Antifungal activity of mango peel and seed extracts against clinically pathogenic and food spoilage yeasts

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ABSTRACT
The antioxidant and antifungal (antiyeast) properties of mango (\textit{Mangifera indica}) peel and seed by-products were investigated. Nine extracts were obtained using three cultivars and two extraction methods. Significant differences between cultivars and extraction methods were detected in their bioactive compounds and antioxidant activity. The antifungal property was determined using agar diffusion and broth micro-dilution assays against 18 yeast species of the genera \textit{Candida}, \textit{Dekkera}, \textit{Hanseniaspora}, \textit{Lodderomyces}, \textit{Metschnikowia}, \textit{Pichia}, \textit{Schizosaccharomyces}, \textit{Saccharomycodes} and \textit{Zygosaccharomyces}. All mango extracts showed antifungal activity. The minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) values were lower for seed than for peel extracts. MICs and MFCs ranged from values <0.1 to 5 and 5 to >30 mgGAE/mL, respectively. The multivariate analysis showed a relationship between antifungal activity, the capacity to inhibit lipid peroxidation and total phenol content. These properties were associated with high levels of proanthocyanidins, gallates and gallotannins in the extracts.

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1. Introduction

Yeasts are the most important microorganisms involved in the fermentation processes of many foods and beverages, such as wine, bakery products and others. Nevertheless, some yeasts (about 20 species) are frequently associated with food spoilage (Fleet 2011). Moreover, yeasts can also cause animal and human infections, and are the most important fungi in the cause of diseases (Cooper 2011). To overcome these problems, antimicrobial compounds (antimycotics) have been routinely used to control animal and human diseases, and a wide range of chemical preservatives are added to foods and beverages to control yeasts spoilage. As a consequence of increasing development of drug resistance in microorganisms pathogenic for human as well as undesirable effects of certain antimicrobial compounds, there is a need to find alternative natural sources to reduce the incidence of important diseases and improve the preservation of foods. In this context, the pharmacological and phytochemical properties, as well as the antimicrobial activity of mango (*Mangifera indica*) by-products, have been extensively studied (Stoilova et al. 2005; Khammuang & Sarnthima 2011; Mura et al. 2015). In this regard, Engels et al. (2009, 2011) describe the antimicrobial mechanism of gallotannins obtained from mango kernels against different bacteria. In addition, the antifungal activity of different flavonoids isolated from mango leaves has been reported against different species of filamentous fungi (Kanwal et al. 2010). However, only a few studies regarding the antifungal activity against yeast species have been developed. Moreover, there are scarce data regarding the minimum inhibitory concentrations (MIC), and there are no available data regarding the minimum fungicidal concentrations (MFC) of the mango extracts against yeasts.

Consequently, the aim of this study was to determine the antifungal activity of mango by-products against frequently occurring food spoilage and human pathogenic yeasts species. The mango by-products were obtained from the peels (using two different extraction methods) and seeds of three cultivars (Keitt, Sensation and Gomera-3). Nine by-products were obtained and after a chemical characterisation (bioactive and antioxidant properties), the *in vitro* effect against 18 reference strains was studied through agar diffusion and broth micro-dilution assays. MIC and MFC were determined. Finally, a principal component analysis (PCA) was performed in order to find a relationship of the mango extracts antifungal activity with the bioactive compounds content, antioxidant activity and phenolic compounds.

2. Results and discussion

2.1. Bioactive compound content

The mango by-products analysed showed high extraction yield of total phenolic compounds, ranging from 11–18 and 8–15 g GAE/100 g DW (GAE: gallic acid equivalents; DW: dry weight) from peel and seed extracts, respectively. These results are in agreement with previous works: 7.38–39.9 g GAE/100 g DW mango (Soong & Barlow 2004; Khammuang & Sarnthima 2011). No significant difference (*p* ≤ 0.05) between the cultivars or extraction processes was detected in the extraction yield of total phenols and tannins from peel extracts. By contrast, seed extracts from the Sensation and Gomera-3 cultivars showed approximately two times higher extraction yield than Keitt cultivar (Table S1A).

Significant difference (*p* ≤ 0.05) between the cultivars or extraction processes was detected in the extraction yield of proanthocyanidins from peel extracts. Extraction process
B (0.24–0.48 mg LEs/100 g DW) (LEs: leucoanthocyanidin equivalents) had higher content than extracts obtained with extraction process A (0.08–0.18 mg LEs/100 g DW), and Sensation and Gomera-3 cultivars had higher extraction yield than Keitt cultivar. In seed extracts, the proanthocyanidins contents were 0.59–0.80 mg LEs/100 g DW, and the Keitt cultivar displayed the highest values (Table S1A). These results seem important since only few works have evidenced the presence of proanthocyanidins in mango by-products.

2.2. Antioxidant activity

No significant difference (p ≤ 0.05) between the cultivars or extraction processes was detected for the ability to scavenge DPPH radicals from peel extracts. However, seed extracts from the Sensation and Gomera-3 cultivars showed higher values than Keitt cultivar. Regarding antioxidant activity coefficient (AAC) values, significant difference (p ≤ 0.05) between the cultivars or extraction processes was detected (Table S1A). Mango peel extracts obtained with process A and seed extracts from Keitt cultivar displayed the lowest capacity to inhibit lipid peroxidation. Interestingly, AAC values of mango peel (extraction B) and seed (Sensation and Gomera-3 cv) extracts, were similar to two synthetic antioxidants used in food industry; butylatedhydroxyanisole (BHA) and butylatedhydroxytoluene (BHT).

2.3. Antifungal activity

The antifungal activity was determined using agar diffusion and broth micro-dilution assays, against 18 yeast species (corresponding to nine genera) associated with food spoilage and pathogens for humans. A total of nine by-products extracts were used with the following concentrations (expressed in mg GAE/mL): KPA, 54; KPB, 230; SPA, 74; SPB, 264; GPA, 72; GPB, 262; KS, 106; SS, 190 and GS, 196 (K: Keitt cv.; S: Sensation cv.; G: Gomera-3 cv.; P: peel; S: seed; A: extraction A; B: extraction B). The agar diffusion assay indicated that all extracts (from peel and seed) had an inhibition effect against all the tested yeasts. The most sensitive species were Candida parapsilosis, C. glabrata and Lodderomyces elongisporus, and the most tolerant were Schizosaccharomyces japonicus and Dekkera anomala. In peel extracts (Table S2), the interaction between extraction process and cultivar (ExC) was not significant (p ≤ 0.05) in the 77.8% of the yeasts tested. Furthermore, significant differences among extraction process (E) were detected in the 92.8% of the yeasts (without interaction ExC). The extracts obtained with process type B showed higher antifungal effect than extracts obtained with process type A. In addition, this result was related to the amounts of total phenols. The extracts obtained with process B had a higher amount of phenols than those obtained with process A (252 and 66 mg GAE/mL on average, respectively). The correlation coefficient between phenolic content (x) and mm of inhibition zones (y) for individual yeast were 0.866 in C. glabrata (minimal value) to 0.994 in Pichia kluyveri (maximum value), with an average of 0.959. Similarly, the correlation coefficient between tannins content (x) and mm of inhibition zones (y) for individual yeast were 0.873 in C. glabrata (minimal value) to 0.992 in P. kluyveri (maximum value), with an average of 0.958. On the other hand, significant differences (p ≤ 0.05) among cultivars (C) were detected in the 21.4% and the 38.9% of the yeasts when peel or seed extracts, respectively, were used (Tables S2 and S3).

For a quantitative evaluation of the antifungal activity, the MIC and the MFC were determined. In this case, all the extracts were diluted to a final concentration between 0.1 and
30 mg GAE/mL. The serial dilution assays confirm that all extracts had antifungal properties, with MICs between <0.1 and 5 mg GAE/mL. In general, the MIC values for seed were lower than for peel extracts (Table S1B). Pichia ohmeri and Zygosaccharomyces bisporus were the most sensitive species, and L. elongisporus was the most resistant. In peel extracts, no differences among extraction process or cultivar were observed in most yeast (66.7%). In seed extracts, MICs differences among cultivars were observed in the 55.5% of the yeasts. In the same way as was observed in the agar diffusion assays, the seed extracts from the Sensation and Gomera-3 cultivars displayed higher antifungal activity (lowest MICs values) than those of the Keitt cultivar. However, no significant correlation was observed between the antifungal activity analysed by both bioassay methods (agar diffusion and micro-dilution). There were no coincidences in the susceptibility or resistance of the species between methods. These results suggest that different variables could affect the sensitivity of the yeasts in in vitro assays. Probably the extract–yeast interaction could be affected by the culture conditions, the physiological conditions of the cells and/or the diffusion capacity of the extracts in each type of bioassay, among others. The MFC assay was performed to confirm cell death in the MIC test. Most of the yeast species displayed a significant difference between the MIC and MFC values, which indicates that the mango extracts have a fungistatic effect. The MFCs range between 5 and 30 mg GAE/mL (Table S1B). This is the first report showing the MFC values and the fungistatic effect of mango peel and seed extracts against different yeasts species.

Different sorts of plants have been used for many years due to their antimicrobial properties in food preservation (condiments or flavourings) and by their antimicrobial and anti-inflammatory effects in traditional medicine. In particular, mango extracts are commonly used in folk medicine to treat a wide variety of ailments. Different potential pharmacological actions, including antimicrobial (especially against bacteria and filamentous fungi) effects, were corroborated in previous studies (Singh et al. 2009; Shah et al. 2010). However, a few numbers of yeasts (C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, Cryptococcus albhdus and Saccharomyces cerevisiae) were tested against mango by-products. The antifungal (against yeasts) properties have been demonstrated on C. albicans using different parts of the plant; stem bark (Singh et al. 2010), leaves, rind (Gupta et al. 2010), seeds (Ahmed et al. 2005; El-Gied et al. 2012) and unripe dried pulp (amchur) (Jain & Nafis 2011). Furthermore, Stoilova et al. (2005) and Singh et al. (2009) showed the antifungal activity of mangiferin and its analogue compounds. Moreover, in some of the reference works the MICs were determined and the results have been diverse. Apparently, the disparity data could be attributed to the different C. albicans strains tested (some of them were not reference strains), the samples origin (leaves, fruit, etc.) and the extraction process (especially type of solvent) of the extracts; MICs = 0.08 mg/mL stem bark (Singh et al. 2010); 7.5–12.5 mg/mL leaves; 7.5–17.5 mg/mL rind (Gupta et al. 2010); 0.3 mg/mL amchur (Jain & Nafis 2011). With regard to the other yeast species mentioned above, no data were available about the MIC, with the exception of the work carried out with C. albhidus by Singh et al. (2010) (MIC = 0.31 mg/mL of steam bark extracts). Taking the above into consideration, it can be seen that the number of yeasts analysed so far is scarce. The MICs values are available only in two yeasts species and MFC data have not been determined. Moreover, it should be noted that in most referenced works (except Singh et al. [2010]) the antifungal analyses were performed using disc agar diffusion assays.
2.4. Principal component analysis

PCA was applied to establish the relationship between the antifungal activity (diameter of inhibition zone) and bioactive compounds content (total phenols, proanthocyanidins and tannins), antioxidant activity (β-carotene and DPPH*) and phenolic compounds (identified in a previous work [Dorta et al. 2014]). The PCA analysis generated two principal components, with a cumulative explained variance of 62.9% of the total variance in by-product extracts samples. The PC1 component (cumulative explained variance 37.5%) was correlated with DPPH* and strongly related with flavonoids (Figure S1a; compounds nº 14, 15, 19, 20 and 21) and benzophenones (4, 7, 8 and 9). Accordingly, the PC1 component was correlated with radical scavenging capacity and the aforementioned compounds are related to this capacity. Furthermore, PC2 (cumulative explained variance 25.4%) was correlated to the diameter of inhibition zone, the capacity to inhibit lipid peroxidation, the total phenols, tannins and proanthocyanidins contents, and was strongly related to gallate and gallotannin compounds (Figure S1a; compounds nº 1, 2, 3, 5, 10, 12, 13, 18, 23 and 24). As a result, the new variable PC2 was clearly associated with the antifungal capacity of the extracts and with the high capacity to inhibit lipid peroxidation. Therefore, we can note that the extracts with high values of AAC have antifungal properties; these extracts were obtained from peel with extraction process B. The corresponding scores of the two first principal components are shown in Figure S1b. As observed, the samples are grouped in three groups. Group 1, corresponded to the peel extracts of the three mango cultivars obtained using the extraction process A (KPA, SPA and GPA) and the seed extracts of the Keitt (KS) and Gomera-3 (GS) cultivars. The group had low PC2 scores, which was related to a low antifungal capacity. Furthermore, this group of extracts has been related with lower content in gallates and gallotannins (Dorta et al. 2014). Group 2, had formed by peel extracts obtained using extraction process B from Gomera-3 cultivar (GPB). This group was characterised as having a high scavenging capacity (high content in flavonoids and benzophenone derivatives) and a moderate antifungal capacity. Finally, Group 3 consisted of the extracts obtained using extraction process B (KPB and SPB) and the Sensation cultivar (SS) seed extracts. This group presented a high PC2 score characterised by high levels of tannins (principally, proanthocyanidins, gallates and gallotannins [Dorta et al. 2014]), high capacity to inhibit lipid peroxidation and high antifungal activity.

The results indicated that the extracts with high antioxidant activity and high content in phenolic compounds (SPB, GPB, KPB and SS) displayed strong antifungal activity. Therefore, we can outline a possible relationship between antioxidant activity and antifungal activity. In this context, several works confirm that the antifungal capacity of natural extracts is related to their tannins content (proanthocyanidins or condensed tannins and hydrolysable tannins) (Patel et al. 2011; Sarnoski et al. 2012). However, another explanation for yeast inhibition due to proanthocyanidins is iron deprivation. Metal ions, such as iron, copper and zinc ions, have important functions in yeast physiology and they may all be chelated by tannic acid (Wauters et al. 2001). Taking the latter into account, it is possible to suggest that in the case of mango extracts, the proanthocyanidins could be responsible for their antifungal activity. In addition, the principal compounds related to the antifungal properties, beyond proanthocyanidins, would be ellagitannins or gallotannins (Figure S1a). In fact, previous studies with gallotannins isolated from mango seed have described iron-binding capacity as the mode of antimicrobial (especially against bacteria) action of these compounds (Engels et
Therefore, the gallates and gallotannins obtained with extraction process B could be the responsible for the antioxidant and antifungal activity.

In conclusion, mango peel and seed extracts rich in tannins obtained from different cultivars in Tenerife (Canary Islands), exhibit good antioxidant activity and inhibitory properties against a large number of yeasts (18 reference strains) associated with food spoilage or pathogenic for humans. The antifungal and/or antioxidant properties have been significantly affected by the extraction process and the cultivar. Low MICs (0.5–1 mg GAE/mL) were observed against important human pathogens (C. bracarensis, C. glabrata, C. nivariensis and C. parapsilosis) and food spoilage (D. anomala, Hanseniaspora uvarum, Z. bailii, Z. bisporus and Z. rouxii) yeast species. Moreover, the MFC was determined and therefore this is the first report showing the fungistatic effect of mango peel and seed extracts. Multivariate analysis allowed observing that the extract with high content in tannins and high capacity to inhibit lipid peroxidation showed high antifungal activities. Therefore, the present study strongly suggested that mango extracts are promising compounds for further development as natural ‘antiyeast’ agents with pharmaceutical and food industry applications. Currently, this is the first report to demonstrate the antifungal properties of mango peel extracts against different yeasts species.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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