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Survival of foodborne pathogens in unripe grape products

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ABSTRACT

Antibacterial effects of unripe grape products (verjuice and sour grape sauce) which are particularly rich in antioxidants and organic acids were evaluated. Survival of *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* Typhimurium and *Staphylococcus aureus* which were inoculated in unripe grape products at different inoculum doses (2 and 6 log CFU/mL) was determined. All the samples were kept at room temperature (approximately 25 °C) for 0, 5, 15 and 30 min after inoculation with pathogens, separately. The unripe grape products which were inoculated at low inoculum dose inhibited all tested pathogens in 5 min, while it took 15 min for high inoculum dose. The pH, titratable acidity (tartaric acid %) and total sugar content values of the samples were between 2.14–2.74, 2.29–7.10% and 1.19–17.65 g/L, respectively. It was concluded that unripe grape products are microbiologically safe products and they have self-protection systems even when they were contaminated with pathogens at high levels.

1. Introduction

In spite of novel techniques and improvements in food processing, unsafe and/or low quality food consumption has caused more than 200 foodborne diseases including diarrhea and cancer (WHO, 2015). Several conventional preservation methods such as drying, cooling, freezing, smoking, salting, canning, adding salt or sugar have been used extensively for reducing the microbial flora and/or preventing the growth of microorganisms in foods and extending the shelf life (Smith & Stratton, 2007). In recent years, novel food preservation strategies such as high-pressure, pulsed electrical field, irradiation, UV application, ultrasound treatment and ohmic or dielectric heating have gained importance in microbial inhibition (Lado & Yousef, 2002; Manas & Pagan, 2005; Pereira & Vicenta; 2010; Raso & Barbosa-Canovas, 2003). On the other hand, both conventional and novel techniques used for safe foods with extended shelf life, could lead to undesirable reactions, culminating in sensorial and/or textural problems in foods (Soliva-Fortuny & Martin-Beloso, 2003).

Food safety and extended shelf life can be ensured by adding preservatives to control the microbial growth (Negi, 2012). Food preservatives can be classified as synthetic or natural sources (Tiwari et al., 2009). However, synthetic preservatives such as benzoic acid, sorbate, hydrogen peroxide, sodium benzoate, sulfides (bisulfite, metabisulfite), nitrite, nitrate, propionic acid and antibiotics (tetracycline, penicillin and erythromycin) could be the cause of hives, itching, asthma, allergies, lung irritation, tumors, antibiotic resistance in human and also could have mutagenic and carcinogenic effects on metabolism (Oms-Oliu et al., 2010; Rangan & Barceloux, 2009; Rico, Martin-Diana, Barat, & Barry-Ryan, 2007). Although the usage of synthetic antimicrobials is approved in many countries, the recent trend is natural foods which contain no synthetic/chemical preservatives. An increasing number of consumers prefer fresh-like, tasty, natural, healthy, safe and functional food products (Wilcock, Pun, Khanonax, & Aung, 2004). Therefore, the consumer demands are increasingly focusing on minimally processed and safe foods produced by using natural preservatives (Allende, Tomas-Barberan, & Gil, 2006; Raso & Barbosa-Canovas, 2003; Señorans, Ibáñez, & Cifuentes, 2003). Furthermore, Western society appears to be experiencing a trend of 'green' consumerism, desiring fewer synthetic food additives and products which have a smaller impact on the environment. As a consequence, the plant based products have been gaining popularity as food preservatives in food industry (Rasooli, 2007).

The antimicrobial effect of various fruit and vegetable juices such as unripe grape, lemon, pomegranate, onion, leek and radish was declared in many studies, and the primary reasons for inhibition of microorganisms are concluded as organic acids and phenolic compounds in these materials (Karapinar & Sengun, 2007; Kıvanç,

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& Kunduhoğlu, 1997; Lucera, Costa, Conte, & Del Nobile, 2012; Saeed & Tariq, 2005). There are several organic acids like benzoic, capric, lactic, malic, tartaric, and acetic acid in fruits and vegetables (Raybaudi-Massilia, Mosqueda-Melgar, Soliva-Fortuny, & Martin-Belloso, 2009). For instance, it was reported that malic acid as minor and citric acid as major compounds which are effective on the inhibitory activity of lemon juice (Tomotake, Koga, Yamota, Kassu, & Ota, 2006). The pH and titratable acidity (tartaric acid %) values of grape juice were determined as 3.21–3.60 and 0.40–0.96%, respectively (Dani et al., 2007), while they were 2.91–2.98 and 2.48–3% for unripe grape berries, respectively (Hayoğlu, Kola, Kaya, Özer, & Turkoglu, 2009). Nikfardjam (2008) tested seven verjuice samples for titratable acidity (%) and the results were between 1.96 and 3.96%.

Antimicrobial effects of fruit and vegetable juices on pathogenic and/or saprophytic microorganisms were studied many times and the results indicated their importance as an alternative and natural antimicrobial source. Antimicrobial activity of raspberry juice against 7 bacteria strains, including *Salmonella* spp., *Shigella sonnei* and *Escherichia coli* was investigated, and it was declared that raspberry juice significantly reduced the growth of tested bacteria (Ryan, Wilkinson, & Cavanagh, 2001). Noni fruit juice has the maximum antibacterial activity against *Mycoplasma* spp. (*Myc. ermentans* ATCC 19989, *Myc. fermentans* P-140, *Myc. pneumoniae* and *Myc. penetrans* HF) which was inoculated (10^6 CFU/mL), and it completely inhibited the pathogens in 2 days (Rivera, Giono, Gonzalez, Rodríguez, & Cedillo, 2011). Aibinu, Adenipekun, Adelowotan, Ogunsanya, and Odugbemi (2007) evaluated the antimicrobial activity of lime juice on 7 Gram negative (*Serratia* spp., *S.* Paratyphi, *Sh. flexnerri*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Citrobacter* spp., *E. coli*), 2 Gram positive (*Staphylococcus aureus, Enc. feacalis*) and 3 anaerobe (*Clostridium* spp., *Bacteroides* spp., *Porphyromonas* spp.) microorganisms, and declared that the diameter of inhibition zones were between 9 and 24 mm; while Onyeagba, Ugbogu, Okeke, & Iroakasi (2004) reported that lime juice caused inhibition zones varying from 11 to 17 mm when treated on *St. aureus, Bacillus* spp. *E. coli* and *Salmonella* spp. Six pomegranate varieties were tested for their antimicrobial activity against seven bacteria strains (*Bacillus megaterium* DSM 32, *P. aeruginosa* DSM 9027, *St. aureus* Cowan 1, *Corynebacterium xerosis* UC 9165, *E. coli* DM, *Enc. faecalis* A10, *Micrococcus luteus* LA 2971), and three fungi (*Kluvyeromyces marxianus* A230, *Rhodotorula rubra* MC12, *Candida albicans* ATCC 1023). It has been observed that the pomegranate had an antimicrobial effect on all tested microorganisms. The diameter of inhibition zones were ranging in 13–26 mm, and the MIC values ranged between 30 and >90 μg/mL (Duman, Ozgen, Dayisoylu, Erbil, & Durgac, 2009). Antimicrobial effects of fresh garlic, onion, leek, red pepper, garden radish and horseradish juice on 11 bacteria (*Bacillus cereus, Bacillus subtilis, Enterobacter aerogenes, E. coli, K. pneumoni, Proteus vulgaris, P. aeruginosa, St. aureus, S.* Typhimurium, *Serratia marcescens* and *Vibrio parahaemolyticus*) (Kıvanç & Kunduhoğlu, 1997); and garlic, lemon, unripe papaya and pomegranate juice on 19 different species of bacteria (*B. megaterium, Bacillus brevis, Bacillus firmus, B. subtilis, Bacillus pumilus, Bacillus pasteurii, St. aureus, St. haemolyticus, M. lutues, Mc. sedenterius, Mc. varians, Mc. nishinomiyaensis, Mc. roseus, E. coli, Neisseria sicca, N. mocosa, N. lactamicus, N. dentrificens* and *N. canis*) were analyzed (Saeed & Tariq, 2005), and the inhibition zones were between 12 mm–30 mm and 17.75 mm–32.66 mm, respectively. There are limited studies on antimicrobial properties of unripe grape products. Unripe grape products were tested against two *S.* Typhimurium strains (*S.* Typhimurium NRRL-B-4420 and *S.* Typhimurium CCM 583) at the level of approximately 10^6 CFU/mL on cucumber and parsley samples. The unripe grape products inhibited the cell number of *S.* Typhimurium NRRL-B-4420 from 6.42–6.92 log CFU/g to 5.02–5.76 log CFU/g for cucumber, and from 6.97–6.99 log CFU/g to 5.06–5.68 log CFU/g for parsley in the beginning of the application. The immediate reduction was also investigated for *S.* Typhimurium CCM 583 from 6.16–6.34 log CFU/g to 4.71–5.33 log CFU/g on cucumber, and from 6.15–6.54 log CFU/g to 4.36–5.72 log CFU/g on parsley (Karapinar & Sengun, 2007). The minimum inhibitory concentrations (MICs) of unripe grape samples on *B. cereus, E. coli, Listeria monocytogenes, S.* Typhimurium, and *St. aureus* were determined and it was found that the MIC values of the samples ranged from 1:2 (50%) to 1:16 (6.25%) (Karabıyıklı & Öncül, 2016).

There are lots of studies on the metabolic activity and effective components of plant based antimicrobials which inhibit or control the growth of target microorganism (Entani, Asai, Tsujihata, Tsukamoto, & Ohta, 1997; Gwayali & Ibrahim, 2014; Lopez & Belloso, 2008; Raybaudi-Massilia et al., 2009; Soomro, Masud, & Anwaar, 2002; Tajkarimi, Ibrahim, & Cliver, 2010; Tiwari, Bharti, Kaur, Dikshit, & Hoondal, 2005). In most of these studies, organic acids and/or phenolic contents of the fruit or vegetable juice were determined as the effective components for antimicrobial activity. The mechanism of antimicrobial activity was associated with the degradation of the cell wall, inhibition of cell membrane and/or proteins and depletion of proton motive force (Kalembra & Kunicka, 2003; Negi, 2012). The organic acid and phenolic content of unripe grape products was determined and it was indicated that these products contain a high amount of these contents (Dani et al., 2007; Hayoğlu et al., 2009; Nikfardjam, 2008; Öncül & Karabıyıklı, 2015). In a previous study, the MIC values of unripe grape products against foodborne microorganisms were tested in unneutralized and neutralized unripe grape samples and the unneutralized samples were effective against all tested pathogens. So, it was detected that the inhibitory effects of unripe grape products mainly depends on the acidity. On the other hand, neutralized samples were also effective against *B. cereus* and *St. aureus* and it was concluded as the antimicrobial effect of neutralized samples was related with their phenolic contents (Karabıyıklı & Öncül, 2016). In another research, the inhibitory effect of neutralized unripe grape products on foodborne pathogen due to their rich phenolic properties and it was indicated that the products could have antimicrobial effects on foodborne pathogens (Öncül & Karabıyıklı, 2016).

Because of this reason, unripe grape products, used as acidifying and flavoring agents in several meals, salads, appetizers and also used as an ingredient in the production of various drinks are important alternatives with their rich antioxidant and organic acid contents. In another aspect, the unripe grape products produced from grape berries that have lower quality as a table grape could be important for natural and functional food production. Because they could be convert to a valuable industrial product such as a salad dressing or a natural antimicrobial and antioxidant additive with their rich contents. Thus, in this study it was aimed to investigate antimicrobial efficiency of unripe grape products on foodborne pathogens.

2. Materials and methods

2.1. Unripe grape products

Ten unripe grape products (five verjuice and five unripe grape sauces) were analyzed in this study (Table 1). Four of these samples were supplied to represent traditional products from local producers and two of them were purchased from local markets to represent industrial ones. The rest of the products were produced in a laboratory, based on the traditional methods.

The main difference in production of verjuice and unripe grape