



Article

# Suitability of the Lebanese “Ace Spur” Apple Variety for Cider Production Using *Hanseniaspora* sp. Yeast

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**Abstract:** In the present research work, the physicochemical and fermentative properties of the “Ace spur” apple variety, obtained from a Lebanese farm, and the “Kermerrien” variety, obtained from a French cider industry, were investigated. The pomological properties were first determined for both varieties showing significant differences in the shapes and sizes. Sugar content, titratable acidity, soluble solids, and pH values were then determined. The potential of the “Ace spur” apple juice was then evaluated using the yeast strain *Hanseniaspora* sp., a major yeast found during the spontaneous fermentation of apples. “Ace spur” apples contained more sugars while the “Kermerrien” cultivar had a highest malic acid and polyphenol concentrations. After 100 h of fermentation, the ethanol percentage (*v/v*) was around 4% in both ciders. Results obtained in this work revealed the chemical potential of the Lebanese apple juice to produce cider.

**Keywords:** Lebanese apple; fermentation; cider; *Hanseniaspora* sp.; physicochemical properties

## 1. Introduction

Apple represents one of the most produced and consumed fruits in the world. It is ranked as the third fruit produced worldwide with 77 million tons during the season of 2016–2017 [1]. It is a major fruit tree crop grown in the Mediterranean region [2]. “Ace Spur”, a dessert apple variety, represents one of the most important cultivars grown in the world [3]. In Lebanon, apple production is one of the active fruit-growing sectors. Despite the fact that most of the apples produced are either sold in the local market or exported, many apples remain unsold. Thus, the processing and fermentation of the apples to produce beverages such as cider could be a promising perspective to exploit the potential of the unused apples. Nonetheless, the apple composition is one of the important factors to be considered as the aroma compounds usually define the quality of the fermented beverages. The content of these compounds could be affected by several factors including the apple variety, the fermentation conditions, and the yeast strain used [4–6]. Determining the physicochemical properties of apples before their use for fermentation processes is thus crucial to obtain a high product quality. Specific varieties of cider apples are grown and dedicated specifically for cider production. Based on their chemical composition, the different varieties of cider apples are divided into several categories. The “Kermerrien” variety is used during this study. The productivity of this variety is around 35–40 tons/ha in France. It is a bitter variety, characterized by a conical shape, and a green to red color. The flavor of the must deriving from is rather bittersweet and slightly fragrant, thus generating a bitter cider [7].

Apple sugar content could change depending on many factors such as the climatic factors including light, temperature, and moisture, as well as the cultivar and the maturity level of the fruit [8,9]. Mature apples are mainly composed of  $\approx 85\%$  water, 12%–14% carbohydrates, 0.3%–1.0% organic acids, 0.3% proteins, less than 0.1% lipids, as well as minerals and vitamins [10]. Fructose, glucose, and sucrose are the main sugars characterizing the fruit quality and the market value of apples [11,12]. Unlike other fruits, fructose content is significantly higher than that of glucose [13,14]. In a study of eight commercially growing apple varieties in China, the average contents of fructose, glucose, and sucrose were reported as 53.9 g/L, 33.8 g/L, and 24 g/L, respectively [15]. Moreover, it was noted that the sensory characteristics of the product could be affected by the ratio of glucose and fructose. For instance, during the fermentation, the yeasts usually prefer to consume more glucose than fructose. Thus, a high fructose content in apple juice may affect the taste of the dry ciders after fermentation [16–18]. A total soluble solid content of 98.8% sugars represents a standard index of fruit ripening [19].

Soluble sugars and organic acids are important constituents contributing to the organoleptic properties of the fruits. They create with the flavoring substances the organoleptic quality related to the fresh apples [20]. L-malic acid is the major organic acid found in apples and its content closely correlates the titratable acidity. It is followed by the citric acid in a very lower concentration [21]. It has been also reported that apple fruit contains at least nine to twelve other organic acids in smaller fractions such as tartaric, quinic, shikimic, and succinic acids [15]. Apple acidity may vary from 0.1 to 2.5 g of malic acid per 100 g of juice [22]. The fruits growing in cool climate regions are usually more acidic than those growing in warmer regions [23].

The phenolic compounds' content has also an important effect on the sensory properties (e.g., color, bitterness, and astringency) of apple ciders. They could be involved in the fermentative process, providing the cider aroma, and as inhibitors of the microorganism growth [24]. In addition, they can influence the sweetness and the sourness, and therefore may affect the overall flavor development during the fermentation process [25,26]. Several types of phenolic compounds are found in apple cultivars, specifically, hydroxycinnamic acid derivatives, monomeric and oligomeric flavan-3-ols, dihydrochalcones, and flavonols [27]. Moreover, differences in the polyphenolic profile may occur between apple varieties and the final phenol content may depend on several factors such as the maturity of the fruit, the harvest year, climate conditions, cultivation, and storage conditions [24,27].

Other important parameters to take into account for apple processing are the fruit size and shape. They are essential for the equipment design for handling, transporting, cleaning, sorting, grading, and processing of the fruit [28].

Besides the chemical composition, the selection of yeast has a significant impact on fermentation. Different yeasts may be present during the first stage of fermentation, giving way to another type of yeast, now called *Saccharomyces cerevisiae* [29]. This yeast is widely used during fermentation due to its controlled and repetitive behavior as well as for the release of its aroma precursors. The role of non-*Saccharomyces* yeasts appears to be important during the fermentation process. These species are characterized by a low fermentation capacity and are sensitive to an alcohol concentration close to 5%–6% [30]. The positive impact of *Hanseniaspora* sp. in alcoholic fermentation has been demonstrated, which may positively contribute to the aroma profile of the fermented apple juice, thereby accentuating the beneficial role of these yeasts [31,32]. The species of the genus *Hanseniospora* are characterized by an apiculate form and are present at the beginning of spontaneous fermentation [33–37].

The review of the literature shows that the information about the physicochemical properties of the Lebanese apples and their potential to produce cider by fermentation is very limited. In this line, this study aimed to determine the physical properties and chemical composition of one Lebanese apple variety; “Ace spur”, as well as its suitability for cider production.

## 2. Materials and Methods

### 2.1. Apples and Chemicals

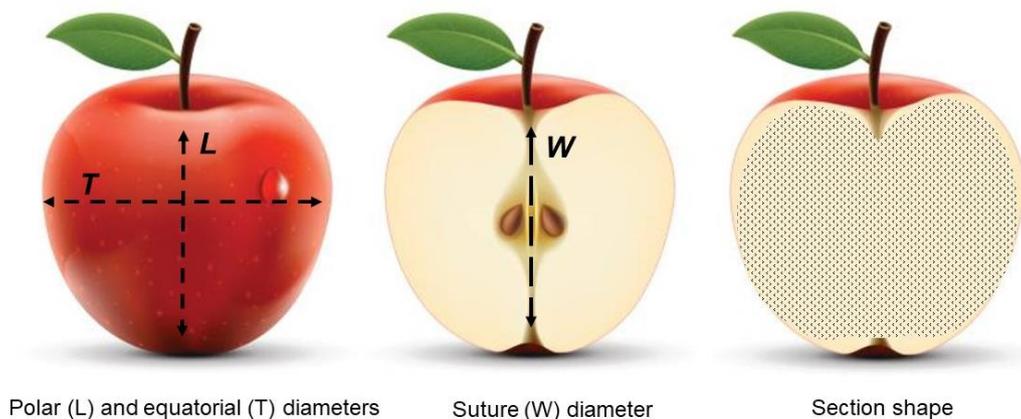
Two apple cultivars (“Ace spur” and “Kermerrien”) were used in this study. The “Ace spur” apples were collected from Bqaatouta village (Keserwan region, Lebanon) and it was chosen due to its high availability in the Lebanese market. Previous studies have shown the great potential of this variety for cider production [38,39]. The “Kermerrien” variety was kindly provided by the IFPC (“Institut Français des Productions Cidricoles”) located in “Le rheu” city (France). This variety is traditionally cultivated for industrial cider production. The apples were brought during the same harvest period (2017/2018 season) to minimize the errors due to the apple storage and ripening. They were stored at 4 °C until use.

Acetonitrile (CH<sub>3</sub>CN), ethanol (C<sub>2</sub>H<sub>5</sub>OH), and peptone were purchased from Fisher Scientific (Illkirch, France). Gallic acid, malic acid, Folin-Ciocalteu phenol reagent, Trolox, and yeast extract were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Fructose, sucrose, and glucose were supplied by Merck Millipore (Guyancourt, France). Sodium carbonate was bought from Prolabo (Fontenay-sous-Bois, France).

### 2.2. Determination of the Apple Properties

#### 2.2.1. Determination of the Size and Shape Properties

The experiments were performed in triplicate over 25 different apples taken from each variety. First, the fruit weight (g) was measured using a monobloc Mettler Toledo PB3002-S precision balance (Aldrich, Germany). After this step, the polar (*L*), suture (*W*), and equatorial (*T*) diameters (Figure 1) of each apple were measured using a digital vernier caliper (Athol, MA, USA).



**Figure 1.** Schematic representation of the apple size and shape determination.

The measurement of these three dimensions allowed the calculation of the following parameters [40,41]. The elongation *E* (Equation (1)):

$$E = \frac{T}{L} \tag{1}$$

The “size” or the arithmetic mean diameter: *Da* (mm) (Equation (2)).

$$Da \text{ (mm)} = \frac{T + L + W}{3} \tag{2}$$

The geometric mean diameter: *Dg* (mm), and the equivalent diameter: *De* (mm) were calculated using Equations (3) and (4) [42].

$$Dg \text{ (mm)} = \sqrt[3]{L \cdot W \cdot T} \tag{3}$$

$$De \text{ (mm)} = \left( \frac{L \cdot (W + T)^2}{4} \right)^{\frac{1}{3}} \quad (4)$$

The surface area:  $S \text{ (mm}^2\text{)}$  was calculated from Equation (5)

$$S \text{ (mm}^2\text{)} = \pi \cdot T \cdot \left( L + \frac{T}{2} \right) \quad (5)$$

The fruit volume:  $V \text{ (mm}^3\text{)}$  was calculated according to Equation (6) [43]:

$$V \text{ (mm}^3\text{)} = \frac{\pi \cdot L \cdot W \cdot T}{6} \quad (6)$$

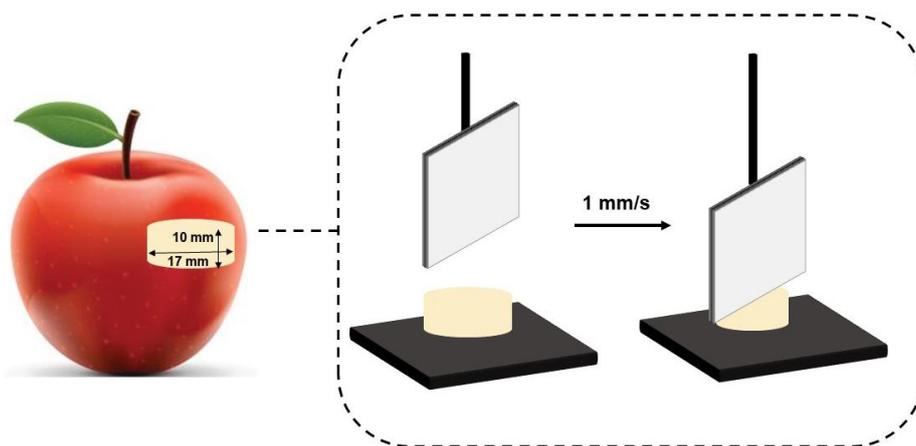
### 2.2.2. Mechanical Analyses

#### Relaxation Test

Relaxation tests were conducted at room temperature in order to quantify and qualify the viscous phenomena and the resistance within the products [44]. The tests were carried out using a texture analyzer (TA-XT plus, Rhéo, Champlan, France) consisting of a force sensor, a pressing piston, and a computer that allows the acquisition, the recording of measurements, and the data processing. The samples were prepared in the sliced form of 1.7 cm diameter and 1 cm thickness using a cylindrical punch tool. A force of 10 N and a displacement speed of 1 mm/s with a maximum deformation of 10% (1 mm) were applied.

#### Cutting/Slicing Test

The goal of this test is to compare the force required for cutting or slicing a product. The device used was the same texturometer described above (TA-XT plus, Rhéo, Champlan, France), equipped with a sharp blade. During this test, a movement speed of 1 mm/s was imposed (Figure 2). The samples were prepared in the sliced form of 1.7 cm diameter and 1 cm thickness. The strength necessary to cut the product to 50% of its initial thickness was delivered.



**Figure 2.** Schematic representation of the cutting test procedure.

### 2.2.3. Determination of the Water Content in Apples

The apples were cut into small cubes ( $\approx 2 \text{ cm}$ ) from different fruit parts (pulp, center cut, pulp, and skin). The water content in apple slices was determined using an infrared dryer (Sartorius MA150, Aubagne, France). The principle consists of heating the sample at  $105 \text{ }^\circ\text{C}$  until obtaining a constant weight. The test was performed in triplicate for each variety.

### 2.3. Determination of the Biochemical Properties

Randomly 5 kg of apples were chosen from ≈300 kg. The apples were first washed and then used to extract the juices at room temperature using a fruit juice centrifuge separator (Moulinex, France). The biochemical properties, detailed in the following sections, were determined immediately after juice extraction. All measurements were repeated at least using three replicates.

#### 2.3.1. Titratable Acidity

The titratable acidity represents the total acid content in apple juice and was expressed as malic acid equivalent. The titratable acidity was calculated as the average of 5 measurements (each of 100 mL diluted juice) with a 0.1 N NaOH solution.

#### 2.3.2. pH Measurement

The pH of the juice was determined with a Mettler Toledo (MP 220) pH meter.

#### 2.3.3. Total Soluble Solids (Brix % (s, w))

Aliquots of both juices were taken to measure the total soluble solids using a digital refractometer (Thermo Scientific, Illkirch, France) [45].

#### 2.3.4. Analysis of the Total Polyphenol Content

The total polyphenol content was determined for both apple juice varieties. The Folin-Ciocalteu colorimetric method was used [46]. First, 1 mL of 10-fold diluted Folin-Ciocalteu reagent was added to 0.2 mL of the juice. Then, 0.8 mL of sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) (75 g/L) solution was added and the mixture was incubated at 60 °C for 10 min. After cooling to room temperature, the absorbance was measured at 750 nm using a UV-vis spectrophotometer (Thermo Spectronic Genesys 20, Thermo Electron Corporation, Waltham, MA, USA). A calibration curve was established using gallic acid solutions (concentrations ranging from 0.2 to 1 mg/mL), and the polyphenols' content was expressed as mg of gallic acid equivalent per 100 g of dry matter.

#### 2.3.5. Determination of the Free Radical Scavenging Activities

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical was used to determine the free radical scavenging activity of each apple juice variety [47]. A juice sample of 50 µL was mixed with 1450 µL of 0.06 mM DPPH free radical prepared in methanol solution. After 30 min incubation at room temperature, the reduction of the DPPH free radical was measured by reading the absorbance at 517 nm. The DPPH free radical scavenging activity was measured using a spectrophotometer UV-vis (Libra S32, Biochrom, France). Trolox, as a synthetic antioxidant, was used as a positive control and the percentage of *inhibition* was calculated according to Equation (7).

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \quad (7)$$

### 2.4. Determination of the Fermentative Capacity

The evolution of the fermentative capacity of the Lebanese apple juice ("Ace Spur" variety) was performed using the yeast strain *Hanseniaspora* sp. This strain belongs to the authors' laboratory collection (Saint Joseph University, Beirut, Lebanon) [48]. This indigenous yeast strain was previously isolated from a spontaneously fermented juice of the Lebanese apple variety "Ace spur". In fact, the genus *Hanseniaspora* was found as a major yeast developed during spontaneous apple juice fermentation [49,50]. The sequencing of the internal transcribed spacer (ITS) domain of ribosomal DNA (SUB6436410 Hsp\_E2MN648725) identified the isolated yeast strain as a member of the *Hanseniaspora* genus. First, a pre-culture was performed by taking one colony of the strain from YPD agar (1% yeast

extract, 2% peptone, 2% dextrose, and 2% agar) plate into 250 mL of liquid YPD (1% yeast extract, 2% peptone, 2% dextrose) medium at pH 3.5 under sterile conditions (20 min at 121 °C in an autoclave HMC HV-110L (HMC Europe GmbH, Tüßling, Germany)). The cultivation was performed during 24 h at 30 °C temperature, and 250 rpm agitation speed in a thermostatic shaker (Thermo Scientific, Illkirch, France). A volume of 1.5 L of apple juice was introduced into a 2 L sterile fermenter and inoculated by the pre-culture to get an initial concentration of  $3.0 \times 10^6$  cells/mL. The cultivation was performed in triplicate at 30 °C and 250 rpm. Samples were periodically taken during the fermentation process for yeast cell counting. Several dilutions were made in ultrapure water, and the cells were counted using a Thoma cell counting chamber (Preciss, France). The biomass concentration  $X$  (cells/mL) was calculated according to Equation (8), where  $n$  is the number of cells counted,  $d$  is the dilution factor,  $N$  is the number of squares counted in the chamber, and 250,000 corresponds to a constant included in the formula of the Thoma cell. To detect the viable cells, the samples were mixed volume to volume with a solution of 0.01% methylene blue.

$$X \text{ (cells/mL)} = \frac{n \cdot 250,000 \cdot d}{N} \quad (8)$$

The ethanol production during the fermentation process was determined by the specific gravity measurements using a 5 mL glass pycnometer (Thermo Fisher, Illkirch, France). For ethanol quantification, a calibration curve was prepared using reconstituted solutions of apple juice/ethanol (1–10%,  $v/v$ ).

The concentrations of carbon substrates (fructose, glucose, and sucrose) were determined using high-performance liquid chromatography instrument LC-20AD (Shimadzu, Japan). The separation was performed in isocratic mode (a mixture of 1:4 (20:80) ( $v:v$ ) water:acetonitrile with a flow rate of 3 mL/min), and using a Luna 5  $\mu\text{m}$   $\text{NH}_2$  250  $\times$  4.6 mm column. The separation temperature was set to 40 °C and the eluted molecules were detected using a low-temperature evaporative light scattering detector ELSD-LT II (Shimadzu, Japan). The samples taken during the fermentation processes were diluted 10 times in ultrapure water, and a volume of 1  $\mu\text{L}$  was injected. The compounds' assignation and quantification were performed using pre-established standard curves of glucose (5–30 g/L), fructose (2.5–30 g/L), and sucrose (2–10 g/L). The kinetics of growth, ethanol production, and substrate consumption were compared to those obtained for the fermented "Kermerrien" juice, performed under the same conditions.

### 2.5. Statistical Analysis

One-way analysis of variance (ANOVA) was used for the statistical analysis of the data using Statgraphics Plus software (version 5.1, Statpoint Technologies Inc., Warrenton, VA, USA). Significance level at 95% confidence was taken for each analysis. The values presented in the figures correspond to the mean value of the repetition, and the error bars correspond to the standard deviations of each measurement.

## 3. Results and Discussion

### 3.1. Apple Pomological Properties

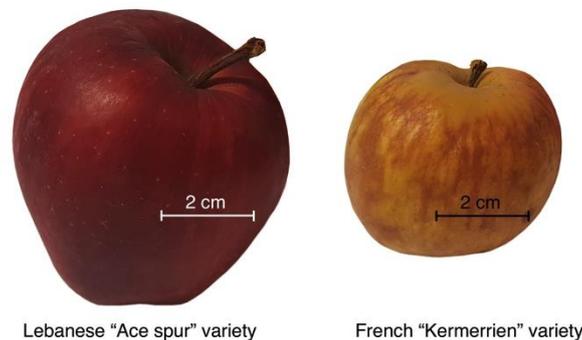
The choice of variety and the knowledge of the cultivar composition allow establishing the sensory quality of the apple juice. The data presented in Table 1 show significant differences in the fruits' properties. The "Ace spur" variety seems to have a bigger size ( $56.72 \pm 3.44$  mm vs.  $44.10 \pm 1.83$  mm), volume ( $174.1 \pm 4.8$  cm<sup>3</sup> vs.  $49.22 \pm 8.2$  cm<sup>3</sup>), and weight ( $117.13 \pm 20.01$  g vs.  $60.57 \pm 8.34$  g) compared to that of the "Kermerrien" apple. Furthermore, the shapes are significantly different between the two varieties: a circular section shape for the "Ace spur" variety ( $E \approx 1$ ), and ovoid for the "Kermerrien" one ( $E > 1$ ) (Figure 3). In fact, it has been reported that the elongation might depend on the cultivar and the orientation of the fruit in the space [51]. In the current study, the two varieties of apples chosen came from two different regions. The "Ace spur" variety is mainly dedicated to daily consumption

where the appearance of the apple has an essential role, whereas the “Kermerrien” variety is a cider apple cultivated to produce cider and is not consumed as a dessert apple. Figure 3 shows that the “Ace spur” variety has a more attractive appearance, which is important for commercial reasons, while “Kermerrien” apples are grown exclusively for processing. Those apples have some imperfections, such as skin blemishes or off shapes (Figure 3), making them undesirable for the fresh market. The fruit size and shape could also be a tool to distinguish the fruits from each other. These properties can also be exploited to determine the parameters for postharvest, sorting, and processing. It should be also integrated in the design of equipment.

**Table 1.** Average fruit weight, linear dimensions, size, elongation, and rheological properties of 25 apples taken from “Ace spur” and “Kermerrien” varieties.

Apple Variety	“Ace Spur”	“Kermerrien”
Average weight (g) *	117.13 ± 20.01	60.57 ± 8.34
Equivalent mean diameter $D_e$ (mm) *	56.72 ± 3.44	44.10 ± 1.83
Mean size $D_a$ (mm) *	56.76 ± 3.34	44.12 ± 1.62
Mean elongation $E$	1.05 ± 0.07	1.14 ± 0.2
Mean Surface area $S$ (cm <sup>2</sup> )	180.14 ± 5.4	119.17 ± 3.7
Mean fruit volume $V$ (cm <sup>3</sup> ) *	174.1 ± 4.8	49.22 ± 8.2
Water content (%)	82.34 ± 2.1	84.15 ± 1.5
Compression force (N) *	64.88 ± 15.7	118.37 ± 20.5
Rupture force (N)	14.85 ± 1.87	14.57 ± 2.5

The \* denotes significant differences.



**Figure 3.** Morphological aspects of the Lebanese “Ace spur” and the French “Kermerrien” apple varieties.

Extracting and milling the apples for apple juice recovery are essential operations during the first steps of the cider production process [52]. Apple pressing and crushing require a certain force, thus the design of the machines and presses will be manufactured according to this force to obtain an optimum yield of juice. The needed force to rupture the “Ace spur” apples was lower than that of the “Kermerrien” one (Table 1). This indicates that the Lebanese variety has a softer tissue compared to that of the French one. Nonetheless, the force required to cut the apples was the same for both varieties. Furthermore, the results in Table 1 show that the “Ace spur” apples had slightly lower water content than the “Kermerrien” apples (82.34% ± 2.1% vs. 84.15% ± 1.5%). Water content is related to the texture and apple firmness. It may depend on the total soluble solids present in each variety. The lowest water content observed in the “Ace spur” apples concurs with the high total soluble solids (20.57 ± 1.80 g/100 mL) found (Table 2).

**Table 2.** Physicochemical apple juice composition of “Ace spur” and “Kermerrien” varieties.

Apple Variety	“Ace Spur”	“Kermerrien”
Glucose (g/L) *	55.69 ± 1.54	39.80 ± 1.60
Fructose (g/L) *	101.41 ± 4.08	57.84 ± 1.98
Sucrose (g/L)	35.98 ± 1.89	34.59 ± 1.23
Total sugar content (g/L) *	193.08 ± 7.51	132.32 ± 4.81
Fructose—Glucose ratio *	1.83 ± 0.08	1.45 ± 0.05
°Brix (g/100 mL) *	20.57 ± 1.80	13.57 ± 0.80
Titrateable acidity (g malic acid/L) *	2.93 ± 0.66	15.28 ± 1.90
Ph *	4.90 ± 0.08	3.59 ± 0.09
Total phenolic content (mg GAE/100 g dry matter) *	419.83 ± 8.32	514.13 ± 13.75
Total antioxidant activity (%) *	93.15 ± 0.87	95.97 ± 0.5

The \* denotes significant differences, GAE denotes Gallic Acid Equivalent.

### 3.2. Apples Composition

The most abundant sugars in apples are fructose and glucose [4]. They represent 81% of the total sugars in the “Ace spur” and 74% in the “Kermerrien” variety. The results obtained (Table 2) showed that the concentrations of glucose and fructose in the two varieties are significantly different. Even more, the fructose/glucose ratio was greater for the “Ace spur” variety ( $1.83 \pm 0.08$ ) than that of the “Kermerrien” one ( $1.45 \pm 0.05$ ). Furthermore, the results showed a strong positive correlation between the total sugar content and the total soluble content (°Brix), though the sucrose concentration was the same for both varieties (Table 2). The fructose concentration in the “Ace spur” variety was relatively high compared to the values found in the literature. The “Kermerrien” variety is considered as a bitter apple cider having a relatively low sugar concentration. In fact, different fructose concentrations were found in the literature. In a study of eight apple varieties obtained from the Polish market, a range of fructose concentration between 82.2 and 59.3 g/L, and an average of 23.15 g/L of glucose concentration were reported [53]. In the Latvian apples, the fructose content ranged from 23.5 to 66.91 g/L, while the glucose concentration ranged from 7.47 and 60.24 g/L [54]. Several authors have investigated the relationship between apple sweetness and its properties. For example, a relationship is demonstrated between apple sweetness and texture properties [55] or apple color [56]. A great difference in the textures and colors of the two varieties is discussed above and shown in Table 1 and Figure 3. The texture differences may justify the significant differences in the sugar concentrations between the “Ace spur” and “Kermerrien” apples.

However for cider production, usually apple cider varieties contain 11% of glucose, 74% of fructose, and 15% of sucrose [4]. Lea and Drilleau, described the “ideal” composition of apple juice for cider production and categorized the apple cider varieties into four groups depending on the concentration of sugars, organic acids, and phenolic compounds [8]. The “Ace spur” variety containing high sugar concentrations should be belonging to the “sweet” apple varieties. Furthermore, the comparison of the sugar content in the “Ace spur” variety to that of the “ideal” apple juice showed that the fructose and sucrose concentrations matched [8]. The glucose concentration was higher in the “Ace spur” variety with a slightly lower acid concentration, which means that the cider from the “Ace spur” variety could be sweeter than the cider from the “Kermerrien” apples. Yet, if an ideal composition is sought for the production of cider, different varieties of apples should be used.

Furthermore, a significant difference was observed between the total phenolic compounds of the two varieties ( $419.83 \pm 8.32$  and  $514.13 \pm 13.75$  mg GAE/100 g dry matter, for “Ace spur” and “Kermerrien” varieties, respectively) (Table 2). This could be related to the fact that the “Kermerrien” variety is specifically used for cider production, which should normally be rich in phenolic compounds [57,58]. Thus, for a good cider quality, it is important to note that the “Ace spur” apples should contain a relatively high phenolic compounds’ content, which was comparable to that found in the “Kermerrien” variety. In fact, the color,

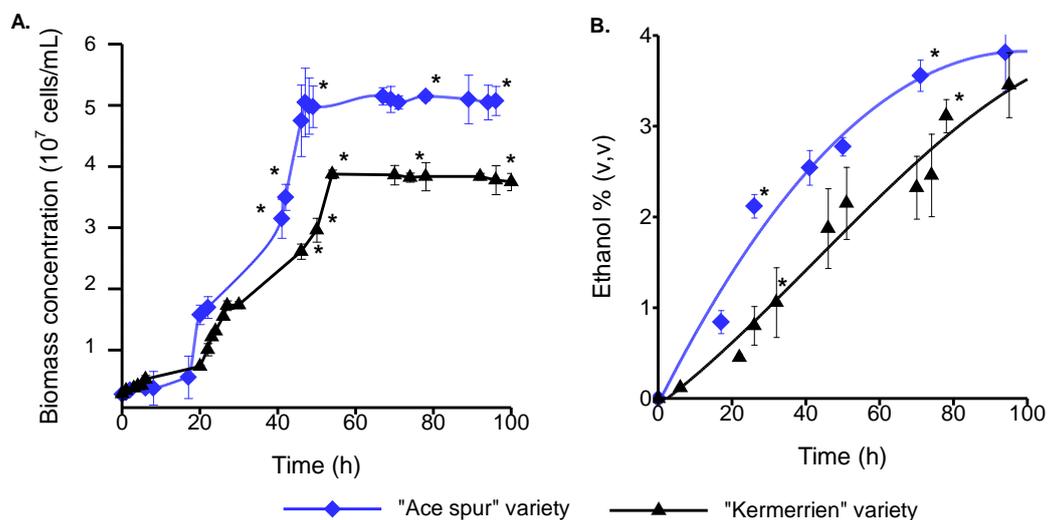
astringency, bitterness balance, and other sensory properties (e.g., aroma and fruitiness) of cider depend on the concentration of phenolic compounds [54].

The antioxidant activity was slightly higher for the “Kermerrien” variety ( $95.97\% \pm 0.5\%$ ) compared to “Ace spur” ( $93.15\% \pm 0.87\%$ ) (Table 2). This could be correlated to the phenolic compounds content, as previously demonstrated [59]. In addition, even though the TPC is lower for the “Ace spur” variety, the polyphenolic compounds present similar antioxidant activity than that of apple cider (Table 2). In another study, it has been reported that a blended cider was ranking higher for acidity and flavor compared to a single cultivar (doesn’t contain the ideal characteristics for a fermented cider). A blend containing both dessert and specific cider cultivars ranked highest for sweetness, appearance, and flavor [60].

### 3.3. Fermentative Potential of the Lebanese Apple Juice

The fermentative capacity of the Lebanese “Ace spur” apple juice was evaluated using *Hanseniaspora* sp. yeast strain and compared to that obtained from “Kermerrien” variety. The kinetics of growth, ethanol production, and substrate consumption were recorded during the fermentation (Figures 4 and 5).

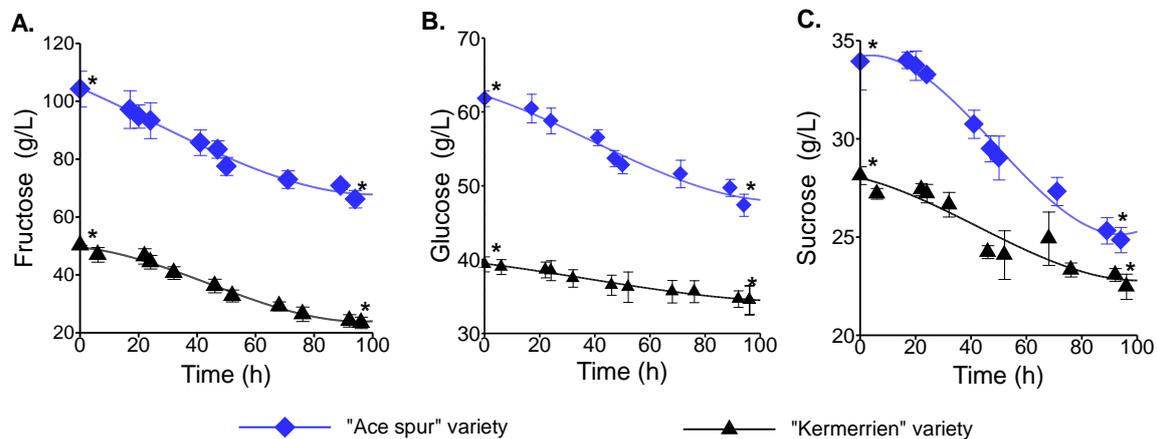
The results in Figure 4A show that *Hanseniaspora* sp. can grow in the apple juice with distinguishable latency, exponential and stationary phases. The evolution of the biomass concentration over time indicates that the yeast growth takes place following a typical fermentative kinetic. The concentration of microorganisms reached a maximum of  $5 \times 10^7$  cells/mL and  $3.8 \times 10^7$  cell/mL for the “Ace spur” and the “Kermerrien” fermentations, respectively. In Figure 4B, the maximum ethanol percentage of  $\approx 3.8\%$  (v/v) reached after 100 h of the “Ace spur” juice fermentation was not significantly different from that of the “Kermerrien” variety ( $\approx 3.5\%$ ). In fact, the final ethanol content in French ciders is usually less than 4%, fixed either by the regulation or by the consumers’ preferences [61,62]. This content is variable from a country to another. In fact, different samples taken from Scotland’s cider varieties were tested and the alcoholic strengths varied in a range of 4.1% to 7.4% volume [63]. Therefore, the ethanol percentage obtained in this work for the “Ace spur” variety seems to be acceptable for cider production.



**Figure 4.** Kinetics of growth (A) and ethanol production (B) of *Hanseniaspora* sp. fermentation in “Ace Spur” and “Kermerrien” apple juices. Continuous lines are provided to guide the eye. “\*” denotes significant differences.

During the Lebanese “Ace spur” fermentation, the yeast consumed 61.5 g/L of the total sugar content after 100 h of fermentation, divided as follows: 38 g/L (36.5%) of fructose, 14.5 g/L (26.51%) of glucose, and 9 g/L (23.35%) of sucrose. For the fermentation of the “Kermerrien” apple juice, the yeast consumed 26.8 g/L (53.28%) of fructose, 5.5 g/L (13.96%) of glucose and 5.7 g/L (20.11%) of sucrose, thus 38 g/L of

the total sugar in the juice was consumed after 100 h of fermentation (Figure 4). The yeast consumed therefore more sugars during the “Ace spur” juice fermentation. Fructose was the most consumed sugar in both fermentations followed by glucose. The fructose–glucose consumption ratio was 2.62 and 4.87 for the “Ace spur” and “Kermerrien” fermentations, respectively. Thus, the indigenous yeast seems to have a fructophilic character. This behavior was observed for the strain *Hanseniaspora uvarum* that didn’t show any preference for glucose consumption [64].



**Figure 5.** Evolution of the fermentative sugars (fructose (A), glucose (B), sucrose (C)) during the fermentation of the juice obtained from the “Ace Spur” and “Kermerrien” varieties. “\*” denotes significant differences between the initial concentrations of the juice and after 100 h of fermentation.

The follow-up of the different fermentative kinetics (Figures 4 and 5) shows that regardless of the initial sugar concentration, the same ethanol percentage is reached in both fermentations after 100 h. A closer look shows that different biomass and ethanol kinetics were obtained. The differences could be due to the significant variations observed in the initial sugar concentrations present in the two juices. In the presence of an excess of sugar, which is the case during the fermentation in the “Ace spur” juice, the yeast cells followed the fermentative pathway from the first hour of fermentation, generating both biomass and ethanol [65] (Figure 4). Nevertheless, for the fermentation of “Kermerrien” apple juice, where the concentration of sugars is practically by half ( $\approx 110$  g/L), the cells were in a respiratory mode generating biomass in the early hours of fermentation. The formation of ethanol seems to increase with the exhaustion of oxygen after hours of fermentation, thus the shift to the anaerobic fermentation mode may occur (Figure 4). Furthermore, the total sugar consumptions were strongly different. The yeast cells used the sugar less efficiently during the “Ace spur” fermentation, which produces a reduced amount of energy by following the fermentative pathway [65]. Thus, the yeast consumed more total sugars during this fermentation (Figure 4). Despite this higher consumption of sugars, the “Ace spur” cider may contain more total sugars, making it sweeter than the “Kermerrien” one.

#### 4. Conclusions

In conclusion, the obtained results showed that the apple varieties “Ace spur” and “Kermerrien” had different pomological properties and chemical compositions. The “Ace spur” variety, a dessert apple, had a bigger size and a circular shape, while the “Kermerrien” variety cider apple had an ovoid shape with some imperfections. The “Ace spur” apples have a high sugar concentration, specifically a high fructose concentration ( $\approx 100$  g/L). The “Kermerrien” apples contained on the other hand high polyphenolic compounds content (514.13 mg GAE/100 g dry matter). The yeast strain *Hanseniaspora* sp. was able to ferment the “Ace spur” apple juice up to  $\approx 3.8\%$  of ethanol after 100 h of fermentation, which was comparable to that of the “Kermerrien” variety. The same ethanol content was reached regardless of the initial sugar concentration and the quantity of sugars consumed during both fermentations. The Lebanese “Ace spur” apple variety has the potential to be used for cider production due to its

suitable chemical composition, it's appropriate texture for industrial processing, and the suitable ethanol content obtained after 100 h of fermentation. Nonetheless, further investigations are required to evaluate the volatile organic compounds and the aromatic profile of the obtained ciders.

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