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Effect of Time and Temperature Storage on the Quality of unpasteurized Prickly Pear Juice Enriched with Hydro-soluble *Opuntia ficus indica* seeds Extract

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ABSTRACT

This study aimed to evaluate the effect of incorporating hydrosoluble *Opuntia ficus indica* seeds extract in unpasteurized prickly pear juice and monitoring its stability. For this purpose, titratable acidity (TA), total soluble solids (TSS), browning index (BI), total phenolic compounds (TPC), total flavonoids (TF), antiradical activity (DPPH), ferric reducing antioxidant power (FRAP) and microbial analysis were monitored for both enriched and controlled juices during different time and temperature storage. Before storage, the enriched juice values were respectively 0.096±0.001%, 14.1±0.01%, 0.756±0.01, 133.3±3.4mgGAE/100ml, 5.58±0.07mgQE/100ml, 95.89±14.27mgGAE/100ml and 59.34±5.52mgGAE/100ml for TA, TSS, BI, TPC, TF, DPPH and FRAP; while 0.16±0%, 14.1 ±0.001%, 1.2±0.01, 88.39±4.2mgGAE/100ml, 3.98±1.003mgQE/100ml, 51.08±14.27 mgGAE/100ml and 50.33±5.16mgGAE/100ml for the control juice. The microbial analysis revealed the absence of microorganisms even the juices were unpasteurized. Moreover, the results revealed that the enrichment attenuated significantly the effect of storage; indeed, the use of the prickly pear seeds extract in combination with the juices can be a good alternative to enhance the shelf life of unpasteurized prickly pear juice, and improve their quality attributes as well as to minimize the unwanted changes in the nutritional and organoleptic properties.

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Introduction

The transformation processes, heat treatments, and storage have deleterious effects on the antioxidant potential of fruits and caused changes in the physicochemical properties and the levels of bioactive substances, which result in alterations in organoleptic, nutritional, and functional qualities (Touati et al., 2014; Touati et al., 2016; Zeeshan et al.,2019). Lately been gaining attention as a way to improve the nutritional value of food due to the increasing demand (Doménech-Asensi et al., 2013); hence, the food industry develops juice enriched with bio-substances to counterbalance the losses during processing and storage (Kaddumukasa et al., 2017).

The fruit of *Opuntia ficus indica* (OFI) is consumed as fresh fruit, juice, jam, and jelly; and it is a good source of bioactive compounds and may protect against human diseases. Moreover, cactus fruit juice positively affects the body's redox balance, and decreases oxidative lipid damage (Dehbi et al., 2014). The seeds of OFI play a key role in the valorization of the fruit because of their high antioxidants content. Their amount varied from 20 to 40% per dry weight of the whole fruit, depending on the cultivars (Habibi et al., 2002).

In Algeria, OFI is developing on the Mediterranean coast; but its culture is marginalized, its production is limited and its consumption remains seasonal. Despite its abundance, the prickly pear arouses little interest; it is not valued. The seeds are even less so since until now they are considered waste and yet their importance continues to grow in other countries such as Mexico, Argentina, and Spain (Habibi et al., 2005).

To our knowledge, several studies have been carried out on prickly pear pulp, seeds, cladodes, peels around the world (Al Juhaimi et al., 2020; Zeghad et al., 2019; Salehi et al., 2019; Smidaa et al., 2017; Chaalal et al., 2013; Habibi et al., 2005); however, there is no study on the monitoring stability of unpasteurized prickly pear juice enriched with its seeds extract.

Materials and Methods

Chemical reagents

DPPH reagent was purchased from Sigma Chemical (Sigma–Aldrich GmbH, Germany), and Folin–Ciocalteu phenol reagent from Biochem, Chemopharma (Montreal, Quebec). All chemicals and solvents used were of analytical grade.

Samples preparation

The fruits were harvested in Ain Hamra, department of Bordj Bou Arreridj (Latitude: 36°15′44’81″S, Longitude: 4°79′13’85″W) towards the end of August 2020. These fruits were stripped of their thorns, washed, peeled then kneaded with an electric mixer (SEB 500 Watt). After separating the seeds from the pulp, the latter was centrifuged (SIGMA 3-30KS) at 3000 rpm for 10 min. The filtrate recovered, constituting the juice, was split into two batches. The first batch was enriched with the hydro-soluble prickly pear seeds extracts (100 mg/l), while the second batch was considered as a control. Both lots were stored at 10,20 and 30°C. Samples for analysis were taken after 2, 4, 6, 8, 10 and 12 days.

Determination of physicochemical parameters

Titratable acidity

The determination of the titratable acidity consists in placing in a Beaker 10 ml of sample (juice) with a few drops of color indicator (0.1% phenolphthalein in pure ethanol). The reaction mixture was titrated with 0.1N NaOH solution until obtained a persistent pink color. The results were calculated according to the following equation:

$$TA\ (\%) = \frac{N_{NaOH}\ x\ V_{NaOH}\ x\ M_{citric\ acid}}{V_{sample}\ x\ 3\ x\ 10}$$

N_{NaOH}: molar concentration of NaOH, V_{NaOH}: volume of NaOH, $M_{citric\ acid}$: molar mass of citric acid, V_{sample}: volume of sample. The divide by 3 because citric acid is triacid (requires three molecules of NaOH to neutralize one molecule of citric acid); while the division by 10 is to express the results relative to 100 ml (Darias-Martín, et al., 2003).

Total soluble solids

The total soluble solids in a solution were measured with a refractometer. After placing a drop of juice on the surface of the glass plate, the value indicated represents the degree of brix expressed in percentage (%).

Browning index

The browning index was determined according to the method reported by Meydav et al. (1977). The samples were centrifuged (824×g, 18°C, 20 min); the recovered supernatants were diluted with ethanol (v/v) and then filtered through Whatman N° 2 paper. The absorbance was measured at 420 nm.

Determination of antioxidant substances

Total phenolic compounds

The total phenolic compounds of the juice samples was determined by the method using the Folin-Ciocalteu reagent (Adesegun et al., 2007). An aliquot of 100 µl of the extract was mixed with 800 µl of Folin-Ciocalteu (10%) and 400 µl of sodium carbonate (7%). After 30 min of incubation at room temperature, the absorbance was measured at 760 nm against the blank. The result was expressed in mg gallic acid equivalent (GAE) per 100 ml of juice by referring to the calibration curve.

Total flavonoids content

The total flavonoid content (TF) of the juice samples was determined by a colorimetric method (Ayoola et al., 2008). A volume of 2 ml juice was added to 2 ml of aluminum trichloride reagent $AlCl_3$ (2% in pure methanol). The absorbance was recorded at 420 nm after 10 min incubation at room temperature against the blank. The result was expressed in mg quercetin equivalent (QE) per 100 ml of juice by referring to the calibration curve.

Evaluation of antioxidant activity

DPPH radical scavenging capacity

The DPPH radical scavenging capacity was evaluated according to the method described by Brand-Williams et al. (1995). A volume of 200 µl of the sample was added to 1 ml of a methanolic solution of DPPH (60 µM). Absorbance was measured at 517 nm after 30 min incubation at room temperature and in the dark. The result was expressed in mg gallic acid equivalent (GAE) per 100 ml of juice by referring to a calibration curve.

Ferric reducing antioxidant power

The ferric-reducing antioxidant power was evaluated according to the method described by Oyaizu (1986). A volume of 2.5 ml of the juice sample was mixed with 2.5 ml of phosphate buffer (0.2 M; pH 6.6) and 2.5 ml of potassium ferricyanide (1%). After 20 min incubation at 50°C, 2.5 ml of trichloroacetic acid solution (10%) was added. A volume of 2.5 ml of the reaction mixture was diluted with distilled water (v/v) and then added with 500 µl of ferric chloride solution (0.1%). The absorbance was measured at 700 nm and the result was expressed in mg gallic acid equivalent (GAE) per 100 ml of juice referring to a calibration curve.

Microbiological analyzes

Preparation of dilutions

From the initial suspension (prickly pear juice), decimal dilutions were carried out under aseptic conditions.

Detection and enumeration of total coliforms

A volume of 1 ml of the sample was placed in empty Petri dishes prepared for this use and numbered. Then about 20 ml of medium (VRBG) was poured in. The tests were carried out in duplicate. A series of dishes were incubated at 37°C for 24 h. This will be used for the search for total coliforms.

Search and enumeration of yeasts and molds

Aseptically, 1 ml of juice was brought to a sterile and numbered petri dish. Then about 15 ml of medium (Sabauraud) was poured. Homogenization of the medium with the sample was made by 8-shaped movements. The tests were carried out in duplicate. The dishes were incubated at 25°C for 5 days.

Statistical analysis

The results (n = 3) were subjected to a two-factor analysis of variance. Mean values were compared using Fisher's test ($P<0.05$). All statistical analyzes were carried out using Infostat® software.

Results and Discussion

Evolution of physicochemical parameters

Titratable acidity (TA), total soluble solids (TSS), and browning index (BI) results of enriched and control juices during storage were shown in figure 1. Before storage, the enriched juice values were respectively 0.096±0.001%, 14.1±0.01%, and 0.756±0.01 for TA, TSS and BI; while 0.16±0%, 14.1±0.001% and 1.2±0.01 for the control juice. The present results were higher than that given by Dehbi et al. (2014), which reported TA values of 0,049%, for Marrocan prickly pear juice (Alkalaa cultivars with spiny, yellow peel, green-yellow pulp); while Medina et al. (2007) reported a rank of TA values between 0.055 and 0.078% for varieties harvested in the various regions of Bejaia. Stintzing et al. (2005) reported TSS values from 10 to 17% for prickly pear fruits of yellow-orange color. Chougui et al. (2013) reported a value of 15% for TSS of the prickly pear fruit. Concerning BI, the results fall in the range reported by Touati et al. (2016) inspite of differents kind of fruits proreported values of between 0.055 and 0.078% orange, pear, and grape nectar, respectively.Statistically, the enrichment before storage has a significant effect on TA and BI ($P<0.05$), contrary to TSS.

As can be seen from figure 1 a and b, TA values after 12 days of storage increased for both enriched and control juices to reach respectively. 1.12±0.003 and 2.08±0.002% at 10°C, 6.72±0.002 and 8.5±0.003% at 20°C, 6.69±0.04 and 8.3±0.02% at 30°C. This fact may be due to the fermentation (Jood et al., 2012). Ilkin et al. (2020) which worked on the stability of enriched orange juice with nettle (*Urtica dioica L)* during storage reported the absence of any statistical difference between the mean values of the samples.

Regarding TSS (figure 1 c and d), the values of enriched juice decreased significantly to achieve at the end of storage the values of 13.5±0.002, 13.0±0.002 and 12.3±0.1%, respectively at 10, 20 and 30°C; while for the control juice, the values were 13.6±0.001, 13.0±0.002 and 13.2±0.001%, respectively.

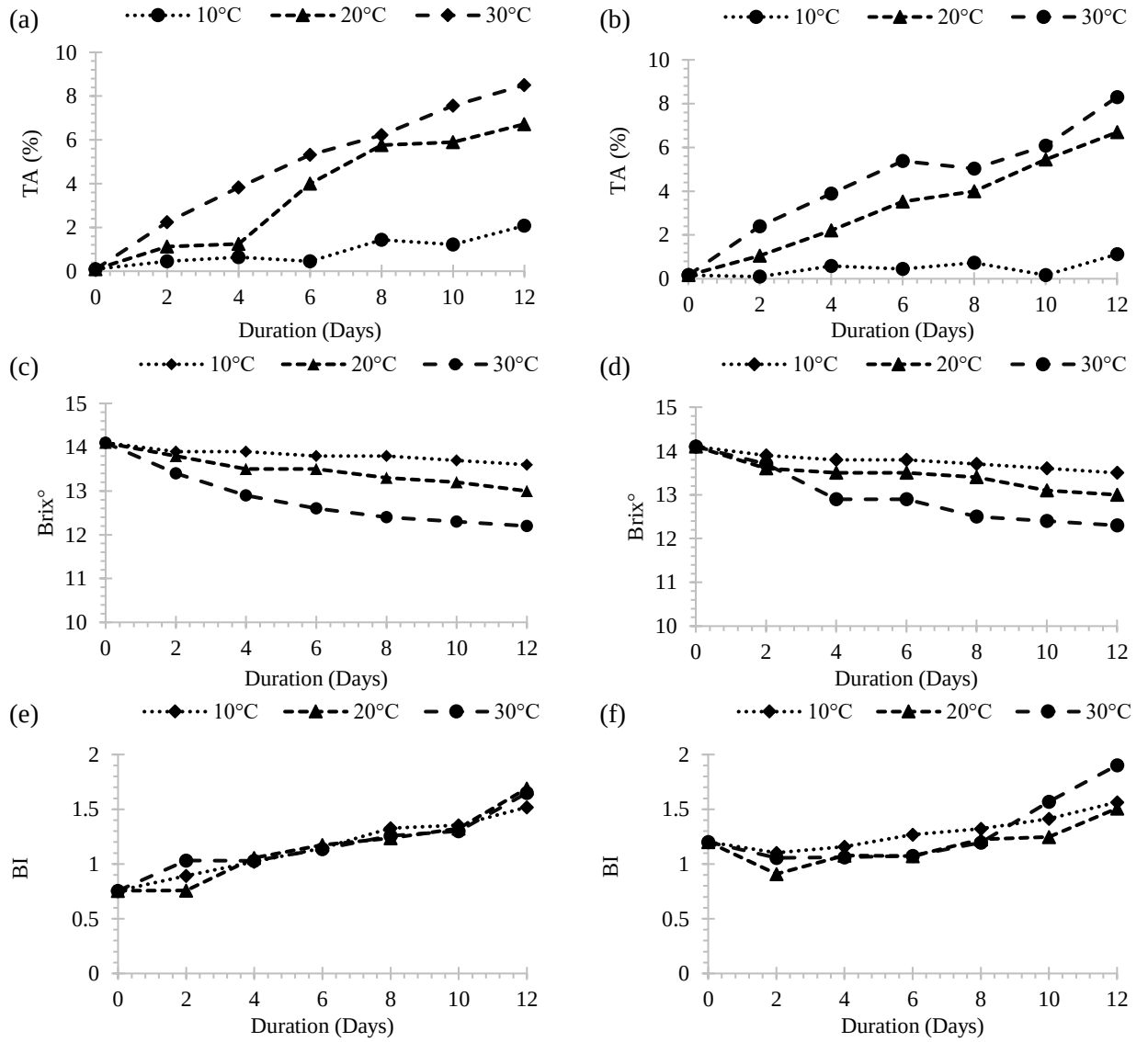

Figure 1. Evolution of physicochemical parameters of enriched and control juices respectively: Titratable acidity (TA), a and b; Total soluble solid (TSS), c and d; and Browning index (BI), e and f.

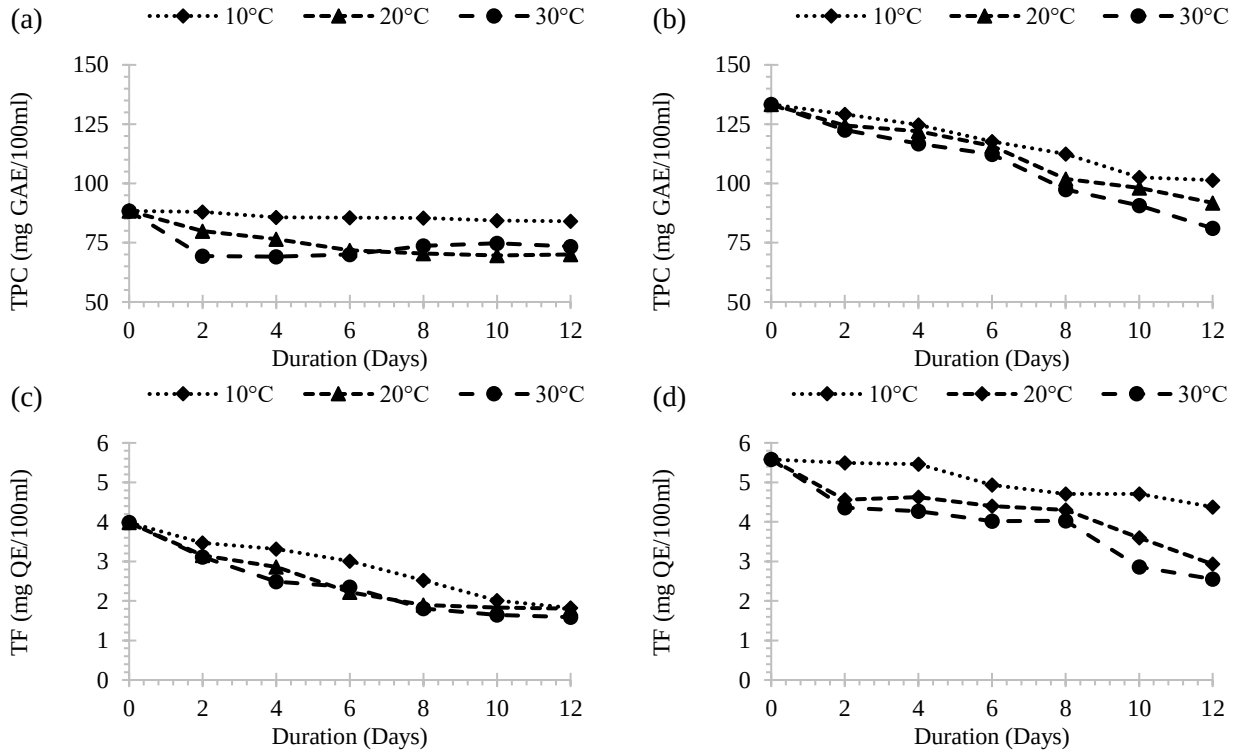

Figure 2. Evolution of antioxidants of enriched and control juices respectively: Total phenolic compounds (TPC), a and b; and Total flavonoids (TF).

The absorption of moisture during storage could be the reason for decreased in TSS values (Dangui, et al., 2014), or it could be due to the increase in the acidity (Sadras et al., 2013). The same results were found by Gao et al. (2018) who reported a decrease in TSS values of navel orange fruits during storage.

Concerning BI during storage at 10, 20 and 30°C (figure 1 e and f), the values increased respectively to achieve 1.519±0.06, 1.649±0.11 and 1.691±0.06 for enriched juice and 1.508±0.03, 1.565±0.01 and 1.902±1.002 for control juice. Touati et al. (2016) noted same trend in different fruits nectars. This augmentation may be due to the results of phenolic compound oxidation that occurred in the plant cell (Inchuen et al., 2010).

Evolution of the antioxidant substances

Total phenolic compounds

Total phenolic compounds (TPC), called secondary metabolites, are among the main antioxidants in plants, alongside vitamin C, vitamin E, and carotenoids. The results of TPC of enriched and control juices as well as their evolution during storage were shown in figure 2 a and b.

Prior storage, the content of TPC in enriched juice was 133.3±3.4mgGAE/100ml against 88.39±4.2mgGAE/100ml in control one. Therefore, the enrichment of juice induced an increase of 50.80% in the yield of TPC. Statistical analysis revealed that there was a significant difference between juices at $P<0.05$. The results obtained were higher than the values (31.0 - 51.1mgGAE/100g) reported by Palmeri et al. (2020). However, our results were lower than those reported by Socorro Santos Díaz et al. (2015) who declared that TPC values in the prickly pear juice were ranged from 630.9 to 880.6mgGAE/100ml. Dehbi et al. (2014) and Chavez-Santoscoy et al. (2009) reported values of 632.11±5.50µgGAE/g for the Marrocan *Opuncia ficus indica*. L juice and 226.3µgGAE/g for Mexican prickly pear juice, respectively.

As can be seen from figure 2a and b, the content of TPC in enriched juice stored at different temperatures exhibited the same decrease tendency, which was important for juices stored at 30°C followed by those stored at 20 and 10°C to reach respectively values of 101.4±0.005, 91.8±0.02 and 81.1±0.005mgGAE/100ml The content of TPC in the control juice stored at 10 and 20°C decreased significantly to reach values of 84.05±1.001 and 70.09±1.003mgGAE/100ml, respectively. In the juice stored at 30°C, a fluctuation was noted; a decrease during the first two days of storage, then stability until the sixth day, followed by an increase until the tenth day, then a decrease to reach the value of 73.42±0.003mgGAE/100ml. This may be due to leaching losses favored by the breakdown of cellular structures occurring as a result of exposure to high temperatures (Al Juhaimi et al., 2005). Several authors have found that TPC appears to exhibit stability during refrigerated storage while a decrease in ambient and high-temperature storage (Touati et al., 2016).

Total flavonoids

The total flavonoid (TF) content was determined using colorimetric method. The results of TF content in both enriched and control juices before and during storage were presented in figures 2c and d.

From figures 2c and d, TF content were 5.58±0.07 and 3.98±1.003mgQE/100ml for enriched and control juices, respectively. This indicates that the enrichment process led to a 40.20% increase in TF yield. Statistical analysis revealed a significant difference in the TF content between the analyzed juices at a significance level of $P<0.05$. The obtained results were higher than those reported by Zeghad et al. (2019) who stated the value of 1.95mgQE\g in prickly pear. On the other hand, our results were in concordance with values reported by Palmeri et al. (2020) who worked on prickly pear juice of different cultivars (4.7 and 5.7mgQE\g as TF in red and yellow cultivars).

The TF content in enriched juice stored at 10°C during the first four days did not exhibit a significant decrease (P<0.05); however, prolonged storage induced a significant decrease which led to reach the value of 4.38±0.008mgQE/100ml. Samples stored at 20 and 30°C showed a significant decrease during the first two days; while during extensive storage, the TF content showed slight stability until the eighth day followed by a significant decrease to reach values of 2.93±0.05 and 2.55±0.02mgQE/100ml for juice stored at 20 and 30°C, respectively. Concerning the control juice stored at different temperatures, the trend of reduction in TF content was greater for samples stored at 30°C followed by those stored at 20 and 10°C to reach the values of 1.82±0.02, 1.81±0,001 and 1.59±0.003mgQE/100ml, respectively. These results were consistent with the literature (Ogodo et al., 2016; Ali et al., 2013). The decline in TF content may be attributed to the breakdown of cell structure which occurred during the storage period (Ali et al., 2013). The decrease of TF content was lower in the enriched juice than the control one. This fact may be due to the addition of hydro-soluble prickly pear seeds extract

Evolution of total antioxidant capacity

Antioxidant contents have been reported to be the main responsible for foods total antioxidant capacity (TAC) (Touati et al., 2016). Therefore, TAC measurement could be a useful indicator of the quality deterioration of fruit juice during storage. For this purpose, DPPH and FRAP values of enriched and control juices were determined before and during 12 days of storage at 10, 20, and 30°C.

DPPH radical scavenging capacity

Results of the antiradical DPPH activity of enriched and control juices during storage was presented in figures 3 a and b.

Prior storage, the antiradical DPPH activity results were 95.89±14.27 and 51.08±14.27mgGAE/100ml for the enriched and the control juices, respectively. Statistical analysis revealed a significant difference between the analyzed juices (P<0.05). The enrichment process led to an increment of 46.73% in the antiradical DPPH activity. The total antioxidant capacity of prickly pear juices evaluated using the DPPH assay has been widely reported in the literature. Palmeri et al. (2020) reported DPPH results ranging from 37.6 to 49.4mgGAE/100ml for prickly pear juices. Smidaa et al. (2017) also noted that the antiradical DPPH activity increased with an increase in the concentration of Opuntia ficus indica.

As can be seen from figures 3a and b, the enriched juice exhibited a significant decrease in antiradical DPPH activity after 6, 8 and 4 days of storage at 10, 20 and 30°C, respectively. Extended storage up to the tenth day was characterized by stability, and then followed by a decrease to reach values of 46.28±1.006, 39.59±0.003 and 40.31±0.07mgGAE/100ml for juice stored at 10, 20 and 30°C, respectively. This might be explained by the decrease of antioxidants which where deteriorated during storage as corroborated by Tudora et al. (2015) who reported that under high temperatures storage some biochemical changes occurred in the fruit's structure. Regarding the control juice, the antiradical DPPH activity results were stable during the first two days of storage at the temperature of 10°C (P<0.05); however, the prolonged storage induced a significant decrease to reach the value of 25.06±0.006mgGAE/100ml. For juice stored at 20°C, the evolution of antiradical DPPH activity showed a decrease reaching a value of 20.79±1.04mgGAE/100ml at the end of storage. Concerning juice stored at 30°C, the values of antiradical DPPH activity showed a decrease during the first four days, and then followed by stability until the end of storage with a value of 23.87±0.06mgGAE/100ml. These findings indicate a degradation of antioxidants during storage, which could be attributed to the effects of temperature and other storage conditions.

Ferric reducing antioxidant power

Results of the ferric reducing antioxidant power (FRAP) of enriched and control juices before and during storage were presented in figures 3 c and d.

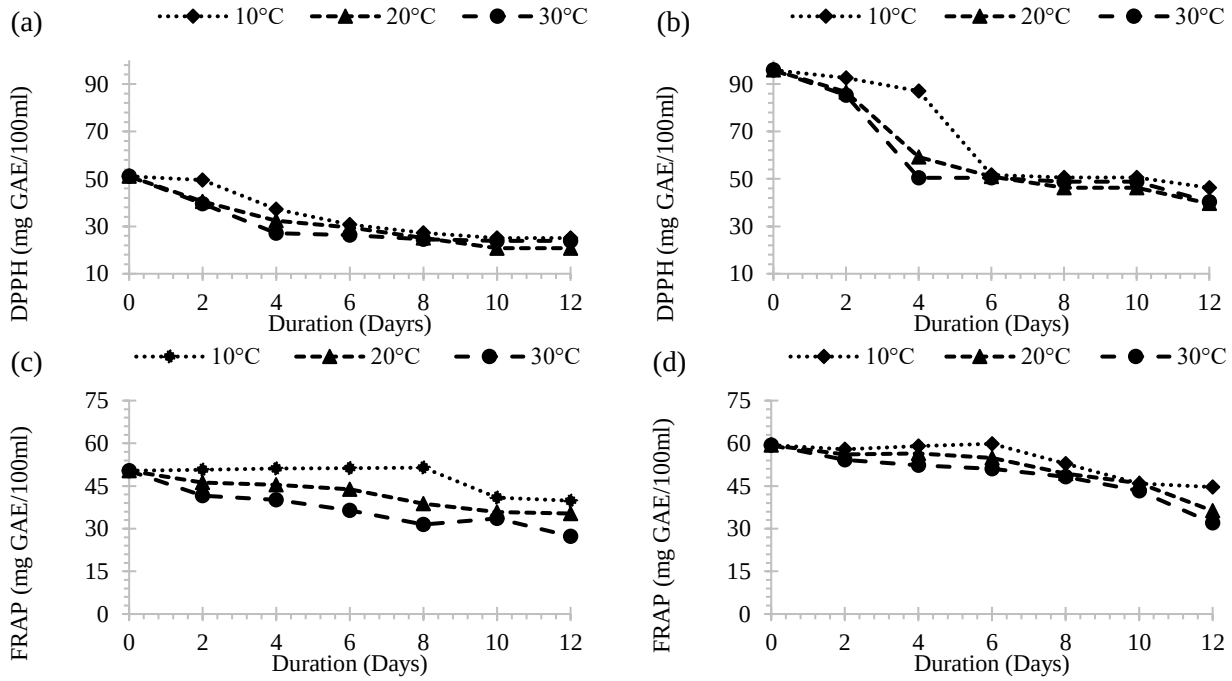

Figure 3: Evolution of antioxidant activities of enriched and control juices respectively: DPPH radical scavenging capacity (DPPH), a and b; and Ferric reducing antioxidant power (FRAP), c and d.

Table 1. Microbiological analysis results of enriched and control juices during storage

Germs	ST	Enriched juices				Control juices				Norms
		Prior storage	2 days	6 days	12 days	Prior storage	2 days	6 days	12 days	
Total coliform	10	-	-	-	-	-	-	-	-	< 10
	20	-	-	-	-	-	-	-	4.5×10^3	
	30	-	-	-	-	-	-	3×10^2	3.9×10^2	
Yeast and molds	10	-	-	-	-	-	-	-	-	10^4
	20	-	-	-	-	-	-	-	-	
	30	-	-	-	-	-	-	-	10.2×10^3	

ST : Storage temperature (°C)

Before storage, FRAP results were 59.34±5.52 and 50.33±5.16mgGAE/100ml for enriched and control juices, respectively. Therefore, the enrichment resulted in a 15.18% increase in FRAP. Statistical analysis showed a significant difference in the antioxidant activity between the enriched and control juices ($P<0.05$).

During storage, the FRAP values fluctuated for both analyzed juices; however, after storage, the temperature of 10°C induced less loss of reducing activity compared to juices stored at 20 and 30°C. The FRAP values at the end of storage under 10, 20 and 30°C were respectively 44.62±0.001, 36.26±0.001 and 31.97±0.003mgGAE/ 100ml for enriched juice, and 39.83±0.03, 35.31±0.005 and 27.32±0.007mgGAE/100ml for control one. The highest value was observed for the samples stored at 10°C

Microbiological analysis

Fresh juice made from fruits and vegetables is highly susceptible to contamination, leading to the deterioration of organoleptic and physicochemical parameters, (Sevindik et al., 2021). Several factors can affect microbial colonization of juices, including redox potential, pH, water activity, nutrients, temperature, antimicrobial agents, and relative humidity (Raybaudi et al., 2009; Pehlivan et al., 2018). The growth and survival of microorganisms in juices depend on their composition and the storage conditions. The results of the microbiological analysis of enriched and control juices, stored at three different temperatures (10, 20 and 30°C) for duration of 12 days, were presented in table 1.

Prior storage, as shown in table 1, results revealed the absence of contaminating germs (total coliforms, yeasts, and molds) in all samples, which perfectly meets the standards required by JORA (2017). These results were similar to those found by Garg et al. (2021) who worked on the Indian enriched gooseberry. Al Amin et al. (2018) reported the absence of total coliforms in orange and apple juice samples. Besides, Asghar et al. (2018) reported that unpasteurized juices such as apple, carrot, orange, and extracted sugar represent a high load of total coliforms.

During storage, the enriched juice showed the absence of total coliforms, which can be attributed to the antimicrobial activity of the aqueous extract of *Opuntia ficus indica* seeds extract. Previous studies have demonstrated the remarkable antibacterial effect of prickly pear seeds extract against various bacterial strains (Xiyu et al., 2020; Shimaa et al., 2022). Similar findings were declared by Al Amin et al. (2018), who reported the absence of total coliforms in commercial pineapple and lemon juice samples. In contrast, the control juices showed the presence of total coliforms, with a count of 3×10^2 CFU/ml after 6 days of storage at 30°C. This result was consistent with the findings of Lewis et al. (2006) and Rahman et al. (2011), which they reported the presence of total coliforms in juice samples. After prolonged storage, the total coliform count increased to 4.5×10^3 and 3.9×10^2CFU/ml for control juices stored at 20 and 30°C, respectively. The metabolic activities of microorganisms during storage can lead to the deterioration of juice samples and reduce their shelf life (Adal et al., 2022). Additionally, the low pH of the juice can promote the growth of acid-tolerant bacteria, further contributing to spoilage (Algari et al., 2016). According to JORA (1998) standards, the total coliform count in juice should be lower than 10CFU/ml.

Regarding yeasts and molds, the enriched juice remained free from their presence throughout the storage period at all temperatures (10, 20 and 30°C). This may be attributed to the high concentrations of secondary metabolites, such as flavonoids and polyphenols, present in the samples. These compounds can penetrate the cell membranes of fungal strains and interact with critical intracellular sites, leading to cell death (Cristani et al., 2007). In the control juice, yeasts and molds were absent in samples stored at 10 and 20°C; however, in samples stored for 12 days at 30°C, the number of yeasts and molds reached 10.27×10^3UFC/ml. The heat promotes the proliferation of yeasts and molds, and 30°C is considered an optimum temperature for their growth (Sevindik et al., 2021). These findings were align with the standards set by JORA (2017), which specify that, the yeast and mold count should be lower than 10^4UFC/ml.

Conclusion

In order to improve the stability of unpasteurized fruit juice, the latter has been enriched with the hydro-soluble extract *of Opuntia ficus indica* seeds. Regarding the physicochemical properties, no detectable difference between enriched and control samples throughout the storage period. In addition, the enriched samples exhibited the highest content of phenolic compounds and total flavonoids; likewise, the enriched juice has a higher antioxidant capacity. Furthermore, the seeds extract was effective in reducing the proliferation of microorganisms. The obtained results demonstrated the effectiveness of enrichment with the hydro-soluble prickly pear seeds extract to increase the nutritional value and improve the stability during storage. This research contributes to the development of innovative strategies in the juice industry by utilizing natural bio conservator and valorizing by-products, ultimately leading to the production of healthier and more sustainable juice products.

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