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Bioconversion of Agro-industrial Food and Wastes by Insects and Microalgae - BIOCOIM

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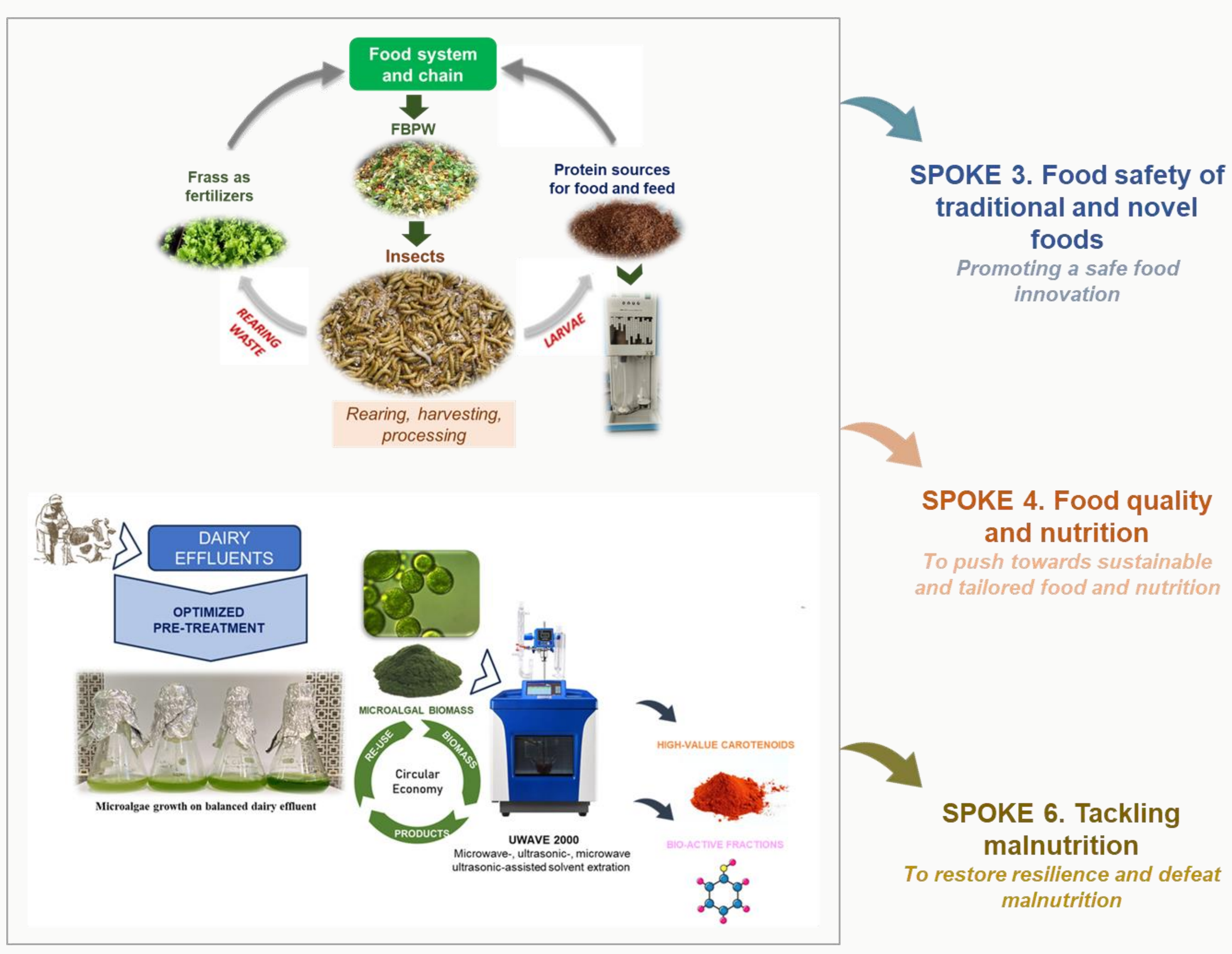
Introduction

As the global population grows by over 9 billion in 2050, food commodity production will rise, resulting in substantial agro-industrial food by-products and wastes (FBPW). These residues can be used as insect feed, providing an alternative protein source for food and feed. Additionally, insect-rearing residues like frass and exuviae have potential industrial applications, such as biofertilizers and chitosan production. Thus, identifying optimal and cost-effective substrates is essential for improving insect growth, and nutritional composition, and ensuring sustainable insect rearing. Using microalgae is another promising approach to valorise FBPW of various origins. According to several studies, microalgae can efficiently remove phosphorus and nitrogen while accumulating valuable biomass. Microalgae biomass is also a valuable source of pigments, starch, and bioactive compounds.

The BIOCOIM project envisages bioconversion of FBPW using insects such as *Tenebrio molitor* (TM) and *Hermetia illucens* (HI) and microalgae such as *Synechocystis* sp. and *Chlorella sp.* Insects will be farming to obtain new protein sources and frass, whereas microalgal biomass will be valorised in high-value products, such as carotenoid and bioactive compounds.



Materials and methods

The TM larvae were fed a standard diet of wheat bran and reared under optimised conditions of temperature, humidity, population density, photoperiod, and water source administration. Insect larvae rearing tests were conducted using diets containing various FBPW (e.g., brewer's spent grain, cocoa husk, bergamot albedo, okara from soy milk, potato and tomato peels, and hemp) at different concentrations, and bran as a complement to 100%. The protein content of the TM larvae and insect diet matrices was mainly determined by the Kjeldahl method. Insect frass concentrations (2-5% and 2.5,5,7.5,10) have been tested as a fertilizer for edible plants after pre-treating at 70°C for one hour.
Hen feed was used as a standard diet for rearing HI larvae, while experimental trials were conducted on different FBPW (e.g., brewer's spent grain, okara from soy milk, potato and tomato peels, and potato waste). By-products and reared larvae were analyzed for their nutritional composition. Different growing parameters of the larvae were measured.
Different strains of cyanobacteria (*Synechocystis* sp. PCC 6803 –SCY- and *Synechococcus elongatus* PCC7942 –SCO-) and green algae (*Chlorella sorokiniana* CCAP 211/8k –CHS-) have been grown on effluents from the dairy sector. Washing waters from dairy plants of hard and soft cheese production lines were assessed as nutrient sources.

First, hydrochemical analysis (COD, macro- and micronutrients) was conducted. Various pre-treatment steps (centrifugation, filtration, pasteurisation and decanting) have been combined to establish the best pre-treatment protocol to minimise steps and energy expenditure. The change in dissolved nutrient content has been assessed before and after pre-treatment. Growing assays at different effluent concentrations (25%, 50%; 75%, 100%) were carried out on the selected strains in scalar volumes, first in well plates, then in flasks and a multicultivator photobioreactor system. A genetic transformation protocol is being developed to implement carotenoid content in *C. sorokiniana*. In parallel, environmentally friendly multi-step extraction protocols are being developed to valorize algal biomass.

Results

The main results of the project are summarized in Tables 1 and 2.

Table 1. FBPW bioconversion by insects

Main results	Species
Larval survival was statistically comparable to the control when the by-products were mixed 50+50%. Used as it, mortality increased on okara, potato waste and brewer's grain. Growth performance for cocoa husk is lower than for other substrates.	TM
The tests with 50% wheat bran show similar growth performance to the control, especially in the first 15 days. In contrast, the tests with the by-products used as such exhibit very different trends.	TM
The lowest FCR was achieved with wheat bran and tomato peels, whereas the highest value was obtained with potato waste.	TM
The crude protein content of the larvae reared on the different substrates was comprised between 39.78% and 48.24%, while the ether extract was 26.41% and 35.56%.	TM
All rearing substrates allowed larval growth and development, but a different number of days was required to complete the trials, especially on potato peels where larvae took longer time.	HI
The survival rate recorded on potato peels was 43.0%, much lower than the other diets (96.80% on brewer's spent grain, 99.87% on okara, and 97.67% on potato selection waste).	HI

Table 2. FBPW bioconversion by microalgae

Main results	Species
The best pre-treatment protocol for minimizing steps and energy expenditure has been established for both dairy effluents (WWs from hard and soft cheese production plants) and the effluent media optimised for all the tested strains.	SCY/SCO/ CHS
From the preliminary growing assays performed in six-well plates and flasks, 75% resulted in the best effluent concentration to sustain algal growth in all the assessed strains.	SCY/SCO/ CHS
The preliminary growing test has been carried out in a Multicultivator photobioreactor (PSI) with raw effluent from soft cheese production plants.	CHS
To establish efficient gene transformation protocols for algal strains producing high levels of carotenoids, resistance assays to several antibiotics (hygromycin and zeocin) were performed, and target genes were selected.	CHS
High-efficiency protocols are being implemented through the use of a multifunctional microwave and ultrasonic extraction station that can speed up extraction processes of bioactive fractions from algal biomass and improve yield, saving energy and time.	SCY

Based on the initial results, it seems possible to use certain FBPW to rear TM and HI larvae for alternative protein source production. This process would help reintroduce these FBPW into the food system and reduce production costs. Similarly, preliminary results show potential for using dairy effluents as a nutrient medium to fully sustain the growth of green algae and cyanobacteria for producing bioactive compounds and recovering water resources.

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