POTENTIAL APPLICATION OF PORTUGUESE PROPOLIS AS 'ROCHA' PEAR POSTHARVEST PRESERVATIVE

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INTRODUCTION

Postharvest fungal diseases in 'Rocha' pear are responsible for considerable economic losses. Synthetic fungicides have been the main method used in managing these postharvest decays. However, the rising concern for health risks and environmental pollution due to the use of chemicals makes necessary the development of new and safer strategies. Propolis is a resinous substance collected by *Apis mellifera* bees from the leaf buds and barks of trees with antioxidant, antibacterial and antifungal properties. Thus, propolis can be a promising natural and safer alternative to chemical fungicides in the control of pears postharvest fungal diseases.

This study aims to:

- Compare the potential of Portuguese propolis and of synthetic fungicides in the control of postharvest diseases in 'Rocha' pear.
- Evaluate the impact of propolis application in 'Rocha' pear sensory and quality attributes.

MATERIALS AND METHODS

Propolis was collected in different Portuguese regions and was extracted with 96% ethanol, in the dark, during 5 days, at room temperature. The insoluble residue was removed by filtration and the ethanolic extract was diluted with water (1:10 v/v). The extract was characterized in what concerns total phenolic content (Folin-Ciocalteu assay) and antioxidant capacity (DPPH and FRAP assays). Immediately after harvest (T0), fruits were treated with the aqueous propolis extract and with commercial fungicides (F1 and F2) and then stored under controlled atmosphere during 4 months. At the end of this period incidence of fruit rot symptoms in all treatments was visually determined and fruits were analyzed in what concerns total soluble solids, titratable acidity, firmness, phenolic compounds (Folin-Ciocalteu assay), color, humidity and pH. The same physic-chemical analyses were performed after 15 days of storage at room temperature (shelf life assay). PPO activity was determined by monitoring the change of absorbance at 420 nm of a 10 mM catechol solution. One unit of PPO activity was defined as the amount of enzyme that caused an increase in absorbance of 0.01 at 420 nm in 1 min under the assay conditions. CAT activity was determined by following the disappearance of H_2O_2 in the enzyme reaction mixture. One unit of CAT was defined as the amount of enzyme that decomposes 1 µmol of H_2O_2 per minute. Protein content in the enzymatic extracts was estimated according to the method of Bradford, using bovine serum albumin as a standard. Sensory attributes (flavor, sweetness and texture) were measured on a scale of 7 points by 30 untrained panelists. Purchase intention was also evaluated.

RESULTS

➢The portuguese aqueous propolis extract applied in 'Rocha' pears showed a high total phenolic content and a high antioxidant activity (Table 1)

Table 1- Propolis extract characterization

Phenolic Compoubds - Folin Ciocalteu	Antioxidant activity - DPPH	Antioxidant activity - FRAP
2255 ± 48,8 mg gallic acid equivalents/L	841 ± 16,1 mg Trolox equivalents/L	34,8 ± 0,4 mmol $FeSO_4$ equivalents/L

➢ After 4 month of storage under controlled atmosphere the incidence of fruit rot in pears treated with propolis was similar to that presented by pears treated with fungicide F1. Both results were significantly lower to those presented by the control group (Table 2 and Figure 1).

Table 2 - Number of infected fruit for each treatment (n=250)

Treatment	Number of infected fruits
Control	21
Propolis	5
F1 + F2	10
F1	5

Figure 1 –Examples of diseases found in pears.

➢Fruits from the 4 different treatments showed similar results in physic-chemical analyses performed after 4 month of storage under controlled atmosphere (Table 3) as well as after an additional storage of 15 days at room temperature (shelf-life assay) (Table 4). After 15 days of storage at room temperature the parameters with greater variation were color (Hue), that changed from greenish yellow to intense yellow, and firmness, that decreased 6 to 7 times.

➢These results showed that the pears treated with propolis presented no changes in physic-chemical attributes in relation to either the control group or the fruits treated with commercial fungicides

Table 3 - Physic-chemical parameters of pears from the different treatments after 4 month of storage under controlled atmosphere

Treatment	L*	Hueº	Firmness (N)	IR (ºBrix)	TA (g malic acid/L)	pH
Control	73,98 ± 0,34	99,03 ± 0,41	49,2 ± 0,1	15,2 ± 0,2	1,4 ± 0,1	4,55
Própolis	71,06 ± 0,38	101,78 ± 0,44	52,4 ± 0,7	11,8 ± 0,2	1,3 ± 0,1	4,67
F1	71,66 ± 0,46	100,68 ± 0,37	49,7 ± 0,9	13,9 ± 0,2	1,3 ± 0,1	4,7
F1+F2	70,57 ± 0,44	103,14 ± 0,34	54,3 ± 0,7	12,6 ± 0,2	1,4 ± 0,0	4,46

Table 4 - Physic-chemical parameters of pears from the different treatments after an additional storage of 15 days at room temperature (shelf-life assay)

Treatment	L*	Hueº	Firmness (N)	IR (ºBrix)	TA (g malic acid/L)	pH
Control	75,31 ± 0,34	87,57 ± 0,31	7,6 ± 1,7	14,6 ± 0,2	1,1 ± 0,3	4,5
Própolis	75,06 ± 0,27	88,21 ± 0,29	8,4 ± 1,2	12,2 ± 0,2	1,5 ± 0,1	4,45
F1	75,13 ± 0,25	88,14 ± 0,22	8,9 ± 0,8	14,2 ± 0,2	1,4 ± 0,1	4,55
F1 +F2	74,87 ± 0,35	88,21 ± 0,31	9,4 ± 0,5	13,1 ± 0,2	1,5 ± 0,0	4,35

➢Sensorial analysis, showed that pears treated with propolis and with F1 + F2 were significant less sweet ($p < 0.05$ according to the t test) than pears from the two other treatments (Figure 2).

➢ Pears treated with propolis had less purchase intent than the other groups. However 51% of the panelist answered yes to the question about purchase intention representing a positive result

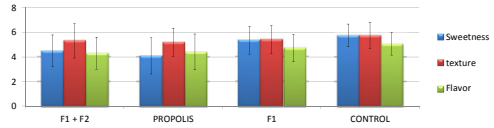

Figure 2 - Results of sensory analysis

➢Phenolic content of the pulp increased after the application of propolis extract (Table 5). However, at the end of the conservation period, this difference becomes much less pronounced.

➢PPO activity increased over storage time in all samples (Table 6, Figure 2), a result that has already been evidenced by other authors. The highest activity of this enzyme in pears treated with propolis did not result in any external or internal browning. This enzyme (PPO) is related to the defense of fruit against the attack of fungi, their greater activity in pears treated with propolis may indicate a greater resistance to infection process.

➢ The enzyme catalase is related to the elimination of H_2O_2. The high enzymatic activity of catalase, control and F1+F2 may mean that pears have been subject to periods of oxidative stress during cold storage. Oxidative stress may be a result of the greater number of pathogens attacks that these pears suffered (Table 2, Figure 3).

Table 5 - Phenolic compounds in the extracts of different samples.

Phenolic compounds (Folin Ciocalteu) mg EAG/100g	
Control T0	17,52 ± 0,7
Própolis T0	26,08 ± 0,88
Control	16,91 ± 0,53
Própolis	22,87 ± 0,92
F1	19,18 ± 0,23
F1+F2	22,42 ± 0,68

Table 6 - Enzyme activity (PPO and CAT) in extracts of different samples.

	PPO (U/mg protein)	CAT (U/mg protein)
Control T0	186 ± 1	4,95 ± 0,26
Própolis T0	157 ± 1	3,70 ± 0,14
Control	426 ± 68	12,61 ± 1,99
Própolis	518 ± 139	4,93 ± 0,80
F1	392 ± 53	3,63 ± 0,70
F1+F2	243 ± 17	15,03 ± 1,27

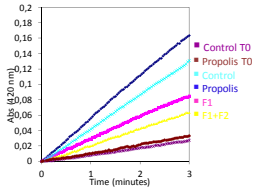

Figure 3- Variation of absorbance at 420 nm obtained in PPO assay with pear extracts from different treatments

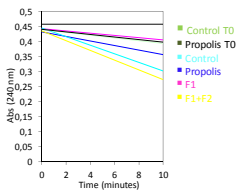

Figure 4 -Variation of absorbance at 240 nm obtained in CAT assay with pear extracts from different treatments

CONCLUSIONS

➢Results obtained showed that propolis has an antifungal efficacy similar to the synthetic fungicide in control 'Rocha ' pear post harvest diseases.

➢The physicochemical analyses showed that the application of propolis had no adverse effects on pears quality attributes.

➢Sensory analysis showed that the pears treated with propolis were well accepted by the tasters.

➢Thus, results obtained suggest that propolis could be a promising, natural and safer alternative to chemical fungicides in the control of 'Rocha' pear postharvest fungal diseases.

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