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Ennouri M *

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Research Article

Development of a Novel Beverage with Cactus Juice (*Opuntia Ficus Indica) and date Palm sap (Phoenix Dactylifera L)*

Ennouri, M [1*], Ennouri, K [2] and Triki, M.A [1]

[1] Olive tree institute, LR16IO01, University of Sfax, Tunisia.

[2] Digital Research Center of Sfax, Technopark of Sfax, University of Sfax, Tunisia.

***Corresponding author:** Ennouri M, Olive tree institute, LR16IO01, University of Sfax, Tunisia.

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Abstract:

This study aimed to evaluate the possibility to produce a beverage combining *Opuntia ficus indica* juice (OFIJ) and date palm sap (*Phoenix dactylifera L.*) (DPS). Beverages (B1, B2, B3) were formulated by mixing the juice and date palm sap at different ratios (40:60%, 30:70%, 20:80%) whereas OFIJ and DPS were considered as the controls. Sensory evaluation using 5-point hedonic scale was performed and showed that the control DPS was the most appreciated in term of aftertaste and overall acceptability. Among the formulated beverages B3 is more appreciated than B2, followed by B1. Physico-chemical analysis showed that the sedimentation and serum separation increase considerably after 28 days of cold storage traducing the instability of beverages. Microbial analysis carried out during 42 days of cold storage revealed the absence of total coliforms, *Staphylococcus and Escherichia coli* for all drinks and that after 28 days of storage the beverages cannot be consumed.

Key words: opuntia ficus indica; phoenix dactylifera l; juice; microbial count; sensory properties; stability

1. Introduction

Beverages based on juices are more and more consumed in regard to their healthy, tasty and refreshing character. Indeed, the average volume of juice consumed per person in 2023 is expected to amount of 4.66L. The volume growth of juices segment is expected to reach 1.8% in 2024 and it is expected to reach amount to 37.633ML by 2027 [1]. The most consumed juices, in volume, are the mixtures and smoothies, orange juice, apple juice, and in equal amounts grapefruit juice, pineapple juice and grape juice [1]. *Opuntia ficus indica* (OFI) presents high nutritional value, social, economic and environment benefits [2]. OFI can be used to stabilize soil and counter soil erosion, it can be used as a forage substitute, and it is considered as a dietary supplements and nutraceutical [3, 4]. *Opuntia ficus indica* juice (OFIJ) is rich in minerals, proteins, and in antioxidants such as polyphenols and flavonoids like reported in the work of Baccouche et al. [5] which evaluated the physical stability of whey-based *opuntia ficus indica* beverages. The study of Aloui et al [6] concerning twenty-two healthy men athletes, reporting the effect of OFIJ supplementation on oxidative stress, cardiovascular parameters and biochemical markers, concluded that supplementation with OFIJ decreases muscle damage caused by endurance exercise and reduces total and LDL-cholesterol. In the same vein, the more recent study of Bellafiore et al [7] has demonstrated that supplementation with OFIJ to women decreased the oxidative stress induced by intense exercise and improved balance in physically active women

Date palm sap (DPS) familiar in Tunisian population and called “legmi” is usually collected by traditional tapping technique, it is consumed in its fresh form as juice or as an alcoholic drink after auto fermentation [8]. DPS is a good source of antioxidant and possess antimicrobial activities particularly against Gram-positive bacteria and possess cytotoxic properties against HeLa human cell line [9]. Abdennabi et al [10] have isolated endophytic fungi from date palm sap and reported antifungal activities. More recently Ben Atitallah et al [11] reported the good potentiality of date palm sap for producing bioethanol.

The combination of *Opuntia ficus indica* juice and palm sap is so evident, since *Opuntia ficus indica* and *Phoenix dactylifera L.* are typical of semi-arid to arid regions and are adapted to growth in severe environment conditions. Each plant offers a juice which is very appreciated by the local population. When consumed separately the two juices are unstable, that’s why we attempt to study the stability of their mixture which has never been reported in the literature.

The aim of our study is on the one hand to test the development of a new natural drink of high nutritional quality and to analyse its organoleptic properties, on the other hand to evaluate physico-chemical, and microbiological stability of drinks during storage at 5°C during 42 days.

2.Materials and methods:

2.1. Beverages:

Ripe cactus fruits (*Opuntia ficus indica*) were bought on the markets of sfax / Tunisia in early August and processed into juice. All the selected fruits are washed with water containing 0.01% chlorine, peeled manually, crushed, the mixed pulp was then passed through a strainer to retain the seeds. The extracted juice is filtered through cheesecloth and stored in the dark at -25°C until use. Exuded sap was obtained by a traditional tapping technique from palm grove (*Phenix doctylifera L.*) and filtered through cheesecloth. The two juices were mixed in different percentage like reported in Table 1 and the beverages were formulation according to the flow chart presented in Figure 1. OFIJ and DPS were considered as controls. The photos of studied beverages were presented in Figure 2.

Beverage	OFIJ	DPS
B1	40%	60%
B2	30%	70%
B3	20%	80%
DPS	0	100%
OFIJ	100%	0

Table 1: Composition of beverages

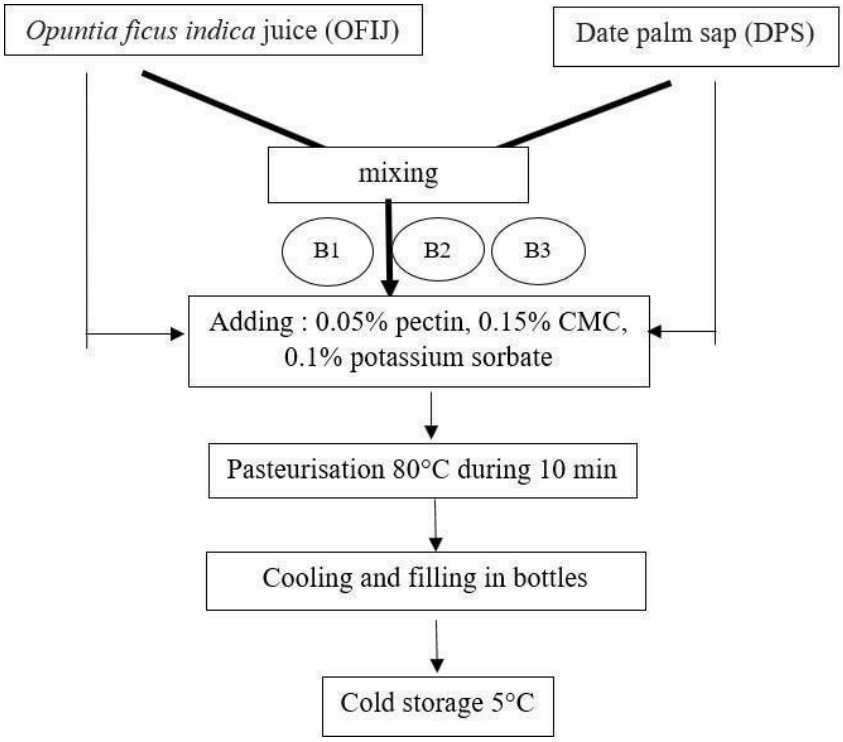

Figure 1: Flow chart of beverages formulation

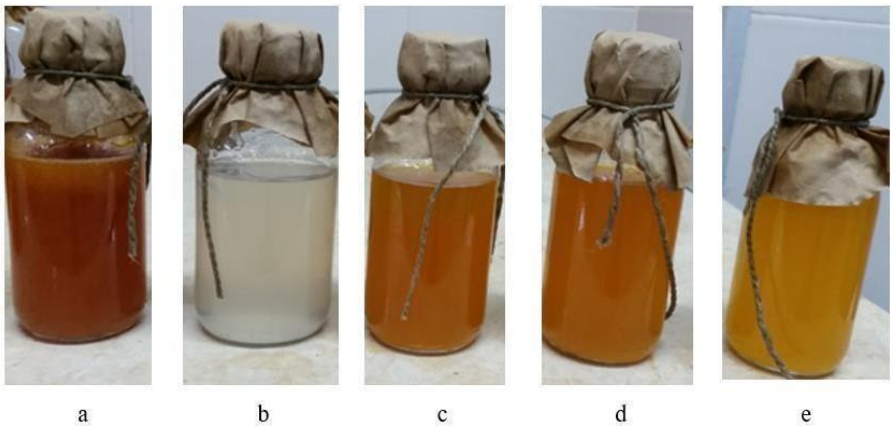

Figure 2: Photos of: *opuntia ficus indica* juice: OFIJ (a), date palm sap: DPS (b), B1 (c), B2 (d) and B3 (e).

2.2. Physico-chemical Analysis

The physico-chemical analysis concerned the determination of pH, turbidity, total soluble solids, sedimentation, and serum separation. The pH was determined by a pH meter (Eutech pH700) at 20°C [12], a turbidimeter (Aqua lytic AL450T-IR) was used for turbidity determination. Total soluble solids were determined at 20°C with a refractometer (ATAGO) and expressed as °Brix.

The sedimentation is carried out by centrifugation of the beverages at 4500 rpm for 20 min with a centrifuge (Universal 320R). The sedimentation corresponds to the pellet, its weight was calculated after elimination of the supernatant.

To evaluate serum separation, samples were placed in 10 ml plastic pipettes sealed at both ends each containing a beverage sample, pipettes were incubated at 5°C to assess serum separation by gravity and were inspected at various times during the 42 days of storage. When sedimentation takes place, a clear zone is observed in the upper part which is the sign of the instability of the drink [13]. All analyses are performed in triplicate.

2.3. Microbiological Analysis

Beverage samples were aseptically removed from the bottles and 10 g from each sample were homogenized in 90 ml of sterile distilled water. Serial dilutions were prepared and 1 ml aliquots were plated in each specific medium and incubated at different temperatures, times and growth media as shown in Table 2.

	Growth medium	Incubation temperature	Incubation duration
Yeasts and molds	Potato dextrose agar (PDA)	28-30°C	72-120h
Total aerobic mesophilic bacteria	Plate Count Agar (PCA)	32°C	48h
Total coliform	Violet Red Bile Lactose (VRBL)	30°C	48h
Staphylococcus	Chapman	37°C	48h
Lactic acid bacteria	MRS agar	35°C	72-90h
E. coli	Lactose Broth	37°C	48h

Table 2: Incubation conditions and media used for microbiological analysis.

2.4. Sensory Analysis

The panel consisted of 25 people accustomed to consume fruit juices and cocktails. The panelists were asked to assess, by scoring on a scale of five, the intensity of seven descriptors; color, taste, smell, sweetness, texture, aftertaste and overall acceptability. During the sensory evaluation each panelist is separated from the others. When presented to panelists, samples are coded with 3-digit codes.

3. Results and discussion:

3.1. Sensory Analysis

The results presented in Table 3 show that DSP is the more appreciated in term of aftertaste and overall acceptability respectively 4.21 and 4.55, whereas the OFIJ obtained the best note for odor. Regarding the formulated beverages, B3 has best notes for color (4.12), sweetness(4.68), texture (4.20) and overall acceptability (4.28). This result is explained by the fact that B3 contains 80% of DSP which was well appreciated by panelists, indeed DSP is appreciated by local population and so, it is recognised when analysed by panelists. We can conclude fromthe sensory evaluation that B3 is the best formulation, but it is important to evaluate the physical and microbial stability of this beverage during cold storage.

	B1	B2	B3	DSP	OFIJ
Color	3.64±0.02	3.68±0.01	4.12±0.04	3.98±0.03	3.31±0.01
Odor	3.95±0.02	2.72±0.02	2.24±0.01	3.59±0.04	3.90±0.03
Taste	3.80±0.01	4.04±0.02	3.96±0.03	4.42±0.05	3.51±0.04
Sweetness	2.86±0.01	3.12±0.02	4.68±0.05	4.53±0.02	3.98±0.03
Texture	3.40±0.02	4.08±0.03	4.20±0.02	3.98±0.03	3.58±0.04
Aftertaste	2.48±0.03	2.56±0.02	3.44±0.03	4.21±0.01	3.95±0.03
Overall acceptability	3.98±0.04	4.10±0.03	4.28±0.04	4.55±0.03	3.29±0.02

Table 3: Sensory properties of formulated beverages and the controls

3.2. Physico-chemical beverages stability:

The evaluation of the physico-chemical characteristics of beverages during storage is presented in Table 4. The initial pH of OFIJ was 6.21 showing a slight decrease with storage time reaching 6.03 after 42 days of storage. The pH of DSP is the more affected by storage, exhibiting a pronounced decrease (pH of 6.80 at day 1 vs. 4.21 at day 42). Regarding formulated beverages, the more affected by storage was B3 containing the highest DPS concentration and the less affected was B2.

The turbidity is influenced not only by storage time but also by the composition of the beverages, the more the percentage of OFIJ in beverage increases and the percentage of DSP decreases, the more the turbidity increases (Table 4). Over time, the turbidity increases more for B1 than for B2 and B3. It can be seen that the soluble solids content decreases for all the beverages during cold storage which is in accordance with the works of Ferreira et al [4] and Quiero et al [14] and can be attributed to the growth of microbial activity consuming thus the sugars.

	Day 1	Day 13	Day 28	Day 42
pH				
B1	6.32±0.02	6.05±0.01	5.83±0.02	5.3±0.01
B2	6.63±0.01	5.51±0.02	5.47±0.00	5.46±0.02
B3	6.56±0.02	5.83±0.00	5.81±0.03	4.27±0.02
DPS	6.80±0.00	5.54±0.01	5.48±0.00	4.21±0.01
OFIJ	6.21±0.01	6.14±0.01	6.13±0.01	6.03±0.00
Turbidity (NTU)				
B1	796±12	1022±10	1024±9	1054±7
B2	727±2	733±1	760±4	786±5
B3	594±3	580±2	568±1	696±4
DPS	102±1	119±0	231±2	930±2
OFIJ	2160±10	2284±9	2386±7	2460±2
Total soluble solids (°Bx)				
B1	10.80±0.00	10.60±0.00	11.30±0.00	10.10±0.00
B2	11.70±0.28	11.70±0.00	11.60±0.10	11.50±0.00
B3	11.80±0.00	11.00±0.10	10.80±0.00	10.80±0.00
DPS	11.10±0.00	10.50±0.00	10.50±0.00	10.40±0.00
OFIJ	12.80±0.00	12.50±0.10	12.20±0.00	12.10±0.28
Sedimentation (%)				
B1	1.38±0.01	4.23±0.02	19.68±0.02	35.78±0.01
B2	3.61±0.02	3.71±0.01	15.62±0.02	34.67±0.02
B3	2.51±0.02	10.13±0.02	25.14±0.04	35.12±0.02
DPS	2.00±0.01	2.58±0.02	15.54±0.02	33.52±0.03
OFIJ	6.84±0.02	8.50±0.03	23.77±0.01	37.61±0.02
Serum Separation (%)				
B1	19±0.71	20±1.41	24.5±0.71	27±0.71
B2	17±0.71	17.5±0.71	20±0.00	23±0.41
B3	14±0.41	15±1.41	15±0.71	16±0.71
DPS	5±0.00	6±0.00	7±0.00	8±0.00
OFIJ	13±0.71	15±1.41	16±0.41	22±0.71

Table 4: Evolution of pH, turbidity, TSS, serum separation and sedimentation during cold storage

Sedimentation and serum separation traduce the physical stability of beverages, these two parameters are very important for consumers and are determinant for purchasing intention. Sedimentation and serum separation of formulated beverages have varied respectively from 2.51 to35.12% and from 14 to 16% for B3 during storage. The observed sedimentation increase is probably due to the complexation between the DPS components and those of OFIJ like reported by Baccouche et al [5] for whey and OFIJ.

3.3. Microbiological Analysis:

The microbiological analysis was carried out on the three formulated beverages and the two controls (DSP and OFIJ) stored for a period of 42 days at 5°C. The microbiological analysis of the different samples showed that total coliforms, *Staphylococcus* and *Escherichia coli* are absent in all beverages.

During storage, the total mesophilic flora and the lactic acid bacteria increase especially during the last 14 days (Table 5). For this, we can say that the presence of these two florae in the drinks after 42 days can lead to the appearance of more or less important enzymatic activities in our product, hence the possibility of its alteration.

	Day 1	Day 13	Day 28	Day 42
Total aerobic mesophilic bacterial count (CFU/mL)				
B1	0.37 $x10^2$	0.81 $x10^2$	8.77 $x10^2$	9.33 $x10^2$
B2	0.75 $x10^2$	0.79 $x10^2$	5.65 $x10^2$	27.90 $x10^2$
B3	0.31 $x10^2$	0.69 $x10^2$	8.77 $x10^2$	108.32 $x10^2$
DPS	0.77 $x10^2$	0.91 $x10^2$	1.67 $x10^2$	26.50 $x10^2$
OFIJ	0.07 $x10^2$	0.27 $x10^2$	8.11 $x10^2$	89.00 $x10^2$
Lactic bacteria (CFU/mL)				
B1	0	9	1.22 $x10^2$	2 $x10^2$
B2	0	5	0.10 $x10^2$	3.57 $x10^2$
B3	0	0	0.10 $x10^2$	0.9 $x10^2$
DPS	0	1	0.37 $x10^2$	2.01 $x10^2$
OFIJ	0	2	0.11 $x10^2$	6.53 $x10^2$
Moulds and yeasts (CFU/mL)				
B1	0	0	0.10 $x10^2$	16.50 $x10^2$
B2	0	0	0.10 $x10^2$	14.50 $x10^2$
B3	0	10	0.90 $x10^2$	2 $x10^2$
DPS	0	0	0.10 $x10^2$	1 $x10^2$
OFIJ	0	0	0.20 $x10^2$	7.10 $x10^2$

Table 5: Total mesophilic bacterial, yeasts and moulds, lactic bacteria count in formulated beverages and controls kept at 4±1°C during 42 days

During the first thirteen days, the number of yeasts and moulds does not exceed 10 CFU in all drinks. This indicates stability probably due to the addition of potassium sorbate. However, between 28 and 42 days, the number of yeasts and moulds exceeded the standard which is 10^2 CFU/mL for pasteurized juices We therefore conclude that from a microbiological safety point of view, that drinks have a shelf life of less than 28 days.

Conclusion

The aim of our work is the elaboration of a new cocktail from two natural resources OFIJ and DPS that are found in our country but are badly exploited because of their rapid alteration. The sensory evaluation has demonstrated that B3, containing 20% *Opuntia ficus indica* juice and 80% date palm sap, is the more appreciated. Physico-chemical analysis showed that after 28 days of cold storage, sedimentation and serum separation increase considerably traducing the decrease of the beverage's stability. From a microbiological point of view, the analysis carried out on the beverages show that the products are safety until 28 days of storage.

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