Volume 89(5). Published July, 01, 2022
www.jnsciences.org
E-ISSN 2286-5314

Assessment of physicochemical, microbiological and sensory quality of new product of cactus fruit (*Opuntia ficus-indica L.*) vinegar flavoured with ginger and cinnamon

Wafa Hassen[1*], Sana Alibi[1], Abdennaceur Hassen[2], Hedi Ben Mansour[1]
[1] *Research Unit: Analysis and Process Applied to the Environment (APAE), Higher Institute of Applied Sciences and Technology Mahdia - University of Monastir, Tunisia.*
[2] *Water Research and Technology Center (C.E.R.T. E), Borj-Cédria Technology Park, Soliman, Tunisia.*

***Corresponding author:** hassen.wafa@gmail.com

Abstract : Cactus fruit (*Opuntia ficus-indica* L.) are known for their virtues, mainly their chemo-preventive properties used in traditional medicines and their various therapeutic benefits including hypoglycaemic, hypocholesterolemic and antioxidant effects. This study aimed at first to add spices and natural aromatic plants to the common manufacturing process of cactus fruit (*Opuntia ficus-indica* L.) vinegar, and secondly to compare afterward the finished product from a nutritional point of view with other kinds of vinegar. The resulting vinegar has undergone a series of physicochemical, nutritional, and microbiological analyses to show its good quality and safety for human consumption. The microbiological analysis showed that the rates of yeast, moulds, and the total cultivable bacteria were below the maximum threshold of acceptability recommended by the Tunisian standards. Besides, cactus fruit vinegar has revealed various interesting activities such as antioxidant, antibacterial, and antidiabetic effects. Furthermore, the cactus fruit vinegar flavouring showed good stabilizing effects of its main physicochemical parameters and thus improved its nutritional quality and biological activity. The present study indicates that vinegar of cactus fruit (*Opuntia ficus-indica* L.) with or without flavouring are a significant source of compounds with interesting biological activities and best sensorial quality, and thus may be useful for chemoprevention via nutraceutical foods.

Key words: Cactus fruit (*Opuntia ficus-indica* L.) vinegar; Vinegar; Flavouring process; Nutritional quality; Biological activity.

1. Introduction

Among various plants of the Tunisian flora, the cactus pear known as *Opuntia ficus indica* is characterised by the uniqueness of its form and abundance in the arid zones. The cactus is an exotic plant from Tunisian soil; it has become acclimatised since its arrival in Spain from Central America. The benefits of Cactus fruit of *Opuntia ficus-indica* L. (CF) are appreciated all around the Mediterranean area. Cactus pear tree is the typical example of plant species perfectly suitable for development in arid and semi-arid areas. Its culture is not particularly demanding in investments and the generally generated income is important. Also, from the environmental point of view and from their roots to their spines, the plant is used in numerous varied fields, especially in traditional medicine, human and animal alimentation, and cosmetics (El-Mostafa et al., 2014; Patel, 2013).

CF is used in the preparation of juice, jam, yogurt, food colouring, cosmetics, alcoholic drinks, etc. (Moussa-Ayoub et al., 2016). Since these products showed in general a pleasant taste and aroma. It has been revealed that it is rich in vitamin C and calcium and its nutritional value is close to that of lettuce and spinach (Özcan and Al Juhaimi, 2011).

The therapeutic benefits of this fruit reside in their control to lower blood sugar and cholesterol levels, as well as their antioxidant power due to their richness in fibres, vitamin C, and polyphenols (Chahdoura et al., 201; Jimenez-Aguilar et al., 20145).

Nowadays, research focusing on developing processes for the valorisation of prickly pears is very advanced. It has resulted in the establishment of technologies for processing cactus fruit into different products. The grains, resulting from the fruit transformation have been used for oil extraction with a very high commercial value. Among the transformations that prickly pears can undergo is the manufacture of vinegar. Currently, the cactus pear tree is the subject of several scientific research all over the world (Abdel-Hameed et al., 2014; Nharingo et al., 2015; Ammar et al., 2018).

Vinegar is an important product in the kitchen. It has several uses, such as making the vinaigrette, mayonnaise, and mustard. It avoids the oxidation of fruit and vegetables and prolongs the shelf life of food. It contributes to soothes stomach pain, prevents tumours, aids digestion, improves appetite, and calms burns. However, its excessive consumption weakens nerve and eyesight and it yellows the colour of the face (Bouazza et al., 2016).

To contribute to the good development of the CF vinegar processing chain, we coupled the traditional manufacturing steps with some flavouring specific operations, and the main physico-chemical and biochemical characteristics of the CF vinegar manufactured were determined. The quality of the finished vinegar was compared with some dates and apple commercial vinegar available in the local market.

2. Materials and methods

2.1. Plant material

This study was performed on cactus pear (*Opuntia ficus-indica* L.) fruit that commonly grow in Tunisia. The CF used are sampled from the Zelfen area situated in the Central West of Tunisia, region of Kasserine. This area is well known and famous for its important plantation and production of the CF. The CF of Zelfen is also well known for its unique juice taste and healthy recognised at the scale of the Tunisian market.

2.2. Diagram of the CFV production

100 kg of CF was collected, washed with tap water and peeled manually. Peeled fruits were cold-pressed and decanted for three hours to ensure the separation of the seeds and juice. The resulting juice was then filtered, packed in polypropylene bins and immediately subjected to alcoholic fermentation under an anaerobic atmosphere and acetic fermentation under an aerobic atmosphere. This final product resulting from the acetic fermentation was followed by an aromatization (Figure 1).

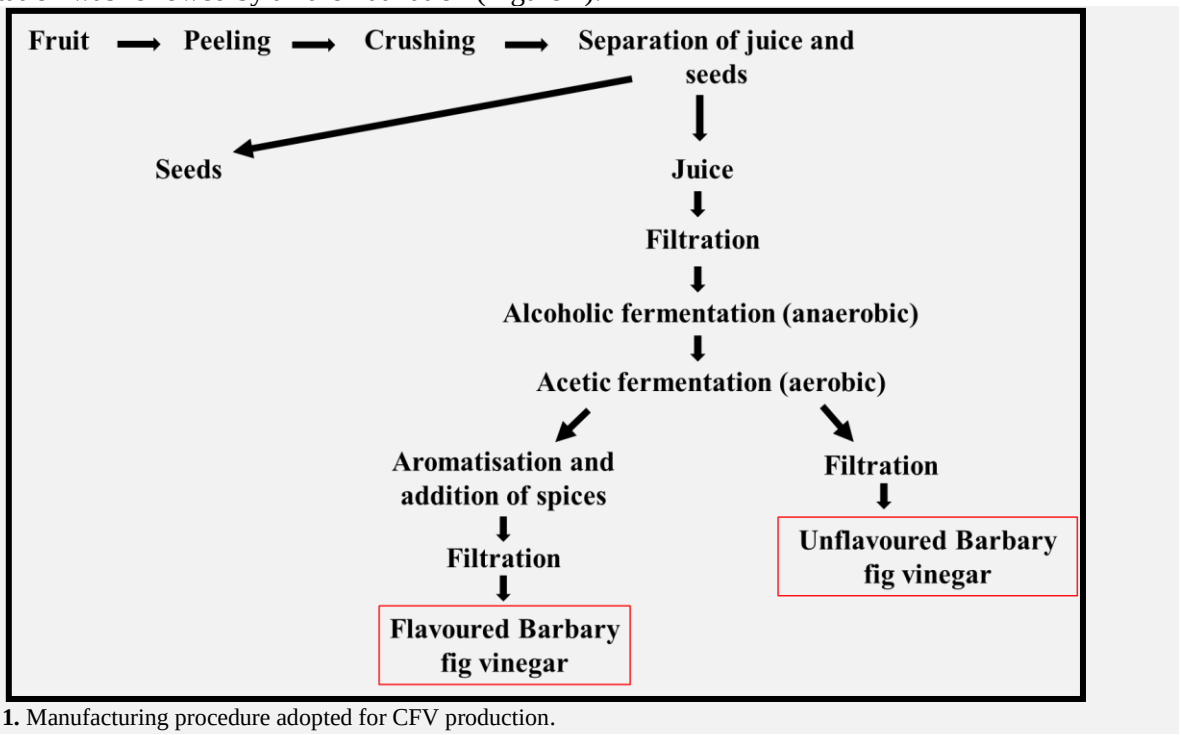

Figure 1. Manufacturing procedure adopted for CFV production.

The CFV manufacturing principle involved a traditional biological double combined processes, anaerobic-aerobic and spontaneous alcoholic and acetic fermentation that will be operated by a multitude of acetic flora naturally present in the original fermentation substrate, involving mainly yeast and acetic bacteria. This double fermentation leads to the production of ethanol that will be immediately transformed into acetic acid. At first and during alcoholic fermentation, almost all the available sugar of CF will be transformed into alcohol, then, this alcohol produced will be converted into acetic acid during the acetic fermentation. According to Wu et al. (2012), all these last processes will be operated under the combined action of oxygen, yeasts, and acetobacters bacteria.

2.3. Vinegar flavouring technique

The common maceration technique was used to flavour the CFV. Two aromatic plants, fresh ginger and cinnamon were hereby added at two different proportions 5 and 10%, respectively. We have added ginger and cinnamon to our vinegar considering the many virtues of ginger and cinnamon known worldwide and which lie in their refreshing power on the general digestive system. In fact, they act as a natural stimulant of our general metabolism and are often used as a highly appreciated food additive.

Depending on the proportion of flavouring, 25 and 50 g of fresh ginger and cinnamon catted in small pieces, were put respectively in four plastic food jars of 500 mL beforehand filled with vinegar (Table 1). The jars

were well closed and incubated for 15 days at room temperature (29 ± 2°C), and flavoured vinegar obtained was filtered.

2.4. Physicochemical parameters of CFV quality

Determination of pH: A calibrated pH metre electrode was introduced into 50 mL beakers containing the vinegar sample and the pH value was read directly. **Determination of electrical conductivity (EC)**: The electrode was introduced in a conductivity meter previously calibrated by KCl (0.02 N) into 50 mL of vinegar and the value of EC (mS/cm) was directly read. **Determination of soluble solids content (SSC)**: The SSC was determined using an Abbe refractometer (Novex, Holland). SSC value was obtained by direct reading on the corresponding scale. **Determination of dry matter content (DMC):** The dry matter of the products was determined by evaporating their moisture without causing the recovery of the constituent substances of the product. It was obtained by drying the vinegar in an oven at 105°C until constant weight. **Determination of ash content (AC)**: It is based on the incineration of the dry matter of vinegar in a muffle furnace at 550°C +/-20°C. **Determination of acetic acid**: The technique is based on the titration of a weak acid (CH_3COOH) with a strong base (NaOH 0.1 N) in the presence of phenolphthalein as a colour indicator.

Table 1. Composition and physico-chemical characteristic parameters of CFV.

Batches code	Nature of the batches	pH	°Brix	EC (mS/cm)	DMC (%)	Water content (%)	Ash content (g/L)	Acetic acid (g/L)
VE1	CFV in alcoholic phase	3.44 ± 0.02^{b}	5.32 ± 0.08^{g}	3.63 ± 0.01^{b}	2.3 ± 0.01^{b}	97.7 ± 0.01^{d}	0.43 ± 0.01^{c}	0.76 ± 0.001^{a}
VE2	CFV in the acetic phase	3.8 ± 0.1^{c}	4.7 ± 0.05^{f}	5.4 ± 0.03^{f}	2.98 ± 0.01^{c}	97 ± 0.01^{c}	0.47 ± 0.001^{d}	16.8 ± 0.065^{f}
VE3	CFV in acetic phase flavoured with 5 % ginger	4.31 ± 0.1^{e}	2.4 ± 0.02^{b}	6.58 ± 0.01^{g}	3.1 ± 0.01^{d}	96.9 ± 0.02^{b}	0.49 ± 0.002^{e}	15 ± 0.03^{d}
VE4	CFV in acetic phase flavoured with 10 % ginger	5.36 ± 0.04^{g}	2.2 ± 0.1^{a}	6.96 ± 0.01^{h}	3.16 ± 0.01^{f}	96.8 ± 0.03^{a}	0.51 ± 0.01^{f}	13.8 ± 0.06^{b}
VE5	CFV in acetic phase flavoured with 5 % cinnamon	4.1 ± 0.2^{d}	3.1 ± 0.02^{d}	4.5 ± 0.01^{d}	3.2 ± 0.001^{g}	96.8 ± 0.01^{a}	0.58 ± 0.01^{g}	15.6 ± 0.05^{e}
VE6	CFV in the acetic phase flavoured 10 % cinnamon	5.12 ± 0.02^{f}	4.4 ± 0.05^{e}	4.65 ± 0.01^{e}	3.24 ± 0.01^{h}	96.8 ± 0.05^{a}	0.61 ± 0.01^{h}	14.4 ± 0.06^{c}
VE7	Date Vinegar	3.16 ± 0.04^{a}	2.6 ± 0.1^{c}	4.3 ± 0.01^{c}	3.14 ± 0.01^{e}	97.9 ± 0.01^{e}	0.36 ± 0.004^{a}	18 ± 0.1^{g}
VE8	Apple Vinegar	3.13 ± 0.07^{a}	2.1 ± 0.01^{a}	3.11 ± 0.01^{a}	2.13 ± 0.01^{a}	97.88 ± 0.01^{e}	0.38 ± 0.01^{b}	19.2 ± 0.1^{h}

Results are presented as means ± SD (n=3). a, b, c...: Within each column, mean values followed by the same letter are not significantly different according to the Student-Newman-Keuls test at $p < 0.05$. CFV: Cactus fruit vinegar; EC: electrical conductivity; DMC: dry matter content.

2.5. Nutritional analysis of CFV

The main nutritional factors of quality of the finished vinegar are assessed as follows: ***Total Proteins***: Total protein colorimetric determination was conducted in an alkaline medium using the Gornall reagent (El Hadj et al., 2001). ***Vitamin C assay***: Vitamin C or ascorbic acid measurement was done according to the method described by Jain et al., (1995). ***Determination of reducing sugars***: Reducing sugars were determined using the DNS (3.5-dinitrosalicylic acid) colorimetric method. ***Determination of total sugar***: The determination of total sugar was carried with the spectrophotometric methods (El Hadj et al., 2001). ***Sucrose content***: The sucrose content was got by the difference between the content of total sugar and reducing sugar present in the sample. ***Determination of total polyphenols***: The total polyphenol content of different vinegar samples was determined by a spectrophotometric method (Kim et al., 2012). ***Determination of flavonoids***: The flavonoid content was determined using the method of aluminium chloride ($AlCl_3$) the spectrophotometric method (Djeridane et al., 2006). ***Determination of condensed tannin***: The condensed tannin was conducted by spectrophotometry (Julkunen-Tiitto, 1985). ***Dosage of hydrolysable tannins***: The hydrolysable tannin method is based on a reaction with ferric chloride (Mole and Waterman, 1987). ***Dosage of pectin***: The pectin determination was carried out according to Zhang et al. (2018).

2.6. Microbiological analysis of CFV quality

Examination of the hygienic quality was only performed by the determination of the total number of microorganisms using conventional methods. ***Total bacteria count***: For the analysis of total bacterial count, 1 ml of CFV samples were added to 9 ml of a physiological saline solution (0.9%), homogenized for 1 min and subjected to a serial dilution. 100 µL of each dilution were spread out on Plate Count Agar (PCA) and incubated at 37°C for 24 hours (AFNOR NFT 90-401 standards). ***Yeast and mould counts***: Enumeration of

yeast and mould was done as recommended by the Directive of Official Methods of Analysis of AOAC International and 100 µL of each dilution were spread out on the Oxy-Tetracycline-Glucose Agar (OGA) and incubated at 25°C for 5 days. Each sample was analysed in triplicate and all the results were expressed as colony-forming units per milliliter (CFU/mL).

2.7. Sensory analysis of vinegar

To check the effect of flavouring on the organoleptic properties of CFV, the sensory study is based on a consumer assessment by considering the level of consumer satisfaction with the finished product, while including colour, smell, acidity, and taste. For the hedonic test, a panel of 60 naïve consumers evaluated the samples and mentioned the most preferred and best-appreciated one. Unflavoured and flavoured finished product of CFV was compared successively with commercial apple vinegar available on the market and with the flavoured CFV by considering the main organoleptic criteria earlier mentioned. Four samples used were labelled with a specific code (100: CFV, 101: CFV flavoured with ginger, 103: CFV flavoured with cinnamon, 104: Apple vinegar). Before conducting the test, dilutions of each product were proceeded by mixing 10 mL of each type of vinegar with 100 mL of mineral water to lower the vinegar acidity to be tested.

2.8. Determination of biological activities

***Evaluation of antiradical activity*:** The 2.2-diphenyl-l-picrylhydrazyl (DPPH) is an unstable free radical that becomes stable by accepting a hydrogen-free radical. The reduction capacity of DPPH was determined by the spectrophotometric method according to Mansouri et al. (2005). The existing antioxidants in the extracts induced the decrease of its maximum absorbance at 515 nm and the colour of the mixture changed from purple to yellow.

Evaluation of the effect of CFV on alpha-amylase activity: Alpha-amylase activity was determined using the DNS reagent (3,5- dinitrosalicylic acid, red colour) that acted as an oxidant. Glucose released as a result of starch degradation which is the reducing agent. The intensity of the red coloration obtained was proportional to the concentration of glucose. The 3,5- dinitrosalicylic acid, the red colour will be reduced to the 3-amino-2-hydroxy-5-nitrobenzoic.

Assessment of the vinegar antibacterial activity: According to Andoğan et al. (2002) with some modifications, the antimicrobial activity of unflavoured and flavoured finished product of CFV was evaluated by the disk diffusion method. Sterile blank disks (with 6 mm of diameter) impregnated with 20 µl of the vinegar were deposited on the surface of Muller Hinton Agar plates previously inoculated with 100 µl of the prepared bacterial suspension (10^8 CFU/mL). Then, the Petri dishes were incubated at 37°C for 24 hours. Inhibition zones around the disks were measured in millimetres. Ampicillin (25 µg/mL) and marketed vinegars were used as a positive control. Reference bacterial strains selected for this test are *Bacillus thuringiensis* BTHD22 (United States Department of Agriculture USDA), *Bacillus thuringiensis entomocidus* HD9 (USDA), *Bacillus thuringiensis* HD2 (Bacillus Genetic Stock Centre BGSC), *Bacillus thuringiensis* HD110 (USDA), *Staphylococcus aureus* 6539 (American Type Culture Collection ATCC), *Methicillin-Resistant Staphylococcus aureus* US300 MRSA (ATCC), *Escherichia coli* 25922 (ATCC), *Salmonella enterica* 14028 (ATCC), *Klebsiella pneumoniae* 10070 (ATCC). All tests were carried out for three sample replications.

2.9. Statistical analysis

Data were analysed by using IBM SPSS Inc. Software (Version 25). One-way analysis of variance (ANOVA) and multiple comparisons were used to check differences between control and treated groups. Data were presented as mean ± SD and P-value were considered to indicate a statistically significant difference ($P<0.05$).

3. Results and discussion

3.1. Physico-chemical characterisation of CFV

To characterise the finished product of CFV and the other kinds of commercial vinegar tested, a series of physico-chemical parameters analyses have been carried out. All results obtained for pH, Brix, and EC; the determination of the DMC, ash, and acetic acid are presented in Table 1.

For acidity, at the beginning of the fermentation and during the alcoholic phase, the pH of CFV (VE1) was around 3.4 and appeared a little lower than the one registered during the acetic phase (VE2) with around 3.8. For flavouring CFV, the measured pH tended to vary somewhat according to the flavour and the percentage of flavour added. Indeed, pH has fluctuated between 4.31 ± 0.1 and 5.35 ± 0.04 for the VFG whose percentage is respectively 5 and 10%. However, VFC showed a lower pH than the one registered for unflavoured CFV. This pH varied between 4.10 ± 0.2 and 5.12 ± 0.02 for 5 and 10% of cinnamon, respectively.

Two marketable samples of dates and apple vinegar were studied in parallel, and they showed two pH values a little lower than the one obtained for CFV, with around 3.16 ± 0.04 for dates vinegar and 3.13 ± 0.07 for apple vinegar. This acidic pH character is mainly involved in inhibiting and eliminating the development of many pathogenic microorganisms and contributed to promoting well-finished product conservation. Besides, this acidity has resulted from the metabolism of acidophilic microorganisms such as acetic, lactic, and lactic acid bacteria. As earlier reported by Lalou et al. (2015), the presence of organic acids in the vinegar such as malic acid, citric acid, and other components might be giving an original acidity and taste to the vinegar.

Electrical conductivity result of CFV increased slightly from 3.63 ± 0.01 mS/cm during the alcoholic phase (VE1) to 5.4 ± 0.03 mS/cm during the acetic phase (VE2). It achieved its highest values in samples VE3 and VE4 that corresponded to CFV flavoured with 5 and 10% of ginger, respectively. These results could be explained by the presence of mineral matter in the CF fruit, or the absence of thorough cleaning of the CF done by the processed water (tap water) generally used in the manufacture of these products. This water is characterised by a significant load of dissolved salts. In return, the two samples of apple vinegar and dates showed a very low EC as compared to one of CFV, whether with or without aroma amendments.

The total soluble solid content (SSC) varied with the concentration of substances in the solution. It appeared clearly that the highest SSC was reached during the phase of alcoholic fermentation (VE1 = 5.32 ± 0.08 °Brix), then it slightly decreased during the phase of acetic fermentation. It was of the order of 4.7 ± 0.05 °Brix for VE2 and higher than the ones registered for date vinegar (VE7 = 2.6 ± 0.01 °Brix) and apple vinegar (VE8 = 2.1 ± 0.01 °Brix), respectively. This result could be explained by the difference in raw materials initially used for vinegar manufacturing. For flavoured CFV, the SSC tended to vary according to the added flavour. Indeed, VFC showed an SSC higher than the one registered for VFG and lower than unflavoured vinegar (Table 1).

The dry matter composition showed a slight increase from the alcoholic phase (VE1= 2.3 ± 0.01%) to the acetic fermentation one (VE2=2.98 ± 0.01%), which exhibited a lower DMC than that registered for date vinegar (VE7=3.14 ± 0.01%) and a higher DMC than that recorded for apple vinegar (VE8=2.13 ± 0.01%), respectively. Thus, the flavouring of CFV looked to increase the DMCs if compared with the unflavoured one. Indeed, the highest dry matter content is observed at VE6 (3.24 ± 0.01%), followed by VE5 (3.2 ± 0.001%), then VE4 (3.16 ± 0.01%), and at last VE3 (3.10 ± 0.01%).

CFV showed comparable ash content with the commercial vinegar of dates and apples tested. Indeed, these ash contents varied and somewhat increased from the alcoholic fermentation phase (0.43 g/L) to the acetic fermentation phase (0.47 g/L), which is higher than the ones determined for date vinegar (0.36 g/L) and apple vinegar (0.38 g/L). This result may be explained by the richness of CF in various minerals as earlier underlined by El Kossori et al. (1998). Moreover, the flavouring of CFV has as effect to increase vinegar ash content as compared with unflavoured one; this increase of ash content may be due to the mineral richness and abundance of the added aromatic flavouring plant. Although, the VFC and VFG showed ash contents very close to each other, ranging from 0.49 g/L for VE3, 0.51 g/L for VE4, 0.58, and 0.61 g/L for VE5 and VE6, respectively. Thirty days after the fermentation of vinegar and corresponding to the acetic fermentation, acetic acid showed a range of 10 to 16 g/L. It was noted that the pH of vinegar increases during this fermentation and reaches values varying between 4.1 and 5.36 on the 30th day. This pH tends to stabilise around this latter value of 5.36 until the finish of the process. According to Akin et al. (2008), yeast metabolism induces perpetual significant changes in the reacting mixture during alcoholic fermentation. Thus, the consumption of carbonaceous and nitrogenous substrates is often coupled and accompanied by the production of acid or alcohol metabolites. Our results showed that the total DMC of the juice changed from 5 to around 3 Brix resulting in the consumption of total dry matter in the yeast. These results were consistent with those found by Bchir et al. (2012) during an experiment on date juice vinegar production. It should be pointed out that the rate of Brix reduction is greater in fermenter inoculated with yeast than the one conducted spontaneously without any yeast inoculation.

On the other hand, acetic acid will arise out of the aerobic oxidation of ethanol by acetic bacteria, known as acetobacters. The acetic acid content of CFV varied from 16.8 ± 0.065; 15 ± 0.03; 13.8 ±0.06; 15.6 ± 0.05 and 14.4 ± 0.06 g/L for VE2, VE3, VE4, VE5, and VE6 respectively (Table 1). Besides, El Hadj et al. (2001) had reported that the unflavoured CFV always showed a low acetic acid content as compared to ones of the date vinegar (18 ± 0.1 g/L) and apple vinegar (19.2 ± 0.1 g/L) tested; this fact makes unflavoured CFV always less acidic than the two other kinds of dates and apples vinegar examined. Similarly, it was observed that there was a reverse relationship between acetic acid and pH, and subsequently, the most acidic vinegar showed a high acetic acid content and a low pH, which was the case for the VE8 experiment. These levels of acetic acid content could be linked to the joint action of fermentation by bacteria and moulds.

In the general industry, the production of acetic acid is held in two separate stages. Fermentation in the case of traditional vinegar manufacture is a combined process in one step. So, at the same time as alcohol is

obtained, acetic acid is produced by oxidation of ethanol. It is a disorderly transformation in which a multitude of microorganisms are engaged. In the same way, the action of the additional effect of yeast, acetobacters, and other microorganisms, gives the medium a more concentrated and cloudy appearance. The fermentation conditions in traditional vinegar production, such as anaerobiosis conditions decrease the fermenting power of acetobacters supported with other microorganism proliferation, and therefore acetic acid could have three different origins: the oxidation of ethanol by acetobacters; the metabolism of lactic acid bacteria; and yeast during fermentation.

3.2. Hygienic characteristics of CFV

Results of total bacteria count and enumeration of yeast and moulds showed that the number of total bacteria was below 5.10^5 CFU/mL, threshold value fixed by the standards of the Good Manufacturing Practices (GMP) of Hazard Analysis and Critical Control Point, or HACCP food safety system (Table 2). Also, their number is not greater than the standards established by the local Quality Control Laboratory of Mahdia, Tunisia with 10^2 cells/g of product. So, the finished product of CFV manufactured appeared free of any yeast and moulds. This result helps to attest that our vinegar is healthy and safe for human consumption.

Table 2. Microbiological characteristics of CFV final products.

Samples	Total bacteria (10^5)	Yeasts (10^2)	Moulds (10^2)
Unflavoured vinegar	$<10^5$	0	0
CFV flavoured with 10% cinnamon	$<10^5$	0	0
CFV flavoured with 10% ginger	$<10^5$	0	0

CFV: Cactus fruit vinegar.

3.3. Nutritional quality of CFV

During acetic fermentation, there is a major increase in the content of protein, vitamin C, phenolic substances, and flavonoids in comparison with alcoholic fermentation (Figure 2). These increased contents are likely due to the ability of acetic bacteria to synthesise and secrete these nutrients in the vinegar. The presence of proteins in vinegar might improve its nutritional value. However, the acidity and the presence of tannin could coagulate and denature some of the proteins (Figure 2A, B). So, a large part of the nitrogenous matter in traditional vinegar production emanated from microbial metabolism. The protein content of the finished product of CFV showed an increase from the alcoholic fermentation phase (VE1=1.46 mg/mL) to the acetic fermentation one (VE2= 3.18 mg/mL). This latter value was right higher than the ones of commercial dates vinegar (VE7 = 1.25 mg/mL) and apple vinegar tested (VE8= 0.74 mg/mL) (Figure 2C).

Besides, the protein contents in the flavoured vinegar seemed very close to each other; they were 4.18; 4.52; 4.89, and 4.74 mg/mL for VE3, VE4, VE5, and VE6 experiments, respectively (Figure 2C). These values appeared higher than the ones achieved in the case of unflavoured CFV, and subsequently, flavouring CFV with ginger and cinnamon looked to enhance the vinegar protein production.

Vitamin C content of CFV showed a tendency to increase from the alcoholic phase (VE1= 1.12 mmol) until the end of the acetic fermentation (VE2= 2.98 mmol). These last two values looked higher than the ones registered for the two commercial vinegars tested VE7 (1.12 mmol) and VE8 (1.35 mmol) (the date and apple vinegar, respectively) (Figure 2D). Also, the vitamin C content of flavoured CFV was higher than the one of unflavoured CFV and appeared to vary according to the percentage of flavour added. The highest percentages of flavours that had presented the highest vitamin C contents are entered in the cases of VE6 (6.085 mmol) and VE4 (5.18 mmol).

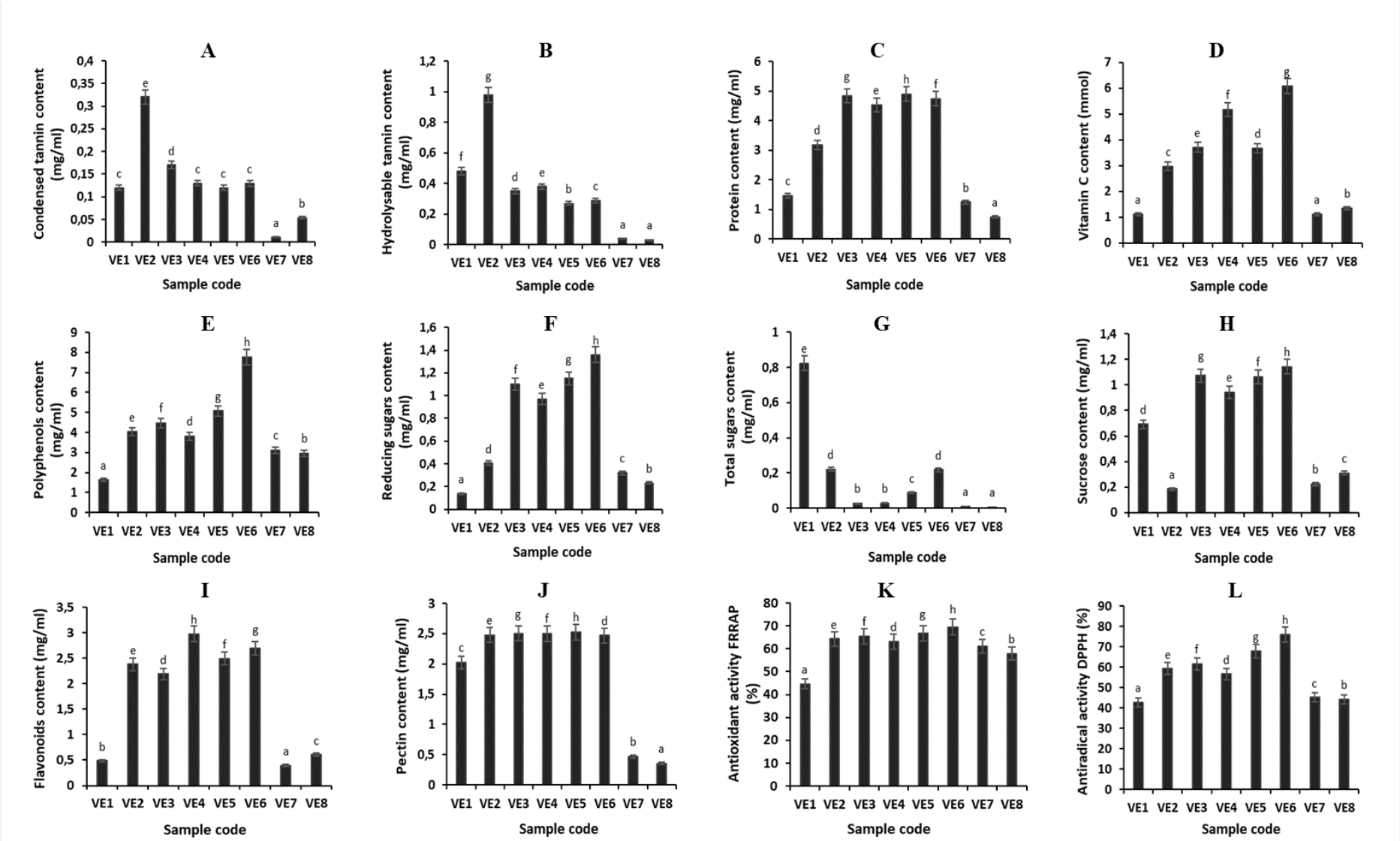

Figure 2. Nutritional parameters analysis of CFV. Values are mean ± SD (n=3). a, b, c...: Within each column, mean values followed by the same letter are not significantly different according to the Student-Newman-Keuls test at $P < 0.05$. (A) Condensed tannin content; (B) Hydrolysable tannin content; (C) Protein content; (D) Vitamin content; (E) Polyphenol content; (F) Reducing sugar content; (G) Total sugar content; (H) Sucrose content; (I) Flavonoids content; (J) Pectin content; (K) Antioxidant activity FRRAP; (L) Antiradical activity DPPH

The polyphenol content of CFV showed a net increase from the alcoholic phase (VE1= 1.62 g/L) to the acetic one (VE2= 4.03 g/L). These polyphenol contents appeared greater than the ones achieved in the case of dates and apple vinegar (Figure 2E). Besides, the polyphenol content of flavoured CFV looked more important than the one achieved in the case of unflavoured CFV. The polyphenol vinegar content closely depended on the kind of flavour and the percentage of flavour added. Also, the highest polyphenol content in flavoured CFV was found in the sample VE6 (7.8 g/L) and the second-highest one in the sample VE5 (5.07 g/L).

In parallel, a sharp decrease in the rate of sugar content, as simple sugars and polysaccharides, was observed (Figure 2F). This decrease in the rate of sugar showed the imperative need for microbial metabolism and activity of sugar for growth and development. This decrease is explained at first by the biotransformation of sugar into alcohol during the first phase of fermentation, and secondly by the biotransformation of alcohol into acetic acid during the second phase of fermentation (Figure 2F, G, H).

The flavonoid content of CFV showed a net increase from the alcoholic phase (VE1= 0.486 mg/mL) to the acetic one (VE2= 2.38 mg/mL); these values appeared greater than for the one determined in the case of commercial vinegar samples tested (Figure 2I). Also, flavonoid content of flavoured CFV revealed higher than the one registered in the case of unflavoured vinegar with VE7= 0.392 mg/mL and VE8= 0.604 mg/mL, registered for dates and apple vinegar. Indeed, this flavonoid content varied and increased according to the flavour and the percentage of flavour added. The highest value was observed at sample level VE4 = 2.98 mg/mL, followed by VE6 = 2.69 mg/mL, and VE5 = 2.49 mg/mL, and finally to VE3 =2.19 mg/mL (Figure 2I).

Pectin contents of CFV presented a slight increase from the alcoholic phase (VE1= 2.026 mg/mL) to the acetic one (VE2= 2.476 mg/mL). These two latter values were higher than the two pectin contents obtained in the case of the two commercial vinegars tested (VE7= 0.465 mg/mL and VE8= 0.351 mg/mL), the date and apple vinegar respectively (Figure 2J). Besides, the pectin content of flavoured CFV was more important than the one recorded for unflavoured one. These pectin contents were very similar to each other and the highest one was observed for VE5 with 2.525 mg/mL, then VE3= 2.503 mg/mL, VE4= 2.497 mg/mL, and VE6= 2.469 mg/mL (Figure 2J). For the antioxidant and antiradical activity, there was an increase from the alcoholic phase to the acetic one (Figure 2K, L). This latter result could be explained by the increase in the rate of antioxidant molecule power in the vinegar during the acetic fermentation, such as vitamin C, phenolic substances, and flavonoids. Indeed, unflavoured CFV showed antioxidant and antiradical activities more important than the ones observed for date and apple vinegar. Moreover, flavoured CFV showed greater values of organic activities rather than for the unflavoured ones. For these two organic activities, the VE6 batch experiment sample showed the highest value while regarding and considering successively the cases of VE5, VE3, and VE4 batch experiments (Figure 2K, L).

It could be concluded that CFV flavoured with ginger and cinnamon presented a higher nutritional quality than unflavoured vinegar one. Also, VFG showed a lower nutritional quality than the one flavoured with cinnamon. Among the flavoured vinegar, VFC at 10% revealed the best nutritive quality because of its richness in proteins, vitamins C, polyphenols, flavonoids, and pectin. This richness allowed to give a definite defensive power against free radicals and reinforces, in general, some biological activities. Studies carried out showed that fermentation also influences the nutritive quality of food, depending on the metabolic activities of the microorganisms involved. Among the beneficial nutritional effects, it could mainly notice the improved digestibility of starch, protein, and increased content of carotenoids and vitamins of the B group (Jimenez-Aguilar et al., 2014; Şanlier et al., 2019). Our results were in agreement with other studies showing that acetic fermentation increases lipids, proteins, and polyphenol levels, as well as antioxidant capacity and therapeutic power (Hamden et al., 2018).

3.4. The antibacterial effect of CFV

The unflavoured vinegar showed lower antibacterial activity than the ones registered for flavoured ginger and cinnamon vinegar, with diameters up to 13 mm and 19.5 mm for *K. pneumoniae* and *E. coli* strains respectively. For VFG 10%, there was an increase in the antibacterial power of the vinegar, noticed by an increase of the diameter of inhibition compared to the unflavoured one, which passed from 13 to 16 mm for *K. pneumoniae* and 18 to 23 mm for *B. thuringiensis entomocidus* HD9. The best antibacterial activity was observed with VFC 10%; however, the VFG 10% gave less antibacterial effect. Indeed, the diameter of inhibition increased from 16 mm for ginger to 19 mm for cinnamon in the case of *K. pneumoniae*. The unflavoured vinegar showed less remarkable antibacterial activity than VFC and VFG, with diameters of inhibition reaching 18 mm in the case of *B. thuringiensis* HD110 strains (Figure 3).

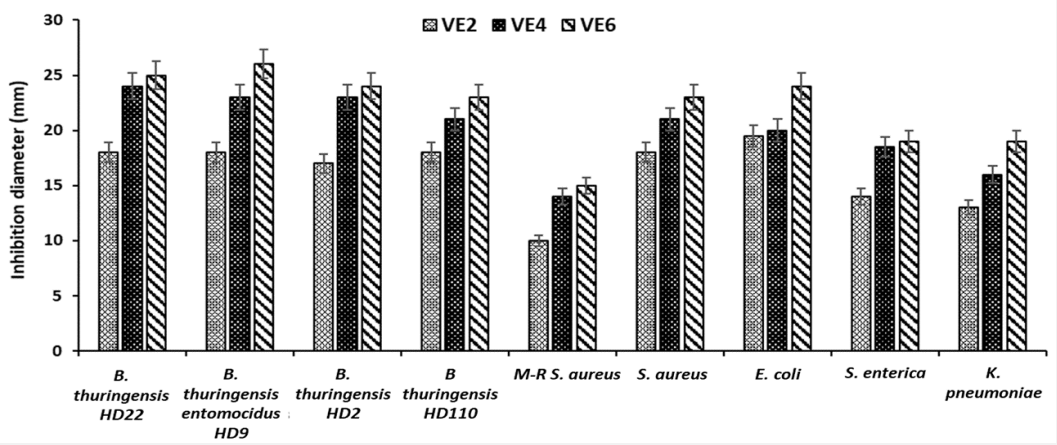

Figure 3. Antibacterial effect of three products of CFV, VE2 (CFV in the acetic phase), VE4 (CFV in the acetic phase flavoured with the 10 % ginger), and VE6 (CFV in the acetic phase flavoured with 10 % cinnamon), after 24 h of incubation. Zones of microbial growth inhibition are indicated by clear zones.

Besides, VFC 10% always showed the highest antibacterial activity as compared to VFG 10% and unflavoured vinegar for all bacterial strains tested. The unflavoured vinegar showed less remarkable antibacterial activity than VFC and VFG, with diameters of inhibition reaching 18 mm for *B. thuringiensis* HD110 (Figure 3). Our results were in perfect agreement with those found by Zhang et al. (2016), who found average variable inhibition diameters of cinnamon extracts depending on the bacterial strain tested. Also, Nabavi et al. (2015) found that *Cinnamomum cassia* extract showed moderate antibacterial activity against various bacterial species tested. For ginger, our results were also in agreement with those of Shareef et al. (2016) and Liu et al. (2017), where ginger gave no antibacterial activity against some of the bacterial strains tested, including some strains like *S. aureus*, *E. coli*, and *Salmonella sp.* But it showed remarkable antifungal activity against *Candida albicans*.

3.5. Effects on alpha-amylase activity

Our study showed that alpha-amylase activity decreased in the presence of unflavoured CFV with around 52% CI50. But, flavouring vinegar with cinnamon at 5 and 10% resulted in increasing the inhibitory effect against alpha-amylase (Table 3).

Table 3. Effects of flavoured and unflavoured vinegar on alpha-amylase activity.

Sample code	Inhibition (%)	IC50 (%)
VE2 (100%)	68	
VE2 (50%)	48	5200
VE2 (25%)	38	
VE3 (100%)	25	
VE3 (50%)	41	6000
VE3 (25%)	53	
VE4 (100%)	31	
VE4 (50%)	49	5100
VE4 (25%)	57	
VE5 (100%)	53	
VE5 (50%)	64	3900
VE5 (25%)	70	
VE6 (100%)	67	
VE6 (50%)	73	3400
VE6 (25%)	89	

Indeed, it is well documented that one of the mechanisms by which polyphenols act on the state of diabetes is the improvement of insulin sensitivity. This improvement consisted in the induction of glucagon-like peptide (GLP-1) synthesis in animal species (Macit et al., 2019). GLP-1 has multiple effects including delaying gastric emptying, improving the cellular insulin-dependent absorption of glucose, inhibiting glucagon secretion, stimulating insulin synthesis, and reducing hepatic glucose production, which collectively may reduce insulin requirements.

3.6. Sensory evaluation of vinegar

The majority of panellists (22 / 60) preferred CFV flavoured with cinnamon. This result was supported by nutritional analyses (Figure 4). Different samples of CFV, whether flavoured or not, showed significant percentages of appreciation than the control vinegar, the apple vinegar. Besides, the sample of unflavoured CFV (100) showed a lower percentage of appreciation with 20% as compared to that flavoured with cinnamon and ginger and greater than the percentage registered in the case of the standard sample with 13.33% (104: apple vinegar). Also, CFV flavoured with ginger (101) showed a lower percentage of appreciation than the one flavoured with cinnamon (103) and greater than the unflavoured one (100). CFV flavoured with cinnamon (103) appeared as favoured by the majority of consumers.

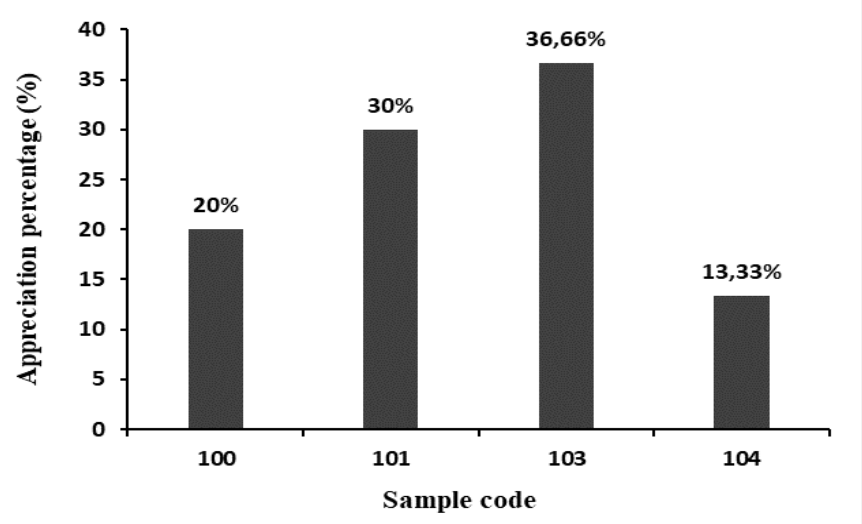

Figure 4. Overall product appreciations. 100 (unflavoured CFV), 101 (CFV flavoured with ginger), 103 (CFV flavoured with cinnamon), 104 (Apple vinegar).

4. Conclusion

Nowadays the research of new processes for the valorisation of CF is very developed through the implementation of new technologies allowing the transformation of the cactus fruit into different by-products such as vinegar from CF juice. Obtained vinegar can effectively presents an interesting biological activity and a good nutritional quality. It could be fortified with other minerals and vitamins to serve as a medicated dietary supplement. This special vinegar product could be introduced and employed as an ingredient in many diets and cosmetic products. Moreover, CFV may be useful for chemoprevention via nutraceutical foods.

Acknowledgements

This work was supported by the UR Analysis and Process Applied to the Environment APAE (Mahdia, Tunisia) and by the Tunisian Ministry of Higher Education and Scientific Research. We are grateful to all staff of the Laboratory of Society Herbéos, Monastir, Tunisia.

Author contributions

WH and HBM conceived and designed the experiments. WH performed the experiments, analysed the data and wrote the manuscript. AH contributed to English editing and some statistical/ methodology verifications. All authors revised and approved the final manuscript.

Conflict of interest The authors have declared no conflict of interest.

5. References

Abdel-Hameed ESS, Nagaty MA., Salman MS, Bazaid SA (2014) Phytochemicals, nutritional and antioxidant properties of two Barbary figs cactus cultivars (*Opuntia ficus indica* Mill.) growing in Taif, KSA. Food chem. 160: 31-38. https://doi.org/10.1016/j.foodchem.2014.03.060

Akin H, Brandam C, Meyer XM, Strehaiano P (2008) A model for pH determination during alcoholic fermentation of grape must by Saccharomyces cerevisiae. Chem Eng Proces: Process Intensification. 47: 1986-1993. https://doi.org/10.1016/j.cep.2007.11.014

Ammar I, Salem MB, Harrabi B, Mzid M, Bardaa S, Sahnoun Z, Attia H, Ennouri M (2018) Anti-inflammatory activity and phenolic composition of Barbary figs (*Opuntia ficus-indica*) flowers. Ind Crop Prod. 112: 313-319. https://doi.org/10.1016/j.indcrop.2017.12.028

Aridoğan BC., Baydar H, Kaya S, Demirci M, Ozbaşar D, Mumcu E (2002) Antimicrobial activity and chemical composition of some essential oils. Arch Pharmacal Res. 25(6):860-4 https://doi.org/10.1007/BF02977005

Bchir B, Besbes S, Karoui R, Paquot M, Attia H, Blecker C (2012) Osmotic dehydration kinetics of pomegranate seed using date juice as an immersion solution base. Food Bioproc Tech. 5: 999-1009. https://doi.org/10.1007/s11947-010-0442-1

Bouazza A, Bitam A, Amiali M, Bounihi A, Yargui L, Koceir EA (2016) Effect of fruit vinegar on liver damage and oxidative stress in high-fat-fed rats. Pharm Biol. 54: 260-265. https://doi.org/10.3109/13880209.2015.1031910

Chahdoura H, Morales P, Barreira JC, Barros L, Fernández-Ruiz V, Ferreira IC, Achour L (2015) Dietary fibre, mineral elements profile, and macronutrient composition in different edible parts of Opuntia microdasys (Lehm.) Pfeiff and Opuntia mycorrhizae (Engelm.). LWT- Food Sci Technol. 64: 446-451. https://doi.org/10.1016/j.lwt.2015.05.011

Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N (2006) Antioxidant activity of some Algerian medicinal plant extracts containing phenolic compounds. Food Chem. 97: 654-660. https://doi.org/10.1016/j.foodchem.2005.04.028

El Hadj MO, Sebihi AH, Siboukeur O (2001) Qualité Hygiénique et Caractéristiques Physico-Chimiques du Vinaigre Traditionnel de Quelques Variétés de Dattes de la Cuvette de Ourgla. Rev Energ Ren: Production et Valorisation-Biomasse 6.

El Kossori RL, Villaume C, El Boustani E, Sauvaire Y, Méjean L (1998) Composition of pulp, skin and seeds of prickly pears fruit (*Opuntia ficus indica sp.*). *Plant Foods Hum Nutr.* 52(3): 263-270. https://doi.org/10.1023/a:1008000232406

El-Mostafa K, El Kharrassi Y, Badreddine A, Andreoletti P, Vamecq J, El Kebbaj MH, Latruffe N, Lizard G, Nasser B, Cherkaoui-Malki M (2014). Nopal cactus (*Opuntia ficus-indica*) as a source of bioactive compounds for nutrition, health, and disease. Molecules. 19:14879-14901 https://doi.org/10.3390/molecules190914879

Hamden K, Boujibiha MA, Ben Abdeljelil N, Njima M, Achour L (2018) Inhibitory Effect of fermented pectin on key metabolic enzymes associated with diabetes, obesity; and Liver-Kidney tissue toxicities. Bioact Carbohydr Diet Fibre. 16: 82-89. https://doi.org/10.1016/j.bcdf.2018.07.002

Jain A, Chaurasia A, Verma KK (1995) Determination of ascorbic acid in soft drinks preserved fruit juices and pharmaceuticals by flow injection spectrophotometry: Matrix absorbance correction by treatment with sodium hydroxide. Talanta. 42(6): 779- 787. https://doi.org/10.1016/0039-9140(95)01477-S

Jimenez-Aguilar MD, Mújica-Paz H, Welti-Chanes J (2014) Phytochemical characterization of Prickly Pear (*Opuntia spp.*) and of its nutritional and functional properties: a review. Curr Nutr Food Sci. 10(1): 57-69. https://doi.org/10.2174/1573401310011403281209 52

Julkunen-Tiitto R (1985) Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. J Agric Food Chem. 33(2): 213-217. https://doi.org/10.1021/jf00062a013

Lalou S, Hatzidimitriou E, Papadopoulou M, Kontogianni VG, Tsiafoulis CG, Gerothanassis IP, Tsimidou MZ (2015) Beyond traditional balsamic vinegar: Compositional and sensorial characteristics of industrial balsamic vinegar and regulatory requirements. J Food Compost Anal. 43: 175-184. https://doi.org/10.1016/j.jfca.2015.07.001

Liu Q, Meng X, Li Y, Zhao CN, Tang GY, Li HB (2017) Antibacterial and antifungal activities of spices. Int J Mol Sci. 18(6): 1283. https://doi.org/10.3390/ijms18061283

Macit MS, Koyuncu S, Kocaadam B, Acar-Tek N (2019) Evaluation of Ginger (Zingiber Officinale Roscoe) on Energy Metabolism and Obesity: Systematic Review and Meta-Analysis. Food Rev Int. 35(7): 685-706. https://doi.org/10.1080/87559129.2019.1608556

Mansouri A, Embarek G, Kokkalou E, Kefalas P (2005) Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (Phoenix dactylifera). Food Chem. 89(3): 411-420. https://doi.org/10.1016/j.foodchem.2004.02.051

Mole S, Waterman P (1987) A critical analysis of techniques for measuring tannin in ecological studies. Oecologia. 72: 137-147. https://doi.org/10.1007/BF00385059

Moussa-Ayoub TE, Jaeger H, Youssef K, Knorr D, El-Samahy S, Kroh LW, Rohn S (2016) Technological characteristics and selected bioactive compounds of Opuntia declines cactus fruit juice following the impact of pulsed electric field pre-treatment. Food Chem. 210: 249-261. https://doi.org/10.1016/j.foodchem.2016.04.115

Nabavi S, Di Lorenzo A, Izadi M, Sobarzo-Sánchez E, Daglia M (2015) Antibacterial effects of cinnamon: From farm to food, cosmetic and pharmaceutical industries. Nutrients. 7(9): 7729-7748. https://doi.org/https://doi.org/10.3390/nu7095359

Nharingo T, Zivurawa MT, Guyo U (2015) Exploring the use of cactus Opuntia ficus indica in the biocoagulation–flocculation of Pb (II) ions from wastewaters. Int J Environ Sci Technol. 12(12): 3791-3802. https://doi.org/10.1007/s13762-015-0815-0

Özcan MM, Al Juhaimi FY (2011) Nutritive value and chemical composition of prickly pear seeds (*Opuntia ficus indica L.*) growing in Turkey. Int J Food Sci. 62: 533-536 https://doi.org/10.3109/09637486.2011.552569

Patel S (2013) Reviewing the prospects of Opuntia pears as low-cost functional foods. Rev Environ Sci Biotechnol. 12: 223-234 https://doi.org/10.1007/s11157-012-9295-6

Şanlier N, Gökcen BB, Sezgin AC (2019) Health benefits of fermented foods. Crit Rev Food Sci Nutr. 59(3): 506-527. https://doi.org/10.1080/10408398.2017.1383355

Shareef HK, Muhammed HJ, Hussein HM, Hameed IH (2016) Antibacterial effect of ginger (Zingiber officinale) roscoe and bioactive chemical analysis using gas chromatography-mass spectrum. Orient J Chem. 32(2): 20-40. https://doi.org/doi:10.13005/ojc/320207

Wu JJ, Ma YK, Zhang FF, Chen FS (2012) Biodiversity of yeast, lactic acid bacteria and acetic acid bacteria in the fermentation of "Shanxi aged vinegar", a traditional Chinese vinegar. Food Microbiol. 30(1): 289-297. https://doi.org/10.1016/j.fm.2011.08.010

Zhang W, Xie F, Lan X, Gong S, Wang Z (2018) Characteristics of pectin from black cherry tomato waste modified by dynamic high-pressure microfluidization. J Food Eng. 216: 90-97. https://doi.org/doi:10.1016/j.jfoodeng.2017.07.032

Zhang Y, Liu X, Wang Y, Jiang P, Quek S (2016) Antibacterial activity and mechanism of cinnamon essential oil against Escherichia coli and Staphylococcus aureus. Food Control 59: 282-289. doi:10.1016/j.foodcont.2015.05.032