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Optimization of ultrasound, vanillin and pomegranate extract treatment for shelf-stable unpasteurized strawberry juice

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ABSTRACT

Optimum combination of ultrasound, vanillin and pomegranate extract to improve quality of strawberry juice was determined using response surface methodology. Samples were stored at 5 °C for 14 days. The optimal conditions to simultaneously minimize native microflora, maximize nutritional parameters and minimize the impact on sensory quality resulted in: 7.5 min of ultrasound treatment, pomegranate extract concentration of 360 μg/mL and vanillin concentration of 0.925 mg/mL. A new batch of strawberry juice was treated at these optimal conditions and stored for validation of the optimization and to evaluate the performance of the optimum treatment on quality parameters throughout storage. Furthermore, a second batch of juice was inoculated with *Escherichia coli* O157:H7 and processed at optimal conditions to evaluate the effectiveness of the treatment on the pathogen survival. The native microflora of the juice, as well as inoculated pathogen, decreased significantly using the proposed hurdle technologies, with no impact on sensory parameters. Ascorbic acid retention was slightly decreased by the optimum treatment; however, DPPH and polyphenolic compounds were significantly higher than those in untreated sample. Overall, a combination of ultrasound, vanillin and pomegranate extract showed interesting potential to enhance quality and safety of strawberry juice, extending the shelf-life of the product.

1. Introduction

Strawberry juice is one of the most popular fruit juices consumed around the world due to their appreciable organoleptical attributes such us attractive color, good aroma and taste of sweet and sour mouth. Additionally, both cloudy and clear juices are considered healthy strawberry products and they are consumed as natural antioxidant drinks (Cao et al., 2012).

In the fruit juice industry, inactivation of microorganisms and enzymes is usually carried out through thermal processing generally at 70–121 °C for 30–120 s (Cao et al., 2011; Zhou, Wang, & Liao, 2009). It is widely known that thermal treatments seriously affect the quality of juices, presenting loss of nutritional components and undesirable changes in sensorial quality. In particular, strawberry juice is highly susceptible to this processing (Cao et al., 2012). Therefore, the application of non-thermal techniques to strawberry juice is gaining popularity. This tendency is also motivated by the increasing consumer demand for minimally processed food products with sensory and nutritional characteristics similar to fresh product.

Sonication is a non-thermal food preservation technique, which uses ultrasound for inactivating food spoilage microorganisms and enzymes, generally at a frequency of 20–40 kHz. The physical phenomenon of cavitation that occurs when applying ultrasound is considered the main cause of microbial inactivation (Vercet, Sanchez, Burgos, Montañés, & López, 2002). Cavitation can break molecules or particles through different mechanisms that can occur individually or combined, generating free radicals in the water sonolysis (H^+ and OH^-), that are responsible of producing oxidative damage, leading to microbial inactivation (Mañas & Pagan, 2005).

Another emerging non-thermal technology is the use of natural compounds as food preservatives, in response to consumers concern about the safety of synthetic compounds used in food.

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Among natural compounds, vanillin (4-hydroxy-3-methoxybenzaldehyde) is a non-toxic and GRAS phytochemical used as food flavoring agent and obtained from vanilla beans. Furthermore, it is known to be antimycotic and bacteriostatic (Fitzgerald, Stratford, & Narbad, 2003). Like many other low-molecular weight phenolic compounds, vanillin displays antioxidant and antimicrobial properties and hence has the potential for use as a food preservative (Tomadoni, Cassani, Moreira, & Ponce, 2015). Fruit extracts have also been identified as novel candidates in the search of natural compounds with antimicrobial properties. In particular, many research studies have focused on the antimicrobial activity of pomegranate (*Punica granatum*) extracts.

The use of these natural compounds as food antimicrobials may have a negative effect on the sensory characteristics of the final product, because of their strong flavor when applied at high concentrations. As a solution to this problem, hurdle technology have been proposed (Leistner, 2000) allowing to reduce the intensity of each hurdle in order to avoid the loss of nutritional and sensory value while maintaining the stability and safety of the product.

In order to optimize the levels of various hurdles, multivariate statistical techniques such as response surface methodology (RSM) have been suggested. RSM is a powerful mathematical tool that presents the advantage of efficiently exploring a particular region on selected ranges of independent variables at low cost, reducing the number of experimental runs (Kuehl, 2000). However, when several responses must be optimized at the same time, the independent optimization of each one can lead to conflicting results, i.e., improving one response may have an opposite effect on another one, failing in the finding of the best solution for all responses simultaneously (Costa, Lourenço, & Pereira, 2011). For these cases, the Desirability function could be a complementary tool to resolve this conflict, allowing finding the optimal experimental conditions to successfully satisfy the optimization of all responses (Costa et al., 2011).

Therefore, the objectives of this study were: (a) to optimize the intensity or levels of three different non-thermal hurdle technologies (vanillin, pomegranate extract and ultrasound) applied on strawberry juice in order to simultaneously improve microbiological, sensory and nutritional quality; (b) to evaluate the effect of the optimum treatment on quality parameters of strawberry juice throughout storage time; and (c) to study the performance of the optimum treatment against a contamination with *Escherichia coli* O157:H7.

2. Materials and methods

2.1. Plant material and juice extraction

Strawberries (*Fragaria x ananassa*) were grown and harvested in Sierra de los Padres, Mar del Plata, Argentine. The strawberries were destemmed and the juices were prepared with a commercial juice extractor. Once the treatments were applied, the strawberry juices were stored for 14 days in sterile polypropylene flasks at 5 °C.

2.2. Treatments and experimental design

The application of hurdle technology on strawberry juice was studied by combining 3 different non-thermal preservation techniques: ultrasound, pomegranate extract and vanillin.

The ultrasound treatments were performed at 40 kHz frequency, using an ultrasonic cleaning bath (TestLab, Argentine), with a rectangular container (290 × 150 × 150 mm) and a maximal tank capacity of 6.5 L. The 40 kHz transducers at the bottom transmit ultrasound waves of 180 W from bottom to above. Temperature in the ultrasonic bath was monitored at 20 ± 1 °C. The juice level in the flasks was 2 cm below the water surface in the ultrasonic bath. The height of the bottom surface of the flasks from the bottom surface of the tank (face of transducers) was 4 cm.

Pomegranate extract used in this study was purchased from PureBulk, USA (35% ellagic acid, 19% gallic acid, 10% punicalagin A, 5% punicalagin B, 2% caffeic acid). Vanillin (>97%) was purchased from Sigma Aldrich (St. Louis, MO, USA). The natural preservatives were applied directly into the juice samples.

Each technology was evaluated at 3 different levels according to the conditions established in the experimental design (Table 1). The ultrasonic processing times selected for this study were: 0, 15 and 30 min, according to previous unpublished experiments. Pomegranate extract was applied at 0, 180 and 360 μg/mL of strawberry juice, while vanillin was applied at 0, 0.625 and 1.25 mg/mL of juice. These concentrations were selected according to Tomadoni, Viacava, Cassani, Moreira, and Ponce (2016).

Response Surface Methodology (RSM) with a Box-Behnken (BB) design was used to establish the effects of the three independent processing parameters namely ultrasonication time (X_1, min), pomegranate concentration (X_2, μg/mL) and vanillin concentration (X_3, mg/mL) on the selected response variables. Fifteen experimental runs were carried out combining the 3 levels of each variable as shown in Table 1, as is suggested by the BB design. Samples were stored at 5 °C for 14 days. On day 14, responses were measured for each trial and a second-order polynomial model (Eq. (1)) was fitted to each response variable using the least-squares regression method.

$$Y_n = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{2} \sum_{j=2, j>i}^{3} \beta_{ij} X_i X_j + \sum_{i=1}^{3} \beta_{ii} X_i^2 \quad (1)$$

where Y_n is the predicted response (Y_1:yeast and molds counts, Y_2: psychrophilic bacteria counts, Y_3: DPPH radical scaveging activity, Y_4: total polyphenol content, Y_5: ascorbic acid, Y_6: hue angle, Y_7: off-odor), β_0 is the model constant, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, $\beta_{ij}$is the coefficient for the interaction effect, and X_i is a dimensionless coded value of the independent variable, x_i.

2.3. Response variables

The effects of combined treatments using ultrasound, vanillin and pomegranate extract applied on strawberry juice were

Table 1
Box-Behnken experimental design matrix.

Exp no.	Independent variables			Coded independent variables		
	x_1 US (min)	x_2 PE (μg/mL)	x_3 V (mg/mL)	X_1	X_2	X_3
1	0	180	0	−1	0	−1
2	0	180	1.25	−1	0	1
3	30	180	0	1	0	−1
4	30	180	1.25	1	0	1
5	15	0	0	0	−1	−1
6	15	360	0	0	1	−1
7	15	0	1.25	0	−1	1
8	15	360	1.25	0	1	1
9	0	0	0.625	−1	−1	0
10	30	0	0.625	1	−1	0
11	0	360	0.625	−1	1	0
12	30	360	0.625	1	1	0
13	15	180	0.625	0	0	0
14	15	180	0.625	0	0	0
15	15	180	0.625	0	0	0