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FULL PAPER

Optimization of heat treatment and pH of red and white pear cactus [*Opuntia ficus-indica* (l.) *mill.*] fruit juice using response surface methodology

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Abstract

The objectives of the study were to determine the effect of heating time and temperature and pH level on the quality of cactus pear juice and to optimize the thermal treatment conditions and the pH for best results using two (red and white) varieties. Response surface methodology was used to optimize the heat treatment conditions and the pH. The heating time and temperature and pH significantly ($p<0.05$) affected the ascorbic acid content, sensory attributes and microbial load. Using graphical optimization, the best combinations for the red prickly juice were 5.5 to 14 mins and 62°C to 82°C with pH of 5.2 or pH 3.3 to 5.0 and temperature of 62°C to 92°C for 15 mins. For white prickly juice, the optimal ranges were 18.7 to 22.6 mins and 83.9°C to 93.4°C with pH of 5.2 or pH of 3.2 to 5.0 and temperature 93.5°C to 108°C for 15 mins. Using numerical optimization, the optimal conditions were 19.2 mins, 100°C and pH of 4 with a desirability value of 0.774 and 22 mins, 100°C and pH of 3.6 with the desirability value of 0.865 for red and white prickly pear juice, respectively.

1. Introduction

Prickly pear (*Opuntia* spp.) is a wild fruit that grows under arid conditions. It is also found in Southern Italy, Central and South America, Israel, South Africa, Sicily and throughout warm and sub-tropical climates (Saenz and Sepulveda, 2001; El-Gharras *et al.,* 2006). The cactus plant grows abundantly in Ethiopia and has adapted perfectly to the arid zones which are characterized by droughty conditions, erratic rainfall, and poor soils subjected to erosion. It contributes in times of drought, serving as a life-saving crop for both humans and animals. It is estimated to cover about 36,000 hectares of land or about 11.2% of the total rangeland of the Tigray region of Ethiopia, with the largest proportion being found in Eastern Tigray (Tesfay, 2004). About 105.26 tons of cactus fruit is consumed daily from June to August in most urban centres in the region, a volume higher than other tropical fruits (Orange, Mango and banana) and has become the major income and food source for about four months of the year. The edible parts of the cactus tree include the fleshy stem also known as a cladode, cactus pad, cactus stem, nopalito (young pad), vegetable cactus, or cactus leaf and the fruits which are highly appealing and nutritious (Rodriguez-Felix, 2002). It is very much part of the culture and livelihood of the people. However, due to the lack of knowledge of its full utilization and processing potential, the fruit is wasted in large quantities during peaks of the harvest period (Mondragon and Tegegne, 2006).

The increasing market demand, the seasonal availability and the increasing knowledge of its nutritional value (Moßhammer *et al.,* 2008; El-Samahy *et al.,* 2009; El-Kharrassi *et al.,* 2016; Melgar *et al.,* 2017; Karabagias *et al.,* 2019) and the presence of disease combating essential photo chemicals present in cactus fruit and stem (El-Gharras *et al.,* 2006; Shedbalkar *et al.,* 2010; Melgar *et al.,* 2017) has stimulated a great interest for food scientists and processing technologists to come up with processed foods to satisfy the need for diversification, increased shelf-life, and convenience of the fruit (Saenz, 2000; El-Gharras *et al.,* 2006; El-Samahy *et al.,* 2008; Kgatla *et al.,* 2011). The application of cactus in the food industry had been reviewed by Shedbalkar *et al.* (2010)

The fairly high sugar content and low acidity of the prickly pear fruit give it a delicious, sweet taste, but at the same time, the high total viable count makes it very

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susceptible to microbial invasion and limits its shelf-life in the fresh state (Sepulveda and Saenz, 1990; Karabagias *et al.,* 2019). This calls for subjecting the fruits to some processing and preservation techniques. Therefore, it is essential that some form of thermal preservation has to be employed when these products are packed. Other preservation methods reported included sugar adjustment to enhance the level of sweetness and the water activity and acidification to lower the pH (Gracía-Gracía *et al.,* 2015), different packaging techniques (Karabagias *et al.,* 2019) and thermo-ultrasound technique (Cruz-Cansino *et al.,* 2016). However, the possibility of combining thermal treatment and pH adjustment has not been reported. The objectives of the study were to determine the effect of heating time and temperature and pH level on the sensory and microbial quality of cactus pear juice and to optimize the thermal treatment conditions and the pH for the best results.

2. Materials and methods

2.1 Raw material preparation

Mature cactus pears (*Opuntia ficus-indica*) were harvested by hand-picking the fruits to reduce mechanical damages from "Erob" and "Adigrat" villages of the Eastern Tigray region, north Ethiopia. Two most commonly known cactus pear cultivars, white (*Sulhuna*) and red (*Gera'o*) (Mulugeta, 2007), were collected from the villages and transported to the Dry land Crop and Horticultural Science laboratory at Mekelle University.

2.2 Juice extraction

Juice extraction was performed after one day of storage of the prickly pear fruits. The cactus pear fruits were washed thoroughly and then manually peeled with a knife. The juice was extracted using a juice extractor (Juicer, Philips, Belgium) and the seeds were separated using an automatic sieve. Prior to the heat treatment and pH adjustment, the juice was homogenized in order to take a representative sample for the determination of total soluble solids, titratable acidity, pH and ascorbic acid contents. The remainder juice was then kept for further treatment and analysis. The ^{o}Bx adjustment was done by adding white sugar to the mass of the prickly pear juice until 17^{o}Bx was attained (Kgatla *et al.,* 2010).

2.3 Juice acidification and heat treatment

Acidification was achieved according to Saenz and Sepulveda (2001) but with slight modification by mixing orange juice and citric acid in volume ratio to the prickly pear juice until it was adjusted to 5.2, 4 and 3.2 pH units. The acidified prickly pear juice samples were then filled in equal portions into sterilized 300 mL bottles and tightly closed to prevent oxidation of the samples. Heat treatment was applied by submerging the bottled samples of prickly pear juice in a water bath set at different time-temperature combinations (Table 1) except for a single treatment sample which was subjected to an autoclave (108°C). After the treatment, the samples were cooled as soon as possible in an ice bath for about 10 mins and then kept in the refrigerator at 5°C for 24 hrs before further analysis.

2.4 Analytical methods and measurements

2.4.1 Juice percentage

A total of twenty cactus pear fruits were randomly selected, weighed and recorded. The fruits were manually peeled with a knife. The peeled fruits were then placed in a juice extractor to separate the seeds from the juice. The juice was weighed and the pulp percentage was determined according to El-Samahy *et al.* (2006).

$$\text{Pulp}(\%) = \left(\frac{\text{Weight of the juice pulp}}{\text{Initial weight of the sample fruits}} \right) \times 100$$

2.4.2 Total soluble solids

The total soluble solids (TSS) content of fruit juices was determined using a handheld refractometer with a range of 0 to 32^{o}Bx and a resolution of 0.2^{o}Bx. The percentage of TSS was obtained from a direct reading of the hand refractometer, which was washed with distilled water and dried with tissue paper prior to its use for the next sample measurement.

2.4.3 pH-value and total titratable acidity

The pH value of the cactus pear fruit juice was measured using a digital pH meter with a glass electrode after homogenizing 10 mL of the fruit juices in 90 mL of distilled water. The pH meter was standardized and calibrated with pH 7.0 and 4.0 standard solutions. The temperature used in measuring the results was 20±1°C (Karabagias *et al.,* 2019). The total titratable acidity was determined by taking 10 mL cactus pear juice sample in distilled water and then titrating it against 0.01N NaOH using phenolphthalein as an indicator. Finally, the result was expressed as the percentage of citric acid which is the predominant acid in cactus pear fruit (El- Kharrassi *et al.,* 2016).

2.4.4 Ascorbic acid

Ascorbic acid (AA) was estimated by the 2, 6-Dichlorophenol indophenol visual titration method according to AOAC (1970). The reagents used for the estimation of vitamin C were metaphosphoric (6%), standard acid solution acid 2, 6-Dichlorophenol indophenol dye.

2.5 Sensory evaluation

A total of fifty untrained panellists were randomly selected from the staff of DCHS and students in Mekelle University who were familiar with the fruit. The prickly pear juice products are scored according to a seven-point Hedonic scale (7 = extremely like, 6 = like very much, 5 = like slightly, 4 = neither like nor dislike, 3 = dislike slightly, 2 = dislike very much and 1 = dislike extremely) while the sweetness was given on a five-point rating scale (5 = very sweet, 4 = moderately sweet, 3 = just sweet, 2 = less sweet and 1 = not sweet) (Gacula, 1984).

2.6 Microbial analysis

Microbial analysis was done following the procedure of (Jay, 2000). Cactus pear juice samples (10 mL) were taken and diluted with 90 mL of 0.1% peptone water solution. Serial dilutions were done in the range of 10^{-1} to 10^{-6} using a sterilized pipette for each dilution by taking a sample of 1 mL to every 9 mL of 0.1 peptone water. After each serial dilution, the test tube of the dilutions was closed and overtaxed using an electronic vortexes device. The samples were homogenized and appropriate dilutions were plated in duplicate on pre-dried surfaces of respective media for the microbial count: total aerobic bacteria (TAB) were counted on Plate Count Agar (PCA) after incubation at 32°C for 48 hrs; Violate Red Bile Agar (VRBA) was used to count coliforms after incubation for 48 hrs at 32°C. Yeasts and moulds were counted on Sabouraud Dextrose Agar (SDA) incubated at 25-28°C for 2-5 days. Microbial shelf life was assessed after 30 and 60 days of storage under refrigeration temperature of 5°C

In order to avoid the acidity of the processed juice, 5 mL original sample was neutralized by adding an adequate amount of 0.1 N NaOH and then the neutralized sample (0.1 mL) was spread onto the surface of a different media. Automatic pipettes with sterile disposable tips were used to transfer the neutralized sample (0.1 mL) to the surface of the appropriately labelled duplicate plates. The inoculums were spread evenly over the entire surface of the plates using a sterile bent glass spreader inside the UV-light cabinet. After incubation, the colonies were counted using a digital colony counter.

2.7 Experimental design and data analysis

Central composite design (CCD) was used involving three factors and five-level combinations. The three factors were processing temperature (62, 70, 85, 100 and 108°C) time (7, 10, 15, 20 and 23 mins) and pH (3.2, 4, 5.2, 6.3 and 6.9). The CCD contained a total of 20 experimental trials (8 factorial points, 6 axial/star points and 6 central points). Optimizations of the independent variables (temperature, time and pH) were carried out using Design-Expert version 7 (Stat-Ease) depending on the responses obtained. The upper and lower levels of the independent variables in this study were based on literature (Saenz, 2000) and preliminary tests. A second-order response surface equation shown below was used to describe the effect of variables on the response variables (Montgomery, 2001)

$$Y = \beta_0 + \sum_{i=1}^{n}\beta_i X_i + \sum_{i=1}^{n}\beta_{ii} X_i^2 + \sum_{i_i<j}^{n}\sum_{j}^{n}\beta_{ij} X_i X_j + \sum_{i}^{n}\sum_{j}^{n}\sum_{k}^{n}\beta_{ijk} X_i X_j X_k + e$$

Where Y = the predicted variable, $X_{i,j,k}$ = the factors, and β's = the coefficients of the linear, quadratic, and cubic terms of the model.

2.8 Optimization

Graphical optimization was used to determine the region of optimal combination of the time, temperature and pH. The contour plots of different parameters were superimposed to come up with an overlay plot that was used to determine the optimal region based on the optimization criteria. Numerical optimization was used where each response y_i was converted into desirability function d_i that varies over the range 0 to 1 (Montgomery, 2001). If the objective or target T for the response Y is a maximum value, the individual desirability functions are structured as:

$$d = \begin{cases} 0 & Y < L \\ \left(\frac{Y-L}{T-L}\right)^r & L \le Y \le U \\ 1 & Y > T \end{cases}$$

If the target T for response y is minimum:

$$d = \begin{cases} 1 & Y < T \\ \left(\frac{U-Y}{U-T}\right)^r & T \le Y \le U \\ 0 & Y > U \end{cases}$$

Where r is weight, L is lower value and U is the upper value.

Then, for *m* responses, the design variables are chosen to maximize the overall desirability D.

$$D = (d_1 \times d_2 \times d_3 \cdots d_m)^{\frac{1}{m}}$$

3. Results and discussion

3.1 Physicochemical properties of red and white prickly pear fruit juice

3.1.1 Total soluble solids and sugars

The total soluble solids were 13.95°Bx for the red and 12.56°Bx for the white raw cactus pear juice. The difference between these two cultivars in their TSS contents may be related mainly to the genetic constitution while many factors, such as environmental effects could also have a contribution (Mulugeta 2007). These results are in agreement with the results of the

TSS of the cactus fruits studied by Inglese *et al.* (2002) which were found to be in the range of 10.23 to 14.66° Bx. TSS values of 12.56, 13.67 and 14.89°Bx have been reported for green, half-ripe, matured prickly fruit juice, respectively (El-Gharras *et al.,* 2006). The TSS contents are important factors in the production of fruit juice. It is well established that the higher the total solids, the better the quality of the juice (El-Samahy *et al.,* 2008).

Sugars are the major soluble solids of the cactus pear fruits. The total sugars of red and white cactus pear pulp were 145.3 g/kg and 108.6 g/kg, respectively. The varietal difference in total sugar may be due to the concentration of nutrients which could be attributed to their genetic makeup and environmental factors (El-Gharras *et al.,* 2006; Dehibi *et al.,* 2014). These results were also comparable with other studies that reported total sugars in the range of 97 g/kg to 165.7 g/kg (El-Samahy *et al.,* 2006; Dehibi *et al.,* 2014; Mohamed *et al.,* 2014)

3.1.2 Titratable acidity and pH

The titratable acidity of red and white pear cactus fruit were 0.04% and 0.03% (as citric acid), respectively. These values are comparable with those reported for Moroccan prickly pear (Dehbi *et al.,* 2014; El-Gharras *et al.,* 2016) but lower than those reported by Mohamed *et al.* (2014) which was 0.22±0.001. The pH values of red and white pear cactus fruit were 6.15 and 6.29, respectively. Titratable acidity and pH value would be of great importance because the ratio of total soluble solids to acidity will affect flavour (Kgatla *et al.,* 2010). This result agreed with other research reports on prickly pear fruit and juice pH where the reported range was 5.72 to 6.38 (Mohammed *et al.,* 2014; ElGharras *et al.,* 2016; Krabagias *et al.,* 2019). A lower pH value ranging from 3.3 to 4.7 has been reported (Kgatla *et al.,* 2010; El Kharrassi *et al.,* 2016) for Moroccan varieties.

3.1.3 Ascorbic acid

Ascorbic acid (vitamin C) contents of red and white cactus pear juice were 30.16 and 27.99 mg/100 g fresh weight basis, respectively. The difference in ascorbic acid of the two cultivars may be their genetic difference as well as the high sugar concentration in the red cactus pear fruit which could serve as a substrate for the synthesis of acids during ripening (El-Guizani *et al.,* 2012). These results agree with earlier reports where ascorbic acid content ranged from 20-40 mg/100 g (Sepulveda and Saenz 1990; Gurrieri *et al.,* 2000; Kader 2002). Karabagias *et al.* (2019) reported ascorbic acid values ranging from 46.36 to 78.11 mg/mL for wild prickly pear juice. Lower values of ascorbic acid ranging from 2.3 g/ 100 g to 24 mg/ 100 g had also been reported (Mohamed *et al.,* 2014; Melgar *et al.,* 2017). Ascorbic acid retention is a good indicator of high food quality because of its nutritional value as well as its contribution to the product's appearance and palatability.

3.1.4 The juice percentage

The mean juice percentages of red and white cactus pear were 54.18% and 49.41%, respectively. The difference might be varietal and/or environmental factors. Plants growing on fertile and moist soil could have higher juice contents. As the samples were randomly taken from different rocky and sandy soils of the mountains, there is a probability that plants grown on dry and less fertile soil could have a low juice yield. However, these results are comparable with Piga *et al.* (2000) who reported pulp percentage in the range of 43-57%. Pulp percentages ranging from 51.93 to 54.73%, 52.8 to 55.97% and 53.99 to 56.36% have been reported for green, half-ripe and ripe Moroccan prickly pear fruit, respectively (El-Gharras *et al.,* 2006).

3.2 Effect of process variables on quality cactus pear juice

3.2.1 Ascorbic acid

The heating time and temperature and pH significantly ($p<0.05$) influenced the ascorbic acid. Among the treated samples, the maximum AA values were 30.06±0.45 mg/100 g and 28.72±1.56 mg/ 100 g treated at 85°C, 15 mins and pH of 3.2 for red and white varieties, respectively. The minimum values were 16.60±0.32 mg/100 g and 16.39±0.75 mg/100 g treated at 108°C, 15 mins and pH of 5.2. Compared to the initial fresh juice the ascorbic acid reduction at these temperature-time-pH combinations were 45.1% and 41.4% for the red and white cultivars, respectively. The trend of changes in ascorbic acid as a function of heat treatment is presented in the response curves (Figure 1). The difference could be due to the initial concentration of nutrients in the juice and their difference in response to the treatments. A decrease in AA with an increase in temperature and heat treatment (Kgatla *et al.,* 2011; Cruz -Cansino *et al.,* 2016) and acidification (Kgatla *et al.,* 2011) have been reported. Vitamin C reductions due to processing heat levels have also been reported in beverages (El-Samahy *et al.,* 2008; Shedbalkar *et al.,* 2010).

The relationship between AA contents heat treatment time and temperature and pH was adequately described by a second-order response surface polynomial equation. The degree of fit and the values of the model coefficients are presented in Table 1 and Table 2. All the linear terms significantly ($p<0.05$) affected the AA content.

Table 1. Model coefficients and the degree of fit of the model fitted to the data for red prickly pear (*Opuntia ficus indica*) juice

Model coefficients	Sensory and microbial (log_{10} CFU/mL) quality attribute										
	AA	Flavour	Colour	Sweetness	Overall Acceptability	TAB30	TAB60	TYM30	TYM60	TCO30	TCO60
β_0	30.85	13.08	100.41	90.54	+86.36	-0.39	+3.12	+4.96	+2.73	+3.08	-0.19
β_1	0.775	-0.107	-2.37	-2.14	-2.13	0.092	+0.063	+0.05	+0.06	+0.07	+0.09
β_2	-0.455	-0.956	-2.48	-1.12	-1.26	0.108	+0.056	-0.141	-0.015	+0.076	+0.51
β_3	-8.91	-1.613	-11.65	-13.81	-11.52	-0.33	-1.008	-1.312	-0.655	-1.148	-0.20
β_{12}	0.012	0.011	0.058	0.022	0.031	-1.07E-003	-1.32E-003	-1.52E-003	-9.33E-004	-1.15E-003	-6.54E-003
β_{13}	0.013	0.038	0.315	0.367	0.33	-4.64E-003	-2.39E-003	+6.16E-003	+2.75E-003	+6.30E-003	-4.20E-003
β_{23}	2.61E-003	0.259	0.149	0.126	+0.089	-5.65E-003	-5.00E-003	+2.82E-003	+6.52E-003	+3.26E-003	-0.062
β_{11}	-6.95E-003	-5.17E-004	0.013	0.012	+0.012	-4.43E-004	-3.17E-004	-5.08E-004	-5.19E-004	-6.78E-004	-4.26E-004
β_{22}	-0.029	-7.32E-003	-9.98E-003	-4.05E-003	-8.74E-003	-7.20E-004	1.53E-003	+7.54E-003	+7.55E-004	-1.72E-003	-2.8E-003
β_{33}	0.571	-0.163	-0.226	-0.212	-0.217	+0.086	0.13	+0.08	+0.037	+0.060	+8.84E-004
β_{123}		-3.17E-003	-1.85E-003	-1.68E-003	-1.17E-003						
β_{112}		+3.76E-005	-2.87E-004	-7.49E-005	-1.37E-004						
β_{113}			-1.72E-003	-2.0E-003	-1.94E-003						
R^2	0.904	0.859	0.905	0.834	0.898	0.857	0.839	0.885	0.893	0.886	0.873
R^2_{adj}	0.817	0.665	0.741	0.549	0.724	0.728	0.694	0.782	0.797	0.782	0.732
p-value	0.0005	0.0220	0.0156	0.0812	0.0190	0.0033	0.0056	0.0012	0.0009	0.0012	0.0057
Lack of fit	0.637	0.0023	0.0005	0.0065	0.0017	0.3798	0.2154	0.2932	0.0017	0.0089	0.0038

Table 2. Model coefficients and the degree of fit of the model fitted to the data for white prickly pear (*Opuntia ficus indica*) juice

Model coefficients	Sensory and microbial (log_{10} CFU/mL) quality attribute										
	AA	Flavour	Colour	Sweetness	Overall Acceptability	TAB30	TAB60	TYM30	TYM60	TCO30	TCO60
β_0	61.07	+5.78	9.05	+0.07	9.56	5.014	-1.70	-3.90	-15.65	-1.166	5.74
β_1	0.205	-0.044797	-0.104	-0.068	-0.12	-0.032	0.125	0.196	0.303	0.135	-0.021
β_2	-1.228	0.085	-0.14771	-0.021	-0.092	+0.07	0.192	0.140	0.683	-0.057	+0.078
β_3	-10.21	-0.51765	-0.224	1.06849	+0.158	-0.317	-0.418	-0.66	0.60998	-0.322	-0.902
β_{12}	8.38E-003	1.07E-003	+1.12E-003	9.05E-004	+1.75E-003	-1.84E-003	-4.36E-004	-3.02E-003	-4.49E-003	9.14E-004	-1.07E-003
β_{13}	-0.0104	-1.45E-003	-5.87E-003	-1.45E-003	-1.95E-003	3.63E-003	5.89E-003	+3.20E-003	1.84E-003	3.47E-003	+0.014
β_{23}	+0.0102	-0.012	+8.97E-004	-0.014	-8.04E-003	+0.015	-0.019	9.08E-003	0.011	0.016	+6.59E-004
β_{11}	-2.57E-003	+4.44E-004	+3.77E-003	4.85E-004	+7.96E-004		-1.06E-003	-1.19E-003	-1.56E-003	-1.19E-003	-3.38E-004
β_{22}	7.53E-003	-2.11E-003	+0.07	5.60E-004	+1.84E-005		-2.48E-003	1.065E-003	-0.013	-5.14E-003	-7.04E-004
β_{33}	+0.92143	+0.063	0.88	-0.079			+0.021	0.0301	-0.077	-9.46E-003	-0.014
β_{123}				1.74E-004							
β_{112}				-8.43E-006							
β_{113}											
R^2	0.844	0.864	0.884	0.602	0.833	0.926	0.888	0.892	0.841	0.901	0.934
R^2_{adj}	0.704	0.742	0.780		0.712	0.891	0.788	0.795	0.697	0.813	0.874
p-value	0.0048	0.0026	0.0012	0.4579	0.0023	< 0.0001	0.0011	0.0009	0.0054	0.0006	< 0.0001
Lack of fit	0.3069	0.9253	0.0545	0.0032	0.4833	< 0.0001	0.1593	0.3985	0.2767	0.0032	0.0423

3.3 Sensory profile

3.3.1 Flavour

The results indicated that temperature, time and pH influenced the flavour score significantly (p<0.05). The highest flavour mean scores were 6.43±0.3 and 6.7±0.12 ("Like very much") for juice processed at 100°C, 20 mins and a pH of 4 and the lowest values were 3.41±0.52 and 3.54±0.2 ("neither like nor dislike" to "slightly dislike") for the non-treated fresh cactus pear juice of white and red varieties, respectively. These scores might be due to the bitter and taint the flavour of the fresh juice or the absence of the heat treatment resulting in no extraction of flavour producing compounds.

For the red prickly pear juice, the score increased with an increase in temperature and time until 15 mins of heat treatment to a score of 5.28 followed by a decrease to 4.78 (Figure 1). For the white cactus pear, the flavour score increased with an increase in temperature whereas the change was insignificant with time and pH (Figure 2). Kgatla *et al.* (2010) reported that non-treated prickly pear juice had a bitter flavour whereas treated juice had a sweet taste. They also reported that heat-treated prickly pear juice samples scored low in the aroma intensity rating sensory profile, which may be attributed to the destruction of the pigments responsible for the aroma. The character-impact compound (unique flavour substances) for a particular flavour or aroma is a unique chemical substance that provides its principal sensory identity. Often, character impact is elicited by a synergistic blend of several aroma chemicals which in turn are dependent on genetic, environmental, cultural practice or processing (Kader, 2002). The relationship between temperature, time and pH and flavour score was adequately described by a reduced cubic response surface polynomial. The values of the model coefficients and the degree of fit are presented in Table 1 and Table 2. For the red variety, the linear terms of temperature and pH and the quadratic terms of pH and the three-way interaction have a significant (p<0.05) influence.

3.3.2 Colour

Temperature, time and pH significantly (p<0.05) affected the colour score. The maximum colour scores were 6.45±1.4 and 5.96±0.12 (between "extremely like" and "like very much") for red and white varieties, respectively treated at 100°C, 20 mins and pH of 4.0. The minimum values were 4.5±0.29 and 3.59±0.35 ("neither like nor dislike" and "like slightly") for the control samples of red and white prickly pear juice, respectively. The trends of changes in the colour score as a function of time, temperature and pH are presented in Figures 3 and 4. The colour score increased with an increase in temperature and time and a decrease in pH. The temperature had the most significant (p<0.05) effect on the colour.

An increase in heating temperature has been reported to increase colour saturation (Cruz-Cansino *et al.,* 2016) and did not affect colour negatively (Moßhammer *et al.,* 2006) during heat treatment and drying. Acidification and heat treatment were reported to improve the brightness index, protect natural colour and exhibit anti-browning of prickly pear juice (Kgalta *et al.,* 2010; Kgatla *et al.,* 2011) which could be the reason for a high colour score by panellists for products treated at high temperature and low pH. Moßhammer *et al.,* (2006) reported that the addition of organic acids (ascorbic acid, iso-ascorbic and citric acids) improved the heat stability of the betaxanthins and betacyanins of cactus pear juice. Moßhammer *et al.* (2006) reported the colour stability of yellow-orange cactus pear juice being more pronounced at pH 4.0 as compared to pH 6.0. The relationship between colour and independent variables was adequately described by a quadratic polynomial. The degree of fit and the values of the model coefficients are presented in Table 1 and Table 2.

3.3.3 Sweetness

The pH and heat treatment had a significant (p<0.05) effect on the sweetness of the processed cactus pear juices. The highest mean values of 4.24±0.09 and 4.42±0.08 ("moderately sweet") were recorded for cactus pear juice processed at 100°C, 20 mins and pH of 6.2 and a temperature of 108°C, 20 mins and pH of 4 for white and red prickly pear juice, respectively. On the other hand, the lowest sweetness values were 2.74±0.25 and 2.69±0.19 ("less sweet") for cactus pear juice processed at temperature, time and pH combinations of 85°C, 15 mins and pH of 3.1 for white and red prickly pear fruit juice, respectively. The trends of changes in sweetness as a function of pH and heat treatment are presented in Figures 1 and 2.

For the white prickly pear juice, an increase in temperature, time and pH generally brought about an increase in sweetness score. For the red variety, an increase in temperature increased the sweetness score up to 15 mins followed by a decrease. Moreover, the sweetness score increased with an increase in pH up to 5.2 followed by a decrease as the pH increased to 6.2. The results of this study are in agreement with Kgatla *et al.* (2010) who observed the acidity to sugar ratio of the cactus juice decreases the juice sweetness due to the citric acid effect of the juice. The relationship between heat treatment and pH and sweetness for both varieties was adequately described by a quadratic polynomial. The degree of fit and the model coefficients are presented in Table 1 and Table 2. The pH and the squared pH terms

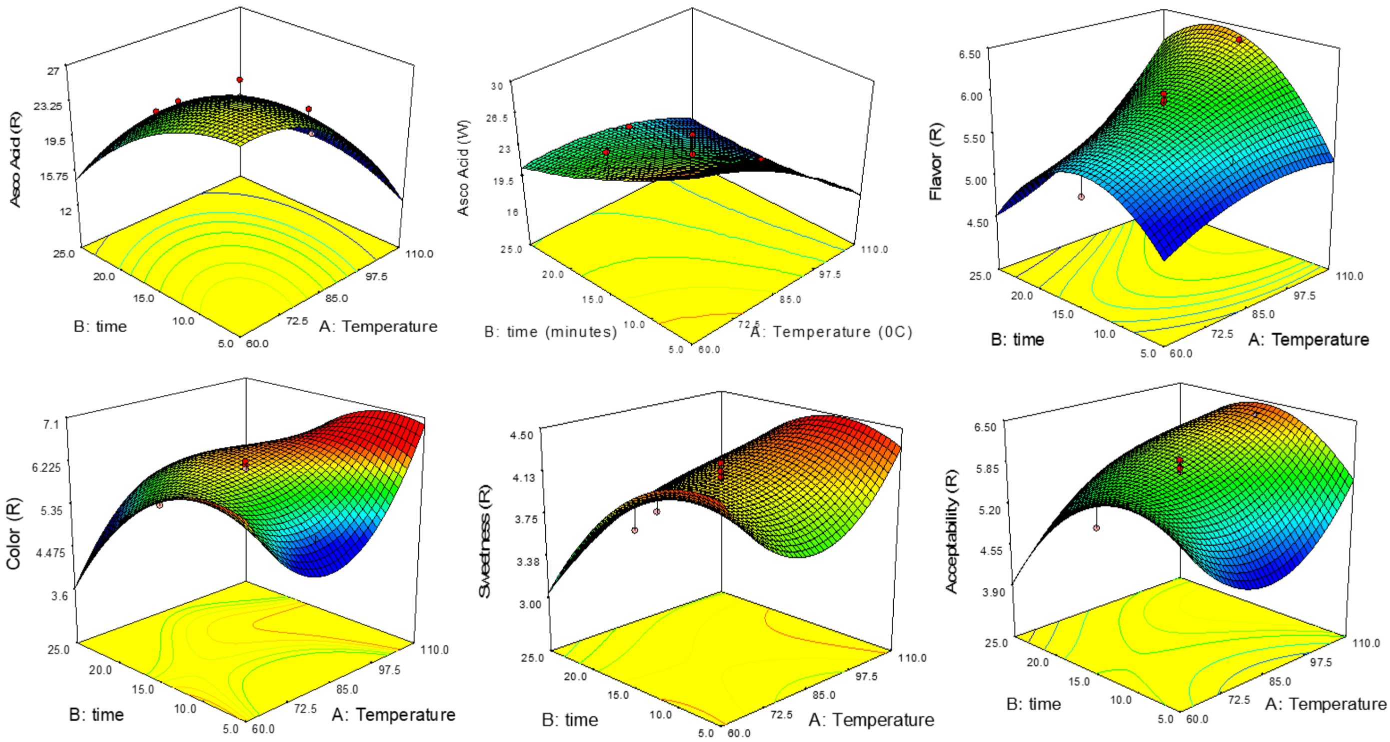

Figure 1. Response surface of ascorbic acid. Flavour, color, sweetness and acceptability of red prickly pear juice as a function of time and temperature at a fixed (5.2) pH value.

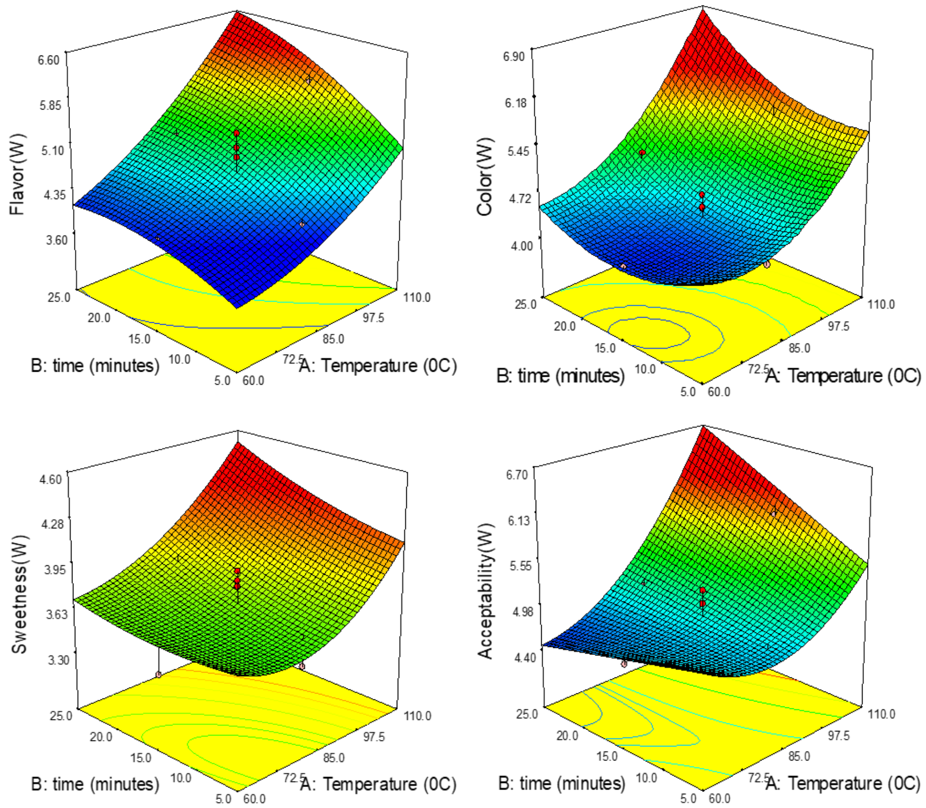

Figure 2. Response surface of flavour, color sweetness and acceptability of white prickly pear juice as a function of time and temperature at a fixed (5.2) pH value.

significantly ($p<0.05$) affected the sweetness score.

3.3.4 Overall acceptability

Overall acceptability of cactus pear juice was significantly (<0.05) affected by heat treatment and the pH modification of the juice. The highest mean scores were 6.07±0.14 and 6.59±0.11 for cactus pear juice processed at temperature, time and pH combinations of 100°C, 20 mins and 4.0, for white and red varieties, respectively representing "like very much". The lowest scores were 3.62±0.2 and 4.4±0.5 ("neither like nor dislike") for non-treated fresh cactus pear juice.

The changes in overall acceptability as a function of heat treatment and pH are shown in Figures 1 and 2. An

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increase in temperature resulted in an increase in overall acceptability whereas the increase in time and pH brought about a slight decrease in overall acceptability. The ascorbic acid content of the juice and acidification of prickly pear juice protected the natural colour and exhibited anti-browning. In general, processing and preservation have positive effects on the organoleptic quality attributes of prickly pear juice and affect the flavour attributes (Kgatla *et al.,* 2010).

A quadratic response surface equation described the relationship between pH and heating conditions with overall acceptability. The model coefficients and the degree of fit are presented in Table 1 and Table 2. For the red variety the coefficients of the linear temperature, the interaction between temperature and pH and the quadratic time and pH terms were significant ($p<0.05$) whereas for the white cultivar, the linear temperature, pH and the quadratic temperature terms were significant ($P<0.05$).

3.4 Microbial quality

3.4.1 Total aerobic bacteria (TAB)

Time, temperature and pH significantly ($p<0.05$) affected the TAB. The initial TAB for the red and white prickly pear juice was 4.48±0.25 log_{10} CFU/ mL and 3.88±0.77 log_{10} CFU/ mL, respectively. After the 30 days of storage, the TAB ranged from 0.83 log_{10} CFU/ mL to 2.94 log_{10} CFU/ mL for the red prickly pear juice whereas for the white variety the TAB ranged from 1.78±0.32 to log_{10} CFU/ mL to 2.92±0.4 log_{10} CFU/ mL. The lowest TAB was for the samples treated at 100°C, 20 mins and pH of 4 whereas the highest values were for samples treated at 70°C, 20 mins and pH of 6.2 which clearly shows the effect of temperature and pH. The lowest values were also comparable to samples treated at 108°C, 15 mins and pH of 5.1. There was a slight increase in the TAB after 60 days of storage. The trends of changes in TAB as a function of temperature, time and pH are presented in Figure 3. An increase in temperature and time coupled with a decrease in pH resulted in reduced TAB.

Karabagias *et al.* (2019) reported a total viable count of about 4 log_{10} CFU/mL, which is comparable with the results in this study. They also reported that the shelf life was only two days in air packages under refrigeration conditions based on the upper limit set at 7 log_{10} CFU/ mL (Riganakos *et al.,* 2017). The TAB count remained well below this limit indicating that the combined effect of heat treatment and acidification extended the shelf life of prickly pear juice to up to 60 days. The relationship between the heat treatment parameters and pH with the TAB was described by the response surface quadratic model. The values of model coefficients and the degree of fit are presented in Table 1 and Table 2. For the red variety, the liner temperature, time and pH terms significantly ($p<0.05$) affected TAB whereas, for the white variety, the linear terms of temperature and pH and the quadratic term of the temperature affected significantly ($p<0.05$).

3.4.2 Total yeasts and moulds

The initial total yeasts and moulds (TYM) of the

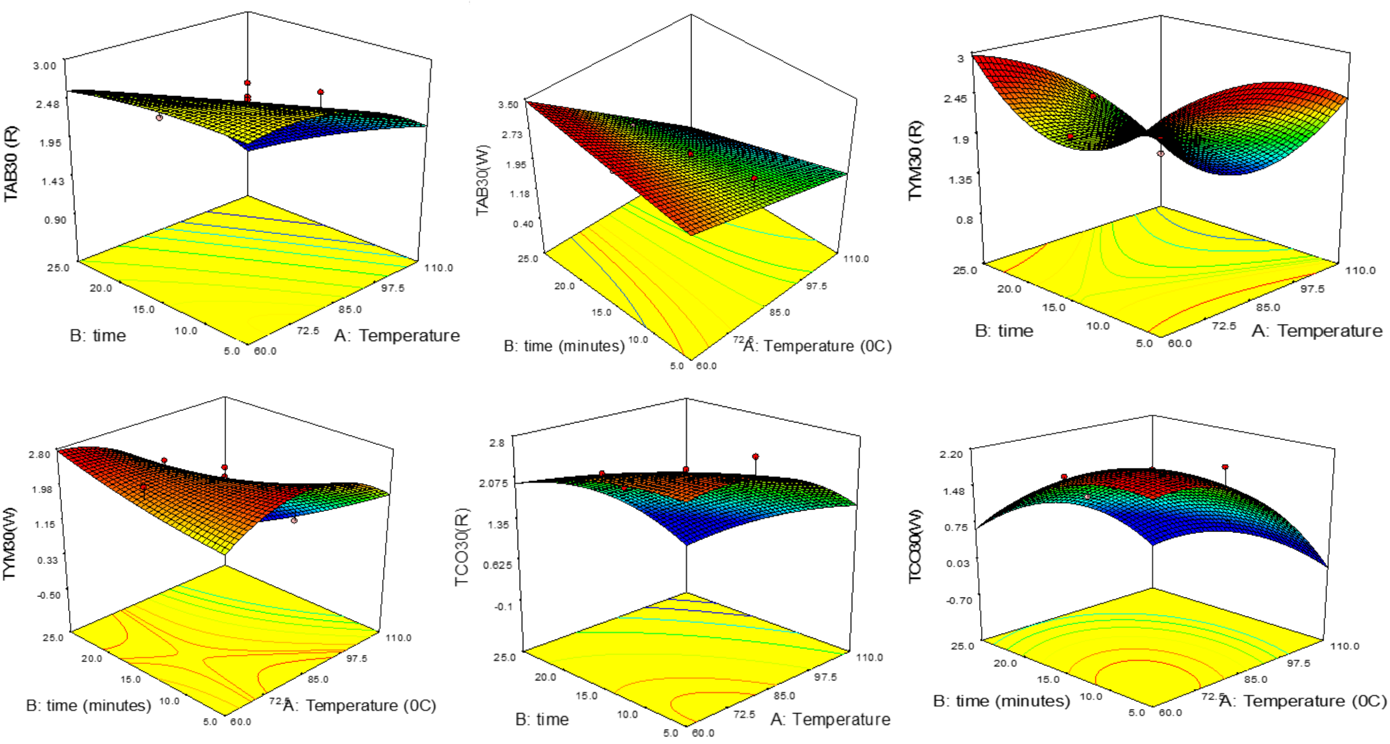

Figure 3. Response surface of total areaobic bacteria (TAB), Total yeast and mould (TYM) and Total coliforms (TCO) of red (R) and white (W) prickly pear juice as a function of time and temperature at a fixed (5.2) pH value.

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fresh sample were found to be 4.31±0.34 $\log_{10}$ CFU/mL and 4.24±0.22 $\log_{10}$ CFU/mL for white and red prickly pear juice samples. Temperature, time and pH significantly ($p<0.05$) influenced the TYM. For the white prickly pear juice after 30 and 60 days of storage, the TYM ranged from 0.45 to 2.59 $\log_{10}$ CFU/mL and 1.4 to 3.06 $\log_{10}$ CFU/mL, respectively. The trends of changes in the TYM as a function of the treatment conditions are presented in Figure 3. TYM, in general, decreased with an increase in time and temperature and a decrease in pH. However, the temperature had the most significant ($p<0.05$) effect compared to time and temperature. Similar trends were reported in earlier studies (García-García 2015; Cruz-Cansino *et al.,* 2016). The total yeast and moulds for prickly pear juice were reported to be about 3.5 $\log_{10}$ CFU/mL (Karabagias *et al.,* 2019) which is comparable with the results in this study. The relationship between temperature time and pH and TYM was adequately described by a quadratic model. The model coefficients and the degree of fit are presented in Table 1 and Table 2 for red and Table 9 for white prickly pear juice. The linear time and temperature and the squared temperature terms exhibited a significant ($p<0.05$) effect in describing the relationship.

3.4.3 Total coliforms

The total coliforms (TCO) for fresh samples that were not treated with heat and not acid-modified were 3.91±0.25 $\log_{10}$ CFU/mL and 3.87±0.32 $\log_{10}$ CFU/mL for white and red prickly pear juice samples, respectively. Temperature, time and pH significantly ($p<0.05$) influenced the TYM. The minimum TCO were 0.33 $\log_{10}$ CFU/mL and 0.9 $\log_{10}$ CFU/mL for samples treated at 100°C, for 20 mins and pH of 4 after 30 and 60 days of storage, respectively under refrigeration conditions for white prickly pear juice. For the red variety, the minimum TCO were 0.82 $\log_{10}$ CFU/mL and 0.56 $\log_{10}$ CFU/mL for samples treated at 100°C, for 20 mins and pH of 4 after 30 and 60 days of storage, respectively. The trends of changes in TC as a function of time, temperature and pH are presented in Figure 3. An increase in temperature and time brought about a decrease in the TCO. The effect of pH was not much pronounced. A quadratic model described the relationship between temperature, time and pH and the response variable TCO. The degree of fit the values of the model coefficients are presented in Tables 8 and 9. For the white prickly pear juice, the liner terms of temperature time and pH, and the interaction between temperature and pH showed a significant ($p<0.05$) effect. However, for the red prickly pear juice only the linear terms of time and temperature were significant ($p<0.05$).

3.5 Optimization

3.5.1 Graphical optimization

The region for optimal combination of heating time and temperature and the pH that gives an acceptable product in terms of the sensory attributes, the AA and microbial counts was obtained by superimposing the sensory attributes, the AA and the microbial load contour plots. The optimum region is depicted in the shaded region in Figures 4a and 4b. This optimum region provides the coordinates of possible optimal levels of temperature, time and pH. The criteria for the optimal region were sensory attributes greater or equal to the "like" intensity, maximized ascorbic acid above 20 mg/100 g and minimized microbial load. The ranges of the three parameters for the red prickly juice were 5.5 to 14 mins and 62°C to 82°C fixing the pH at 5.2 or pH 3.3 to 5.0 and temperature 62°C to 92°C fixing the time at 15 mins. For white prickly juice, the ranges of the three parameters were 18.7 to 22.6 mins and 83.9°C to 93.4°C fixing the pH at 5.2 or pH of 3.2 to 5.0 and temperature 93.5°C to 108°C, fixing the time at 15 mins.

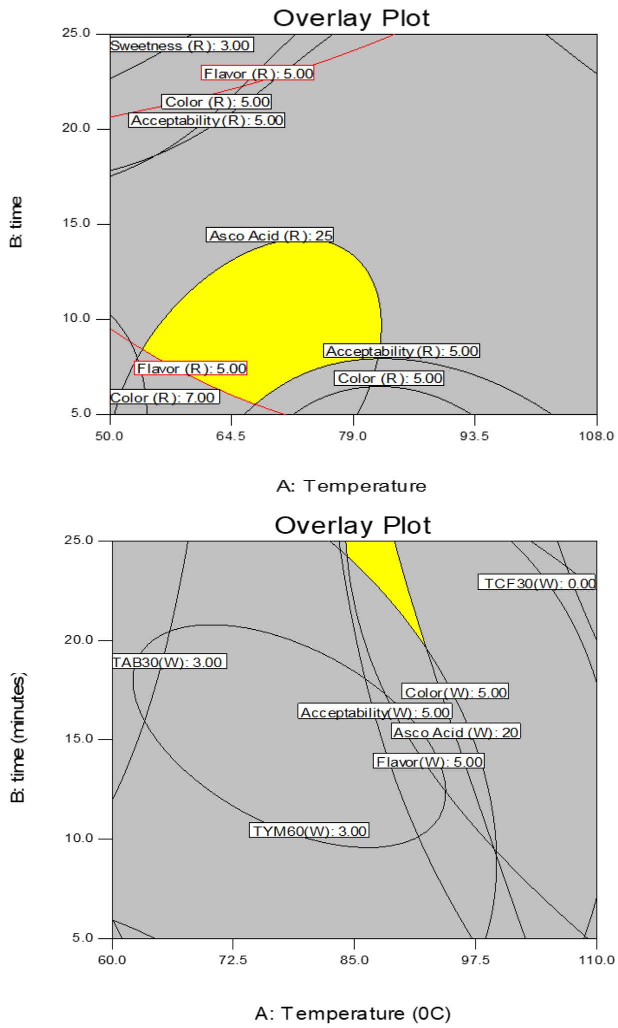

Figure 4. Overlay (superimposed) plots for red (a) and white (b) prickly pear juice showing the optimal range of heat treatment

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3.5.2 Numerical optimization

Using the criteria of maximizing all the sensory attributes and ascorbic acid and minimizing the microbial load, the optimal proportions which gave the best result were 19.2 mins, 100°C and pH of 4 with desirability value of 0.774 and 22 mins, 100°C and pH of 3.6 with the desirability value of 0.865 for red and white prickly pear juice respectively.

4. Conclusion

The study revealed that it is possible to extend the shelf life of prickly pear juice by the combined effects of heat treatment and pH adjustment. Heat treatment and pH had a significant ($p<0.05$) influence on the ascorbic acid content, sensory attributes and microbial load of the prickly pear juice. The microbial load of the heat-treated and pH adjusted cactus pear juice was within the acceptable limit by the end of 60 days of storage. The optimal heat treatment conditions and pH levels found in the study using numerical and graphical optimization could be used to produce a shelf-stable prickly pear juice.

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