Antimicrobial potential and chemical and bioactive compounds in agroindustrial by-products from peach

Potencial antimicrobiano e compostos químicos e bioativos em subprodutos agroindustriais de pêssego

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ABSTRACT

The objective was to determine the antimicrobial potential and evaluate the chemical and bioactive compounds in the by-products of peach syrup production, which were "substandard peaches" and the peach kernel almond and its extracts. The analyses included total soluble solids, pH, total titratable acidity, centesimal composition, vitamin C, carotenoids, total antioxidant activity, total phenols, antifungal potential, antibacterial activity, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The most concentrated extracts from the peach and peach kernel almond presented higher antioxidant activity in IC50, with levels of 2.66 and 7.88 µg. mL^{-1} respectively. The total phenol content was 253.4 mg GAE.100g^{-1} for the peach and 29.3 mg GAE.100g^{-1} for the almond. The extracts tested showed no antifungal potential; however, the extracts of peach and almond presented antibacterial potential against *S. Typhimurim* and *S. aureus* bacteria. The MIC for kernel almond and peach extracts against *S. aureus* was 0.75 mg.mL^{-1} for both, but these did not present CBM. The extracts obtained from the peach by-products did not present antifungal activity, but the peach almond showed an inhibitory effect on S. aureus. The peach and peach kernel almond presented significant antioxidant activity and phenolic compound contents, owning high levels of bioactive compounds.

Keywords: minimum inhibitory concentration, kernel, almond, fruit.

RESUMO
Objetivou-se determinar o potencial antimicrobiano e avaliar os compostos químicos e bioativos dos subprodutos da produção do xarope de pêssego, que foram “pêssegos abaixo do padrão” e da amêndoa do caroço do pêssego e seus extratos. As análises incluíram sólidos solúveis totais, pH, acidez total titulável, composição centesimal, vitamina C, carotenóides, atividade antioxidante total, fenóis totais, potencial antifúngico, atividade antibacteriana, Concentração Inibitória Mínima (MIC) e Concentração Bactericida Mínima (MBC). Os extratos mais concentrados do pêssego e da amêndoa do caroço do pêssego apresentaram maior atividade antioxidante no IC50, com níveis de 2,66 e 7,88 µg. mL^{-1} respectivamente. O conteúdo total de fenóis foi de 253,4 mg GAE.100g^{-1} para o pêssego e 29,3 mg GAE.100g^{-1} para a amêndoa. Os extratos testados não apresentaram potencial antifúngico; entretanto, os extratos de pêssego e amêndoa apresentaram potencial antibacteriano contra as bactérias S. Typhimurim e S. aureus. A CIM dos extratos de amêndoa e pêssego contra S. aureus foi de 0,75 mg.mL-1 para ambos, mas estes não apresentaram CBM. Os extratos obtidos dos subprodutos do pêssego não apresentaram atividade antifúngica, mas a amêndoa do pêssego apresentou efeito inibitório sobre S. aureus. O pêssego e a amêndoa do caroço do pêssego apresentaram significativa atividade antioxidante e teores de compostos fenólicos, possuindo elevados teores de compostos bioativos.

Palavras-Chave: concentração inibitória mínima, núcleo, amêndoa, fruta.

1 INTRODUCTION

The search for natural substances that have biological functions has not only led to the use of extracts from plants and fruit, but also the use of agroindustrial by products such as bark, kernels, bagasse and seeds (Melo *et al*., 2011; Pereira & Cardoso, 2012). They can contain vitamins, minerals, fibers, and in some cases antioxidant and antimicrobial compounds (Padinha & Basso, 2015). Moreover, the direct exposure of the waste generated by the agroindustry to the environment represents one of the main causes of environmental pollution, in addition to a loss of biomass that could be used for the production or recovery of different metabolites with commercial value (Filho & Franco, 2015; García & Del Bianchi, 2015).

The production of peach in syrup generates innumerable by-products, including small peaches with a lesser diameter than the ideal one for processing (above 40 mm), is usually discarded. Peach (*Prunus pérsica* (L.) Batsch) is classified as a drupe with a woody endocarp, belonging to the family *Rosaceae* (D'Ávila *et al*., 2015). It is a fruit rich in antioxidants, which include phenolic compounds, carotenoids, and ascorbic acid, among others (Segantini *et al*., 2012; Betemps, 2010).

The peach core from the cutting and ginning stages during the production of peach in syrup is an agroindustrial by-product that corresponds to about 10% of the fruit's weight. The kernel in this core contains the cyanogenic amygdalin glycoside, which is a precursor of hydrocyanic acid; therefore, it presents toxicity and can be used in fumigants (D'Ávila *et al*., 2015).

The antimicrobial effect of plant extracts can be attributed mainly to the presence of bioactive compounds such as phenols, proanthocyanidins, caffeine, catechins, cinnamaldehyde and camphor (Bonila & Sobral, 2017), besides tannins, and ferulic, cinnamic and vanillic acids (Souza *et al*., 2010). The hydroxyl groups present in these compounds can form hydrogen bonds with enzymes of the microbial metabolism, deactivating them and inhibiting the development of the fungal biomass (Souza *et al*., 2010).

Considering the importance of the use of agroindustrial by-products, the objective of this study was to determine the antimicrobial potential of the chemical and bioactive compounds in the by-products of peach production in syrup: "sub- standard" peaches and peach kernel almond.

2 MATERIAL AND METHODS

Raw material, physical-chemical analyses, bioactive compounds, obtainment of plant extracts, determination of antifungal activity - Well diffusion test

The raw material used was "substandard" peaches (2015/2016 crop) with a diameter of approximately 3.5 mm and dry peach pits (2014/2015 crop), both by-products resulting from the production of peach in syrup and that would be destined for disposal. These werekindly provided by Frutos da Terra Ltda - Pelotas, Rio Grande do Sul state, Brazil. The analyses of chemical and bioactive compounds were carried out on the uns kinned peach (without its pit). As a result, dry kernels were obtained (submitted to natural drying at the company from which they originated) and fresh kernels were obtained from the substandard peach crop of 2015/2016. The peaches were stored in an ultra-freezer at -80°C, and the pits and kernels were packed in 1 kg polyethylene packs and stored at room temperature until the time of analysis.

Fungal isolates of the genera *Rizhopus* sp. and *Trichoderma* sp., were both kindly provided by the Chemistry and Food School Laboratory of the Federal University of Rio Grande (FURG), Rio Grande/RS, Brazil, were used for antifungal determination.

The bacteria used for Minimum Inhibitory Concentration and Minimum Bactericidal Concentration were *Listeria monocytogenes* (ATCC7644); *Salmonella Typhimurium* (ATC13311); *Escherichia coli* O157: H7

(ATCC 43895) and *Staphylococcus aureus* (ATCC 25923). Microbiological analyses were performed at the Laboratory of Microbiology of the School of Nutrition of the Federal University of Pelotas (UFPel), Pelotas/RS, Brazil.

Moisture, ash, protein, crude fiber, lipids, Total Titratable Acidity (TTA), vitamin C, Total Soluble Solids (TSS) and pH were performed in peach and peach kernel according to the methodology described by AOAC (2012). The carbohydrate content was determined by difference, subtracting from 100 the values obtained for moisture, proteins, ashes, lipids and fibers. The Atwater conversion factors were used to determine the Energy Value (TEV). Results were expressed as mean ± standard deviation.

The carotenoid content was performed according to the methodology of Krumreich *et al.* (2015). The results were expressed in µg total carotenoids.g^{-1}.

The Total Antioxidant Activity (TAA) was determined by the DPPH (2.2- 93 diphenyl-1-picryl-hydrazyl) free radical capture method, following the methodology described by Rufino *et al.* (2007) (5%, 10%, and 20%). The results were expressed as IC 50.

The concentration of total polyphenols was determined according to the Folin-ciocalteu colorimetric method described by Swain & Hills (1959). The result was expressed in milligrams of Gallic Acid Equivalents (GAE) per 100 g of sample (mg GAE.$100g^{-1}$).

The extracts were obtained from the peach and the peach kernel, following the methodology described by Scapin (2014) with modifications. The obtained samples were weighed, separately crushed in a Walita® RI2087/90 600 blender and then added to 400 mL of 80% (v/v) hydrous alcohol solution to obtain extracts of dry peach kernel at concentrations of 2.5%, 5% and 7.5% and peach and fresh peach kernel at the concentration of 7.5%. The obtained extracts were stored in an ultra-freezer at -80°C.

The tests for antifungal determination were performed by the well diffusion method according to the methodologies described by Gurgel *et al*. (2005) and Fontenelle *et al*. (2007) with adaptations. The antifungal potential of peach extracts at concentrations of 7.5% and peach kernel at concentrations of 2.5%, 5% and 7.5% were both tested against fungi *Rizhopus* sp. and *Trichoderma* sp. Fungal concentrations were standardized on the McFarland scale, in which scale 3 ($9.0x10^8$ CFU.mL^{-1}) was obtained for the fungus *Rizhopus* and scale 5 ($1.5x10^9$ CFU.mL^{-1}) for *Trichoderma*.
The plates were incubated at 28°C for 7 days. Inhibition was verified by the formation of a characteristic translucent halo around the cavity related to each fungus tested. Inhibition halos ≥ 10 mm were considered satisfactory (Silva *et al*., 2012).

Determination of antibacterial activity

The antibacterial activity was evaluated in 7.5% peach extract and 2.5%, 5% and 7.5% peach kernel extracts, which were tested against the bacteria *E. coli*, *S. aureus*, *L. monocytogenes* and *S. typhimurium*. The disc diffusion test was performed according to the protocol proposed by the Clinical and Laboratory Standard Institute Manual (Clsi, 2005).

Subsequently, with the aid of a platinum loop, they were suspended in tubes containing 0.85% (w/v) saline solution and standardized to the concentration of 0.5

($1.5x10^8$ CFU.mL^{-1}) by the McFarland scale. The plates were incubated at 37°C for 24 h, and inhibition was verified by the formation of a characteristic translucent halo around the disc. The analyses were performed in triplicate.

Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined by the microplate dilution assay according to the methodology described by Cabral *et al*. (2009). After reactivation, the inoculum concentration was adjusted to correspond to the turbidity of 0.5 of the Mc Farland scale ($1.5x10^8$ CFU.mL^{-1}), and a volume of 100 µl of the bacterial suspensions was inoculated into 30 mL of the BHI broth to give a bacterial concentration around 1-$2x10^5$ UFC.mL^{-1}.

The technique was developed in 96-well microplates. The microplates were incubated at 37°C for 24 h. For the determination of MBC, aliquots of 10 µL of culture medium from the wells considered to have inhibitory effect were seeded on BHI agar and incubated at 37°C for 24 h. MBC was considered as the lowest concentration in which there was no growth on the surface of the culture medium. The tests were performed in triplicate.

3 RESULTS AND DISCUSSION

Physical-chemical analyses, antioxidant activity, phenols, antifungal and antibacterial activity (peach and peach kernel almond)

From the data in Table 1, it can be observed that the average total soluble solids, pH and peach acidity found were 11.06°Brix 3.15 and 1.08% in citric acid, respectively. When analyzing peach pulp, Rodrigues & Moretti (2008) found values of 11.42 °Brix and 4.05% of acidity. The literature reports that citric acid levels in peaches decrease as fruits ripento the detriment of the increase in sugar content (Chapman & Horvat, 1990). According to the Food Composition Table (Taco, 2011), the fresh peach of the Aurora cultivar presents 89.3% of humidity, 0.8% of proteins, traces of Lipids, 1.4% of dietary fiber, 0.5% of ash, 9.3% of carbohydrates and total energy value of 36 kcal. Therefore, the peach analyzed in the present study had a composition similar to that described in the literature.

Table 1: Physical-chemical composition of "substandard" type peach

Parameters	Peach*
Total Soluble Solids (°Brix)	11.06 ± 0.11
pH	3.15 ± 0.00
Acidity (% in citric acid)	1.08 ± 0.10
Humidity (%)	88.78 ± 1.63
Proteins (%)	1.25 ± 0.03
Lipids (%)	0.43 ± 0.11
Crude fiber (%)	1.01 ± 0.01
Ash(%)	0.50 ± 0.14
Carbohydrates (%)**	8.9 ± 0.87
Total energy value (Kcal)	44.41 ± 0.08
Vitamin C (mg. 100g^{-1})	6.17± 2.02
Carotenoids (ug.100g^{-1})	107 ± 1.02

* Mean ± standard deviation; **Determined by difference

The small variations in the values of the parameters can be attributed mainly to the differences in cultivars, genetic factors and degree of maturation (Krumreich *et al.*, 2015).

Regarding the fiber content, Anvisa Resolution Nº. 54/2012 (Brasil, 2012) recommends that for food to be considered as a source of fiber it must have at least 3g of fiber/100g of solid food. Therefore, the analyzed peach sample is lower than that recommended by the legislation to be considered as a fiber source, presenting a value equivalent to 1.01%.

The vitamin C content of the peach was 6.17 mg.100g^{-1}. The result found in the present study is higher than that described by Gil *et al.* (2002) when analyzing the peach of the Granada cultivar (3.6 mg 100.g^{-1}). The study by Barcia *et al.* (2010) evaluated the vitamin C content of various fruits including peaches of the varieties Eldorado, Sensation and Granada, in which they identified levels of 2.17, 6.12 and 1.12 mg.g^{-1} respectively. The highest value of vitamin C found in the present study can be attributed to the fact that the peach taken as an agro-industrial by-product consists of a mixture of several distinct cultivars, which in turn can differ in vitamin C levels.

In the present study, the carotenoid levels were 10^{7} ug.100g^{-1}. This value was higher than that found by Segantini *et al.* (2012) when analyzing yellow-flesh peaches. In that study levels of carotenoids ranged from 35.94 to 81.58 ug.100.g^{-1}. The difference in carotenoid content may be related to several factors such as genetic variety, maturation stage, post-harvest storage, processing and preparation (Ribeiro *et al.*, 2020).

Analyzing Table 2, it can be observed that the peach kernel stands out as a source of fibers and because it presents significant amounts of lipids, proteins and ash. Analyzed peach kernels and obtained moisture, protein, ash and lipid contents of 26.1%, 21.5%, 3.36% and 25%, respectively, while the fiber and carbohydrate contents totaled 24% (Mezzomo, 2008). The differences observed in the present study may be related to the fact that the kernels analyzed belong to peaches of different cultivars, since small variations in the values in the physical-chemical parameters can be attributed mainly to the cultivar differences (Krumreich *et al.*, 2015).

Table 2: Physical-chemical composition of the peach kernel

Parameters	Peach*
Humidity (%)	8.46 ± 0.33
Proteins (%)	26.10 ± 0.36
Lipids (%)	42.73 ± 2.42
Crude fiber (%)	3.63 ± 0.01
Ash(%)	4.10 ± 0.06
Carbohydrates (%)**	14.98 ± 0.02
Total energy value (Kcal)	548.9± 0.03
Acidity (% in citric acid)	1.14 ± 0.01

*Mean ± standard deviation**Determined by difference

The antioxidant activity of the peach at concentrations of 5%, 10% and 20% was 11.43μg.mL^{-1}, 3.40μg.mL^{-1} and 2.66μg.mL^{-1}, respectively.

Considering that the lower the IC50, the higher the antioxidant activity (García & Del Bianchi, 2015), it was observed that the antioxidant activity of the peach was higher in the 20% concentration extract. This value, which is higher than found by Sousa *et al*. (2011) when analyzing residues of tropical fruit pulps, such as guava (142.89 μg.mL^{-1}), acerola (308.07 μg.mL^{-1}), pineapple (3293.92 μg.mL^{-1}), graviola (612.37 μg.mL^{-1}), bacuri (2506.6 μg.mL^{-1}) and cupuaçu (554.87μg.mL^{-1}). The antioxidant activity of the peach may possibly be related to its vitamin C and carotenoid content (Table 1), but is mainly due to the presence of phenolic compounds, considering that these are the major compounds in the fruit (Vieira *et al*., 2011). This is in agreement with many studies that have shown that the antioxidant activity of fruits is mostly related to the content of the phenolic compounds present in them (Silva, 2015; Rocha *et al*., 2011)

The antioxidant activity of the kernel was also higher at the concentration of 20%, presenting an IC50 value equivalent to 7.88 μg.mL^{-1} Arbos, Stevani & Castanha (2013) evaluated the kernel of mango fruits and found IC50 values of (1,330 μg.mL^{-1}); thus, the antioxidant activity of peach kernel is higher than that found in mango kernels.

The total peach phenols were 253.4±0.05 mgGAE.100g^{-1}, and in the peach kernel the values were 29.3±0.17 mgGAE.100g^{-1}. Regarding the total phenol content of the peach, values of 253.4 mgGAE.100g^{-1} were observed. The results found in this study were higher than those found by Costa & Fachinello (2014) when analyzing peaches of the cultivar Eldorado produced in different systems of plant trellising, finding values of 139.41, 169.69 and 169.49 mgGAE.100 g^{-1} for the leader, V-shaped and vase systems, respectively.

Segantini *et al.* (2012) found levels of polyphenols ranging from 53.38 to 141.77 mg GAE.100g^{-1} of pulp when analyzing yellow peaches, and 'Douradão' presented the lowest content, while 'Big-Aurora' showed the highest content of polyphenols. Martins *et al*. (2004) observed differences in the total phenol content of peaches during ripening, noting that there was an increase in the content of these compounds according to the degree of ripening, as peaches in the semi-ripe and ripe stage presented higher phenol content than the fruits harvested in the green stage. Therefore, the content of phenolic compounds found in the peach in the present study may be related to the degree of fruit ripeness and, consequently, with soluble solid content and acidity (Table 1).

Table 3 presents the results of the microorganisms sensitivity to the five extracts tested: peach (7.5%), dry peach kernel (2.5%, 5% and 7.5%) and fresh peach, 5.0%). It is observed that *Rizhopus* sp. and *Trichoderma* sp. did not show sensitivity to the different concentrations of the peach extracts and peach kernels. The literature reports the antifungal activity of other plant extracts in relation to different fungal species, but no reports were found regarding extracts of peach agroindustrial by-products against *Rizhopus* sp. and *Trichoderma* sp.

Table 3: Antibacterial and antifungal activity of peach and peach kernel

Microorganisms	Inhibition Halo (mm)				
	Extracts				
	Peach (7.5%)	Fresh Kernel (7.5%)	Kerneldry (2.5%)	Kerneldry (5.0%)	Kerneldry (7.5%)
Bacteria					
E. coli	A	A	A	A	A
S. Typhimurium	A	7.0 ± 0.05	5.0 ± 0.00	4.5 ± 0.06	3.8 ± 0.05
L. monocytogenes	A	A	A	A	A
S. aureus	A	8.3 ± 0.28	5.0 ± 0.05	A	A
Fungi					
Rizhopus sp.	A	A	A	A	A
Trichoderma sp.	A	A	A	A	A

*Results expressed as mean ± standard deviation; A = absence of inhibition halos

Table 3 shows that the extracts of fresh kernel (7.5%) and dry peach kernels at concentrations of 2.5%, 5.0% and 7.5% presented antibacterial activity against S. *typhimurium*. The inhibitory effect reached its maximum value (7.0 mm) with fresh kernel extract (7.5%).

It is observed in the present work that the *S.aureus* microorganism presented greater sensitivity, evidencing that the largest inhibition halo formed from the fresh kernel extract 7.5% was 8.3 mm, and for the dry kernel 2.5% it was 5.0 mm. The literature reports that inhibition halos should be 8 to 13 mm if the extract is to be considered to have moderate antimicrobial power, and inhibition halos >14 mm for extracts with high antimicrobial power (Mothana & Lindequist, 2005). In this context, it can be inferred that the extract of the fresh peach kernel (7.5%) presented moderate antimicrobial activity against *S. aureus.*

Arbos, Stevani & Castanha (2013) analyzed the antibacterial action of mango skin and kernels, and found antibacterial activity from both against four strains bacteria including *S.aureus*, with inhibition halos of 19 mm and 14.9 mm, respectively.
The antimicrobial activity observed in the peach kernel is probably attributed to the phenolic compound content and also due to the presence of cyanide, since it presents high toxicity (Mezzomo, 2008). However, the fresh kernel presented higher antibacterial activity when compared to the dry kernel, which can be explained by the instability of the phenolic compounds against the application of high temperatures (Campos *et al*., 2008) and by the high volatility of cyanide (D'Ávila *et al*., 2015).

In relation to *E. coli* and *L. monocytogenes*, it was observed that none of the extracts tested was able to inhibit the growth of these microorganisms through disc diffusion and well diffusion tests, demonstrating a higher resistance of these bacteria to antimicrobial agents in these tests.

The peach is mentioned in the literature as a potential source of phenolic compounds, presenting antioxidant activity and also notable for its antifungal and antibacterial potential (Souza *et al*., 2010). However, in the present work, the peach extracts presented no antibacterial activity against any of the analyzed bacteria (*E.coli,* *S.typhimurium, L. monocytogenes,* and *S. aureus*). This fact that can be attributed to the higher resistance of these bacteria to the peach extracts tested, problems of diffusion of the peach extracts against these microorganisms in the disc diffusion test, or due to the physical-chemical composition of the peach, which by presenting high moisture content, protein and carbohydrates (Table 1) favors the development of some microorganisms.

The literature evidences the antibacterial activity of other fruits, including in a study by Djipa *et al*. (2000) which verified the antimicrobial activity of jambolan extracts on several microorganisms, among them *S. aureus*. This property is attributed to the high concentration of tannin present in this fruit. In the present study there may have been a difference in the chemical composition of the plant extracts, since the inhibitory action of the phenolic compounds against spore germination, mycelial growth and microbial enzyme production/activity varies among the different groups of phenols (Stangarlin *et al*., 2011).

Therefore, observing the results of the total phenol analysis of the present work, it can be inferred that there was no relationship between antifungal and antibacterial activity and the total phenol contents for these peach extracts. However, it is important to note that peach kernel extracts presented antibacterial activity against *S. aureus* and *S. typhimurium,* indicating the efficiency of these extracts against the microorganisms that are more frequently involved in outbreaks of food poisoning and food infection, respectively.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC results of peach (7.5%) and almond peach (7.5%) extracts showed that they had no inhibitory activity against *E. coli, S. typhimurium* and *L. monocytogenes* at the tested concentrations. However, it was observed that the peach and almond peach kernel extracts showed activity for *S. aureus* (MIC = 0.75 $mg.mL^{-1}$) for both extracts. Although the peach extract (7.5%) did not present an inhibitory effect in the disk diffusion technique, its antimicrobial effect was detected in the MIC test. This may have happened because of the difficulty in diffusion of the extract on agar in the disc technique, which may be attributed to the lipophilic characteristics of some samples and/or the chemical nature of the isolates (Alves *et al*., 2008). The absence of an inhibition zone may not necessarily mean that the extract is inactive against the tested microorganisms, but rather that the diffusion was not complete especially for less polar compounds, which diffuse more slowly in the culture medium (De Bona *et al*., 2014).

The literature reports similar results to that found in the present study, as was described by Al-Zoreky (2009). When analyzing hydroalcoholic extracts of pomegranate peel, the authors also found antibacterial activity against *S. aureus* with a minimum inhibitory concentration of 2 $mg.mL^{-1}$.

Therefore, the results of MIC for *S. aureus* of both peach and peach kernel almond can be considered significant, considering that a lower concentration was obtained when compared to the studies of the analyzed authors. However, it was observed that none of the extracts tested in the present study showed minimal bactericidal concentration (MBC), indicating that the extracts only have a bacteriostatic effect against *S. aureus*.

4 CONCLUSION

The "substandard" peach is an important source of carbohydrates, vitamin C and carotenoids. The peach kernel almond stands out because of its content of fibers, lipids, proteins and ash, as mineral content. In addition, peach and peach kernels almond showed antioxidant activity and phenolic compound contents, demonstrating that they have high levels of bioactive compounds. Extracts from by-products (almond and peach) did not show antifungal activity, and only the fresh peach kernel extrac (7.5%) presented moderate antibacterial activity against *S. aureus*.

The fresh kernel presented higher antibacterial potential when compared to dry kernel. Peach and dry kernel (7.5%) extracts presented bacteriostatic effect for *S. aureus*, but none of these showed bactericidal activity.

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