPP 20/Foil6.5/PE40 Films](image)

**PET 12/CPS 40**

Figure 8 shows the GPC chromatogram of non-irradiated and irradiated PET 12/CPS 40. Small peaks almost indistinguishable from noise were seen in the chromatogram of the non-irradiated film. With irradiation, appearance of four (4) peaks with an intense peak at an MW of 1,196 kDa was observed.
Figure 8. GPC of Non-irradiated and Irradiated PET 12/CPS 40 Films

PET 12/PE 50

Figure 9 shows the GPC chromatogram of non-irradiated and irradiated PET 12/PE 50. Minor peaks at MW range of 500 kDa to 1,000,000 kDa and a major peak at < 1 kDa were observed in the non-irradiated film. Considering that the major peak has an MW < 1 kDa, this compound may not be a polymer but could be a non-polymer additive e.g. plasticizer. Irradiation of the film had produced more leachates at the MW range of 350-500 kDa, giving more intense peaks. New peaks were also observed at an MW of 50-100 kDa. Irradiation, likewise resulted in the disappearance of the peak found at MW < 1 kDa.

Figure 9. GPC of Non-irradiated and Irradiated PET 12/PE 50 Films

Laminated PET/PE

Figure 10 shows the GPC chromatogram of non-irradiated and irradiated laminated PET/PE. Similar to the results of the PET 12/PE 50 films, it has small peaks at an MW range of 500 kDa to 1,000,000 kDa and a strong peak at a very low Mw of less than 1 kDa. Appearance of a strong intense peak at MW = 830 kDa and a very broad and strong peak at an MW of 34 kDa with a shoulder peak at 22 kDa were observed with irradiation. Disappearance of the low MW peak of < 1kDa was also observed.
Figure 10. GPC of Non-irradiated and Irradiated Laminated PET/PE Films

**Nylon/PE**

Figure 11 shows the GPC chromatogram of non-irradiated and irradiated Nylon/PE. Very few minor peaks were observed in the non-irradiated film. A very slight increase in the intensity of peaks was observed at MW > 500 kDa with irradiation. New small peaks were also observed at MW of 10-50 kDa. The results indicate that Nylon/PE film is relatively stable with irradiation.

Figure 11. GPC of Non-irradiated and Irradiated Nylon/PE Films

**Nylon 15/PE 50**

Figure 12 shows the GPC chromatogram of non-irradiated and irradiated Nylon 15/PE 50. Similar chromatogram with that of the Nylon/PE film was observed in both non-irradiated and irradiated Nylon 15/PE 50. Slight increase in intensity in peaks > 500 kDa and new small peaks at MW 10-50 kDa were also observed.
In summary, among the commercial films tested, VMPET12 /PE70; Nylon/PE; and Nylon 15/PE 50 showed the least components of radiolytic products formed at 10 kGy.

3.5. Gas Chromatography of Volatile Components from Packaging Material

Gas chromatography has been employed as an analytical procedure for determining volatile compounds in irradiated packaging materials. Several studies reveal hundreds of volatiles from irradiated LDPE, PP, OPP films, polyester, PP-copolymer film, etc. The identified compounds are predominantly hydrocarbons, alcohols, aldehydes, ketones and carboxylic acids [8]. In this study, the volatile compounds from the irradiated packaging materials were sucked using a syringe and dissolved in tetrahydrofuran (THF). The samples were then analyzed for GC-MS. Among the packaging materials, only PET/FOIL/PE indicated the presence of volatile material which was identified as 3,3-Dimethylheptane and 3,3-Dimethylhexane (Table 3). This material was also present in non-irradiated PET/FOIL/PE with no indication of increase in quantity. The procedure may have to be refined further to detect more volatile radiolytic products from the packaging material.

Table 3. Volatile compounds identified by GC-MS on non-irradiated and irradiated (10kGy) PET/FOIL/PE films.

<table>
<thead>
<tr>
<th>No.</th>
<th>Retention Time</th>
<th>Compound</th>
<th></th>
<th>Non-Irradiated</th>
<th>Retention Time</th>
<th>Compound</th>
<th></th>
<th>Irradiated at 10kGy</th>
<th>Retention Time</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.50</td>
<td>3,3-Dimethylheptane</td>
<td>1</td>
<td>8.50</td>
<td>3,3-Dimethylheptane</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>9.97</td>
<td>3,3-Dimethylhexane</td>
<td>2</td>
<td>9.97</td>
<td>3,3-Dimethylhexane</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.6. Isotope Ratio - Mass Spectrometry

Stable isotopic technique is now a much sought methodology to establish the authenticity and origin of foods, and other materials, where provenance and traceability are of importance. It relies in natural abundance measurements of the bio-elements (HCNOS). While this technique has been quite useful in studies related to hydrology and provenancing e.g. differentiating products of various geographical origins, food adulteration, source attribution of packaging materials, etc.; this technique may find new application in determining food contaminants from packaging materials.

Stable isotope techniques are based on the principle that materials – natural or synthetic will reflect the isotopic composition / signature specific to the origin of the material. Plastics which are of petrochemical in origin will have different relative abundance of the stable isotopes of carbon, 13C and 12C (δ13C), Oxygen (18O and 16O, or δ18O), and Hydrogen (2H and 1H, or δ2H) compared to food or fruits which are of natural origin. Additionally, if pure water was irradiated inside the plastic packaging material, volatile radiolytic products from plastics may mix with water, resulting in
isotopic exchange that could ultimately change the isotopic ratio of δ2H and δ18O of water. Thus, this method could possibly determine migration of packaging material.

18O/16O (δ18O) and (2H /1H or δ2H) have been determined in packaging materials which were filled with water coming only from one source. A plot of δ18O against δ2H is shown in Figure 13. The figure shows that δ18O and δ2H values for all packaging materials both from non-irradiated and irradiated water samples indicate increasing trend. Although differences with the blank were quite minimal, there is a probability of δ18O and δ2H being enriched by leachates coming from the packaging material as suggested by the slight increase in δ18O and δ2H. Results also show that there is no significant difference between non-irradiated and irradiated samples. Data obtained from this experiment are quite preliminary which need further refinement of procedure. The results may only indicate a positive probability of enrichment by leachates from the packaging material. The method of using the stable isotopic technique for determining migration of leachates has to be developed further to increase its sensitivity.

![Figure 13. Stable Isotope Ratio (δ18O ‰ and δD ‰) of the Different Non-Irradiated and Irradiated Packaging Materials.](image)

4. CONCLUSION

Tensile Strength of the different packaging materials being tested suggests no changes with irradiation at an absorbed dose of 10 kGy. Elongation at break increased at this absorbed dose. Although migration test did not reveal any quantifiable leachates from the water solvent, several high and low molecular weight components have been detected in both non-irradiated and irradiated packaging films using the gel permeation chromatography. Leachates from packaging materials increased in quantity after irradiation. Laminated PET/PE films indicated high leachable components after irradiation. VMPET12/PE70, Nylon/PE, and Nylon 15/PE 50 were the most resistant packaging materials to irradiation with the least effect in terms of quantity of radiolytic products.

Stable isotope technique for δ18O ‰ and δD ‰ is a promising methodology to determine possible contamination of leachates from packaging materials.
Acknowledgement:

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REFERENCES


BASED ON STARCH - PVA SYSTEM AND CELLULOSE REINFORCED PACKAGING MATERIALS PREPARED USING OF RADIATION MODIFICATION

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Abstract: The aim of the present work was to modify the properties of the composite packaging materials based on starch – PVA system. This concerns the trials of optimization the films properties by modification of the composition of the basic components and by introducing a cellulose fibres characterised by various size (till nano scale) as a reinforcing agent, followed by irradiation with gamma rays (in vacuum) or E-beam (in air). In relation to the possible dedication of the newer materials for packing the products predicted for radiation decontamination, the resistance of these materials to irradiation was also tested. Moreover, the basic studies dealing with the modification occurring under influence of irradiation were carried out.

It was found that application of the PVA with the highest molecular mass enable to obtain the films with the best properties directly after the synthesis as well as after the further irradiation. Appropriate introduction of nanocellulose into the PVA films leads to the essential improvement of the films properties. Nanocrystalline cellulose appeared more sensitive to irradiation as compared to the microcrystalline cellulose and microfibrinal cellulose. The effect of irradiation on the properties of the films depends on the sample composition and on the condition applied during the films synthesis and storage. It has appeared possible to obtain the films with the improved properties (in particular with decreased hydrophilicity) due to the irradiation processes. Some films appear resistant to irradiation with an absorbed dose as high as 25 kGy. Degradation is the major process induced by irradiation, however it seems to be accompanied by crosslinking. Addition of the selected additives into the films composition enable to stabilize the polymer against irradiation, and to modify the physicochemical properties of the films. The changes in the films properties due to irradiation can be attributed to modification of the surface properties of the films and to their modified microstructure.

1. INTRODUCTION

The studies are connected to the increasing interest in substitution of traditional packaging (films and foams) by the materials prepared basing the natural and biodegradable polymers and in increase the safety of food. Variety of commercial biodegradable plastics are proposed at present on the market as packaging materials for food products. Preparation of the films in the mixed systems composed from variety of natural polymers as well as containing polysaccharide – artificial polymer seems to be one of the abilities to obtain the better and more friendly for environment packaging material. The composition of these materials is sometimes based on starch and polyvinyl alcohol (PVA).

Starch is an abundant and cheap biopolymer with a good film forming ability and therefore it appear to be an appropriate source for preparation of the cheap biodegradable packaging [1-4]. However, although starch forms relatively strong films, mechanical properties of these
materials are still worse as compared to the artificial plastic, in particular that the higher strength of these films is connected to their insufficient elasticity. Serious disadvantage of such material is its’ high affinity to water. Therefore, in purpose to modify the properties of starch films various modification methods are applied for the starches alike degradation, crosslinking, oxidation, etherification or esterification. Development of the methods of biopolymers modification that apply ionising radiation is observed also last decades [3-11].

The other ability is to blend starch with the other natural polymer or with the artificial biodegradable polymer. PVA is biocompatible polymer with an excellent film forming ability and an excellent compatibility with starch [9-13]. PVA forms films with good mechanical and gas barrier properties.

The next solution to improve the properties of the starch or PVA based plastics is introducing of the reinforced agent, alike inorganic particles or natural fibre [12-16]. It is known, that presence of the cellulose fibres in artificial polymers is capable to improve the functional properties of the plastics and that it has also appear useful in the case of both starch and PVA. It was proved moreover that introduction of the nanosized cellulose (nanofibres NFC, nanocrystalline NCC and bionanocellulose) enable to produce the plastics with the better properties as compared to those produced using the macro-scale cellulose fibres. Several reports concerns till now also preparation of such materials basing the biodegradable or natural polymers, including PVA-starch blended films with the cellulose fibres [7, 12-15]. These materials are oftentimes addressed for food packaging.

Increasing interest in production of the more safe and more durable food of the higher quality induces the interest in radiation decontamination of the “ready –to-eat” products. Accordingly, evaluation of the effect of irradiation on the possible packaging material is highly important.

The present research concerns elaboration of the methodology and optimization of the conditions for preparation of the films in the starch-PVA and starch-cellulose/nanocellulose systems in relation to the possible application as food packaging, evaluation of the ionising radiation effects (gamma and electron) and the trials for the radiation supported modification of the polymer. Simultaneously, evaluation of the effect of irradiation performed at the conditions applied in INCT for sterilization of packaging materials (with E-beam) was done.

2. EXPERIMENTAL

Materials, irradiation and the films preparation.

Four PVA-s (products of Sigma and of Alfa Aesar) characterized by various molecular masses (PVA1: 145000; PVA2: 90000; PVA3: 60000, and PVA4: 15000-30000) as well as two cornstarches: SC1 (by Sigma) and SC2 (by Cerestar) and two potato starches, SP1 (Sigma product) and S7 (commercial, local market) were selected for the films preparation. Moreover, the starches SC1$_d$ and S8$_d$ degraded on the way of irradiation with a dose of 10 kGy (in purpose to reduce their viscosity [1,5]) were prepared and applied. The celluloses applied were: microcrystalline cellulose (MCC), microfibrinal cellulose (MFC), nanocrystalline cellulose (NCC) and nanofibrinal cellulose (NFC).

Films were prepared by solution casting method after addition of glycerol as a plasticizer at the level of 0, 20 and 30 % (in relation to the starch-PVA mass). The films were dried, peeled from the substrate and conditioned during couple of days at the relative humidity of 43 % before testing. The films thickness was equal to ca 100 ± 15 µm.
Irradiations were carried out at room temperature with Co-60 gamma radiation in vacuum or in air in the Gamma Chamber 5000, as well as in E-beam Elektronika 10/10 (10 MeV/10 W) in air placed in the Centre for Radiation Research and Technology, INCT.

Methods

Mechanical tests (determination of tensile strength, elongation at break \( \Delta l \) and Young Modulus) were carried out using of Inström testing machine. In purpose to evaluate the hydrophilic/hydrophobic properties the wetting angle measurements (\( \theta \) values) were done using the instrument constructed in the Laboratory of Material Research, INCT. Beside, capability of the films for water uptake and the dynamics of this process was evaluated for the complementary studies of interaction the films with water. For this purpose the increase in mass of the film stored at RH = 100% (at room temperature or at 4 \( ^\circ \)C) during several time intervals was determined. Moreover, swelling in water was determined as a mass of water absorbed in the films after storage in water for 24 h ((100xW - Wd)/Wd; W and Wd means weight of the sample after water sorption and after drying, respectively). Scanning electron microscopy (SEM) studies were conducted applying magnifications from 100x to 100000 x.

Basic studies of the processes taking place under gamma irradiation were carried out by means of electron spin resonance EPR, gas chromatography and DRS spectroscopy (Diffuse Reflectance Spectroscopy). The processes of free radicals formation taking place in the substances were carried out for the basic substances and for the films irradiated with the absorbed dose of 5 kGy (room temperature) and efficiency of hydrogen formation and oxygen consumption was done for the films irradiated with the \( \gamma \)-rays with 5, 10, 30 kGy and e-beam with a dose of 30 kGy. The content of gel fraction was also determined for the resulting films. Examination of the occurring processes were completed by thermogravimetry (TGA) and differential scanning calorimetry (DSC).

3. RESULTS AND DISCUSSION

Basic processes taking place under influence of irradiation
None particular differences between the EPR patterns connected to free radicals resulting after irradiation were found between the particular PVAs. The pattern presents triplet with \( g = 2.003 \) and \( a = 36 \) G). The intensities of the signal connected to free radicals (located at C atom interfering with two hydrogen atoms) differs, however in the case of particular PVAs and can be expressed as the following values (arbitrary units: PVA1: 0.9/mg, PVA2: 0.4/mg, PVA3: 0.6/mg and PVA4: 0.7/mg. The difference were found in the case of the radicals formed in the cases of particular starch preparations. It can be stated, however, that the signal recorded for cornstarch CS1 is more intensive as compared to that of cornstarch CS2.

The difference were observed between the EPR signals detected for the various celluloses. In particular, high reactivity was noticed in the case of nanocellulose (3.15/g) as compared to microfibrinal cellulose (1.16/g). High reactivity of NCC was confirmed by the gas chromatography data. For example, efficiencies of the hydrogen formation and of the oxygen consumption were equal to 0.438 and 0.422 (\( \gamma \), 30 kGy) respectively, as compared to the values in the range of 0.259-0.291 found in the cases of the micro-sized celluloses. These results can be related to the microstructure of these preparations, in particular to the smallest specific surface area of the large granules of MCC, more expanded in the case of MFC and to a strongly developed specific surface area of the NCC crystallites (Fig. 2).

Moreover, the EPR studies were carried out for the selected films. All the patterns of the films containing 30% of glycerol with the following compositions: PVA, starch:PVA (50:50), PVA:NCC (90:10) and starch:PVA:NCC (45:45:10) represent similarities to that of PVA with the strong evidence of the free radical that can be attributed to PVA.

Different patterns were observed in the cases of the irradiated PVA-starch films with the modified compositions using of DRS spectroscopy. The patterns of both starch and PVA indicates presence of the peroxides, hydroxyperoxides and probably aldehyde groups, while oxidation products of the films prepared of all the mixed systems indicates strong presence of
C=O groups inside the polymer chain (Fig. 3a). This indicates strong interaction of the components in the mixed system. The intensity of the bands in DRS arising from the oxidation products increases with the increase of the irradiation dose applied (Fig. 3b).

**Fig. 3: DRS spectra recorded for the starch-PVA films containing 30% of glycerol: a) characterized by a various starch content (shown in percentage) and irradiated with the dose of 25 kGy; b) for the starch:PVA film (50:50) irradiated with the several absorbed doses.**

**The films prepared basing the starch-PVA system**

**Optimization of the methodology for preparation of the PVA, starch and starch-PVA films**

In the first stage the experimental conditions enabling to prepare homogeneous, good quality PVA, starch and starch-PVA films were optimized. This concerns conditions applied for dissolution of the PVAs, gelatinization if starch, addition of plastificator and conditions of drying that enable to avoid phase separation during this process. Accordingly, radiation pretreatment has appeared useful for this purpose. This is because that due to the high viscosity of the gels prepared basing the native starches it was not possible to obtain homogeneous films applying the procedure leading to the appropriate gel formation. Therefore, the experiments were done dealing with the films preparation applying the starches subjected to degradation induced by irradiation with gamma rays. The dose of 10 kGy was found appropriate to obtain the starch gels with sufficiently lowered viscosity and our preliminary experiments have shown that the films prepared basing the starch irradiated with a 10 kGy dose reveal better properties as compared to the films prepared basing the non-irradiated starch. Optimization of the preparation procedure for the mixed films were done basing the composition characterized by starch:PVA ratio equal to 50:50 and glycerol content of 30%. Application of various preparations of PVA and starch was tested in this step.

PVA films have appeared highly flexible. Tensile strength of the PVA films was the higher and elasticity was the lower when Mw of the substrate was the higher. Increase in elasticity and decrease in tensile strength was connected to the increase in the glycerol content in the films. All the PVA films shown limited hydrophilicity (contact angle ca. 70°). The hydrophilicity of those films was the higher when plastificator content was the higher. This was confirmed also by the results of moisture uptake experiments.

To the contrary, starch films appeared highly stiff. Similarly as PVA films, starch films have also revealed limited hydrophilicity with a contact angle varying in the range 60 – 81°, in the case of the films containing native starches and 30% of glycerol. The films obtained basing the irradiated starches have revealed higher contact angle as compared to those obtained using of the non-irradiated specimens. For example, the contact angle reach value of ca. 90° in the case of the films obtained basing the irradiated SC1 cornstarch as compared to the value of ca. 81° obtained in the case of native SC1 starch.
In the cases of the starch-PVA compositions the films characterized by the high content of PVA has appeared flexible while those containing large amount of starch revealed strong brittleness. Simultaneously, the films containing high amount of starch were generally characterized by the lower values of the wetting angle and lower swelling as compared to those containing high amount of PVA.

### TABLE 1: THE PROPERTIES OF THE STARCH:PVA FILMS (50:50, 30% OF GLYCEROL) PREPARED USING PVAS CHARACTERIZED BY THE VARIOUS MOLECULAR MASS, NON-IRRADIATED AND IRRADIATED.

<table>
<thead>
<tr>
<th>PVA</th>
<th>Irradiation</th>
<th>Dose kGy</th>
<th>Tensile strength MPa</th>
<th>EB %</th>
<th>Wetting angle (W&lt;sub&gt;i&lt;/sub&gt;,W&lt;sub&gt;d&lt;/sub&gt;)</th>
<th>(W&lt;sub&gt;i&lt;/sub&gt;,W&lt;sub&gt;d&lt;/sub&gt;)&lt;sub&gt;W&lt;sub&gt;d&lt;/sub&gt;x100%&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVA1</td>
<td>none</td>
<td>0</td>
<td>15.14±1.85</td>
<td>148±17</td>
<td>96.97±2.62</td>
<td>321</td>
</tr>
<tr>
<td>PVA1</td>
<td>γ, vacuum</td>
<td>25</td>
<td>14.77±1.30</td>
<td>178±20</td>
<td>92.89±5.49</td>
<td>290</td>
</tr>
<tr>
<td>PVA1</td>
<td>EB, air</td>
<td>25</td>
<td>14.95±0.55</td>
<td>125±12</td>
<td>91.64±3.23</td>
<td>275</td>
</tr>
<tr>
<td>PVA2</td>
<td>none</td>
<td>0</td>
<td>11.22±0.86</td>
<td>137±8</td>
<td>88.63±4.54</td>
<td>394</td>
</tr>
<tr>
<td>PVA2</td>
<td>γ, vacuum</td>
<td>25</td>
<td>13.45±1.76</td>
<td>136±18</td>
<td>73.28±6.86</td>
<td>383</td>
</tr>
<tr>
<td>PVA2</td>
<td>EB, air</td>
<td>25</td>
<td>15.22±1.73</td>
<td>206±18</td>
<td>78.76±8.27</td>
<td>385</td>
</tr>
<tr>
<td>PVA3</td>
<td>none</td>
<td>0</td>
<td>13.28±1.14</td>
<td>88±16</td>
<td>95.64±7.29</td>
<td>398</td>
</tr>
<tr>
<td>PVA3</td>
<td>γ, vacuum</td>
<td>25</td>
<td>9.43±0.52</td>
<td>113±8</td>
<td>90.51±5.41</td>
<td>479</td>
</tr>
<tr>
<td>PVA3</td>
<td>EB, air</td>
<td>25</td>
<td>14.75±1.57</td>
<td>122±42</td>
<td>91.75±2.99</td>
<td>425</td>
</tr>
<tr>
<td>PVA4</td>
<td>none</td>
<td>0</td>
<td>13.67±0.87</td>
<td>112±25</td>
<td>68.71±4.69</td>
<td>396</td>
</tr>
<tr>
<td>PVA4</td>
<td>γ, vacuum</td>
<td>25</td>
<td>12.81±2.41</td>
<td>74±7</td>
<td>61.51±1.80</td>
<td>369</td>
</tr>
<tr>
<td>PVA4</td>
<td>EB, air</td>
<td>25</td>
<td>9.58±0.69</td>
<td>100±7</td>
<td>59.63±5.06</td>
<td>386</td>
</tr>
</tbody>
</table>

Specific interaction of the PVA and starch-PVA films with moisture was observed (Fig. 7). In particular, in numerous cases after the initial increase in mass of the sample connected to the water uptake, the fall down in mass was observed. Moreover, the curves presenting water uptake often has not achieved plateau even after a long period (till 21 days). Hydrophilicity (shown by the contact angle data) decreases during storage of the films.

Basing the preliminary results, it was decided to apply 30 % of glycerol content and to add glycerol to the solution before the starch gelatinization in the next systematic experiments. Simultaneously, PVA1 (Mw=145 kDa) and pre-irradiated cornstarch CS1 (absorbed dose of 10 kGy) were selected and a number of the films syntheses were done accompanied by investigation of the effect of irradiation on the films properties (presented in the next chapter). However, as it seems possible that the other substrates might appear better for synthesis of the films in such cases when the synthesis is supported or followed by irradiation, the effect of introduction of particular PVAs into the starch:PVA composition (50:50) and of the irradiation effect on the obtained films were re-investigated (Table 1).

The results (Table 1) have confirmed that application of the PVA1 characterized by the highest molecular mass enable to obtain the starch:PVA films (50:50) with the best properties as compared to application of the other components, as well after synthesis as after the
subsequent irradiation. In particular, decrease in swelling parameter was observed in this case after radiation treatment.

**Studies of the irradiation influence on the starch-PVA films properties.**

The preliminary studies were conducted for a series of the starch, PVA and starch:PVA (50:50) films prepared applying various PVA and starch preparations. Furthermore, the series of the films was prepared using of the PVA1 and the SC14 and characterized by the various starch:PVA ratio. Evaluation of the films properties was done and the influence of irradiation on these properties and on the occurring physico-chemical changes was studied. Irradiations of the PVA films were conducted with a dose of 25 kGy and of 100 kGy. The dose applied for the starch and starch-PVA films was equal to 25 kGy using both gamma (vacuum) and electron (air) irradiation (Fig. 4).

The composition of starch:PVA (45:55) was then selected for the studies of the effect of the absorbed dose on the films properties (Fig5 a-c, Table 2). It was found that application of the dose of 5 kGy enable to improve tensile strength and hydrophobicity with the acceptable change in elasticity (Table 2) and relatively small degree of degradation (Fig.5c).

![Fig. 4: Mechanical properties of the starch-PVA films prepared basing various compositions (glycerol content 30%), non-irradiated and irradiated with γ-rays (vacuum) or electrons (air) applying a dose of 25 kGy.](image)

The effect of irradiation on the films’ properties depend on the films composition and the preparation conditions (The examples in Figs. 4a,b and 5a-c). In the majority of cases none particular effect on the tensile strength of irradiation carried out a well in vacuum (gamma rays) as in air (EB) was noticed. It was stated, however, that irradiation induces decrease in elasticity of the films (Fig. 4b). In majority of cases it was accompanied by an increase in wetting angle (Fig. 5a). This can be explained by the probable oxidation processes occurring on the surface of the films (DRS, Fig. 3). However, it seems that the water uptake of the number of the irradiated films is higher as compared to the non-irradiated ones (Fig.7).
Fig. 5: The values of wetting angle (a), absorbed water (swelling) (b) and gel content (c) determined for the films starch:PVA films, non-irradiated and irradiated with γ-rays (vacuum) or electrons (air) applying a dose of 25 kGy

Decrease in the gel fraction content was found after irradiation in majority of the samples showing the occurring degradation processes (Fig. 5c, 8c). However, these processes seems to be accompanied by crosslinking, as decrease in the gel fraction content is much lower than can be expected taking into consideration starch content in the samples. Therefore gel fraction reach in the irradiated samples 70 – 90% of that determined for the non-irradiated reference sample, while in the case of starch substrate (Sc1a) irradiated under the same conditions these value was on the level of ca. 30%. TGA and DSC data also suggest simultaneous occurrence of degradation and crosslinking processes.
Fig. 8: Dependence on the absorbed dose (γ-rays) of properties of the cornstarch-PVA films (45:55): tensile strength (a), wetting angle (b) and gel content (c).

TABLE 2: DEPENDENCE ON THE ABSORBED DOSE OF THE ELONGATION AT BREAK OF THE CORNSTARCH-PVA FILMS (45:55)

<table>
<thead>
<tr>
<th>Dose [kGy]</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>50</th>
<th>75</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elongation at break, [%]</td>
<td>98 ±6.8</td>
<td>76 ±12.1</td>
<td>91 +6.5</td>
<td>85 ±7.7</td>
<td>61 ±8.0</td>
<td>48 ±6.4</td>
<td>47 ±5.1</td>
</tr>
</tbody>
</table>

SEM observation indicates the improvement of the homogeneity of the films after irradiation (Fig. 9).

Fig. 9: The examples of images of the starch-PVA films (SC1, d-PVA1, 30 % of glycerol). A – initial, B – irradiated in vacuum with a dose of 25 kGy.

Fig. 10: DRS spectra recorded for the starch:PVA(40:60) films containing: a) 8 % of the antioxidants A1 or A2, and b) 1 % of NFC.

The effect of the addition of the two selected antioxidants (A1 and A2) on the modification taking influence under influence of gamma and electron irradiation was studied in the next
step. These compounds were introduced into the film composition on the level of 8 and 1%. The results showed strong resistance of the mechanical properties, as well as hydrophilic/hydrophobic properties (contact angle and swelling) of the films containing 1% of antioxidant against irradiation (Tables 3, 4). Moreover, an essential decrease in hydrophilicity was noticed after irradiation. This result can be related to the protective effect of the presence of antioxidants in the films, discovered by DRS (Fig. 10a).

**TABLE 3: THE EFFECT OF ADDITION OF ANTIOXIDANTS ON THE PROPERTIES OF THE STARCH:PVA FILMS (40:60) CONTAINING THE TWO ANTIOXIDANTS**

<table>
<thead>
<tr>
<th>Antioxidant addition</th>
<th>Irradiation</th>
<th>Dose kGy</th>
<th>Tensile strength MPa</th>
<th>EB %</th>
<th>Wetting angle o</th>
</tr>
</thead>
<tbody>
<tr>
<td>8% A1</td>
<td>none</td>
<td>0</td>
<td>11.09 ±1.90</td>
<td>146 ±24</td>
<td>60.91±11.16</td>
</tr>
<tr>
<td>8% A1</td>
<td>γ, vacuum</td>
<td>25</td>
<td>12.89 ±1.72</td>
<td>99 ±28</td>
<td>83.09±3.37</td>
</tr>
<tr>
<td>8% A1</td>
<td>EB, air</td>
<td>25</td>
<td>11.20 ±2.10</td>
<td>115 ±18</td>
<td>83.85±5.5</td>
</tr>
<tr>
<td>8% A2</td>
<td>none</td>
<td>0</td>
<td>7.22 ±0.73</td>
<td>120 ±23</td>
<td>43.90±5.77</td>
</tr>
<tr>
<td>8% A2</td>
<td>γ, vacuum</td>
<td>25</td>
<td>6.73 ±0.54</td>
<td>78 ±10</td>
<td>52.55±4.57</td>
</tr>
<tr>
<td>8% A2</td>
<td>EB, air</td>
<td>25</td>
<td>7.12 ±0.75</td>
<td>93 ±28</td>
<td>54.64±4.37</td>
</tr>
<tr>
<td>1% A1</td>
<td>none</td>
<td>0</td>
<td>15.49 ±1.63</td>
<td>225 ±17</td>
<td>89.11±3.41</td>
</tr>
<tr>
<td>1% A1</td>
<td>γ, vacuum</td>
<td>25</td>
<td>14.91±1.14</td>
<td>259±13</td>
<td>88.59±3.95</td>
</tr>
<tr>
<td>1% A1</td>
<td>EB, air</td>
<td>25</td>
<td>14.47±0.58</td>
<td>223±10</td>
<td>89.37±5.36</td>
</tr>
<tr>
<td>1% A2</td>
<td>none</td>
<td>0</td>
<td>13.25±0.68</td>
<td>181±17</td>
<td>89.11±4.09</td>
</tr>
<tr>
<td>1% A2</td>
<td>γ, vacuum</td>
<td>25</td>
<td>14.34±1.51</td>
<td>197±10</td>
<td>89.05±6.33</td>
</tr>
<tr>
<td>1% A2</td>
<td>EB, air</td>
<td>25</td>
<td>11.88±0.90</td>
<td>182±19</td>
<td>76.55±6.33</td>
</tr>
</tbody>
</table>

**TABLE 4: SWELLING IN WATER 100% X (Wf-W0)/W0 OF THE FILMS PREPARED WITH ADDITION OF THE TWO ANTIOXIDANTS**

<table>
<thead>
<tr>
<th>Addition</th>
<th>0 kGy</th>
<th>25 kGy, γ, vacuum</th>
<th>25 kGy, EB, air</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>335</td>
<td>357</td>
<td>334</td>
</tr>
<tr>
<td>A2</td>
<td>345</td>
<td>377</td>
<td>360</td>
</tr>
</tbody>
</table>

The films prepared basing the starch-PVA-cellulose/nanocellulose system

The methods for incorporation of the cellulose and nanocellulose fibrils or crystallites into the films were adjusted. The composition of cornstarch CS1 and PVA1 (50:50) was selected for examination of the effect of introduction of the microsized and nanosized celluloses and the effects of irradiation on the properties of the obtained films was studied. Addition of the nanosized cellulose induced modification of the oxidation processes taking place under gamma irradiation in relation to the samples containing only starch and PVA (Fig. 10b).
Introduction of micro-sized celluloses into the films induced decrease in tensile strength and in elasticity, while appropriate introduction of nanocellulose might lead to some improvement of the mechanical properties of the films. This result might be related to the differences in microstructure of the films, in particular to the high homogeneity of the films containing nanocellulose and non-homogeneity of the films containing micro-sized cellulose (Fig. 11). Irradiation induces increase in homogeneity of the films, both containing microcrystalline (as well as nanocrystalline celluloses (Figs 12, 13) and changes distribution of the nanocrystallites in the films matrices (Fig.13).

None particular effect of addition of NCC into the films composition on tensile strength was observed in the case of the experiment shown in Table 5. However, elasticity of the films decreased in the case of composition containing the higher amount of NCC. Simultaneously, mechanical properties of the starch-PVA films containing NCC (Table 5) are less sensitive to irradiation as compared to pure starch-PVA films.

Table 5: Mechanical properties of PVA-starch- nanocellulose films.

<table>
<thead>
<tr>
<th>NCC content Wt%</th>
<th>0 kGy</th>
<th>25 kGy, vacuum</th>
<th>25 kGy, air</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tensile strength MPa</td>
<td>EB %</td>
<td>Tensile strength MPa</td>
</tr>
<tr>
<td>0</td>
<td>27.3 ±2.5</td>
<td>120</td>
<td>22.6 ±1.2</td>
</tr>
<tr>
<td>1</td>
<td>28.7 ±1.7</td>
<td>128</td>
<td>27.8 ±0.8</td>
</tr>
<tr>
<td>2.5</td>
<td>24.8 ±1.8</td>
<td>80</td>
<td>23.4 ±1.6</td>
</tr>
<tr>
<td>5</td>
<td>24.1 ±1.4</td>
<td>157</td>
<td>22.9 ±0.3</td>
</tr>
<tr>
<td>7.5</td>
<td>24.5 ±1.6</td>
<td>68</td>
<td>25.0 ±1.5</td>
</tr>
<tr>
<td>10</td>
<td>26.5 ±0.9</td>
<td>66</td>
<td>27.2 ±2.1</td>
</tr>
</tbody>
</table>

Fig. 11: SEM images of the PVA1 films containing 10 % of MCC, non-irradiated and irradiated (25 kGy, \( \gamma \), vacuum).

Fig. 10: SEM images of the surfaces of PVA films containing 10 % of: MCC (A) or NCC (B).
4. SUMMARY AND CONCLUSION

The methods were elaborated of the films preparation basing various preparation of the PVA, cornstarch, potato starch and the systems starch-PVA as well as for incorporation of the micro- and nano-sized cellulosics into the films prepared basing the studied systems. Simultaneously, the methods were elaborated for the measurements enabling appropriate evaluation of the obtained films’ properties.

It was found that application of the PVA with the highest molecular mass enable to obtain the films with the best properties directly after the synthesis as well as after the further irradiation while the films prepared basing the low molecular PVA are characterized by poor properties and irradiation results in deterioration of these properties.

Appropriate introduction of nanocellulose into the PVA films leads to the essential improvement of their elasticity, without the important decrease in the tensile strength, while introduction of the microcrystalline cellulose (at a level of 10%) causes decrease in the tensile resistance and in elasticity. Starch films containing nanocellulose revealed significantly higher tensile strength as compared to the films containing microfibrinale cellulose.

Nanocrystalline cellulose appeared more sensitive to irradiation as compared to the microcrystalline cellulose and microfibrinale cellulose. The differences were also observed in the potential reactivity of the various starch preparations induced by irradiation by means of EPR and gas chromatography. To the contrary, differences between the particular PVAs were not very specific. Formation of C=O groups on the surface of the films was found by DRS. The processes taking place under gamma irradiation in the starch-PVA blended films characterized by modified composition leads to formation of the other oxidation products as compared to the films formed basing separately starch or PVA. This indicates the strong interaction between the particular components in the blends. The protective effect of the presence of the selected additives in the films composition was discovered.

The effect of irradiation on the properties of the films depends on the sample composition and on the condition applied during the films synthesis and storage. Irradiation causes decrease in hydrophilicity of the selected compositions prepared basing starch-PVA-glycerol or starch-PVA-glycerol-nanocellulose systems. None particular effect after irradiation on mechanical properties or even their improvement was found in the cases of some composition, although deterioration of these properties was noticed for the majority of starch-PVA films. Degradation is the major process occurring under influence of irradiation. However, it can be considered that this process is accompanied by crosslinking. Introduction of the selected additives into the films’ composition enable to improve their physico-chemical properties and might result in the improvement of these properties after irradiation or in the increased resistance against irradiation. Irradiation carried out in vacuum seems to improve of the films homogeneity and compatibility of their components as well as distribution of the nanocellulose particles. Relatively small effects of irradiation carried out with the sterilization.
dose and improvement of the properties of the selected films after irradiation suggest that this type of material might be promising for packaging the food predicted for radiation decontamination.

BIBLIOGRAPHY

COMPARATIVE STUDY ON THE EFFECT OF γ-IRRADIATION AND COLD PLASMA EXPOSURE ON THE VARIOUS POLYMERIC SUBSTRATE MODIFIED TO IMPART MULTIFUNCTIONAL PROPERTIES FOR ACTIVE/BIOACTIVE FOOD PACKAGING MATERIALS

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Introduction

Because of the inert nature of most commercial polymers, they must undergo surface functionalization prior to attachment of a bioactive compound. Surface functionalization techniques are applied in order to introduce the desired type and quantity of reactive functional groups which are able then to covalently attach a bioactive compound. Bioactive compounds can be natural or synthetic and are defined as compounds which catalyze or elicit a specific response within a given biological system. [Goddard J.M., Hotchkiss J.H. (2007), \textit{Progress in Polymer Science}, 32, (7), 698–725; Goddard J.M.. Hotchkiss J.H. (2008), \textit{Journal of Applied Polymer Science}, 108, 2940–2949]

Cold plasma chemistry (cp) appears as a useful and suitable technique for \textit{in situ} polymer synthesis and surface modification of both synthetic and natural polymers. The main advantage of plasma treatments is that the modification turns out to be restricted to the uppermost layers of the substrate, thus not affecting the overall desirable bulk properties. On polymer surfaces the main effects, which depend on the treatment conditions, are: a cleaning effect, an increase of microroughness, the production of radicals and finally implementation of various functional groups, the surfaces becoming hydrophilic with acidic, basic or amphiphilic character. [Biederman H., Osada Y. (1992) \textit{Plasma polymerization processes}, Elsevier Science Publishers, pp 1–5; Totolin M. (2007), \textit{Plasma Chemistry and Natural Polymers}, Pim Publisher, Iasi, p. 15] The exposure of polymers to the action of \textbf{high energy radiation} causes bulk modification of properties and even the backbone scissions. [Spinks J.W.T., Wood R.J. (1990), \textit{An Introduction to Radiation Chemistry}, Wiley, New York]. The most important feature of irradiated polymers is the possibility of grafting for certain structures, because the radical sites are available for coupling.

1. Experimental

The surface modification was achieved for two biodegradable polymer substrates as poly (lactic acid) (PLA) and cellulose-based materials (CC) and a synthetic polymer, namely polyolefins (PO) by ionizing radiation/plasma gas discharge and optimization of experimental conditions and further coating with active/bioactive compounds like chitosan (CHT), lactoferrin (LF), vitamin E (VE), vitamin C (VE) and essential oils (rosehip seeds (RO) and Tea Tree (TT)). For obtaining stable layers onto polymeric
different coupling agents were employed in covalent linking of active/bioactive formulation to the pre-treated polymeric surfaces.

Cellulose-based materials pre-treated as above mentioned were grafted with different synthetic phenolic compounds (p-hydroxybenzoic acid (HBA), gallic acid (GA), eugenol (Eu)) and vegetable oils (grape seeds (GO) and RO) by impregnation and/or coupling reaction. These fibers are a kind of modified cellulose fibers which is produced by the special chemical way of adding natural anti-bacterial high molecular weight chitin-polymer. By grafting all polymeric substrates, with mentioned bioactive compounds, antimicrobial and antioxidant properties have been imparted, by developing environmental friendly methods for food packaging applications.

2. Pre-treatment Methods

To optimize the surface functionalization conditions of polymer substrates a variation of the discharge parameters were performed. Optima conditions for cold-plasma surface functionalization: the polymeric films were exposed to high-frequency plasma (nitrogen or air), which was created inside a glass reactor, using a 0.4 mbar (40 Pa) vacuum. Outside the reactor were two electrodes connected to a source of high-frequency (1.3 MHz) discharge of 100 W. During plasma treatment and further air exposure various nitrogen and oxygen-containing groups are incorporated onto polymer surfaces.

The exposure to ionizing radiation was performed in air at room temperature inside of a γ-irradiator M-38 GAMMATOR (USA) which has a $^{137}$Cs source. The dose rate was 0.4 kGy/h. The samples were continuously rotated for homogenous irradiation. The values of pre-irradiation γ-dose were different, because the radiation resistances of poly (lactic acid), cellulose and polyethylene (PE), are unlike; poly(lactic acid) was exposed to 10, 20 and 30 kGy, cellulose fibers were irradiated at 5, 10 and 20 kGy while for polyethylene the applied doses were 20, 30 and 50 kGy. At the end of irradiation, polymer samples were preserved in refrigerator for avoiding the decay of long life radicals.

Changes in the outer layer of the polymeric substrates activated by plasma discharge and/or γ-irradiation, and the subsequently applied modification were proved by contact angle measurements, ATR FTIR spectroscopy, X-ray photoelectron spectroscopy, scanning electron microscopy, atomic force microscopy, chemiluminescence.

3. Functionalization

3.1. Poly(Lactic Acid) (PLA) surface functionalization

The poly(lactic acid) used in this study was type 2002D from NatureWorks LLC, having a density of 1.24 g/cm$^3$ and melt flow index (MFI) of 5−7 g/(10 min) (at 210 °C/(2.16 kg)). The PLA films were obtained by melt processing and pressing using a Carver press at 175 °C (3 min pre-melting and 3 min pressing). Surface modification of PLA was done using different active/bioactive compounds such as: lactoferrin (LF) from bovine milk (Sigma Aldrich) and high weight molecular chitosan (CHT) (Sigma Aldrich). After N$_2$ or/and air plasma exposure and γ-irradiation the PLA films were removed from the treatment chamber and then immersed into a lactoferrin solution of 136 μg/mL concentration or chitosan solution of 1.5 mg/mL concentration. The films
modified with LF were then washed with phosphate buffer saline solution (PBS, pH 7.4) and dried in nitrogen flow and the films modified with chitosan were excessively rinsed with water and further vacuum dried at 50°C. For covalent bonding of LF and CHT onto PLA surface were used two coupling agents, EDC (1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride) and NHS (N-hydroxysuccinimide) (ratio 75 mM/15 mM).

I. Lactoferrin immobilization onto PLA surface

The water contact angles of the PLA surfaces decreases by increasing the N2 plasma exposure time, the surface wetting being enhanced by incorporating polar groups at PLA surface. By increasing the plasma exposure time was observed also a direct proportional mass loss. It was noticed that plasma exposure determines a more significant functionalization of PLA surface than γ-irradiation.

The ATR-FTIR spectra confirmed the presence of lactoferrin onto PLA plasma treated sample, especially when the immobilization was done by covalent coupling and not by physical adsorption. The absorption bands specific to lactoferrin (Amide I at 1645 cm\(^{-1}\), Amide II band at 1540 cm\(^{-1}\) and at 3400 cm\(^{-1}\) the O-H and N-H stretching vibrations) were more evident for N2 plasma pretreated PLA, meaning that this functionalization is more suitable for anchoring the protein onto PLA surface than gamma-irradiation. Typical chemical compositions after exposure to nitrogen plasma, determined using surface-sensitive XPS, showed on survey spectra an emission signal assigned to nitrogen atoms (2.0 at%), which does not appear in the native PLA spectra. After lactoferrin coating the nitrogen atomic percentage increases even more (8.2 at%), the highest content being determined for the sample obtained by lactoferrin immobilization onto PLA surface using coupling agents (EDC+NHS) (9.7 at%).

Gamma irradiation determines functionalization of PLA substrate by oxygen and nitrogen-containing group incorporation. After lactoferrin immobilization the nitrogen atomic percentage significantly increases when using coupling agents (5.7 at%), which suggests that a better protein-functionalization of the PLA substrate is achieved. It was observed also a slight decrease of the oxygen atomic percentage (from 38.1 at% to 36.6 at%) with increasing the γ-irradiation dose. Comparing the two functionalization methods is noticed that the plasma exposure is more efficient in terms of functional groups incorporation at PLA surface than γ-irradiation.

The surface morphology of nitrogen plasma and γ-irradiated PLA samples were determined by AFM in tapping mode on a 1 × 1 μm\(^2\) area. The average surface roughness increases with treatment time and irradiation dose (from 6 nm to 22.5 nm and 11.6 nm, respectively) hence it can support the adhesion improvement. After lactoferrin immobilization the polymer surface become smother. The protein molecules fill the gaps between the two “hills”, which leads to a more homogenous surface. Chemical immobilization of the lactoferrin onto gamma irradiated PLA (PLA/30kGy/EDC+NHS/LF) induces a roughness increase, which is associated with protein-clusters formation – Figure 1.
Figure 1. AFM 2D (A) and phase (B) of N₂ plasma treated and gamma-irradiated PLA with immobilized lactoferrin onto surface.

The thermal stability of γ-irradiated PLA is lower, the overall activation energies of thermo-oxidative degradation decreasing. But after lactoferrin grafting its stability is improved accordingly with the nature of reacted moieties. Non-isothermal CL curves for PLA/10kGy/EDC+NHS/LF have shown the presence of peroxides on the same temperature range as for PLA/10kGy/LF. The thermal stability of grafted PLA is higher than the pristine material as activation energies of thermo-oxidative degradation comparison revealed (from 58.53 kJ/mol for PLA to 121.97 kJ/mol for PLA/10kGy/EDC+NHS/LF).

The percentage of radical scavenging activity (RSA%) of each sample was assessed by DPPH (1,1-diphenyl-2-picryl-hydrazyl) free radical assay. The changes in color (from deep violet to light yellow) were read with a UV-vis spectrophotometer. The decrease of the absorbance at 517 nm is associated with the reaction of DPPH with an antioxidant compound, which can donate hydrogen. As revealed in Figure 2(a) the sample obtained by lactoferrin grafting imparts antioxidant activity to PLA substrate (RSA 10%). The obtained samples were tested for inhibiting the growth of three different bacteria Escherichia coli, Listeria monocytogenes and Salmonella typhymurium. In the case of lactoferrin modified PLA substrate the gamma pre-irradiated sample has more pronounced antioxidant (Table 1) and antibacterial activities (Table 2) when compared with nitrogen pre-treated sample, even though the chemical grafting was significantly more in the last case (as FTIR and XPS analyses showed).
**Figure 2.** UV-Vis spectra for radical scavenging activity (RSA) determination by DPPH assay of lactoferrin-modified (a) and chitosan-modified (b) PLA by plasma and gamma irradiation.

**Figure 3.** Aspects of the apples (a1-a3) and apple juice in contact with the PLA (b1), PLA/Ag (b2), PLA/Ag/Vitamin E (b3) membranes after 48 hours.

**II. PLA surface modification by chitosan grafting**

Chitosan immobilization onto PLA substrate leads to an increase in water contact angles (from 19° for PLA/cp N₂ to 79° for PLA/cp N₂/EDC+NHS/CHT), which may be explained by the almost hydrophobic nature of chitosan. ATR-FTIR spectra revealed that surface immobilization of chitosan onto PLA substrate, through grafting method, is achieved both by nitrogen plasma pre-treatment and gamma pre-irradiation (evident characteristic bands at 3250 cm⁻¹ for –OH stretching, 1597 cm⁻¹ NH₂ bending
and 1657 cm\(^{-1}\) for amide I C=O stretching). In the last mentioned case are observed slightly more intense absorption bands, meaning that the radicals, formed by exposure of the polymer to gamma radiation, are suitable as anchoring sites for chitosan functional groups. Chitosan immobilization onto polymer surface impart antioxidant properties to poly(lactic acid) substrate, with 100% radical scavenging activity (RSA) (Figure 2(b) and Table 1) for gamma pre-irradiated sample, which is significantly higher than the RSA determined for plasma pre-treated PLA (11.8%). The γ-pre-irradiated and nitrogen plasma pre-treated PLA substrate modified with chitosan presents the same bacterial inhibitory activity (Table 2) (100%) but the pre-irradiated sample is distinguished by a more pronounced antioxidant activity.

### 3.2. Cellulosic substrate modification

After activation, the cellulosic substrate were removed from the treatment chamber and immediately immersed in the treatment chloroform solutions (10 wt%) of Eu, GO and RO for 60 minutes, on mechanic stirring. The solutions used for grafting contain two chemical coupling agents, namely EDC and NHS. The cellulosic substrates were then dried at 60 °C, and after that extracted for 25 h in a Soxhlet extractor with chloroform respectively, in order to remove the physically adsorbed unreacted chemicals.

**Figure 4. Normalized ATR-FTIR spectra for CC (●), CC/Eu - plasma activated (●), CC/Eu - irradiated (●), CC/GO - plasma activated (●), CC/GO - irradiated (●), CC/RO - plasma activated (●), CC/RO - irradiated (●)**

ATR-FTIR spectra of the most representative bands for cellulosic material treated with eugenol (1701 cm\(^{-1}\) for C=O stretching, 1232 cm\(^{-1}\) for C-O stretching), grape seed oil and rosehip seed oil (2926 - 2971 cm\(^{-1}\) for OH stretching, 1765 – 1770 and 1545 - 1569 cm\(^{-1}\) for C=O stretching, 1437 - 1459 cm\(^{-1}\) for -CH\(_2\)- deformation, 1230 - 1235 cm\(^{-1}\) and 1051 - 1055 cm\(^{-1}\) for C-O stretching) under cold plasma discharge or by γ-irradiation are evidenced. There are also important shifts of the bands position to lower or higher wave numbers which indicate that the new compounds are formed after two step treatment process. The modification took place after activation in all plasma discharge gases and for all γ-irradiation doses. However, it seems that the efficiency was higher when using air as discharge gas and 20 kGy dose for γ-irradiation.
The surface morphology analyzed by SEM of untreated was quite homogenous and that the individual fibers were intact (Figure 5a) while modified cellulosic substrates - Figure 5b-d - with Eu, GO and RO show a more rough surface and also a thin layer of deposits seems to cover the whole surface.

![Representative SEM images of the samples](image)

Figure 5. Representative SEM images of the samples (a) CC, (b) CC/cp air/Eu, (c) CC/cp air/GO, (d) CC/cp air/RO

The radical scavenging activities of cellulosic substrate after modification with phenolic compounds were about 100%, excepting CC/10kGy/GO and CC/20kGy/GO samples -Table 1.

**Table 1.** Radical scavenging activity (RSA) of untreated, plasma and/or irradiated polymeric substrates further modified with different compounds.

<table>
<thead>
<tr>
<th>Sample</th>
<th>RSA (%)</th>
<th>Sample</th>
<th>RSA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA</td>
<td>0</td>
<td>CC/10kGy/GO</td>
<td>54.3</td>
</tr>
<tr>
<td>PLA/cp N₂</td>
<td>11</td>
<td>CC/20kGy/GO</td>
<td>49.6</td>
</tr>
<tr>
<td>PLA/cp air</td>
<td>12</td>
<td>CC/5kGy/RO</td>
<td>100</td>
</tr>
<tr>
<td>PLA/N₂/EDC+NHS/LF</td>
<td>10</td>
<td>CC/10kGy/RO</td>
<td>100</td>
</tr>
<tr>
<td>PLA/10kGy</td>
<td>6</td>
<td>CC/20kGy/RO</td>
<td>100</td>
</tr>
<tr>
<td>PLA/20kGy</td>
<td>8</td>
<td>PE</td>
<td>0</td>
</tr>
<tr>
<td>PLA/30kGy</td>
<td>8</td>
<td>PE/20kGy</td>
<td>5</td>
</tr>
<tr>
<td>PLA/cp N₂/EDC+NHS/CHT</td>
<td>11.8</td>
<td>PE/30kGy</td>
<td>13</td>
</tr>
<tr>
<td>PLA/20kGy/EDC+NHS/CHT</td>
<td>100</td>
<td>PE/50kGy</td>
<td>50</td>
</tr>
<tr>
<td>CC/cp air/Eu</td>
<td>100</td>
<td>PE/20kGy/EDC+NHS/TT</td>
<td>100</td>
</tr>
<tr>
<td>CC/cp N₂/Eu</td>
<td>100</td>
<td>PE/30kGy/EDC+NHS/RO</td>
<td>92</td>
</tr>
<tr>
<td>CC/cp air/GO</td>
<td>100</td>
<td>PE/20kGy/EDC+NHS/CHT</td>
<td>15.1</td>
</tr>
<tr>
<td>CC/cp N₂/GO</td>
<td>100</td>
<td>PE/30kGy/EDC+NHS/CHT</td>
<td>24.3</td>
</tr>
<tr>
<td>CC/cp air/RO</td>
<td>100</td>
<td>PE/50kGy/EDC+NHS/CHT</td>
<td>25.7</td>
</tr>
<tr>
<td>CC/cp N₂/RO</td>
<td>100</td>
<td>PE/cp air/CHT</td>
<td>0</td>
</tr>
<tr>
<td>CC/5kGy/Eu</td>
<td>100</td>
<td>PE/cp air/EDC+NHS/CHT</td>
<td>100</td>
</tr>
<tr>
<td>CC/10kGy/Eu</td>
<td>100</td>
<td>PE/cp air/CHT+VC</td>
<td>9.2</td>
</tr>
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</table>
Hydroxyl groups present on their structure are believed to be associated with their antioxidant activity. The radical-scavenging activities of irradiated and phenolic compounds treated samples depend on the irradiation dose and to the different abilities of the individual phenolic compounds to react with DPPH giving a stable non-radical compound.

Both plasma activation and γ-irradiation have great influence on the antimicrobial activity - Table 2, being known that they are relatively simple and quite safe microbial sterilization techniques that are utilized in a variety of applications for its low operating costs and non-polluting capabilities. Using plasma or irradiation treatment, the surface of cellulose/chitin mix fibers is cleaned and etched and so more chitin is available at the surface to impart better antimicrobial properties to the fibers. Further modification with phenolic antioxidants also improves the antimicrobial properties. The antimicrobial activity was up to 100 %.

We have determine the following order of the antimicrobial properties: CC/cp air/RO > CC/20 kGy/GO > CC/ cp air/Eu/> CC/20 kGy/RO > CC/20 kGy/Eu > CC/cp air/GO.

The best antioxidant and antimicrobial properties were found for cellulose/chitin mix fibers modified with rosehip seeds oil.

Table 2. Antimicrobial activity (%) of untreated, plasma and/or irradiated polymeric substrates further modified with different compounds

<table>
<thead>
<tr>
<th>Sample</th>
<th>Escherichia coli</th>
<th>Listeria monocytogenes</th>
<th>Salmonella enteritidis</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA</td>
<td>52</td>
<td>40</td>
<td>55</td>
</tr>
<tr>
<td>PLA/cp N₂</td>
<td>91</td>
<td>82</td>
<td>97</td>
</tr>
<tr>
<td>PLA/cp N₂/LF</td>
<td>75</td>
<td>71</td>
<td>87</td>
</tr>
<tr>
<td>PLA/cp N₂/EDC+NHS/LF</td>
<td>62</td>
<td>65</td>
<td>60</td>
</tr>
<tr>
<td>PLA/20K Gy</td>
<td>97</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>PLA/20K Gy/EDC+NHS/LF</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>PLA/20K Gy/CHT</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>PLA/20K Gy/CHT</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>PLA/20K Gy/CHT</td>
<td>84</td>
<td>96</td>
<td>100</td>
</tr>
<tr>
<td>CC/cp air</td>
<td>47</td>
<td>54</td>
<td>75</td>
</tr>
<tr>
<td>CC/cp air/Eu</td>
<td>79</td>
<td>100</td>
<td>94</td>
</tr>
<tr>
<td>CC/cp air/GO</td>
<td>48</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>CC/cp air/RO</td>
<td>85</td>
<td>100</td>
<td>97</td>
</tr>
<tr>
<td>CC/20k Gy</td>
<td>60</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>CC/20k Gy/Eu</td>
<td>72</td>
<td>87</td>
<td>100</td>
</tr>
<tr>
<td>CC/20k Gy/GO</td>
<td>82</td>
<td>100</td>
<td>97</td>
</tr>
<tr>
<td>CC/20k Gy/RO</td>
<td>64</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>PE</td>
<td>23</td>
<td>26</td>
<td>32</td>
</tr>
<tr>
<td>PE/20k Gy</td>
<td>91</td>
<td>87</td>
<td>99</td>
</tr>
<tr>
<td>PE/30k Gy</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>PE/20k Gy/EDC+NHS/TT</td>
<td>95</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
### 3.3. Non-degradable substrate (PE) functionalization

After plasma exposure and γ-irradiation the PE films were removed from the treatment chamber and then immersed into a chitosan solution of 1.5 mg/mL concentration (prepared in aqueous solution of 2% acetic acid) or 4% rosehip seeds oil or tea tree oil in chloroform. The films modified with chitosan were excessively rinsed with water and further vacuum dried at 40°C and the ones modified with natural oils were rinsed with chloroform. For covalent bonding EDC and NHS (ratio 75 mM/15 mM) coupling agents were used.

The increase of the carbonyl index (determined by ATR-FTIR spectroscopy) with the irradiation dose (from 0.23 for PE/20kGy to 0.38 for PE/50kGy) indicates that the sample undergoes an oxidative degradation. The unstable species (generally free radicals) formed by γ-irradiation determines grafting or cross-linking of polyethylene. By air plasma treatment new oxygen and nitrogen-containing functional groups (–OH, C=O, C-O-C, N-H) are implanted onto PE surface, which determines an increase of polymer’s wettability.

When make comparison between irradiation and air plasma treatment of PE and further chitosan grafting is noticed that both samples have good antibacterial activity but plasma pre-treated sample has higher antioxidant activity.

The successful immobilization of tea tree oil (TT) onto gamma pre-irradiated polyethylene takes place when using 20 kGy irradiation dose and coupling agents while for rosehip seeds oil (RO) higher irradiation doses are needed, as ATR-FTIR spectra has revealed the bands at 3382 cm⁻¹ –OH stretching, 1712 cm⁻¹ C=O stretching, 1640 cm⁻¹ and 1564 cm⁻¹ alkene functional group (C=C), 1224 cm⁻¹ C-O stretching for TT and 3009 cm⁻¹ OH stretching from COOH, 1751 cm⁻¹ C=O stretching, 1163 cm⁻¹ C-OH stretching and 719 cm⁻¹ CH stretching for RO.

The **thermal stability** determined by chemiluminescence analysis of natural oils-grafted PE is higher than the pristine material (the activation energies were 63.93 kJ/mol for PE/20kGy and 84.05 kJ/mol for PE/20kGy/EDC+NHS/TT). This feature recommends these modified materials as packaging products, which exhibit higher thermal resistance than neat polymer. The application of radiation technique is suitable for the grafting polymers through which the addition of grafted molecules takes place under “clean” conditions.

Gamma irradiated PE presents some antioxidant activity which is amplified by the modification with tea tree oil. The samples functionalized with TT presents radical scavenging activity of 100%, no matter the irradiation dose used. The gamma irradiation confers antibacterial activity to PE substrate, which increases by increasing the irradiation dose (Table 2).
PE films modified with TT present higher antioxidant activity (100%) than the sample modified with RO (92%), but the last one has more pronounced antibacterial activity (Table 2).

Conclusions

All results confirm the morphological and structural changes after treatments which determine the modification of the polymeric substrates. Covalent bonding onto polymeric substrates of some bioactive components (chitosan and vitamin E and C and various vegetable oils) was achieved by a two step procedure: I) cold plasma or γ-irradiation as pre-treatment method for surface functionalization; II) coupling reaction with EDC+NHS. Two biodegradable substrates (polylactic acid and cellulose/chitin mixed fibers) and one non-biodegradable (polyethylene) were chosen. For all modified substrates by plasma activation or gamma irradiation the following orders were established:

PLA packaging material antioxidant activity: PLA/cp N₂/EDC+NHS/LF < PLA/cp N₂/EDC+NHS/CHT < PLA/20kGy/EDC+NHS/LF << PLA/20kGy/EDC+NHS/CHT, while in respect with antibacterial activity: PLA/cp N₂/EDC+NHS/LF < PLA/20kGy/EDC+NHS/LF ≈ PLA/20kGy/EDC+NHS/CHT ≈ PLA/cp N₂/EDC+NHS/CHT.

In case of CC packaging materials the following order of the antimicrobial properties was established: CC/cp air/RO > CC/20kGy/GO > CC/cp air/Eu > CC/20kGy/RO > CC/20kGy/Eu > CC/cp air/GO.

For PE packaging materials the antioxidant activity varied in the following order: PE/20kGy/EDC+NHS/CHT < PE/30kGy/EDC+NHS/RO < PE/cp air/EDC+NHS/CHT ≈ PE/20kGy/EDC+NHS/TT and for the antibacterial character: PE/20kGy/EDC+NHS/TT < PE/30kGy/EDC+NHS/RO ≈ PE/20kGy/EDC+NHS/CHT ≈ PE/cp air/EDC+NHS/CHT.

In most cases rosehip seeds oil imparts the best antioxidant and antimicrobial properties.

As γ-irradiation affects also the bulk properties of the selected polymers and for a proper functionalization longer time is needed, it has been concluded that the most efficient procedure is plasma treatment. Choosing two biodegradable polymers as functionalizable substrates, the environmental concerns are avoided.
Thailand: Country Report

DEVELOPMENT OF NATURAL AND SYNTHETIC POLYMERS AS SUITABLE PACKAGING MATERIALS FOR FOOD PRODUCTS STERILIZED BY RADIATION PROCESSING

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Abstract

The beginning part of this research aims to study the effects of gamma irradiation on commercially available polymers currently being used for food products sterilized by radiation processing at Thailand Irradiation Center (TIC). The chosen product is fermented pork sausages. The effects of gamma irradiation on packaging material of fermented pork sausages were investigated using FTIR, DSC and universal testing machine. FTIR and DSC revealed changes in chemical and thermal properties of the packaging material, respectively, while the universal testing machine showed changes in mechanical properties. The results showed that the high fat content, the direct contact between acidic fermented pork sausages and the packaging material as well as irradiation led to changes in chemical and mechanical properties of the packaging material.

1. OBJECTIVE OF THE RESEARCH

The specific objective of this research is to first study the effects of gamma irradiation on commercially available polymers currently being used for food products sterilized by radiation processing at Thailand Irradiation Center (TIC). After irradiation, changes in mechanical and thermal properties and chemical structure will be investigated. The obtained information will be used to determine the most suitable packaging materials for food irradiation as well as to develop biodegradable packaging materials suitable for food irradiation. Radiation processing will be utilized to improve the properties and enhance the performance of biodegradable packaging materials.

2. INTRODUCTION

Radiation sterilization of food and medical products, including radiation pasteurization of foods, is one of the most important applications of ionizing radiation [1]. During the radiation, packaging materials of these products are exposed to radiation as well. Most of these packaging materials are synthetic polymers. During radiation, energy transferred to polymer molecules can induce chemical as well as structural changes through the formation of various reactive intermediates such as free radicals, ions and atoms in excited states [2]. These reactive intermediates will finally lead to disproportion, hydrogen abstraction, rearrangement and/or formation of new bonds, depending on structure of the polymers and conditions, both before, during and after irradiation. Two of the most important changes for polymers, after irradiation, are crosslinking and degradation. As a result, controlling undesired changes, such as deterioration of mechanical properties and color changes has become an important subject for radiation sterilization of commercial products. The success of radiation sterilization using packaging materials made from synthetic polymers is due to two major reasons. The first
reason is simply the convenience of radiation sterilization of products in various shapes and sizes. The second reason is the fact that most of the synthetic polymers tend to crosslink rather than degrade, upon irradiation [1-2]. On the contrary, natural polymers are likely to undergo degradation, upon irradiation [3-4]. For example, cellulose, which is the main component of paper, degrades upon irradiation, resulting in decline of mechanical properties of paper. Therefore, choosing the right natural polymers for food packaging materials is an important factor to be taken into consideration, in order to maintain the desired properties as well as to ensure the safety and suitability in food packaging of these natural polymer-based packaging materials. Biodegradable polymers have been attracting a lot of attention due to their environmental friendliness. They have become perfect candidates for food packaging materials. Some of these food packaging materials may be applied for food products to be sterilized by radiation processing as well. Hence, the investigation of the effects of radiation on their properties is very important and must be done in order to ensure their suitability and safety as packaging materials for food irradiation.

Thai Irradiation Center (TIC) is a part of Thailand Institute of Nuclear Technology (Public Organization) (TINT). TIC’s main mission is to offer radiation service to both government and private organizations, mostly for commercial purposes. TIC offers radiation service for various products from food and agricultural products to non-food products such as medical products and cosmetics. Examples of food and agricultural products include spices, fruits, frozen seafood, onion and garlic. Currently, TIC offers radiation service using a gamma irradiator with Co-60 as a source of gamma radiation which offers high-penetrating capability, therefore making it suitable for irradiation of packaged food as well as medical products. Each year, TIC hosts an annual seminar for the customers to offer basic knowledge of radiation as well as to discuss about the customers’ needs and problems. In recent years, TIC has received questions from a number of customers concerning the effects of radiation on plastic packaging of food products. One customer complained that after irradiation, properties of the packaging are lower than standard values. The customer would like TINT to do a research work on this problem to determine scientific reasons causing this problem, so that they can develop suitable packaging materials for their products. A private company offering radiation service in Thailand is also facing a similar problem, having a difficult time to find an optimum dose for each product. To solve these problems, TINT proposes this research project to investigate the effects of gamma and electron beam irradiation on properties of commercial packaging materials for food irradiation as well as to use the obtained knowledge to develop bio-based polymers as suitable packaging materials for food irradiation.

3. MATERIALS AND METHODS

3.1. Materials
Unirradiated and irradiated fermented pork sausage was purchased from Sutthiluck Innofood Co., Ltd, Thailand.

3.2. Irradiation process
Fermented pork sausages were irradiated using a (60)Co multi-purpose irradiator (JS8900, MDS Nordion, Canada) installed at TIC plant in Patumthanee, Thailand, to achieve 2 kGy absorbed doses. Dosimetry was done using Nylon thin film type FWT-60-00 dosimeters. The average dose rate was 2.09 kGy/h. The minimum dose measured was 2.33, while the maximum dose measured was 3.05, with dose uniformity ratio (DUR) of 1.309.
3.3. FTIR analysis

Infrared spectra of non-irradiated and irradiated packaging films for fermented pork sausages were obtained using a Fourier transform infrared spectrometer (Tensor 27, Bruker, Germany). FTIR spectra within the range of 650-4000 cm\(^{-1}\) were recorded in reflection mode using an attenuated total reflectance (ATR) accessory equipped with ZnSe crystal. Spectra were collected with 16 co-added at a resolution of 4 cm\(^{-1}\).

3.4. DSC Measurements

Thermal properties of non-irradiated and irradiated packaging films were characterized by a differential scanning calorimeter (DSC 822\(\text{e}\), Mettler Toledo, Thailand). All experiments were done with a sealed empty pan as a reference under nitrogen purge with a flow rate of 60 ml/min. For the first run, the sealed pans with samples (3-5 mg) were first cooled to -50°C, held isothermally for 1 min and then heated at a heating rate of 10°C/min to 190°C. During the second run, the whole process was repeated one more time to obtain the DSC thermograms.

3.5. Mechanical Properties

Mechanical properties were measured using a universal testing machine (AG-100kNG, Shimadzu, Japan). The samples were cut with test specimen cutter (Dumbbell, Japan) with known dimension according to ASTM test method D638 type IV. The samples were mounted between machine’s grips, which were separated at a constant speed of 200 mm/min. The data reported are the average values (with the related error bars) obtained through ten tests per sample.

3.6. Color Measurement

The Hunter lab color parameters were measured using a Chroma Meter (CR 300, Minolta) in terms of lightness (L) from black to white, “a” from green (-) to red (+) and “b” from blue (-) to yellow (+). The lightness was measured in Hunter scale ranging from 0 to 100, while “a” and “b” values varied from -60 to +60. Five specimens were used for each set of sample and average value is reported.

4. RESULTS AND DISCUSSION

4.1. FTIR Analysis

Figure 1 shows pictures of Suddhiluck’s fermented pork sausages and their 3-layer packaging. Since the packaging materials are of commercial origin, the information regarding the type of polymers is not known. FTIR was used to identify the type of polymers used for the packaging. The FTIR spectra of each layer of the packaging are shown in Figure 2. It can be seen that the FTIR spectra of the packaging materials from the middle and inside layers are similar, while that of the outside layer is different. In order to identify the type of polymers for each layer, spectrum search program was used to match the FTIR spectra of each sample with the library included in the analysis program of the FTIR spectrometer. The results showed that the packaging for the outside layer is made of polyethylene (PE), while that for the middle and inside layers is made of polypropylene (PP).
FIG. 1. Suddhiluck’s fermented pork sausages (left) and their 3-layer packaging (right).

The FTIR spectra of the packaging materials from the middle PP and inside PP look similar, but a closer look in Figure 3 reveals that, in addition to a small peak at about 1639 cm\(^{-1}\) which is also present in the middle PP, the inside PP showed an extra peak at 1745 cm\(^{-1}\). This extra peak at 1745 cm\(^{-1}\) corresponds to the absorption of carbonyl groups (most likely C=O stretching in ester), indicating the formation of oxidation products [5]. Note that these IR spectra were taken from the middle PP and inside PP of unirradiated fermented pork sausages. In this case, the formation of carbonyl group is hence not the result of radiation-induced oxidation reactions. The only difference between the middle PP and the inside PP is that the inside PP is in direct contact with fermented pork sausages, while the middle PP is not. Generally, meat is susceptible to oxidative deterioration due to the oxidation of polyunsaturated fatty acids in phospholipids. One of the main ingredients of Thai’s fermented pork sausages is pork fat. Therefore, the premature oxidative degradation of the inside PP is most likely due to the high fat content of fermented pork sausages as well as the direct contact between fermented sausages and the inside PP.

FIG. 2. FTIR spectra of each layer of the 3-layer packaging material.
After irradiation at 2 kGy, the effects of irradiation on the chemical structure of both the middle and the inside PP were followed by FTIR. The results are depicted in Figures 4. Figure 4 shows that irradiation at 2 kGy did not induce any further changes for the inside PP, even after 1 or 2 months. On the contrary, Figure 4 also shows that for the middle PP, upon irradiation at 2 kGy, there was a regular increase, with time, in the intensity of the bands at 3355, 3178, 1739, 1654 and 1631 cm\(^{-1}\). The first two bands indicate the formation of –OH in alcohols and/or in carboxylic acids, while the last three bands imply the formation of C=O in esters. The formation of these functional groups is most likely the result of radiation-induced oxidation reactions with atmospheric oxygen, mediated through free radicals [6].
Fermented sausages are produced from a mixture of pork, pork fat, salt, curing agent (nitrate and/or nitrite) and spices with lactic acid starter culture. At the end of the ripening process, fermented sausages are characterized with accentuated acidity and slight sourness [7]. In order to measure the acidity, 300 g of water were added to 100 g of fermented sausages. The mixture was ground using a blender. The ground mixture was filtered to separate the solid part from the liquid part. The acidity of the liquid part was measured using a pH meter, as shown in Figure 5. pH value of unirradiated fermented sausages was 4.30, whereas that of fermented sausages irradiated at 2 kGy was 4.37. The results showed that fermented pork sausages are relatively acidic. In addition to the high fat content, the acidity of fermented pork sausages is also most likely one of the main reasons explaining the premature oxidative degradation of the inside PP which is in direct contact with the fermented sausages.

4.2. DSC

Figure 6 shows the DSC thermograms of the middle PP and the inside PP, both before and after irradiation at 2 kGy. Typical PP shows characteristic peaks for a glass transition temperature ($T_g$) at about -33 °C and a melting ($T_m$) at approximately 165 °C. As seen in Figure 6, DSC thermograms of both the middle PP and the inside PP show $T_g$ at roughly -33 °C. However, their $T_m$ splitted into two peaks. A closer look at their $T_m$ peaks is shown in Figure 7.
As seen in Figure 7, $T_m$ of unirradiated middle PP splitted into two peaks at about 159 °C and 165 °C. The middle PP irradiated at 2kGy showed similar $T_m$ peaks, except that the peak at lower temperature shifted a little further to 158 °C. The shift to lower of irradiated middle PP agreed very well with the results from FTIR. Radiation-induced oxidative degradation in PP led to the formation of new functional groups, such as esters and alcohols, in PP molecules. These irregularities have different heat of fusion from pure PP molecules, hence resulting in the shift of $T_m$. As for the inside PP, both unirradiated and irradiated samples showed two $T_m$ peaks at 152 °C and 158 °C. The shift to even lower temperature of both $T_m$ peaks of the inside PP (compared with the middle PP) once again agreed well with the results from FTIR and pH measurement. That is the high fat content as well as the direct contact between acidic fermented sausages and the inside PP led to the premature oxidative degradation of the inside PP.

4.3. Mechanical Properties
Mechanical properties of the middle PP and the inside PP, both before and after irradiation at 2 kGy, were studied to investigate the radiation-induced changes. Changes in tensile strength, elongation at break, modulus and yield strength are illustrated in Figures 8. The results showed that for unirradiated samples, the mechanical properties of the inside PP slightly decreased from those of the middle PP. This can be explained from the fact that oxidative degradation (as confirmed by results from FTIR, DSC and pH measurement) of the inside PP, due to the high fat content and the direct contact between acidic fermented sausages and the inside PP, led to reduction in its mechanical properties. After irradiation, both the middle PP and the inside PP also showed further decrease in their mechanical properties. The trend is most obvious for modulus and yield strength. Figure 8 clearly showed that the modulus of unirradiated middle PP was 549 kg/cm², whereas that of unirradiated inside PP was 450 kg/cm². Upon gamma irradiation at 2 kGy, the modulus of irradiated middle PP decreased to 483 kg/cm², while that of irradiated inside PP dropped further to 385 kg/cm². These results once again confirmed that both the high fat content and the direct contact between acidic fermented sausages and the inside PP led to the premature degradation of the inside PP.

4.4. Color Measurement

The effects of irradiation on the color changes, in terms of “Hunter” color parameters (L, “a” and “b”), of both the middle PP and the inside PP are depicted in Figures 9. In figure 9, Hunter L value, which is the function of lightness (apparent proportion of incident light reflected by an object) increased with irradiation for the middle PP, but decreased with irradiation for the inside PP. Hunter “a” values followed a similar trend. The Hunter “b” values of the unirradiated middle PP was similar to that of the irradiated middle PP. On the contrary, Hunter “b” values of the irradiated inside PP was higher than that of the unirradiated inside PP. Both of them have higher Hunter “b” values than the middle PP samples. Increase in Hunter “b”
values indicated that irradiated samples become more yellowish. These results agreed well with the appearances of the irradiated samples.

FIG. 9. Hunter L (left), Hunter “a” (middle) and Hunter “b” (right) values of of the middle and inside PP, before and after irradiation at 2 kGy.

5. CONCLUSION
The effects of gamma irradiation on packaging material of fermented pork sausages were investigated. FTIR results showed that the middle and inside layers of the packaging materials were made of PP. FTIR analysis revealed that the chemical changes of the inside PP, even before irradiation processing, was caused by the high fat content and the direct contact between the inside PP and acidic fermented pork sausages. The results from mechanical tests also implied that the direct contact between acidic fermented sausages and the inside PP led to the premature degradation of the inside PP, whereas irradiation led to further decrease in mechanical properties of both the middle and the inside PP.

Work Plan for Coming Year
Synthetic and natural polymers will be blended to form films to be processed as packaging materials. The effects of radiation on properties of the blends will be studied, using different parameters such as type of radiation (gamma and electron beam), radiation dose, radiation atmosphere and the blend ratio. Radiation-induced changes in chemical, physical and mechanical properties of the blends will be investigated. Optimum conditions, including types of polymer and the blend ratio, will be determined to identify the most suitable material as a candidate for food packaging materials to be used for food irradiation.
REFERENCES

INVESTIGATION OF THE EFFECTS OF GAMMA-IRRADIATION ON SOME BARRIER PROPERTIES OF FOOD PACKAGING POLYMER FILMS

(Research contract no: 17761)

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Abstract
The incorporation, immobilization, surface modification of antimicrobial agents directly into polymeric packaging is an exciting development, which allows industry to combine the preservative functions of antimicrobials with the protective functions of preexisting packaging concepts. There are other synthetic and naturally occurring compounds that may be exploited by the packaging industry. These include organic acids, bacteriocins, spice extracts, chelating agents, antibiotics and enzymes, etc. The use of bacteriocins and other biologically derived antimicrobials in packaging materials is attracting increasing interest in recent times. In this study, the objective of the present work was to graft some food additives onto commercial packaging film polyethylene (PE) by gamma-irradiation under O₂ and N₂ atmosphere. With this aim, we tried to graft some food additives (FA) with antimicrobial properties such as fumaric acid (FAc), grafting acrylic acid (AAc) and then loaded natamycin (Nat) onto PE film. We investigated the irradiation dose and concentration of FA on grafting yield. The grafting yield of FAc onto PE film was very low. The antimicrobial activity of FAc grafted PE films was investigated. There was no grafting of natamycin onto PE film. In addition to this study we have been working on the grafting of acrylic acid (AAc) onto PE films and AAc grafted PE film can be then used to bind antifungal agents, such as natamycin, and the antifungal properties of the films were investigated. It was found that this process results in highly conformal and uniform PAAc grafts on the surface PE films. The synthesized PE-g-PAAc copolymers were characterized by Grazing FTIR spectroscopy, X-ray photoelectron spectroscopy, elemental analysis, scanning electron microscopy. The results of various techniques confirmed the existence of well-defined PAAc chains in copolymer composition.

Introduction
Most plastic containers currently used for food packaging are made up of petroleum-based polymeric materials. Their use is widespread in this and many other applications, due to their numerous advantages, including large scale availability, relatively low production cost, lightweight, versatile and good mechanical and barrier properties (1,2). However, these materials have certain disadvantages since, in addition to being synthesized from a non-renewable source, they are not biodegradable, proving a major source of generation and accumulation of residues (3). Polymers play important roles in a wide range of applications such as membranes, biomaterials, sensors, etc. For polymers used in different fields, surface hydrophilicity is one of the most important properties (4-9). However, most of the polymers are naturally hydrophobic; the improvement of the hydrophilicity of polymer surfaces is thus an important research task. The hydrophilicity of the polymer surfaces can be improved (10-15) by using the surface modification techniques such as plasma treatment, irradiation with gamma-rays, corona discharge, ion beam treatment, UV radiation, etc., (10,15,16).

Microbial contamination and subsequent growth reduces the shelf life of foods and increases the risk of food borne illness. Traditional methods of preserving foods from the effect of microbial growth include thermal processing, drying, freezing, refrigeration, irradiation, MAP and addition of antimicrobial agents or salts (17). However, some of these techniques cannot be applied to food products such as fresh meats (18). Antimicrobial food packaging materials have to extend the lag phase and reduce the growth phase of microorganisms in order to extend shelf life and to maintain product quality and safety (19). Comprehensive reviews on antimicrobial food packaging have been published by Appendini and Hotchkiss (20) and Suppakul et al. (21). To confer antimicrobial activity, antimicrobial agents may be coated, incorporated, immobilised, or surface
modified onto package materials (21). A comprehensive list of antimicrobial agents for use in antimicrobial films, containers and utensils is presented in a review by Suppakul et al. (21). The classes of antimicrobials listed range from acid anhydride, alcohol, bacteriocins, chelators, enzymes, organic acids and polysaccharides (22). Antimicrobial films can be classified into two types: those that contain an antimicrobial agent which migrates to the surface of the food and, those which are effective against surface growth of microorganisms without migration.

Antimicrobial agents, either mixed directly or applied on the surface of foods, can be used to prolong the shelf-life of packed foods. However, it could also be possible to incorporate antimicrobial agents into packaging materials which then migrate in small amounts into the food. This may be especially effective with vacuum packaging because of the intimate contact between the packaging material and the food surface. Substances with antimicrobial effects can be, for example, nisin produced by Lactococcus lactis (23), organic acids, esters and sorbates. Some preservatives can also be incorporated into or onto polymeric packaging materials to provide antimicrobial activity. These agents can be applied by impregnation, mixing or using various coating techniques. A more sophisticated concept is the use of immobilised enzymes, and materials having chemically bound antimicrobial functional groups on the material surface. Some functional groups that have antimicrobial activity have been introduced and immobilised on the surface of polymer films by modified chemical methods. Haynie et al. (24) prepared a series of antimicrobial peptides covalently bonded to a water-insoluble resin which proved to have antimicrobial activity. Laser-induced surface functionalisation of polymers has recently been found to be an effective way of imparting functional groups to food packaging polymer surfaces to improve adhesion, to modify barrier properties and to give the polymers antimicrobial activity. Paik et al. (25) recently presented the use of 193 nm UV irradiation using a UV excimer laser to convert amide groups on the surface of polyamide plastic to amines which have an antimicrobial effect. The antimicrobial active packaging reduces, inhibits or delays the growth of the microbiota present mainly on the surface of the packaged food, where most deterioration reactions take place (26-27). Active packaging is produced by the addition of volatile and non-volatile antimicrobial agents directly to the polymer, through antimicrobial adsorption on its surface, antimicrobial immobilization into the polymer by ionic or covalent bonds, and by the use of polymer with antimicrobial activity (20).

The incorporation of antimicrobial agents directly into polymeric packaging is an exciting development, which allows industry to combine the preservative functions of antimicrobials with the protective functions of preexisting packaging concepts. There are other synthetic and naturally occurring compounds that may be exploited by the packaging industry. These include organic acids, bacteriocins, spice extracts, chelating agents, antibiotics and enzymes, etc. (28,29). The use of bacteriocins and other biologically derived antimicrobials in packaging materials is attracting increasing interest in recent times (30-32). A wide range of antimicrobial substances have been studied in order to evaluate their potential application in antimicrobial biodegradable films. These substances include organic acids (sorbates, benzoic acids), bacteriocins (nisin, pediocin), enzymes (lysozyme), natural extracts (garlic oil, rosemary extract, propolis, etc.) and fungicides (33-36). Each antimicrobial substance exhibits a specific mechanism of action against a particular class of microorganisms (37).

The choice of an FAc is often restricted by the incompatibility of that agent with the packaging material or by its heat instability during extrusion (38,39). Coating of films with food additives can result in effective antimicrobial activity.

Nat is a natural antifungal agent produced by the bacterium Streptomyces nataelensis during fermentation and is widely used in the food industry for the prevention of mold contamination (40). This compound is approved by the Food and Drug Administration (FDA) as a food additive and classified as GRAS (Generally Regarded As Safe). Natamycin was also assigned as a natural preservative by the European Union (35). The direct application of Nat on food surfaces by spraying, dipping or coating has shown questionable results due to unsuitable adherence and also because the diffusion into the bulk of the food will reduce the surface concentration (41,42).
Furthermore, the maximum Nat concentration at the surface of final products should be 1 mg/dm² and it should not be detectable at 2 mm depth (41,43). The use as active films wrappings could be more efficient, by maintaining a critical concentration on the food surface and preventing its migration or loss (42). Active biofilms are usually prepared by the direct loading method, adding the antimicrobial to the film-forming solution, containing biopolymer, plasticizer and cross-linkers.

However, due to the large molecular weight and the conjugated double bond structure, Nat has very low water solubility (0.052 mg/mL) and the films obtained by this method are usually opaque and heterogeneous (43). The poor distribution of the active agent in the film matrix may also cause changes in the structure and in the functional attributes of the films. Effects on the barrier and mechanical properties of biopolymers films were reported by several studies (44-46).

Nat is a polyene macrolide antibiotic that is used as an antifungal agent in food preservation. In the U.S., Nat may be applied on cheese in amounts not to exceed 20 ppm in the finished product and is no longer restricted in its method of application, which may include dipping, spraying, or as a dry mixture with safe and suitable anticaking agents (47). Nat has the chemical formula C_{33}H_{47}NO_{13}, a molecular mass of 665.725, and the Chemical Abstract Service Registry Number CAS 7681-93-8 (48). This antymycotic compound consists of a large lactone ring of 22 carbon atoms linked to a mycosamine moiety, an amino sugar, by a glycosidic linkage. Based on the presence of four conjugated double bonds, Nat belongs to the polyene antibiotic group (49).

In this work, the heterogeneous surface modification of polyolefin films such as polyethylene (PE) was undertaken via the gamma-radiation grafting of fumaric acid (FAc) and acrylic acid (AAc). The influence of different reaction parameters on the graft yield such as irradiation dose and concentration of FAc, Nat and AAc was analyzed. The antimicrobial activities of PE-g-FAc, PE-g-PAAc and PE-g-PAAc/Nat were investigated. Then, the active functional groups present in the surface were used for binding Nat to impart to the films antifungal activity. The surface modification of the films with the treatment time was characterized by gravimetric measurements and also by chemical analysis, such as Grazing Fourier Transform Infrared (FTIR) spectroscopy, volumetric titration and dye adsorption. In addition, the topographical evolution of the film surfaces was followed with Scanning Electron Microscopy (SEM) and X-ray Photoelectron Spectroscopy (XPS). Finally, it was investigated whether FAc and Nat is electrostatically bonded to the functionalized films, their antimicrobial and antifungal properties are verified.

**MATERIALS AND METHODS**

The irradiation of samples with ⁶⁰Co-γ-rays was performed using a "Gammacell 220" with a dose rate of 0.26 kGy/h. The following chemicals were commercially acquired: AAc (Sigma), fumaric acid (Sigma), acetone (Merck), ethyl alcohol (Merck), acetic acid(Merck), sodium acetate (Sigma), NaOH (Sigma), NaH₂PO₄.H₂O (Sigma), Na₂HPO₄.12H₂O (Sigma), dye crystal violet (Sigma), and natamycin (Sigma), lactose (Sigma). All chemicals were used as received, without further purification. Deionized distilled water was used in the preparation of FAc and Nat solutions without contaminants. The chemical structures of fumaric acid, natamycin and acrylic acid utilized for surface modification of PE in this work are presented in Figure 1. Commercially polyethylene (PE) films were used in this study. The PE films were cut into 3-cm squares and placed in a sterile bottle. Then PE films were washed with acetone and then dried. Nitrogen gas was bubbled and 0.25 and 0.50 % w/v fumaric acid solutions were prepared and nitrogen gas was also bubbled in fumaric acid solutions in bottle. PE films were dipped in fumaric acid solution. They were irradiated between 0-150 kGy irradiation doses. After the irradiation the treated films were washed with distilled water to remove the remaining food additives. Prior to characterization, the films were dried at ambient temperature. For the Nat solutions (25 ppm) and PE films were followed the same way. The grafting yields which calculated by gravimetric measurement were very low (Table 1 and Figure 2). For unirradiated and irradiated and grafted PE films, Grazing Angle-FTIR spectra for all films were recorded. Grazing Angle-FTIR spectroscopy technique was utilized for characterization of the PE films. Grazing Angle-FTIR spectra were collected in the 400-4000 cm⁻¹ region with resolution 4 cm⁻¹. The grafting yield of food additives onto PE films as a function of irradiation dose and food additive solution concentrations were determined. The antimicrobial activity of PE-g-FAc films was investigated.
Nat was not grafted onto PE film by gamma-irradiation. Nat is a polyene macrolide antibiotic that is used as an antifungal agent in food preservation. The permissible dosage in the foods is 40ppm. At this concentration, Nat may be used effectively against a variety of microorganisms especially yeast and fungi. The very low aqueous solubility (30-50 mg/L) of Nat does not allow the preparation of a concentrated stock solution. Therefore, it is applied as an aqueous suspension to the shredded cheese surface, which results in clogging of spray nozzles and a heterogeneous distribution to the cheese surface. Solubility may be a limiting factor in the bioavailability of active Nat, since the dissolved fraction must diffuse to the site of action and bind to the target organism (50). Preliminary study indicated that it is impossible to coat nisin, the other important natural not chemical antimicrobial, directly onto PE films, and therefore a coating solution is needed as a carrier for nisin. Nat is more advantages than nisin. We decided to use Nat (antifungal agent) as food additive. Samples were characterized by using different techniques.

Grafting of PAAc on PE

Irradiation

Prior to γ-irradiation PE films were cut into approximately 3 cm × 3 cm dimensions with a weight of ~0.06 g. Each film was washed in acetone for 24 h and dried under vacuum till constant weight. The irradiation was performed using 60Co source with the dose rate 0.26 kGy/h in N2 and O2 atmosphere for predetermined time intervals.

Grafting

In a typical irradiated grafting, N2 purged grafting solution containing the monomer (AAc) in 10% and 40% v/v solutions in water at room temperature transferred into glass bottles containing preirradiated PE films under N2 atmosphere and in air. The polymerization solution in purgeable glass bottle was then put into a thermostated bath at 40° and 60°C for different time intervals. All grafted samples were extensively washed before analysis with a NaOH solution (pH 8) and finally with distilled water to remove traces of the unreacted monomer and the homopolymer that formed. The samples were dried in vacuo at room temperature until a constant weight and finally characterized. PE-g-PAAc films irradiated with a reaction time over became brittle, sticky and hard to manipulate, so they could not be characterized.

Film characterization

Gravimetric measurements

The degree of grafting (DG, wt.%) was calculated by the weighing of the films before and after the grafting reactions were carried out. The degree of grafting was estimated as follows:

\[ DG = \left( \frac{W_2 - W_1}{W_1} \right) \times 100 \]

where \( W_1 \) (g) is the weight of PE film and \( W_2 \) (g) is the dry weight of the PE-g-PAAc film.
Chemical analysis

Volumetric titration The COOH groups grafted onto the surfaces of the films were determined by volumetric titration with a 0.01M NaOH solution standardized against 0.01 M oxalic acid dihydrate.

Dye adsorption Light absorption of the crystal violet was carried out in a Carry 100 recording spectrophotometer. The adsorption of the dye was performed as follows. The films were immersed in an aqueous crystal violet solution (2.5x10^{-5} M) and buffered to pH 4.6 (by the addition of acetic acid and sodium acetate), and the dye adsorption was measured as the difference in the absorption in the crystal violet solution before and after contact with the films with ultraviolet-visible spectrometry at 584 nm (with the previous performance of a calibration curve).

FTIR spectroscopy The FTIR spectra were made with a Perkin Elmer, Spectrum Two. The FTIR spectra were performed on films in the transmission mode with a resolution of 4 cm^{-1} and 2 scans.

Natamycin loading The adsorption of natamycin was performed as follows. The grafted films were immersed in an aqueous dispersion of a 50/50 natamycin/lactose blend (0.25% W/V) buffered to pH 8.0 (by the addition of a phosphate buffer). After 24 h, the films were removed from the dispersion and exhaustively rinsed with phosphate buffer and finally with distilled water to remove the natamycin not ionically bonded to the modified films. At last, the films, modified with both antifungal agents, were dried in vacuo at room temperature until a constant weight, and their activity was verified through microbiological assays in vitro.

Surface analysis

Scanning electron microscopy (SEM) SEM pictures of the samples before and after grafting and loading were taken using a FEI Quanta 200FEG microscope at various magnifications. Samples were sputter-coated with gold-palladium. Electron micrographs of each sample were recorded at different magnifications.

XPS analysis X-ray photoelectron spectra were recorded on a Thermo spectrometer with a mono-chromatized Al Kα X-ray source (1486.6 eV photons) at a constant dwelling time of 100 ms for several scans and a pass energy of 20 eV for region scan spectra and 100 eV for survey scan spectra. The anode current was 20 mA. The pressure in the analysis chamber was maintained at 2x10^{-9} Torr or lower during each measurement. The PE- g-PAAc/Nat films were mounted on the standard sample studs by means of double-sided adhesive tapes. The core-level signals were obtained at the photoelectron take off angle (a, with respect to the sample surface) of 90°. All binding energies (BEs) were referenced to the C1s hydrocarbon peak at 285 eV. In peak analysis, the line width (full width at halfmaximum) for the Gaussian peaks was maintained constant for all components in a particular spectrum. Surface elemental stoichiometries were determined from peak-area ratios, after correcting with the experimentally determined sensitivity factors, and were reliable to ±5%. The elemental sensitivity factors were determined using stable binary compounds of well-established stoichiometries.

Evaluation of the antimicrobial activity To evaluate the antimicrobial activity of the PE films modified with AAc and natamycin, discs of the modified films were placed onto a dish of nutrient agar previously inoculated with a suspension of yeast and E-coli, S.-aureus, P.aurantiorium, A.versicolor. The dish was then incubated at 20±1°C for 5 days in darkness.

RESULTS and DISCUSSIONS
The investigation of grafting of FAc on the surface of PE film

The grafting yields which calculated by gravimetric measurement were very low (Table 1 and Figure 2). For unirradiated and irradiated and grafted PE films, Grazing Angle-FTIR spectra for all films were recorded. Grazing Angle-FTIR spectroscopy technique was utilized for characterization of the PE films. Grazing Angle-FTIR spectra were collected in the 400-4000 cm\(^{-1}\) region with resolution 4 cm\(^{-1}\). The grafting yield of food additives onto PE films as a function of irradiation dose and food additive solution concentrations were determined. The antimicrobial activity of PE-g-FAc films was investigated.

**Table 1** The grafting yield of food additives onto PE films as a function of irradiation dose and food additive solution concentrations at 25°C.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Irradiation dose (kGy)</th>
<th>[FAc]=0.25%w/v</th>
<th>[FAc]=0.50%w/v</th>
<th>[Nat]=25 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Grafting (%)*</td>
<td>Grafting (%)*</td>
<td>Grafting (%)*</td>
</tr>
<tr>
<td>PE</td>
<td>2.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>0.2</td>
<td>0.2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>24.0</td>
<td>-</td>
<td>0.7</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>40.3</td>
<td>0.7</td>
<td>0.7</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>58.5</td>
<td>0.7</td>
<td>0.9</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>84.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>150.0</td>
<td>0.9</td>
<td>1.2</td>
<td>-</td>
</tr>
</tbody>
</table>

* Determined by gravimetric measurements

FAc: Fumaric acid  Nat: Natamycin
The comparison of the Grazing Angle-FTIR spectra of PE films untreated and treated with FAc as a function of irradiation time is shown in Figures 3 and 4. The spectra of the treated films show a similar pattern to the untreated film in Figure 3 for low FAc concentration. We can say that the effect of irradiation dose is not notable on Grazing Angle-FTIR spectra of PE-g-FAc films 0.25 \% w/v FAc concentration and for 40 and 150 kGy irradiation doses. The grafting yields which calculated by gravimetric measurement were very low. As shown, for untreated PE, the absorption peaks 2916, 2848, 1463 and 719 cm\(^{-1}\) are attributed to methylene nonsymmetry stretching vibration, methylene symmetry stretching vibration, methylene nonsymmetry changing angle vibration, and methylene swing in plane vibration, respectively. Newly formed absorption band for treated PE, centered at around 680, 810, 960 and 987, 1410 cm\(^{-1}\) should be assigned to C–H bend (51). Other characteristic peaks, observed in treated PE films are overlapped by untreated PE bands. Evidently a new peak at around 1580 cm\(^{-1}\) appeared on the treated surface of the PE films. Figure 4 shows the Grazing Angle-FTIR spectra of the treated PE films with FAc whose concentration is higher than the other as a function of irradiation time. Clear evidence for the successful attachment of some active groups on the films surface is appearance of all peaks come from FAc. This contribution of surface modified to the spectra of the PE film can explain important intensity of the new bands. It means that new bands, appearing in the spectra result apparently from the interaction of FAc with PE surface. Irradiation time has favorable effect on the process of surface modification. It is obvious that the longer irradiation time increases the intensity of new bands, characteristic for groups attached to the film surfaces. This effect is clear from Table 1, where the grafting yield of the FAc onto from untreated PE film increases. The efficiency of the chemical modification was evaluated by gravimetric measurements and confirmed by the determination of –COOH groups grafted onto film surfaces. Antimicrobial activities of FAc grafted PE films are illustrated in Figures 5 and 6. Inhibitory zones were detected for PE-g-FAc 1.2% grafting at 150 kGy irradiation dose and 0.7 % grafting at 40 kGy irradiation dose and untreated PE film after 24 and 48 hours against E.coli. PE-g-FAc, 1.2 % grafting at 150 kGy irradiation dose, and untreated PE film did not inhibit any growth of tested
E. coli. PE-g-FAc, 0.7 % grafting at 40 kGy irradiation dose, generated significantly larger inhibitory areas.

Figure 3 Grazing Angle-FTIR spectra of Polyethylene (PE) and grafted PE films. [FAc]=0.25%w/v, a) unirradiated PE, b) PE-g-FAc (40 kGy), c) PE-g-FAc (150 kGy), d) FAc.

Figure 4 Grazing Angle-FTIR spectra of Polyethylene (PE) and grafted PE films. [FAc]=0.50%w/v, a) unirradiated PE, b) PE-g-FAc (150 kGy), c) FAc
Synthesis of PE-g-PAAc Copolymers via gamma-irradiation

Application of γ-radiation (from a 60Co source) generates radicals on the PE film surface and then at different temperatures in the monomer solution. Subsequently, monomer radicals and radicals formed on the surface initiate propagating chains. The chemical modification of the surface of PE film were carried out by radical grafting polymerization initiated by gamma-rays. AAc was used as the grafting comonomer. For chemical modification, variables such as the irradiation dose, concentration of comonomer, temperature, and the time of reaction, were examined. The experimental conditions and optimum conditions to use some characterization and analysis are shown in Tables 2-4 for PE. Figure 7 shows the general modification found on PE film. The efficiency of the chemical modification was evaluated by gravimetric measurements and confirmed by the determination of the –COOH groups grafted onto the film surface through volumetric titration and dye adsorption. The quantification of the –COOH groups grafted onto the surfaces of the film showed the same trend observed for gravimetric measurement.

Dye adsorption

The available carboxyl groups were evaluated by the ionic bonding of a dye, such as violet crystal. This is a cationic and voluminous dye that was strongly adsorbed by the carboxylic groups of the film surfaces (Fig. 8). As shown in Table 3, the concentration of the –COO dye was lower than those found by titration. This result explains why only the carboxylic groups with no steric hindrance were able to bond to the dye, and indeed this bond ability was lower than that shown with Na+. These kinds of carboxylic groups probably are available and active sites for further applications and for the bonding of organic compounds with a specific function. The complex formation between PAAc and MV, the interaction of PAAc and Nat were investigated and The UV spectra for complex formation between PAAc and Methyl Violet, PAAc and Natamycin are given in Figure a and b part, and the change of the Nat loading with time is given in c part, respectively.
Table 2 The grafting yield of food additives onto PE films as a function of irradiation dose and food additive solution concentrations, reaction times and temperatures.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample</th>
<th>Dose rate (kGy/h)</th>
<th>Dose (kGy)</th>
<th>Grafting (%)</th>
<th>[AAc]=%v/v</th>
<th>t (°C)</th>
<th>Reaction time (h)</th>
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<tr>
<td>PE (N₂)</td>
<td>AAc (N₂)</td>
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<td>10</td>
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Table 3 Reaction parameters and -COOH equivalents for the reactions with PE

<table>
<thead>
<tr>
<th>Sample</th>
<th>Microequiv of -COOH (cm²)**</th>
<th>Grafting(%)***</th>
<th>Nanoequiv of violet crystal (cm²)</th>
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<tbody>
<tr>
<td>PE-g-PAAc</td>
<td>7.11</td>
<td>3.6</td>
<td>0.1990</td>
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**Equivalents of -COOH determined by titration
***Determined by gravimetric measurements.

Table 4 Loading of Nat on PE-g-PAAc at 25°C

<table>
<thead>
<tr>
<th>Sample</th>
<th>Reaction time in Nat sol.(675 ppm) (h)</th>
<th>Loading(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE-g-PAAc (3.6% grafting)</td>
<td>24</td>
<td>1.6</td>
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</table>
Figure 7 General scheme for the modification of PE film.

Figure 8 General scheme of dye adsorption for modified PE film.
Figure 9 UV spectra for complex formation between a) PAAc and Methyl Violet, b) PAAc and Natamycin.
FTIR spectroscopy

FTIR spectra of the films was performed using a spectrometer Perkin Elmer at 2 scans and with 4 cm\(^{-1}\) resolution, between 400 and 4000 cm\(^{-1}\). Films without natamycin were also evaluated as control. The modified films were characterized with FTIR spectroscopy. Representative spectra are shown in Figures 10-14 for PE, PE-g-PAAc, PE-g-PAAc/Nat, at different irradiation doses, at different grafting percent, different reaction times. FTIR spectra for PE grafted with AAc for different reaction times are given in Figure 10. As shown, for PE, the absorption peaks 2916, 2848, 1462 and 719 cm\(^{-1}\) are attributed to methylene nonsymmetry stretching vibration, methylene symmetry stretching vibration, methylene nonsymmetry changing angle vibration, and methylene swing in plane vibration, respectively. Other typical characteristic bands of base polymers were observed, such as PE peaks at 1472 and 1462 cm\(^{-1}\) (CH\(_2\) and CH\(_3\) bending) and at 719 and 729 cm\(^{-1}\) (CH\(_2\) rocking). Typically, intense C=O absorption (1718 cm\(^{-1}\)) characteristic of poly(acrylic acid) was seen in the spectra. The absorption of this band increased when the percentage of grafting increased with the reaction times. On the other hand, AAc homopolymerization could occur outside the film surfaces. According to this finding, the FTIR spectra of films before the removal treatment showed a larger signal from C=O groups of AAc at 1613 and 810 cm\(^{-1}\), revealing the presence of unreacted monomer. The structures of PAAc grafted and ungrafted PE film were studied by FTIR spectroscopy. Figure 10 compares the FTIR spectra of PE and PE-g-PAAc with various DG. Inspection of the spectra demonstrates the grafting of PAAc by the appearance of a new peak at ~1710 cm\(^{-1}\) corresponding to C=O stretching of carboxylic acid groups of PAAc. The intensity of this peak increases proportionally with the amount of PAAc grafted. C-O stretching of PAAc can be seen in the range of 1150-1270 cm\(^{-1}\) (52). New absorption bands related to the acid group of PAAc associated by hydrogen bonding in different chemical environment are also observed. A broad band at around 3100 cm\(^{-1}\) is attributed to acid groups intermolecularly bonded while a second broad band at around 2800 cm\(^{-1}\) results from acid dimers (51). The comparison of the FTIR spectra of PE, PE-g-PAAc, PE-g-PAAc/Nat, Nat are shown in Figures 11-14. Peaks around 3230, 1598, 1418, and 1025 cm\(^{-1}\) can be observed, attributable to O-H, COO\(^-\) (asymmetric), COO\(^-\) (symmetric), and C-O-C stretching of natamycin, respectively (53). No relevant changes occurred in any of the characteristic structural peak of films after natamycin loading. Peaks around 3500 to 3300 cm\(^{-1}\) are also attributed to N-H stretch, characteristic of natamycin, and probably were overlapped by O-H peaks. For films processed by loading, bands close to 2940 cm\(^{-1}\) that correspond to the C-H stretching vibration shifted to lower wavenumbers (2920 cm\(^{-1}\)) and bands of O-H shifted to higher numbers (3270 cm\(^{-1}\)).

![Figure 10 FTIR spectra for PE grafted with AAc for different reaction times.](image-url)
Figure 11 FTIR spectra of PE, PAAc, PE-g-PAAc, PE-g-PAAc/Nat, Nat.

Figure 12 FTIR spectra of PE, PAAc, PE-g-PAAc.
Surface analysis

N20082 Scanning Electron Microscopy. The surface microstructure of the films were evaluated using scanning electron microscopy (SEM). Figures 15-17 show scanning electron micrographs of the outer surface of the PE film and PE-g-PAAc, PE-g-PAAc/Nat at different magnifications. The structural features of the PE films are different than that of the PE-g-PAAc, PE-g-PAAc/Nat since the grafting occurs mainly at the surface of PE film and the loaded Nat. The surface structure of the PE film was compact with a smaller uniform polymer network than those in the PE-g-PAAc and PE-g-PAAc/Nat. The SEM microphotographs of PE-g-PAAc and PE-g-PAAc/Nat
showed bright marbling on the film surface, which was uniformly distributed. These white areas could represent the deposits of AAc and Nat particles in the surface of PE film.

Figure 15 SEM microphotographs of PE film
Figure 16 SEM microphotographs of PE-g-PAAc, 3.6 % grafting:
X-ray Photoelectron Spectroscopy (XPS) Analysis
In order to get detailed information about surface chemical composition XPS experiments were performed. The elemental surface composition of PE-g-PAAc is calculated from XPS spectra and presented in Figure 18. In Figure 18-a, three characteristic peaks corresponding to C1s at 285.1 eV and O1s at 533.0 eV, N1s at 400.1 eV were observed. Small N content of PE-g-PAAc/Nat is attributed to the amine that may exist in natamycin. With the grafting of PAAc to the structure, surface composition changes significantly as can be seen from the elemental percentages. More detailed chemical analysis on XPS will obtained from the C 1s, O1s and N1s spectra can be curved-fitted with three peak components.
a) C1s = 84.54 %
O1s = 12.07 %
N1s = 1.74 %

b) 

b) 

c) 

d)
Figure 18 a) XPS survey wide scans of PE-g-PAAc/Nat copolymer with DG of 3.6 %, b) CIS core level XPS, c) O1S core level XPS, d) N1S core level XPS.

Evaluation of the antimicrobial activity

To evaluate the antimicrobial activity of the PE films modified with AAc and natamycin, discs of the modified films were placed onto a dish of nutrient agar previously inoculated with a suspension of yeast and E. coli, S. aureus, P. aurantiorium, A. versicolor. The dish was then incubated at 20±1°C for 5 days in darkness. The appearances of the antimicrobial activities of the PE, PE-g-PAAc, PE-g-PAAc/Nat against two bacteria and two molds are given in Figures 19-21.

Antibacterial effect: Two bacteria strains were tested;
1. E. coli ATCC 35218 was used for the determination of antibacterial effect of the PE film and PE films modified with AAc and natamycin. “Agar diffusion method” was used for this determination. Modified films were cut as circle with 16 mm diameter. PE film and PE films modified with AAc and natamycin were used. Each of them was placed on the inoculated agar medium (TSA agar) with E. coli ATCC 35218 culture containing about 10³ cell/ml in Petri dish. Then the petri dish was incubated at 37°C for 24 hours. After incubation the inhibition zone around the films were measured as mm.

2. Staphylococcus aureus ATCC 6538 was used for the determination of antibacterial effect of the PE film and PE films modified with AAc and natamycin. “Agar diffusion method” was used for this determination. Modified films were cut as circle with 16 mm diameter. PE film and PE films modified with AAc and natamycin were used. Each of them was placed on the inoculated agar medium (TSA agar) with Staphylococcus aureus ATCC 6538 culture containing about 10³ cell/ml in Petri dish. Then the petri dish was incubated at 37°C for 24 hours. After incubation the inhibition zone around the films were measured as mm.

Antifungal effect: Two mold strains were tested;
1. Penicillium aurantiorium 501588 was used for the determination of antifungal effect of the PE film and PE films modified with AAc and natamycin. “Agar diffusion method” was used for this determination. Modified films were cut as circle with 16 mm diameter. PE film and PE films modified with AAc and natamycin were used. Each of them was placed on the inoculated agar medium (PDA agar) with Penicillium aurantiorium 501588 spore suspension containing about 10³ spore/ml in Petri dish. Then the petri dish was incubated at 25°C for 5 days. In each incubation day, the inhibition zone around the films were measured as mm.

2. Aspergillus versicolor 200853 was used for the determination of antifungal effect of the PE film and PE films modified with AAc and natamycin. “Agar diffusion method” was used for this determination. Modified films were cut as circle with 16 mm diameter. PE film and PE films modified with AAc and natamycin were used. Each of them was placed on the inoculated agar medium (TSA agar) with Aspergillus versicolor 200853 spore suspension containing about 10³ spore/ml in Petri dish. Then the petri dish was incubated at 25°C for 5 days. In each incubation day, the inhibition zone around the films were measured as mm.

- There is no antibacterial effect of the PE film and PE films modified with AAc and natamycin against E. coli ATCC 35218 and S. aureus ATCC 6538. Because there is no any inhibition zone around the film circle.

- There is no any inhibition zone around "number 1 film" PE and "number 2 film"PE-g-PAAc. There is antifungal effect of the PE films modified with AAc and natamycin against Penicillium aurantiorium 501588 because there is inhibition zone around "number 3 film" or PE-g-PAAc/Nat. After 24 hours Inhibition zone diameter is 25 mm.
- There is no any inhibition zone around "number 1 film" PE and "number 2 film" PE-g-PAAc. There is antifungal effect of the "number 3 film" PE-g-PAAc/Nat against Aspergillus versicolor 200853. There is inhibition zone around "number 3 film". After 24 hours Inhibition zone diameter is 30 mm.

Microbiological inhibitory assays of the films prepared with the active agents natamycin (antifungal) on mold, bacteria (E.coli and S.Aureus, P.aurantiorium) were performed on Petri dishes using the agar diffusion method. Different quantities of antibacterial and antifungal agents were used; however, the best results were obtained with 2 % w/w of natamycin. As shown in Figure 18, the antifungal action of natamycin was verified, since films containing this active agent inhibited the growth of mold. This result was obtained using a very low amount of natamycin (2 % w/w). A small inhibition zone (about 25 mm in diameter) could also be observed. This suggests that the active agent diffuses through the film, producing inhibition of fungal flora in a region larger than that covered by the film. Determinations with films containing a higher amount of natamycin showed a wider inhibition zone (results not shown). As expected, this film did not inhibit the growth of bacteria (Ramos et al., 2012).

Preparation of the mold spore suspension:
Aspergillus versicolor 200853 and Penicillium aurantiogriseum 501588 isolated from the Turkish Cashar cheese were obtained from TUBITAK (The Scientific and Technological Research Council of Turkey) Marmara Research Center, Turkey. These two mold strains were used to investigate antifungal activity of PE with AAc samples. The spore suspensions of them were prepared as follows (Temiz et al., 2013). The test mold strain was grown on the slope surface of Potato Dextrose Agar (PDA)(Merck, Darmstadt, Germany) medium at 25° C for up to 7 days. The mycelium of the test strain was suspended in 10 mL of sterile saline containing 0.05% (w/v) Tween 80. After dispersing the fungal clumps, the suspension was filtered through a filter paper and centrifuged at 3000 x g for 15 min. The washing procedure was repeated two times and then spore suspension was prepared in saline containing 0.05% (w/v) Tween 80. The spore counting was carried out by using a Thoma counting chamber. The spore densities of the suspensions were 2.40x10^6 and 1.44x10^7 spores/mL for A. versicolor 200853 and P. aurantiogriseum 501588, respectively. The spore suspension of each mold strain is diluted to the spore densities of about 10^3 spore/ml (54).
Figure 19 Antibacterial activity of PE, PE-g-PAAc, PE-g-PAAc/Nat against a) *E. coli* and b) *S. aureus* after 0 and 24 hours. 1) PE film, 2) PE-g-PAAc, 2 % grafting at 42 kGy irradiation dose, 3) PE-g-PAAc/Nat, 1.16 % loading Nat.

Figure 20 Antifungal activity of PE, PE-g-PAAc, PE-g-PAAc/Nat against *P. aurantiurom* after 0, 24, 48 and 72 hours. 1) PE film, 2) PE-g-PAAc, 2 % grafting at 42 kGy irradiation dose, 3) PE-g-PAAc/Nat, 1.16 % loading Nat.
CONCLUSIONS
- FAc was grafted on PE films by post-irradiated method but Nat couldn’t grafted on PE films by the same method.
- AAc was grafted on PE films by pre-irradiated method in O₂ medium.
- For chemical modification, variables such as the irradiation dose, concentration of comonomer, temperature, and the time of reaction, were examined. The experimental conditions and optimum conditions to graft PE films were determined.
- Nat was bounded to modified PE films.
- The presence of AAc and Nat on the surface of PE film was explained by gravimetric, titrimetric and adsorption studies, surface analysis, XPS spectra.
- PE-g-FAc film was antibacterial effect against E.Coli. Although PE-g-AAc/Nat samples were not antibacterial against E.Coli and Staphylococcus aureus, they were antifungal effect against Penicillium aurantium and Aspergillus versicolor. The antifungal effect against Aspergillus versicolor after 0, 24, 48 and 72 hours. 1) PE film, 2) PE-g-AAc, % grafting at 42 kGy irradiation dose, 3) PE-g-AAc/Nat, 1.16 % loading Nat.
versicolor is more than Penicillium aurantiocrumar. The inhibition zone diameter in Asperillus versicolor is bigger than those of Penicillium aurantiocrumar.

References
Application of phase separation induced fractionation of polymer blends in aqueous solutions: gum arabic-hyaluronan systems

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Key words: polysaccharides, phase separation, molecular weight, blends.

Abstract

This paper reports the application of a novel method to modify hydrocolloids in the aqueous state using phase separation induced fractionation. The method is applicable to all types of polymers irrespective of structure and origin. The functionality of the polymer (natural or synthetic) can be controlled by adjusting the extent of the fractionation. In this report, gum arabic of high molecular weight prepared either by irradiation in the solid state (see Report F2-22063-CR-1) or by maturation treatment was used in the aqueous mixture with hyaluronan. The molecular weight of gum arabic in the bottom phase was increased from ~4 million to 6 million while the arabinogalactan fraction (AGP) was increased from 28% to 55%. As a result the functionality of the fractionated materials improved the emulsification performance and stability compared to the unfractionated gum. The method reported here could be potentially applied to a range of polymers to enhance the required functionality in food coating or packaging.

1. Introduction

Recent literature gives examples of considerable efforts by various researchers to improve the mechanical properties and water resistance of polysaccharides to match those offered by synthetic (non-biodegradable) counterparts. Method such as radiation treatment, chemical cross-linking, radiation grafting, thermal or UV curing have been proposed for various systems. Another approach to extend the application of natural hydrocolloids and utilise their biocompatibility, low toxicity is the production of biocomposite through blending with synthetic polymers in order to obtain the desired functionality as well as cost reduction. In doing so there seems to be either an assumption that the mixture will be stable or in some cases phase separation is acknowledged and reported as an obstacle to further the study.

When a solution of polymer A is added to a solution of polymer B, two things might happen: the two solutions will mix or separate into two phases. Which of these two processes will happen depends on the sign of the change of the Gibbs free energy of mixing, $\Delta G_{\text{mix}}$, which is given by:

$$\Delta G_{\text{mix}} = \Delta H_{\text{mix}} - T \Delta S_{\text{mix}}$$ (1.1)

where $\Delta H_{\text{mix}}$ and $\Delta S_{\text{mix}}$ denote the enthalpy and entropy of mixing respectively. $T$ denotes the absolute temperature. If $\Delta G_{\text{mix}} \leq 0$, the system will mix, whereas in the case of $\Delta G_{\text{mix}} > 0$, the system will separate into separate phases.
One of the widely acknowledged applications of phase separation induced fractionation is its application for purification and bioseparation (1), for which there is an extensive literature. Interactions between food macromolecules can be either attractive or repulsive, underlining two opposite phenomena: complex formation and biopolymer incompatibility (2, 3). An example of the former case is that polymers with an opposite charge interact and form soluble or insoluble complexes. The insoluble complexes concentrate in liquid coacervate drops, leading to a phase separation of the mixture into two liquid layers (4).

2. Methodology

The GPC-MALLS system used in this study has been previously described (5, 6). The phase diagram for EM10/HA system was prepared as follows. Stock solutions of EM10 (40wt%) and HA (1wt%) were prepared by dispersing dry powders in distilled water containing 0.005% NaN₃ as a preservative. The solutions were left to tumble mix for 24h at room temperature to ensure complete dissolution of the biopolymers. Subsequently, an appropriate weight was taken from each solution and mixed with distilled water to total weight of 10g in 14ml capacity tube. The solution mixtures were then left on a rotating mixer. The mixture was then centrifuged for 3 h at 25°C and 4000r/min using a Heraeus Megafuge 1.0R centrifuge. Centrifugation for 3hrs was found to be the optimum and longer time had no effect on the phase separation. Phase separated solution with two phases was stored in the centrifuge tubes at room temperature. The top phase and bottom phase solutions were separated using a long syringe needle and diluted to concentration range of 0.5mg/mg-2mg/ml, respectively. The diluted solutions were then injected into GPC-MALLS system for establishing the phase diagrams of each binary mixing system.

3. Results and Discussion

Table 2 gives the molecular weight characterisation of gum Arabic and hyaluronan used in this study as described previously. Gum arabic was processed as one peak and also processed as two peaks where peak 1 is assigned to the arabinogalactan protein fraction and peak 2 corresponds to the arabinogalactan and glycoprotein fractions. Hyaluronan elution profile gives only peak and was processed to determine the weight average molecular weight, Rg and polydispersity value.

<p>| Table 1. Molecular parameters of gum Arabic and hyaluronan determined by GPC-MALLS. |
|---------------------------------|-------------------|------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Sample</th>
<th>Processing</th>
<th>(M_a (10^5 \text{Da}))</th>
<th>Polydispersity ((M_w/M_n))</th>
<th>Rg (nm)</th>
<th>% mass recovery</th>
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<tbody>
<tr>
<td>EM10</td>
<td>(whole gum)</td>
<td>40.7</td>
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<td>HA</td>
<td>(1 peak)</td>
<td>16.8</td>
<td>1.06</td>
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</table>

The most widely used method to determine the concentration of a biopolymer in a mixed system is by spectroscopic measurements. The method relies on of selecting a peak in the
absorption spectrum which is free from interference so that it can be used to establish a respective calibration curve to determine each biopolymer. Another method is by visual determination of the cloud point. The concentration of one biopolymer is fixed while increasing the concentration of the second biopolymer was varied and subsequently the cloud point in the mixed solution was observed following centrifugation for 3h at 4000r/min 25°C. We increased the concentration of HA from 0.02% to 3%. While the concentration of EM10 was fixed from 0.2 wt% to 10 wt%. By increasing the concentration of HA in these solutions a clear cloud point was observed after centrifugation. The phase diagram is shown in Figure 1 wherein a pair of points joined by a dashed line (tie line) represents the composition of two layers of a phase separated mixture. Therefore, the solid line joining the composition points is the phase boundary which marks the compatible region and the phase separated region. According to the phase diagram in Figure 1, EM10 and HA are only compatible when the EM10 concentration is below around 2.0% by weight or, when the HA concentration is extremely low, that is, less than around 0.05% by weight. Consequently, to achieve phase separation induced fractionation in this system EM10 concentration needs to be greater than 2% and HA to be greater than 0.05%.

![Figure 1. Cloud point phase diagram of EM10/HA mixtures at 25°C by visual observation method.](image)

Figure 2 shows EM10 and HA mixture solutions at varying mixing ratios, the solutions contain a fixed concentration of HA at 0.25% and decreasing concentration of EM10 from left to right: 5%; 4%; 3% and 2.5 wt%. The solutions were stained with Direct Red dye to increase the contrast between layers. Visual inspection of the phase volume of separated systems did not show any difference in the presence and absence of Direct Red dye.
It has been widely acknowledged that phase separation is strongly influenced by the molecular weight of biopolymers which results in a higher tendency to phase separation with increasing the molecular weight (7). EM10 and HA samples used in the study have different molecular weight with polydispersity index values of 8.02 and 2.54 (see Table 1). Consequently, the fraction of higher molecular weight tend to segregate while lower molecular weight fraction has relatively a higher tolerance to coexistence (7). This is the basis of phase separation induced fractionation. Furthermore, GA is heterogeneous polymer consisting of three molecular species namely: AGP, AG and GP (8,9). These three components have distinct chemical structures and molecular weight. HA is also an extracellular matrix component and a high molecular weight glycosaminoglycan composed of disaccharide repeats of N-acetylglucosamine and glucuronic acid (10). The phase separation of EM10/HA should, therefore, be treated as a multi-component system rather than a classic binary system.

The results given above demonstrate that the extent of phase separation can be adjusted by varying the mixing ratio of EM10 and HA. The extent of phase separation was demonstrated using GPC-MALLS system. The upper and bottom phases were separated carefully by using a syringe needle to withdraw a sample from the respective layer. The sample was then diluted and injected into GPC-MALLS system and compared with the control. Figure 3 shows the proportion of the first peak, for samples obtained from the bottom phase, increases with decreasing EM10 concentration as compared to the control. Control EM10 has two distinct peaks in the RI signal, located at 7 mL and 12.4 mL, respectively. The first peak can be assigned to AGP and the second to AG and GP fractions (11). The intensity of peak 2 decreases with decreasing EM10 concentration when HA concentration is fixed, indicating a decreased proportion of the low molecular weight fractions and increased proportion of the high molecular weight fraction AGP. The results demonstrate that the AGP content can be controlled by adjusting the extent of phase separation in EM10/HA system. On the other hand, the comparison of the RI profiles of upper and bottom phases for each solution mixtures is shown in Figure 4. The results show that there is clear molecular weight distribution difference between the two phases in all solution mixtures at a fixed concentration of HA. In the upper phase the second peak (i.e. AG and GP fractions) in general increases with decreasing EM10 concentration, implying an increased proportion of the lower molecular weight fractions in the upper phases. Additionally, the combined peak for AG and GP (i.e. peak 2) shifts slightly to lower elution volumes with decreasing EM10
concentration, indicating a molecular fractionation for AG and GP with the higher molecular weight retained in the bottom phases.

Figure 3. RI profiles in GPC-MALLS measurements for the control EM10 and a series of AGP-rich EM10 products obtained via different extents of phase separation with HA.

Figure 4. RI elution profiles of the upper phases and bottom phases of phase separation systems containing 0.25% HA with different concentrations of EM10.

The results given above demonstrate that segregative phase separation takes place in aqueous mixtures of EM10 and HA. This phase separation results in AGP rich phase (bottom phase) and AG and GP rich phase (upper phase). The AGP content and molecular parameters of each phase were determined using GPC-MALLS technique. The results are tabulated in Table 2. The AGP content increases from 28.7% for control EM10 to 54.6% while its molecular weight slightly changed. Thus the increase in Mw of AGP rich phase
mainly results from the increased proportion of the high molecular weight fraction AGP while the AG and GP fractions are concentrated in the upper phase, rather than any chemical change or physical aggregation occurring in the samples.

**Table 2.** Molecular weight parameters of AGP rich phases prepared from phase separation of EM10 and HA.

<table>
<thead>
<tr>
<th>Product (obtained from bottom phase)</th>
<th>AGP (%)</th>
<th>Mw of AGP (x10^6) Da</th>
<th>Mw of whole gum (x10^6) Da</th>
</tr>
</thead>
<tbody>
<tr>
<td>EM10</td>
<td>28</td>
<td>10</td>
<td>3.3</td>
</tr>
<tr>
<td>5wt %EM10/0.25wt % HA</td>
<td>35</td>
<td>10</td>
<td>4.1</td>
</tr>
<tr>
<td>4wt %EM10/0.25wt % HA</td>
<td>41</td>
<td>11</td>
<td>4.9</td>
</tr>
<tr>
<td>3wt %EM10/0.25wt % HA</td>
<td>47</td>
<td>11</td>
<td>5.8</td>
</tr>
<tr>
<td>2.5wt %EM10/0.25wt % HA</td>
<td>55</td>
<td>11</td>
<td>6.9</td>
</tr>
</tbody>
</table>

Gum arabic is used mainly in confectionary, clarification of wine, thickener, stabiliser and emulsifier in variety of food stuff. Gum arabic's role as an emulsifier is achieved as a consequence of its amphiphilic character due to the presence of protein and polysaccharide moieties. It reduces the oil–water interfacial tension, thereby facilitating the disruption of emulsion droplets during homogenization. The peptide moieties (2-3% present in gum arabic) are hydrophobic and strongly adsorb onto the surface of oil droplets, while the polysaccharide chains are hydrophilic and extend out into the solution, preventing droplet flocculation and coalescence through electrostatic and steric repulsion forces [8]. The increase in the AGP fraction achieved by utilising phase separation induced fractionation resulted (data not shown) in enhancing the emulsification performance and stability by providing better interfacial properties and thus formation of an elastic film around the oil droplets.

### 4. Conclusion

The phenomenon of polymer phase separation seems either reported as problem in the design of formulations containing more than one polymer or ignored. Blending of polymer solutions to increase a given functionality could greatly benefit from careful selection of the starting materials as well as establishing a phase diagram. The phase diagram can then be used to prepare fractions with desired properties. The results given in this study on gum Arabic/ hyaluronan mixture solution demonstrate the application of phase separation to fractionate polydisperse hydrocolloids and to concentrate functional component in order to increase their functionality (12).

### References


Modified Atmosphere Packaging and Polymer Integrity under Electron Beam Irradiation Conditions: Mechanical and Bioactive Stability Studies

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Portions of this text are from previously published presentations, articles and reports by the authors

Abstract

Food encompasses more than just nutrition and has to meet society’s needs for portion sizes, quality, convenience, and sustainable packaging. Electron beam (eBeam) technology for cross-linking polymers is finding increasing applications in the development of bioplastics as sustainable packaging material. Our ongoing studies with bioplastics such as PLA and clay-amended PLA have shown that eBeam technology even at 10 kGy does not adversely affect polymer properties. These findings open up opportunities for sterilizing bioplastics used in aseptic packaging. Sterilization by eBeam is already replacing hydrogen peroxide in aseptic packaging thereby reducing chemical usage and allowing significant energy savings. The bioplastics industry is expected to grow by 500% over the next couple of years. Bioplastics as pouch material in combination with Modified Atmosphere Packaging (MAP) for fruits and vegetables will further expand bioplastic applications. Bioplastics with customized gas permeability characteristics can be particularly useful for MAP applications.

The activities over the past 18 months that could be attributed to the CRP were varied and could be categorized into separate activities. This report, therefore, has been organized into sections that detail the different activities that were undertaken.

Activities Performed under the CRP

1. Providing technical consulting regarding eBeam doses to be used when evaluating structural characteristics studies of biopolymers

Drs. Clare Silvestre and Donna Duraccio from the Institute of Chemistry and Technology of Polymers CNR, Pozzuoli, Naples, Italy contacted us to explore the possibility of providing eBeam dosing of certain biopolymers that they had developed. Specifically, they had developed polylactic acid/montmorillonite (PLA/MMT) and polypropylene/montmorillonite (PP/MMT) nanocomposites at 1%, 3% and 5% (by weight). The original idea was to deliver relatively high doses to these materials. However, in order to be responsive to the overall objectives of the CRP and to enhance the commercial applicability of the results, it was decided that the eBeam doses should not exceed 10 kGy. Thus, 1 kGy, 10 kGy and 0 kGy (control) were employed in this study. The rationale for choosing 1 kGy was that currently 1 kGy is approved by the US FDA to be used on all fresh produce in the United States for extending the shelf-life of fruits and vegetables. Given the value that biopolymers can have on serving as packaging material for fruit and vegetables in vending machines, it was decided that 1 kGy should be a target dose. The maximum dose that was employed was 10 kGy because this dose limit is at the upper limit for all foods.
that can be treated with irradiation in the US. The decision to focus on these two critical dose points has in our opinion significantly enhanced the commercial success of the potential research outcomes.

2. **Providing eBeam dosing studies for collaborations with COST researchers in Italy**

The biopolymers were shipped as flat sheets from Italy via courier as “research samples”. The samples were placed in a single layer in Ziplock™ bags and exposed to eBeam irradiation conditions. Test sample bags (“speed check samples”) were employed to initially calibrate the conveyor belt speeds to defined target doses. The doses were measured using alanine dosimeters and read using a calibrated dosimetry system [1]. The samples after irradiation were shipped by courier mail back to the researchers in Italy for their material and barrier property characterizations.

3. **Collaboration with biopolymer scientist in Mexico**

There is an untapped business opportunity in Mexico and the United States to positively influence nutrition and health by positioning fruits and vegetable in the vending machine channel, particularly in schools, universities, workplace, and in public areas. Mexico has a number of federal research institutes that are well equipped in terms of expertise and instrumentation. One such institute is CIAD (Centro de Investigación en Alimentación y Desarrollo, A.C.) in Hermosillo, Sonora, Mexico that has, as one of its research focus, biopolymers. The lead scientist is Dr. Tomas Madera-Santana who has significant expertise in biopolymers. A proposal titled, “Combining Electron Beam and Biodegradable Modified Atmosphere Packaging for Fruits and Vegetables to Develop Healthy Vending Food Items” that focused on the application of biodegradable packaging material was prepared and submitted to the Texas A&M-CONACYT program for funding. Even though the proposal was not selected for funding, Dr. Madera-Santana has offered to provide a variety of biodegradable polymers that he prepares in his laboratory for us to use in our laboratory for microbiology studies and eBeam dosing studies. A variety of blown films and sheet films have been provided (Table 1). Preliminary studies are currently underway in our laboratory to evaluate whether these films can be used to perform eBeam dosing experiments for shelf-life extension studies.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Polymer Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDPE-EVOH</td>
<td>Blown film of LDPE 90%+EVOH 10%. A non-biodegradable polymer</td>
</tr>
<tr>
<td>LDPE-TPS-10</td>
<td>Blown film of LDPE 90% + TPS 10%. The sample has partial biodegradability due to the content of TPS. The film can be blown to lower thickness. Can be sealed easily.</td>
</tr>
<tr>
<td>LDPE-TPS-20</td>
<td>Blown film of LDPe 80% + TPS 20% Film remains some properties of the previous film, however, the opacity of the sample is higher. This film is also partially biodegradable.</td>
</tr>
<tr>
<td>PLA</td>
<td>Cast extruded flat film of PLA. The sample is transparent but fragile and prone to tear in longitudinal direction. This film is completely biodegradable.</td>
</tr>
<tr>
<td>PLA 60%+TPS 40%</td>
<td>This film contains PLA 60% + TPS 40%. Appears rigid but flexible when rolled. The film is completely biodegradable.</td>
</tr>
</tbody>
</table>

4. **Performing microbial pathogen reduction studies on strawberries at low eBeam dose**

Studies were performed to demonstrate utility of eBeam as a platform technology at 1 kGy to enhance the shelf-life of strawberries, cherry tomatoes, grapes, and watermelon when combined with Modified Atmosphere Packaging (MAP). Additional studies were performed to demonstrate that quantifiable reduction of a significant enteric pathogen such as nonO157 toxigenic *E.coli* when strawberries are contaminated can be achieved. Fresh strawberries, cherry tomatoes and grapes (50g) were packaged under ambient and MAP (O₂/CO₂/N₂: 5%:15%:80%) conditions and exposed to 1 kGy eBeam dose. The samples were maintained at 4°C for up to 21 days. At periodic intervals, Plate Count Agar (PCA) plates were used for bio-burden analysis. Firmness and objective sensory analyses were also performed. Additionally a cocktail of the “Big 6” nonO157 *E.coli* strains were inoculated into the pulp of 25 g of strawberries that were contained within a heat-sealable bag at an inoculation dose of ~
108 CFU/g. The inoculated samples were exposed to a target dose ≤ 1 kGy eBeam using a 10 MeV linear accelerator. The eBeam treated samples and the untreated samples were plated on modified MTEC agar and TSA plates to enumerate all possible E. coli survivors and all heterotrophic bacterial populations. The use of eBeam even at a low dose such as 1 kGy in combination with MAP is able to decrease the bio-burden on the fruits (Fig. 1) The extent of reduction does depend on the initial bio-burden. It is significant that the effect of eBeam processing is reflected even after 21 days of MAP storage of the fruits. The shelf-life extension is as expected more pronounced in certain fruits.

Figure 1. Enhanced shelf-life of selected fruits when exposed to ambient conditions and MAP conditions (green highlights)

Consumer acceptability studies were also performed using treated and 1 kGy eBeam treated fruits mentioned above. Overall appearance and odor in tomato sample were all rated similarity and acceptable by consumers (Fig. 2). These result suggested that the joint application of eBeam and MAP resulted in better flavor of grapes by the consumers who participated in these studies.

Figure 2. Consumer acceptability scores of 1 kGy eBeam treated tomato and grapes
The use of eBeam even at low doses at 1 kGy was able to achieve a 3-log (99.9%) reduction in the levels of 6 strains of he toxigenic non O157 E.coli (data not included due to publication requirements) This result was significant in that it demonstrated that even at 1 kGy dose fresh produce can be treated to eliminate significant numbers of the microbial pathogens. The reduction that was observed in both the TSA plates and MTEC plates confirms that the use of 1 kGy eBeam dose will not only result in bio-burden reduction but in the collateral reduction of any pathogens that may be present. Thus, the ability of a non-thermal technology to extend shelf life and achieve 99.9% reduction of a bacterial pathogen can be a game changer for the fresh produce industry. These specific studies showed that it is possible to switch to biopolymers that meet all structural and functional properties and yet be able to allow eBEam doses as low as 1 kGy to inactivate bacterial pathogens on fresh produce. Similarly, at least a 2-log reduction of the total bacterial bio-burden was achievable when exposed to eBeam doses ≤ 1 kGy. Thus, these studies showed that if appropriate biopolymers that meet appropriately bench-marked structural and functional characteristics are available they could be used to substitute current polymers for food packaging that involved fresh fruits. The ability to utilize biopolymers can open up a potentially large market that caters to packaging materials for vending machines.

5. Outreach Activities
There is significant interest in importing fruits and vegetables into the United States after eBeam treatment (to address phytosanitary requirements). These shipments are expected to exceed 13,000,000 kg in 2014. All these shipments contain significant amount of PET polymer based materials. In order to reduce the usage of polymers such as PET, the National Center for Electron Beam Research has been trying to advocate the importers and exporters to utilize biodegradable polymers to the maximum extent possible. These activities have included direct face to face meetings with the leading importers as well as with grower commodity groups primarily from Mexico.

References

Research Outputs
Books

Book Chapters

Oral Presentations
4. Pillai S, Shaynafar S (2014). A holistic approach of ensuring public health, animal health, and environmental helath using electron beam as a biooprocessing technology platform, IFIBOP, 7-10 Sep, Lille, France.

Poster presentations


Research proposals submitted
1. Combining Electron Beam and Biodegradable Modified Atmosphere Packaging for Fruits and Vegetables to Develop Healthy Vending Food Items – submitted to TAMU-CONACYT program (not funded)
2. Combination of Multiple Non-Thermal Microbial Control Technologies for Ensuring Safety, Quality and Nutritional Content of Berries, the Antioxidant Superfruit Group – submitted to United States Department of Agriculture –Coordinated Activities Project (USDA-CAP) grant program (under review)
3. Transformative Impacts on One-Health using Electron Beam as a Platform Technology – submitted to TAMU One-Health Initiative (funded)
4. Combining Electron Beam and Biodegradable Modified Atmosphere Packaging for Fruits and Vegetables to Develop Healthy Vending Food Items – submitted to USDA-Foundation Grants Program (under review)