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APPLE PULP INHIBITION PROPERTIES CONCERNING *STAPHYLOCOCCUS AUREUS* ATTC 25923 POPULATIONS

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Abstract: The purpose of this study was to evaluate *S. aureus* inhibition by phytoncides present in apple pulp. The apple fruit used in the tests were of the cultivars: Grey Rennet and Cox's Orange Pippin. The behaviour of the staphylococcus population in its interaction with apple pulp was tested after 30 and 120 minutes of storage at 37°C. *S. aureus* growth was determined on an RPF Baird-Parker substrate. The tests demonstrated biocidal properties of Grey Rennet pulp against *S. aureus*. The other apple cultivar displayed weak biostatic properties, reducing the staphylococcus population by only 22.6% after two hours of incubation.

Keywords: apple, pulp, *S. aureus,* biostatic properties.

1. INTRODUCTION

Coagulase-positive *Staphylococci* of the *S. aureus* species can be present not only in food of animal origin, but also in fresh and thermally-preserved products of plant origin [Nipa et al. 2011; Steinka 2018]. Moon et al. 2007 in evaluating the levels of staphylococcus contamination in selected fresh vegetables, isolated *S. aureus* from 15% of sprout samples, 13% of spinach samples and 4% of napa cabbage samples. The tests indicated that *Staphylococcus aureus* bacteria were present in 73.1% of the test vegetable samples. The strains were characterised by the ability to produce a coagulase and an SE toxin. The results enabled the conclusion that bacteria isolated from vegetables exhibited a high resistance to antibiotics, such as penicillins [Moon 2007]. Tests of frozen vegetables confirmed the presence of methicillin-resistant *staphylococci* (MRSA) in 100% of the test spinach samples, indicating the ineffectiveness of the blanching process that precedes freezing [Steinka 2018].

Staphylococci isolated from many food products are the most commonly antibiotic-resistant strains, immune to many antibiotics [Normano et al. 2007; Crago et al. 2010; Gbonjubola, Jibo and Agu 2011; Hassan et al. 2011; Hammad et al. 2012; Steinka et al. 2013; Steinka 2018]. The plant material cultivation methods

and harvesting conditions are the likely sources of the *staphylococci* isolated from the finished products [Khachatourians 1998]. This constitutes a reason to seek methods of preventing the growth of these bacteria in food.

The purpose of this study was an attempt to evaluate *S. aureus* inhibition by phytoncides present in apple pulp.

2. TEST MATERIAL AND METHODS

The tests were conducted in a model system. Fruits of *Malus domestica* apple trees of the cultivars Polish Grey Rennet and English Cox's Orange Pippin were used in the study. After disinfecting the skin, a fragment of the skin was peeled with a scalpel from the sterile fruit. Using a cork borer, the pulp was sampled, weighed and 1 g collected. All actions were performed under sterile conditions, in a laminar flow cabinet. 1 g of the pulp was added to two test tubes, each containing 9 ml of *Staphylococcus aureus* ATTC 25923 broth culture.

The control samples were *Staphylococcus aureus* broth cultures, whose titre after dilution was determined on Merck RPF Baird-Parker substrates. The titre of the test *Staphylococcus aureus* cultures used in the mixtures ranged from 6.75 to 7.02 log cfu/ml. The behaviour of the *staphylococci* populations interacting with the pulp was tested after 30 and 120 minutes of incubation. For this time, the mixtures were stored at 37^{o}C, then 1 ml portions were streaked on Petri dishes. The streaking was performed using the pour plate method on Agar + RPF Baird-Parker substrates. Incubation lasted 48 hours at a temperature of 37^{o}C. *Staphylococci* determinations were performed in conformity with PN-EN ISO 6888-2:2001.

The results were subjected to statistical analysis using Excel version 8 software. Linear correlation equations describing the changes in *staphylococcus* population during incubation with the fruit macerate and the coefficient of determination were calculated.

3. RESULTS

Evaluation of the Grey Rennet inhibition properties showed that for a *S. aureus* inoculum of 7.02 log cfu/ml, the bacteria count after 30 minutes of incubation changed by 1.66 log cfu/g. Further incubation doubled the reduction in *S. aureus* cell numbers, to the level of 4.13 log cfu/g. 120 minutes of Grey Rennet fruit incubation with the *staphylococci* reduced the population by 2.89 log cfu/g, which meant a drop in cell numbers by 58.8%. The trend in *staphylococcus* population changes can be described with a linear equation (Tab. 1). The results indicated that 99.2% of the variation was determined by the duration of the interaction between the test bacteria cells and the substances contained in the fruits.

For *S. aureus* under the effects of Cox's Orange Pippin pulp, after 120 minutes there was a reduction in bacteria count by 1.53 log cfu/g, compared to the initial inoculum (Chart 2).

An initial drop of 2.89 in the population count was observed after 30 minutes of storing the mixture, followed by an increase by 1.06 log cfu/g. The trend of changes in *S. aureus* count under the effects of Cox's Orange Pippin pulp was accurately described by a second degree polynomial equation. The coefficient of determination R^2 of the linear correlation equation showed that the interaction time accounted for only 34.5% of the causes behind the drop in *staphylococcus* count. The reduction in *staphylococcus* cell count, observed after 120 minutes, was 22.6% compared to the inoculum. This indicated a more than twice as low level of inhibition of these bacteria than when using the Grey Rennet.

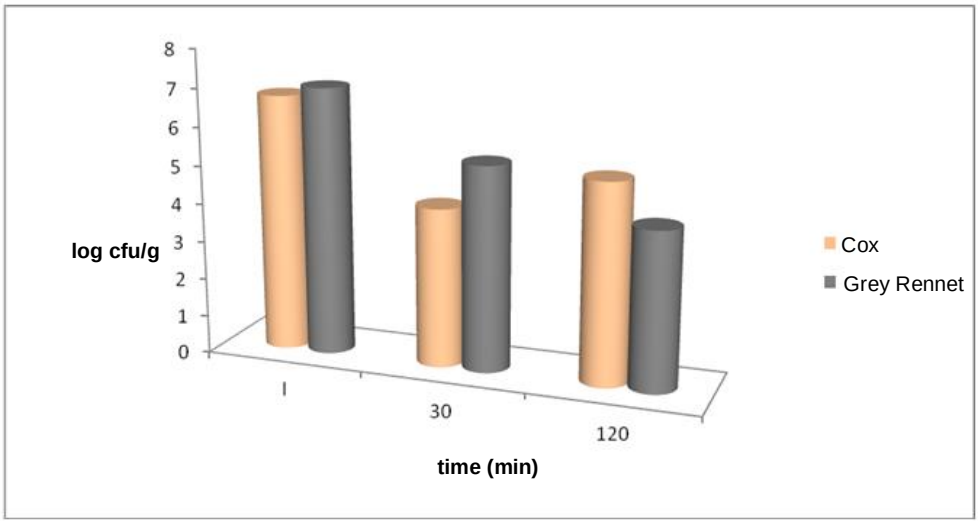

Fig. 1. Comparison of the biostatic properties of the apple pulps

Table 1. Equation describing *S. aureus* ATTC 25923 population variation

Apple cultivar	Equation describing population changes	Coefficient of determination
Malus domestica vs. Grey Rennet	$y = -0.765x + 6.90$	$R^2 = 0.345$
	$y = -0.765x + 6.906$	$R^2 = 0.345$
Malus domestica vs. Cox's Orange Pippin	$y = 1.825x^2 - 8.065x + 12.99$	$R^2 = 1$

The results of the apple pulp interactions with *S. aureus* population demonstrated the varied impact of the phytoncides contained in apples, depending on the cultivar of the fruits used. This is likely caused by the different content of the aqueous phase in the fruits of the two cultivars. Cox has a more cohesive tissue than Grey Rennet. There is a likelihood that maceration of the softer Grey Rennet tissue during test preparation contributed to the presence of a higher phytoncide concentration in the aqueous phase. Easier penetration by the juice-dissolved phytoncides, with significant biostatic properties, causing the destruction of *staphylococcus* cells was the most likely cause of the *S. aureus* count reduction by almost 60% after 120 minutes of the pulp's interaction with the microorganisms.

Apples are a type of fruit having a significant phytoncide content, e.g. flavonoids and other phenolic compounds (epicatechins) with significant biostatic properties. The content of these compounds may range from 13 ± 2 to 29 ± 7 mg/100 g of fruit, depending in the cultivation method [Kschonsek et al. 2018] and apple cultivar [Francini and Sebastiani 2013]. Among the compounds mentioned, catechins exhibit a major inhibition effect against *S. aureus*. Additionally, the organic acids contained in the pulp of these fruits, caffeine and coumarin, are additional substances with inhibition effects on *staphylococci*. Studies indicate that their concentration depends on the fruit cultivar [Eisele and Drake 2005]. The inhibition properties of both tested apple cultivars may have been affected both by the duration and conditions of fruit storage, which are important for the concentration of polyphenolic compounds.

4. CONCLUSIONS

1. Grey Rennet had significant inhibition properties affecting *S. aureus*, which caused a reduction in the population of these bacteria by 58.8% on average after 120 minutes of interaction, compared to the initial inoculum.
2. The use of apple pulp in treating diarrhoea during *staphylococcus* poisoning may be less effective if apples of cultivars with more cohesive tissues are utilised.

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