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COMPARING PHYSICOCHEMICAL, NUTRITIONAL AND BIOCHEMICAL PROPERTIES OF THREE TRADITIONAL FIG VINEGARS PRODUCED IN ALGERIA

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ABSTRACT

Several fruits, including figs, can be used to produce vinegar, which has been recognized for its therapeutic uses. However, limited studies are available on Algerian fig vinegar production and properties, despite increased research interest in this product. The aim of this study was to compare the physicochemical, nutritional and biochemical properties of three vinegars produced from three fresh fig varieties of Beni-Ourtilane region (Setif - Algeria). Various physicochemical parameters (pH, conductivity, °Brix, etc.), nutritional properties (proteins, sugars, and lipids), and biochemical parameters (acetic acid content and alcohol) were measured. The traditional homemade vinegars produced had alcohol contents below 1% in accordance with the Codex Alimentarius standards, while the levels of acetic acid ranged between 1.05 and 2.79 °, not meeting the required standards. The chemical properties including pH value, conductivity, °Brix, moisture, dry extract, density, ash and organic matter of vinegar samples were determined as 3.9 to 4.05, 392 to 451 µS/cm, 7.6 to 10.2%, 90.29 to 93.58%, 6.42 to 9.71%, 1.01 to 1.02, 0.09 to 0.20% and 6.32 to 8.9%, respectively. Furthermore, the energy intake from the three vinegar samples was not significant as the values obtained varied between 3.54 and 4.47 Kcal per 100g of product. In conclusion, the homemade vinegars produced from three varieties of fresh figs exhibited comparable physicochemical, nutritional and biochemical properties and this transformation constitutes a new way to utilize the fruit of the fig tree.

1.Introduction

The fig (*Ficus carica*) is the fruit of the fig tree cultivated since the dawn of time by humans, especially in warm, dry climates and can be eaten fresh, dried (peeled or unpeeled) or as jam. Many studies show that figs are an important source of nutrient such as minerals, vitamins and dietary fiber; they are fat, sodium and cholesterol-free and contain a high number of amino acids (Solomon *et al.*, 2006; Veberic *et al.*, 2008) and bioactive molecules (Shahidi *et al.*, 2008). However, figs have a limited post-harvest shelf life, which can cause important economic losses. So, to increase the shelf life of figs, they can be dried or processed to vinegar for obtaining very special taste for flavouring. Vinegar is a liquid, fit for human consumption, produced exclusively from suitable products containing starch and/or sugars by the process of double fermentation, first alcoholic and then acetic. Acetic acid, the principal organic component of vinegar, is known for its preservative and flavoring properties (Sholberg *et al.*, 2000). Vinegar may contain optional ingredients such as herbs, spices, fruit and honey. According to Budak et al. (2014), the Babylonians produced and sold vinegars flavored with fruit, honey, and malt until the 6th century. References in the Old Testament and from Hippocrates indicate vinegar was used medicinally to manage wounds. Vinegar has many health promoting effects as antimicrobial, satietogenic effect, hypolipidemic, hypoglycemic and seems to prevent atherogenic risk (Shishehbor *et al.*, 2008; Beheshti *et al.*, 2012). Several fruits can be used to produce vinegar since they all contain sugar. The traditional production of fig vinegar is based on a spontaneous fermentation. This spontaneous fermentation occurs generally for 6 to 14 weeks, according to the ambient temperature, until desired acidity (at least 4%, w/v) and flavour is obtained. The dominant component in vinegar is acetic acid. It is commonly known that the production of fig vinegar is not an easy work. Low acidic value of fig fruit (0.18 to 0.48%, w/v) provides a suitable condition for uncontrolled microbial growth during fermentation process (Sengun, 2013). To the best of the authors' knowledge, there has been no study done regarding the properties of Algerian traditional fig vinegar. Hence, the objective of this study was to investigate the physicochemical, nutritional and biochemical properties of homemade fig vinegar produced traditionally. For this, three varieties of fresh fig (Thaamriwth, Aberkan and Azandjar) from the region of Béni-Ouartiène, District of Sétif (Algeria) known for its fig culture, were chosen for this study to produce Thaamriwth vinegar (V1), Aberkan vinegar (V2) and Azendjar vinegar (V3).

2. Materials and methods

2.1.Materials

2.1.1.Samples

The fig fresh varieties used were harvested from the region of Béni-Ouartilan (Sétif) at august 2018. The varieties are Thaamriwth (green variety), Aberkan and Azandjar (purple variety) (Fig 1). For each variety, 1kg was taken for vinegar preparation.

Figure 1. The three fresh fig used for preparation of vinegar; a): Aberkan variety; b): Thaamriwth variety; c): Azandjar variety

2.1.2. Vinegar preparation

After washing, sorting and crushing the figs, they are fermented in appropriate containers according to the modified adapted diagram of Sungen (2013) (Fig 2). Traditional vinegar production is based on a double aerobic and anaerobic combined spontaneous fermentation. This bioconversion uses yeasts and acetic bacteria naturally present in the fig. These lead to the production of ethanol which is converted into acetic acid. The first fermentation stage (alcohol production), which took place under anaerobic conditions, lasted 15 days at room temperature. During this stage, the sugars of the figs were transformed into ethanol by the action of yeasts. The second stage (production of acetic acid), which took place under aerobic conditions lasted 30 days at room temperature. During this stage, the alcohol was converted into acetic acid by the action of acetic bacteria. After fermentation, vinegars produced were directly analyzed.

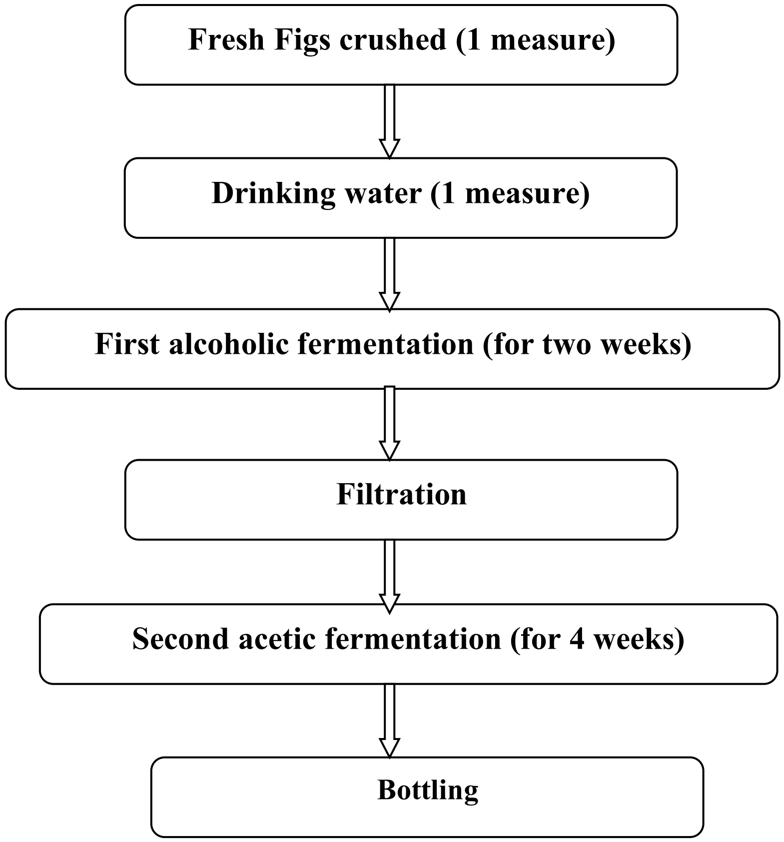

Figure 2. Traditional vinegar manufacturing diagram

The vinegars produced were studied to determine their physicochemical, nutritional and biochemical quality by applying protocols using classical methods. All analyses were conducted after the fermentation process.

2.2 Vinegar analysis

2.2.1. Physicochemical parameters

The pH value of fig vinegar samples was measured by using previously calibrated pH-meter, while conductivity was measured by electrometry according to the Algerian Standard (NA 749), results were expressed as µS/cm. Brix degree (°Brix) of the samples were measured using refractometer at 20 °C and results were reported as percentage. Moisture percentage was estimated according to the method described in the Algerian Standard (NA 1132). Ash content was determined by incineration of the sample at 525°C to constant weight according to the international standard (ISO 2171) and the results were expressed as percentage. As for the dry residue, it was estimated after drying vinegar samples in an oven (105 °C) to constant weight, whereas the vinegar density was estimated using a densimeter. The organic matter content was calculated knowing the moisture and the ash content.

2.2.2 Nutritional parameters

It is the determination of the rate of the energy elements content in vinegar. The dosage of the sugars was carried out according to the *Bertrand* method. Its principle is based on the reduction of CuO (cupric oxide) into small brick red grains-Cu_2O (cuprous oxide). A table gives the correspondence between the mass of copper and the mass of glucose. The result was expressed as g / 100g. The protein content was estimated using the *Kjeldahl* method, which is based on the mineralization of the sample in sulfuric acid with the presence of a catalyst. During this process, the organic nitrogen in the sample is converted into ammonium. Subsequently, in an alkaline medium, the ammonium ions are converted into ammonia. The ammonia is then released by steam and quantified using acid / base volumetric titration. The fat was determined by weighing after hot extraction with petroleum ether as solvent by Soxhlet. The result was expressed as g/100g of product. finally, energy intake is determined by the contribution of each of these elements

2.2.3 Biochemical parameters

It's about the determination of the two parameters characterizing a vinegar; the alcohol level, which was obtained by directly reading the alcohol content using an alcoholimeter after distillation. The result was expressed as percentage. Additionally, the acidic acid content was determined through titration, using a strong base (NaOH) to titrate the weak acid (acetic acid). The result was expressed as acetic degree.

2.3. Statistical analysis

Correlation among physicochemical fermentation parameters was analyzed by Pearson's correlation using the Excel 2007 software.

3.Results and discussions

3.1. Physicochemical Properties of homemade fig vinegars

Table 1 summarizes the results related to physicochemical parameters. There are a few studies considering these parameters for different kinds of vinegars, especially on the fig vinegar. From Table 1, pH values ranged from 3.9 to 4.05. This low pH makes vinegar a product with antimicrobial properties that make it useful for a number of applications. Vinegar is considered as disinfectant product since the ancient Greece era, it has been commonly used as an antifungal and antimicrobial element because of its very low pH and the presence of acetic acid as a major component (Ali *et al.*, 2017). In the work of Sengun (2013), the pH of fig vinegar produced in Turkey ranged from 3.05 to 3.73. the recorded values in the present study are slightly higher than those reported by Sengun (2013). This difference can be attributed to the vinegar preparation process, specifically, fermentation time. It has been observed that vinegars with longer fermentation times tend to have highly acidity, resulting in lower pH levels. However, the pH obtained makes it possible to inhibit the development of pathogenic microorganisms and to permit a good preservation of the product. Some other researchers reported that the pH values of different kind of vinegars ranged between 2.64 and 3.21 (Jang *et al.*, 2015) and 3.12 and 3.65 (Ould El Hadj *et al.*, 2001). However, as noted by Golivari et al. (2015), commonly commercial vinegar has pH 4.2. This value is depended on the acetic acid content.

°Brix is used as an index for the amount of soluble solid content including sugars, acids and minerals. From Table 1, vinegar V1 exhibited a significantly higher °Brix value (10.2%) compared to the other vinegars with °Brix values of 8.5% and 7.6 % for V3 and V2 vinegars, respectively. The measurement of °Brix is important in all drinks including vinegar. The °Brix of pineapple peelings vinegar was 5.3% as recorded by Sossou et al. (2009). In a study by Çaliskan and Polat (2008) that examined eight cultivars of fresh fig from Turkey ('Sarilop', 'Bursa Siyahi', 'Goklop', 'Yediveren', 'Yesilguz', 'Morguz', 'Sari Zeybek' and 'UfakYesil') °Brix values ranging from 22.7 to 27.2 % were reported. Therefore, the results of this study indicate that after approximately 30 days of acetic fermentation, the percentage of sugar in the vinegars studied decreased compared to the initial °Brix of fresh figs and juice. The recorded values ranging from 6.7 to 10.2 %, highlighting the reduction in sugar content during the fermentation process. This proves that the fermentation process runs properly. The results of this study were close to those recorded by Ould El Hadj et al. (2001) on date vinegars with values ranging from 7 to 10 %. Moreover, these differences recorded in the results are a function of the raw material used for the production of the vinegar. Indeed, according to Ait Haddou et al. (2014), the level of soluble solid (° Brix) is closely related to the content of dry matter, mineral, organic matter, total fiber, total protein, glucose and fructose.

Table 1. Physico-chemical composition of vinegar samples

	pH	Conductivity (µS/cm)	°Brix	Moisture (%)	Dry extract (%)	Density	Ash (%)	Organic matter (%)
Thaamriwth (V1)	4.05	392	10.2	90.29	9.71	1.0186	0.20	8.9
Aberkan (V2)	3.9	451	7.6	92.64	7.36	1.0120	0.09	7.27
Azandjar (V3)	4.01	432	8.5	93.58	6.42	1.0128	0.10	6.32

Table 1 also shows the relative density values for the produced vinegar samples: 1.02 for V1 and 1.01, for both V2, V3. The results obtained in this study closely align with the findings of Abdullah (2016) regarding some vinegars, where values ranged from 1.015 to 1.025 g/cm^3 and are slightly higher than those reported by Golivari et al. (2015) in their investigation of three types of Iranian vinegars (ranging from 1.004 to 1.007). This high density of solutions studied may be attributed to the presence of large amount of colloidal materials suspended in vinegars under investigation.

The percentage of moisture for samples of the produced vinegar was 90.29, 92.64, and 93.58 % for V1, V2 and V3, respectively (Table 1). The moisture content is crucial in vinegar production as water constitute the major component of this beverage. An increase in dissolved moisture enables yeasts to produce a larger amount of alcohol during alcoholic fermentation in the first stage. This, in turn, facilitate the subsequent production of a greater amount of acetic acid by the acetic acid bacteria present in the vinegar mother during the second stage of ferme,tation (Al-Asadi and Abdullah, 2005 cited *by* Abdullah, 2016). These findings closely align with the results obtained in the same study for samples vinegar produced by the malt of some varieties of maize, Zehdi dates, and grapes as values ranged between 94.721 and 96.107 % (Abdullah, 2016).

Results in Table 1 indicate that the dry extract (%) values for the produced vinegar samples were 6.42, 7.36 and 9.71 for V1, V2 and V3, respectively. The richness of these traditional vinegars in microorganisms (double fermentation) explained, in part, the significant dry residue recorded in samples. Mbungu et al. (2016) found a dry matter content of 2.27 % in their study on mangoes vinegar. Bakir et al. (2016) reported a range of values in grape vinegar (3.8 – 8.25 %) and apple vinegar (4.3 – 8.8 %) in their research.

It was also observed from Table 1, that the ash contents of the vinegar samples were 0.20 %, 0.09 % and 0.10 % for V1, V2 and V3, respectively. Furthermore, the percentage of organic matter in the three traditional homemade vinegars samples, were 6.32 %, 7.27 % and 8.9 % for samples V1, V2 and V3, respectively. Ash determination is important to support vinegar characterization and quality evaluation. Results from this study were close to the findings of Mbungu et al. (2016) as the percentage of total ash in filtered mangoes vinegar was 0.20 %. The obtained results were also close to those obtained by Abdullah (2016) in his work on some vinegars as the ash content ranged between 0.26 – 0.52 %. According to the same study, the overall percentages of ash are affected by several factors, including the nature and quality of the raw material used in production, as well as factors that impact the proportions of total solids. The rate of the organic matter recorded in our study, were higher than those reported by Akakabe et al. (2006) as they found amounts estimated to be 2.3 to 4.6 % (w/w) in bamboo vinegar. These differences may be due to the nature and components of the raw materials.

The conductivity values for the produced samples were 392, 451 and 432 (µS/cm) for samples V1, V2 and V3, respectively (Table1). Conductivity is the measure of the soluble salts content in the product. The obtained results differ from those found by Ould El Hadj et al. (2001) in their study on three Algerian date vinegars, where values ranged between 4.88 and 6.29 (mohms/cm). The fruit used and the preparation mode of vinegars may explain these differences. Furthermore, the contribution of tap water used in the vinegar preparation should not be overlooked, as it significantly influences the product's conductivity due to its dissolved salts content.

3.2. Nutritional properties of homemade fig vinegar

The content of energetic elements (total sugars, proteins and fat) is shown in Figure 3.

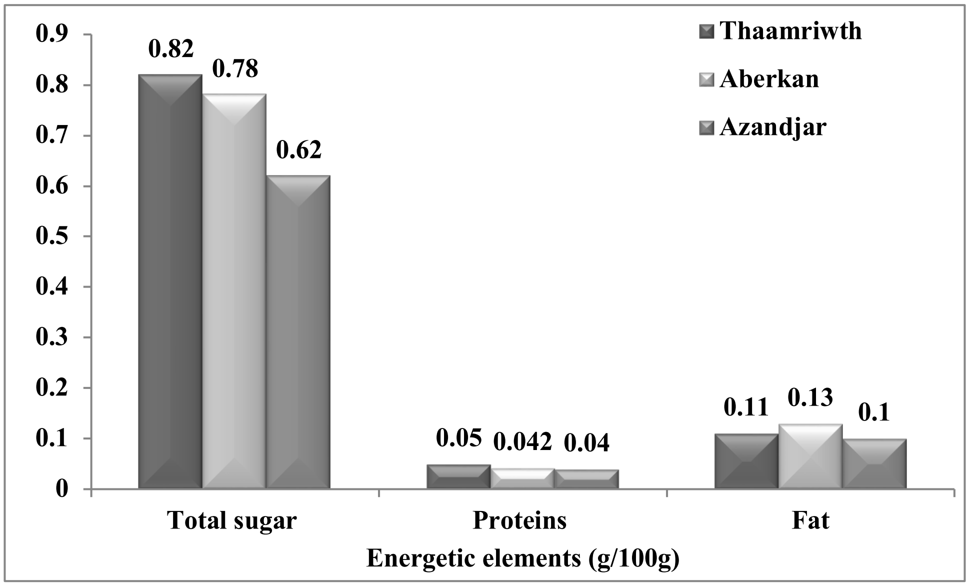

Figure 3. The energetic elements content of the vinegar samples

As it can be seen (Fig. 3), the protein contents of the vinegar samples, namely V1, V2 and V3 were found to be 0.05, 0.042 and 0.040 (g/100 g), respectively. The protein levels obtained were ten times lower than those recorded by Zakaria and Mokhtar (2014) for samples of Kelubi vinegar, Rambutan vinegar and Dokong vinegar as the values were 0.45 %, 0.42 % and 0.44 %, respectively. Additionally, the results also indicated lower protein content compared to the findings of Mokhtar et al. (2016) for Rambutan vinegar (0.27 %), Dokong vinegar (, 0.18 %), apple cider vinegar (0.13 %) and Nipa vinegar (0.25 %). It is worth noting that the high acidity and the presence of tannins in vinegars can potentially coagulate and denature some of the proteins.

After fermentation, the content of total sugars in vinegars were 0.82, 0.78 and 0.62 (g/100 g) for samples V1, V2 and V3, respectively (Fig. 3). It should be noted that total sugars include reducing and non-reducing sugars. The levels of sugars recorded were significantly higher when compared to the findings of Tanaka et al. (2016) on banana pulp vinegar as they found 10.27g/L and by Matloob and Hamza (2013) for artisanal manufactured and unrecorded dates vinegars where the total sugar content varied between 0.88 % and 4.07 % (w/v). These differences can be attributed to variation in raw materials used and the specific protocol followed during the vinegar production process. The low levels recorded in this study could also be interpreted by the complete utilization of sugar present is the figs by yeasts, which was subsequently transformed into ethyl alcohol. The crushing of figs during the process facilitated the efficient diffusion of sugars trapped within the cells of the pulp, making them readily accessible and usable by the microorganisms involved.

The fat contents of the samples V1, V2 and V3 were measured at 0.11, 0.13 and 0.10 (g/100g), respectively. The lipid contents in the vinegar sample are particularly low, which is expected since vinegar is primarily an aqueous product. The small amount of fat can be explained by the fruit used, which, like other fruits, is not rich in lipids. Limited data are available in the literature concerning the lipid content of vinegar, especially fig vinegar. In the study conducted by Mokhtar et al. (2016) on various vinegar, the fat content ranged between 0.07 % and 0.59 %. As stated previously, fig like most fruits, contain negligeable amount of lipids. According to Favier et al. (1993), the average lipid content of fresh figs is estimated to be around 0.2 g / 100g, which explains the low lipid content observed in fig vinegar.

Based on Figure 4, the energy intake of the three homemade vinegar samples (V1, V2 and V3) was measured at 4.47, 4.46 and 3.54 (Kcal/100g), respectively. The low content of energy elements (sugars, proteins and lipids) in vinegars results in a correspondingly low energy input, as these elements are the primary source of energy. However, the presence of acetic acid in vinegars can enhance biological energy consumption by increasing myoglobin levels and upregulating the expression of genes related to the synthesis of fatty acids (Yamashita *et al.*, 2009; Hattori *et al.*, 2010).

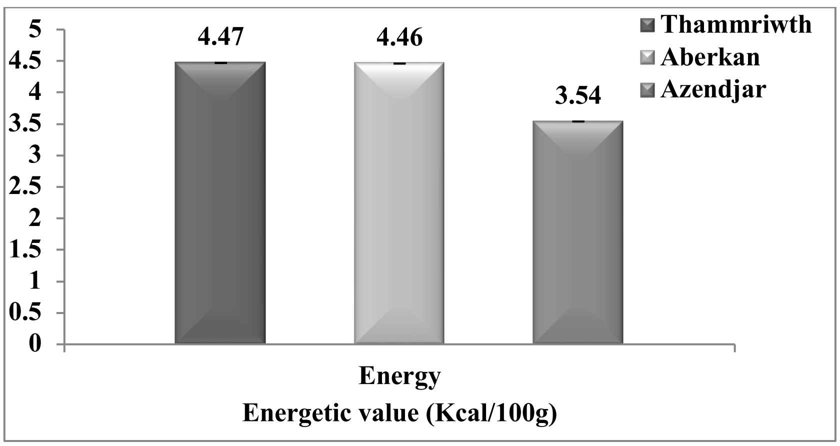

Figure 4. The energy value of the vinegar samples

3.3. Biochemical Characteristic of traditional fig vinegar

Alcohol and acetic acid contents of homemade fig vinegars are presented in Figure 5. Alcohol is the main metabolite of yeasts; it is used as a source of carbon for acetic acid bacteria during the first stage of vinegar production. Alcohol levels recorded were 0.18 %, 0.16 % and 0.17 % for vinegars V1, V2 and V3, respectively. While the conversion of sugar to alcohol occurs during the first stage of fermentation, the conversion of ethanol to acetic acid occurs during the second stage of fermentation by the action of the acetic bacteria.

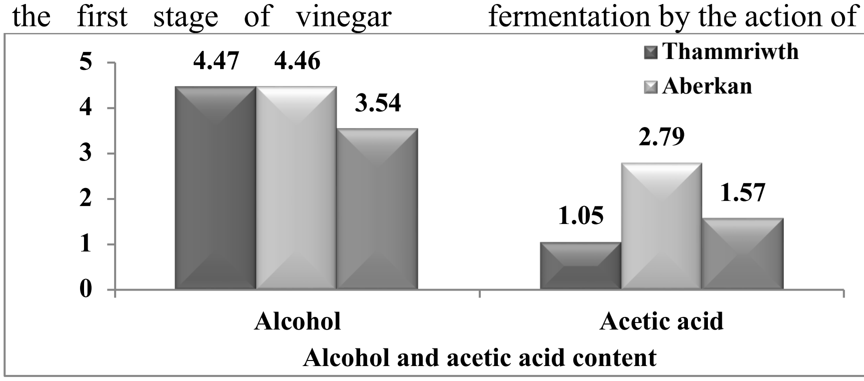

Figure 5. Acetic acid and alcohol content of homemade fig vinegars

Results are expressed as % for alcohol and as acetic degree (°) for acetic acid

Saccharomyces cerevisiae has been commonly recognized as the most prominent microorganism historically used for bioethanol production. It is capable in fermenting hexose sugars / sucrose and can yield ethanol concentration as high as 18 % in the fermentation broth. As result, it remains the preferred choice for the most of ethanol fermentation processes. *Saccharomyces cerevisiae* is also generally recognized as safe (GRAS) as a food additive for human consumption. Therefore, it is an ideal option for producing alcoholic beverages and for leavening bread (Lin *et al.*, 2006; Sossous *et al.*, 2009; Balakrishnaraja *et al.*, 2017). The alcohol content obtained corresponds to that of the unprocessed alcohol, in other words, to residual alcohol. The unprocessed alcohol level is regulated by the Codex Alimentarius (2000) standards, which stipulate that wine vinegar should not contain more than 0.5 % (v/v) residual alcohol or 1.0 % (v/v) for other types of vinegars. The results obtained in the current study were higher than those reported by Grégrová et al. (2012), who recorded residual alcohol contents ranging from 0.15 to 3.40 g/L in twenty samples of spirit vinegar. According to Jamaludin et al. (2016), after five days of fermentation, the content of ethanol in grape, apple and orange was 7.42 %, 6.53 % and 6.79 %, respectively, close to the findings of our study. Based on the same analysis, the authors found that, overall, during the initial stage of fermentation, the alcohol content is influenced by various factors, including pH, percentage of sugar and amount of acid present.

Acetic acid bacteria are group of Gram-negative bacteria that are strictly aerobic. They are well known for their ability to rapidly and incompletely oxidize carbon substrates, especially sugars and alcohols. These bacteria are widely distributed in nature and play an important role in the production of various food and beverages, such as vinegar (Gullo *et al.*, 2008; Sossous *et al.*, 2009; Gomes *et al.*, 2018).

The concentrations of acetic acid in the analyzed samples are depicted in Figure 5. The acetic acid contents in vinegar samples were found to be 1.05°, 2.79° and 1.57° for samples V1, V2 and V3, respectively. However, the levels of acetic acid obtained during acetification were significantly lower than the values recommended by the Codex Alimentarius (2000) standards which requires a minimum of 5 % or 50 g/L of acetic acid. This may be attributed to several factors. Firstly, the fermentation time may have been insufficient for the acetic bacteria to convert all the alcohol produced during the initial stage of fermentation into acetic acid. Additionally, it is possible that the production rate of alcohol was low, or the activity of yeast and/or acetic bacteria was inadequate. According to Ho et al. (2017), this low content of acetic acid might be due to inadequate oxygen levels during the acetic fermentation process. In fact, acetic acid bacteria require aerobic conditions to produce acetic acid effectively. The low oxygen concentration could influence the production of acetic acid and the speed of the fermentation process (Dabija and Hatnean, 2014). It has been reported by Buyuksirit and Kuleasan (2014) that the low production of acetic acid during the aerobic acetic fermentation could be due to the presence of toxic-secreting strains of *Saccharomyces cerevisiae* (yeast), that may inhibit the growth of Acetobacter species, the bacteria responsible for acetic acid production. Therefore, in order to enhance the acetic acid concentration, it is suggested to increase the oxygen levels through aeration during the acetic fermentation process. This can promote the production of acetic acid in the fig vinegar. Morales et al. (2001) reported a range of acetic acid levels between 71.8 and 94.4 µg/mL in their work on sherry vinegars. Similarly, Abdullah (2016), found varying values for different vinegars ranging from 27.890 µg/mL and 78.962 µg/mL.

3.4. Pearson correlation analysis

The correlation matrix (Table 2) reveals significant correlations between the various fermentation parameters. A high positive correlation was observed between the pH and the alcohol level (r = 0.97). Conversely, a negative correlation was found between pH and acetic degree, representing acidity (r=-1.00). The °Brix exhibited an inverse relationship with the alcohol level (r= -0.81). Finally, a highly significant negative correlation was identified between the alcohol level and the acidity (r = -0.97). These different relationships between the parameters elucidate the progression of the fermentation. The decrease in the °Brix during the fermentation is accompanied, in fact, by an increase in the alcohol content due to yeast utilizing sugar as a carbon source to produce alcohol. This alcohol is then metabolized by acetic acid bacteria to generate acetic acid, resulting in a decrease of alcohol level and an increase in acidity. This transformation is reflected in the decreasing pH of the medium. In the study of Ho et al. (2017), it was found that a higher pH of 5.5 significantly ($p<0.05$) increase acetic acid production in vinegar, while pH did not affect significantly ($p>0.05$) the ethanol production.

Table 2. Correlation between the different physicochemical parameters of fermentation

	pH	Brix (%)	Alcohol	acetic acid
pH	1,00			
Brix (%)	-0,62	1,00		
Alcohol	0,97*	-0,81*	1,00	
acetic acid	-1,00*	0,65	-0,97*	1,00

** Significant correlations marked at p <0.05.*

4. Conclusions

The present study is the first one that represents the initial exploration of the physicochemical, nutritional and biochemical properties of traditional homemade fig vinegar, produced in Algeria. The obtained results indicate a comparable quality profile among the three vinegar samples. The alcohol content of vinegars met the standards set by the Codex Alimentarius, while the level of acetic acid did not comply with these standards. The acidic pH of vinegars serves as a natural barrier against the proliferation of many pathogenic bacteria. Further investigations are warranted to examine the influence of factors such as raw materials, fermentation time, and fermentation temperature on the physicochemical, nutritional and biochemical properties of vinegar. Additionally, research should include the determination of bioactive molecules level, assessment of product acceptance among Algerian consumers and the evaluation of the microbiological quality under laboratory conditions.

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