PROTECTING FRUITS WITH PROPOLIS

The New University of Lisbon introduces Propolis, a natural phytopharmaceutical agent to reduce pesticide use and improve fruit quality

Fruit losses in pre- and post-harvest have increased significantly in recent years, causing an important negative impact on the activity of producers and distributors. The climatic conditions from flowering to fruit maturation condition the physiological state of the fruits at the time of harvest, which in turn affects their susceptibility to fungal and bacterial infections and influences the evolution of their quality during shelf life after harvest.

The probability of fungal or bacterial infection during the pre-harvest period is strongly correlated with the extent of micro-organism proliferation in orchards during the vegetative cycle. Adoption of preventive cleaning operations may reduce the microbial load of soil and biomass, but temperature and humidity conditions are still critical factors for fungal development and dissemination.

On the other hand, over time, some micro-organisms have developed resistance to traditional pesticides that have therefore lost effectiveness. Furthermore, the high toxicity of some plant protection agents and the concerns about their migration through the food chain led to the introduction of legislative limits on their use. Thus, there is now a great interest in the identification of new agents with fungicidal or bactericidal action, effective in inhibiting the proliferation of micro-organisms that infect fruits at different stages of the production cycle, and that do not show the stability and ecotoxicity of synthetic pesticides.

Save the bees, save the world

Propolis is a bee product known for its high phenolic content and relevant biological properties, such as antioxidant, antimicrobial and anti-tumoural activity. Bees produce propolis to insulate the beehive and prevent the loss of heat, as well as the entrance of wind, rain and eventual predators. Propolis is also used to inhibit fungal and bacterial growth within the hive as well as to prevent the putrefaction of any small animals that die inside it.

The antimicrobial properties of propolis have been tested against numerous Gram-positive and Gram-negative bacteria and motivate the use of propolis extracts in numerous hygiene and disinfection products.

The main objective of the PROFRUTA project was the development of a new phytopharmaceutical product derived from propolis, and its evaluation *in vitro* and *in vivo* using pears as a fruit model. The project activities involved propolis producer BeeCaramulo Lda. - a centre of interface and technology transfer to the fruit production sector (COTHN – Operational and Technological National Centre of Horticulture) and two research units (FCT-UNL – Faculty of Science and Technology, New University of Lisbon and INIAV - National Institute for Agricultural and Veterinary Research). Individual producers and companies involved in propolis production and in fruit production, distribution and transformation also participated by supplying test samples and facilities for field tests.

The project tasks were developed over a period of three years and included:

1) Collection of 30 propolis samples representative of the different production areas in Portugal and characterisation of the chemical composition and *in vitro* antioxidant activity and antimicrobial activity of the corresponding hydroalcoholic extracts;
2) Fractionation of the crude extracts and detailed characterisation of the hexane, ethyl acetate and water fractions;
3) Evaluation of antioxidant activity and antimicrobial activity of propolis fractions and of their main 26 individual components;
4) Development of aqueous propolis formulations adequate for use in fruit treatment;
5) Evaluation of antioxidant activity *in vivo* by the treatment of pears and strawberries with aqueous propolis extracts and determination of physiologic parameters, including internal browning, during storage;
6) Antimicrobial *in vivo* tests against *P. expansum*, *S. vesicarium* in pears, using crude extracts and the corresponding fractions;
7) Production and characterisation of aqueous propolis extracts adequate for large-scale tests;
8) Treatment of olives and pears in pre-harvest (in the orchard) with aqueous propolis;

9) Treatment of table olives with aqueous propolis during storage;

10) Field tests of the treatment of pears with aqueous propolis and storage under different modified atmospheres. Tests were performed in two subsequent years and the treated pears were sampled three times, every two months after storage;

11) Use of aqueous propolis extracts to treat fruits before dehydration; and

12) Physiological and sensorial evaluation of pears treated with aqueous propolis.

Main conclusions

Propolis is a heterogeneous material with a composition that depends on the flora available, which varies with the geographical origin and the period of the year. The main components of Portuguese propolis are flavones, chalcones, hydroxycinnamic acids, terpenes, waxes and fatty acids; the qualitative composition is rather constant, but the concentrations of active components are higher in samples collected in the central region of Portugal (Coimbra, Castelo Branco, Aveiro, Viseu e Guarda districts) and in the period from April to September.

Aqueous dispersions of ethanolic concentrated extracts in water to a final ethanol concentration of 10% resulted in the formation of stable emulsions that preserve a significant amount of bioactive propolis components and allow a good wettability of the fruit surface, as a result of the ethanol effect on the water superficial tension.

Propolis extracts, their fractions and most of the main individual components presented high responses in the tests of antioxidant activity: total phenols, antiradicalar activity and ferric reduction activity.

The *in vitro* antimicrobial activity of propolis extracts was confirmed for the micro-organisms: six Gram-positive bacteria (*Staphylococcus aureus, Staphylococcus aureus* resistant to methicilin, Staphylococcus epidermidis, *Listeria monocytogenes, Bacillus cereus* and *Enterococcus faecalis*); one yeast (*Candida albicans*); five phytopathogenic fungi (*Penicillium expansum, Colletotrichum gloeosporioides, Stemphylium spp*, *Stemphylium vesicarium* and *Botrytis cinerea*).

The *in vivo* tests showed that propolis extracts inhibit the proliferation of fungi in pears, so these extracts may be used as preventive disinfectant agents. Nevertheless, the application of those extracts in combination with another phytopharmaceutical agent may be required to achieve a low percentage of affected fruits. Simultaneous use of propolis extracts and another natural fungicide or a lower concentration of a traditional pesticide could be exploited.

Another conclusion was that the pre-harvest application reduced fruit infection and therefore a combination of pre-harvest and post-harvest treatments may improve the degree of protection.

The *in vivo* antioxidant activity tests showed a reduction of the pears internal browning during the first months of storage.

The bioactive components of propolis were detected in the peel and pulp of treated pears, so this treatment can also be used as a tool for the fortification of fruits with phenolic components.

The physiological and sensorial evaluation of pears treated with propolis extracts showed no significant changes in the physical-chemical parameters and a good acceptance by the testing panels.

The propolis extracts were also used in the treatment of fruit slices to be dehydrated (pears, apples and pineapple), in order to evaluate their effect in the surface oxidation and browning, total phenolic content and sensorial quality. Positive results were obtained in what concerns total phenols concentration and organoleptic acceptance.

In conclusion, the application of propolis extracts in pre- and post-harvest, may contribute to a more sustainable protection of fruit products, by limiting losses due to infections by fungi or bacteria while decreasing the application of toxic and persistent phytopharmaceutical agents. Besides the environmental and food safety advantages of this approach, this strategy will reinforce co-operation between two agricultural sectors and is expected to stimulate the industrialisation of propolis production.

Furthermore, fruit fortification with propolis components may contribute to an improvement of its nutraceutical value given the reported antioxidant and anti-tumoural effects of propolis extracts and their components.

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