PhD DISSERTATION PROJECTS

**Development of an active bio-based coating for corrugated cardboard with waterproofing and antimicrobial properties**

Joel Armando Njieukam (joelarmando.njieuk2@unibo.it)

Dept. of Agricultural and Food Sciences, University of Bologna *Alma Mater Studiorum*, Cesena, Italy

Tutor: Prof. Rosalba Lanciotti

Co-tutors: Dr. Lorenzo Siroli and Dr. Claudio Dall'Agata

The present research project aims at developing, during the three-year period of the industrial PhD, a corrugated cardboard packaging coated with a bio-based film which is obtained from bacterial cellulose produced by selected strains of acetic acid bacteria and functionalized thanks to the addition of natural antimicrobial agents, capable of reducing microbial proliferation and increasing the shelf-life of fruits and vegetables.

**Messa a punto di un coating attivo bio-based per cartone ondulato ad azione impermeabilizzante e antimicrobica**

Il presente progetto di tesi si propone di mettere a punto, nel triennio del dottorato industriale, un imballaggio in cartone ondulato rivestito da un film bio-based, ottenuto a partire da cellulosa batterica prodotta da ceppi di batteri acetici selezionati, e attivato grazie all’addizione di antimicrobici naturali rilasciati nel tempo, in grado di ridurre la proliferazione microbica e di incrementare la shelf-life di prodotti ortofrutticoli confezionati.

# **1. State-of-the-Art**

# The reduction of food wastes and the negative environmental impact of fossil-based plastic used in the food packaging industry are certainly among the most debated topics nowadays. The emphasis was placed on these key issues by including them in the 2030 Agenda for Sustainable Development of the United Nations. In fact, more than 40% of the global production of plastic materials are used for packaging and approximately 96% of this material is converted into waste and is not recycled (Alshehrei, 2017; Ncube *et al*., 2021). There is then, a huge and urgent need for more sustainable materials with added properties (such as antimicrobial activities, improved mechanical and thermal properties).

# In order to reduce the dependence of the food industry on non-renewable resources such as Petroleum based polymers, research efforts have been developed at different levels to find alternative solutions which include the production of more sustainable packaging using biodegradable and renewable polymers (Cazón and Vázquez, 2021; Wang *et al*., 2022). For instance, biopolymers such as alginate, chitosan, lipid-based compounds or those based on proteins (egg white, casein, soy protein, collagen, gluten, whey protein, fish gelatine, myofibrillar proteins) have been successfully tested and the results highlighted the great potential of these biomaterials to replace synthetic polymers in the food packaging industry (Wang *et al*., 2022). However, among the bio-based materials, polysaccharide-based polymers have received tremendous attention due to their low production cost, biodegradability, wide availability and broad application (Xu *et al*., 2016). On the other hand, numerous studies have highlighted the possibility of using compounds deriving from microbial biomass or microbial metabolism such as bacterial cellulose, produced by some strains of acetic acid bacteria such as *Komagataeibacter* spp. and *Novacetimonas* spp., to produce food packaging and coatings with technological properties almost similar to those of traditional packaging and with the great additional advantage of being biodegradable (Kolesovs *et al*., 2022; Cazón and Vázquez, 2021). Furthermore, bio-based packaging intended to come into contact with food can, in some cases, be functionalized through the incorporation of natural components with antimicrobial or antioxidant activity which can be gradually released into the food product or its environment, allowing an increase in the shelf-life of the product and consequently the reduction of food wastes and losses (Almasi *et al.*, 2021). Despite the large number of studies completed on this topic, the development of sustainable and effective functional biobased coating for food packaging applications is still far to be reached. For this reason, the present industrial PhD research project, in partnership with Bestack (Italian consortium of corrugated cardboard packaging), is aimed to develop an active bio-based coating for cardboard packaging, obtained from bacterial cellulose produced by selected acetic acid bacteria strains, and activated by the addition of natural antimicrobials released over time, able to reduce the microbial proliferation and increase the shelf-life of packaged fruits and vegetables.

# **2. PhD Thesis Objectives and Milestones**

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Bibliography research**

A2) **Selection and production of bio-based films using acetic acid bacteria**: this first step aims to isolate and identify acetic acid bacteria from different food matrices, especially wastes or by-products (A2.1) and then, screen and characterize them for bacterial cellulose production (A2.2). The cellulose production by the selected strains will be improved and optimized by the modulation of culture parameters (A2.3) and the optimal protocol for bacterial cellulose production will be defined (A2.4).

A3) **Selection of natural antimicrobials for the activation of bio-based films**: the second phase aims at screening and characterizing natural antimicrobial agents obtained from different sources such as essential oils and antimicrobial peptides (A3.1). In particular, the considered compounds will be characterized for organoleptic compatibility and for their antimicrobial activity against foodborne pathogens and spoiling microorganisms associated with fresh fruits and vegetables. Those of greatest interest will be selected (A3.2) and used for packaging activation.

A4) **Activation and characterization of bio-based films**: definition of protocols and ingredients for the production of biopolymers integrated with natural antimicrobials (A4.1), characterization of the antimicrobial activity of the active bio-based film (A4.2) and technological characterization of the bio-based film obtained.

A5) **Application on corrugated cardboard and evaluation of the effect on a packaged product**: definition of the optimal protocol for the coating of corrugated cardboard with the obtained functional bio-based film (A5.1), evaluation of the effect of the active packaging on the shelf-life, safety and organoleptic properties of packaged fruits and vegetables (A5.2) taking into consideration the final requirements of the industrial partner.

A6) **Preparation** of the final PhD thesis, scientific articles, posters and/or oral presentation.

***Table 1***Gantt diagram for this PhD thesis project.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Activity | Months | **2** | **4** | **6** | **8** | **10** | **12** | **14** | **16** | **18** | **20** | **22** | **24** | **26** | **28** | **30** | **32** | **34** | **36** |
| A1) | ***Bibliography research*** |   |   |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |
| A2) | ***Bio-based films by acetic acid bacteria***  |   |   |   |  |  |  |  |   |   |  |  |  |  |   |   |   |  |   |
|  | 1) Acetic acid bacteria isolation  |   |   |   |  |  |  |  |   |   |  |  |  |  |   |   |  |  |  |
|  | 2) Screening and characterization |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |   |  |   |
|  | 3) Performance optimization |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 4) Optimal protocol definition |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A3) | ***Natural antimicrobial agents selection***  |   |  |   |   |   |   |  |   |   |   |   |   |  |   |  |  |  |  |
|  | 1) Screening and characterization |   |  |   |   |   |  |  |   |   |   |   |  |  |   |  |  |  |  |
|  | 2) Antimicrobial activity evaluation |   |  |   |  |  |   |  |   |   |  |  |   |  |   |  |  |  |  |
| A4) | ***Bio-based film activation and characterization*** |   |  |   |  |  |   |   |   |   |  |  |   |   |   |  |  |  |  |
|  | 1) Optimal protocol definition |   |  |   |  |  |   |   |   |   |  |  |   |   |   |  |  |  |  |
|  | 2) Antimicrobial effect evaluation |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |
| 3) Technological effect evaluation |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A5) | ***Corrugated cardboard coating and evaluation*** |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |  |   |
|  | 1) Optimal protocol definition |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Effect evaluation on real products |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A6) | ***Preparation of the final thesis, scientific articles, posters and oral presentation*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

# **3. Selected References**

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