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Prediction of Ethanol Content and Total Extract Using Densimetry and Refractometry

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Abstract: This study investigated the interrelationships between the parameters of density, optical refraction, the volume fraction of ethanol and the total extract, using model solutions and samples of wine materials. The regularities of changes in refractometer readings in the process of alcoholic fermentation have been studied. The functional dependence of the value of the volume fraction of ethanol in the finished wine products on the density and scale of refractometer values has been established. A technique is proposed for controlling the process of alcoholic fermentation of grape must, based on the use of refractometry. Finally, we present an algorithm to calculate the composition (volume fraction of ethanol, mass concentration of the total extract) of the fermentation product from its physical properties (density, refractive index), the coefficient of determination was R^2 = 0.975.

Keywords: densimetry; refractometry; process control; ethanol content; sugar content; refractive index

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1. Introduction

To determine the concentration of sugars (extract) in the wort before fermentation, as well as monitoring the decrease in their concentration during alcoholic fermentation in winemaking, a densimetric (hydrometric) method based on the linear dependence of the wort density on the concentration of sugars is used. The density of freshly squeezed grape must before fermentation uniquely, with an accuracy of 5 g/dm^3, determines the mass concentration of sugars [1]. During alcoholic fermentation, the density of the wort decreases in proportion to the amount of fermented sugars. To estimate the concentration of sugars during fermentation by the densimetric method, it is necessary to know the initial density of the wort before fermentation. The relationship between the wort density and the mass concentration of sugars is shown by the formula [2]:

$$C = C_0 - \frac{p_0 - p}{0.453} \quad (1)$$

where C and C_0 are the determined and initial concentration of sugars in the wort, g/dm^3, p_0 and p are the initial and desired density of the wort (in kg/m^3), and 0.453 is a coefficient showing the decrease in the density of the wort during the fermentation of 1 g/dm^3 of sugar.

Previously, we proposed and justified a method for determining the volume fraction of ethanol and the mass concentration of the extract in grape wines by measuring the refractive index and density of the product, with further calculation of the composition indicators based on a developed mathematical model [3]. However, due to their complexity, the previously reported algorithms and models cannot be the basis for creating a draft guidance document (on methods for determining the volume fraction of ethanol), followed by its metrological certification and introduction into the register of methods of analysis generally accepted for the industry. This publication aims to fill this gap in two ways:

1. Establish patterns of changes in refractometer readings during the fermentation of grape must and develop a way to control the fermentation process by registering changes in °Brix refractometer readings;
2. To develop a rapid, non-destructive method for determining the volume fraction of ethanol in liquid homogeneous products of grape winemaking, based on measuring the density and refractive index. This will be conducted using a device suitable for implementation in production conditions (i.e., using standard laboratory equipment that would be available at a winery's laboratory) and based on a clear algorithm of actions and calculations that ensures sufficient accuracy and unambiguity of the determined result.

2. Materials and Methods

2.1. Materials

The material for the study was the white must of Sabash and Muscat grapes harvested in 2018 with a sugar content of 150 g/dm^3 to 300 g/dm^3. A total of 8 wort samples were available. In clarified wort, sulphated to 75 mg/dm^3, density, mass concentration of sugars and mass fraction of dissolved substances in terms of sucrose were determined by densimetry and refractometry.

2.2. Fermentation Process and Monitoring

Fermentation of the samples was carried out using the yeast strain 47-K. After the appearance of signs of fermentation, the fermentation process was monitored at intervals of 2–3 days according to the following indicators:

- Density—by the areometric method
- Mass concentration of sugars—by the Bertrand method according to Magwaza and Opara [4]
- The volume fraction of ethanol—by distillation according to Iland et al. [5] and Gayda et al. [6]
- Refractive index according to the °Brix readings of a URL-1 (LLC "Spektro Lab", Ukraine) refractometer at a temperature of (20 ± 0.5) °C [7]

2.3. Production and Measurements of Model Solutions

Model solutions were produced by mixing varying amounts of wine materials of various types and wine distillates and water. The use of model solutions made it possible to obtain sufficient accuracy and unambiguity in determining the volume fraction of ethanol based on refractometry and densimetry data. The model solutions were prepared with a volume fraction of ethanol from 0 to 30% and a total extract concentration from 0 to 350 g/dm^3 in increments of 3% vol. by volume fraction of ethanol and 10–25 g/dm^3 by extract. In the obtained model solutions, the refractive index (in °Brix) was measured by the refractometric method [8], density by the areometric method, and the total extract mass concentration according to Iland et al. [5]. Preliminary metrological certification of the non-destructive express method for determining the volume fraction of ethyl alcohol for wines and wine drinks prepared on the basis of grape winemaking products, based on measuring the density and refractive index of liquid media was carried out according to Taylor [9].

2.4. Data Analysis

Mathematical processing of experimental data and metrological evaluation of the method were carried out using Microsoft Excel with an analysis package—Visual Basic for Applications (VBA) and a solution search module.

The experimental data obtained from the model solutions were processed using two-dimensional interpolation methods by Newton polynomials [10–12] in order to construct a regular, tabulated function of the volume fraction of ethanol depending on the density and the refractometer readings.

3. Results and Discussion

3.1. Background

Refractometry can also be used to determine the volume fraction of ethanol in water-alcohol solutions with between 0 and 50% vol. ethanol [13,14]. In the case of ethanol solutions, the volume the solution occupies has a non-linear relationship with its concentration; consequently, the density function of water-alcohol solutions also has a non-linear character. In the process of alcoholic fermentation of wort, there is a decrease in the concentration of reducing sugars with a concomitant increase in the concentration of ethanol. Two molecules of ethanol and two molecules of carbon dioxide are formed from one hexose molecule. Taking into account the molecular weights of hexose and ethanol, it is therefore theoretically possible to obtain 0.5114 g (0.6479 cm^3) of pure ethanol from 1 g of sugar. However, the actual yield of ethanol is always lower than this due to the influence of various factors, principally the formation of fermentation by-products, the entrainment of alcohol with carbon dioxide, and the use of a portion of the sugars in yeast biomass accumulation. A value of 0.6 cm^3 of ethanol formed from 1.0 g of sugars is commonly used in technological calculations [2,15]; this is a normative value, rather than reflecting the actual alcohol output at each current time. If we consider the process simplistically, it can be assumed that for each unit of reduction in the wort sugar concentration, a certain volume of alcohol is formed, which should theoretically lead to a linear change in the refractive index of the wort during fermentation in proportion to the amount of sugar fermented.

One of the main components of grape wine and its processing products is endogenous natural ethanol, which is formed during the fermentation of must and pulp, as well as exogenous ethanol, obtained artificially from sugar-containing food raw materials and used in the production of liqueur wines and wine drinks. The certified method (Method OIV-MA-AS312-01A) for determining the concentration of ethanol in the wine industry is distillation of a certain volume of product, followed by determination of the density of the distillate [5]. The use of this method is mandatory in case of disputes with manufacturers or suppliers of products, and is also indispensable for determining the ethanol content in products with a heterogeneous structure, for example, pressing, yeast precipitation, when rationing in winemaking, etc. However, in most cases, the winemaker deals with homogeneous liquid products in the winemaking process, such as wort, wine material, mistelle, etc., all of which have the properties of a true solution. Consequently, it would be beneficial to promptly obtain information about the content of ethanol in these products without conducting an extended analysis. A separate problem is the need to determine the volume fraction of ethanol when the volume of the sample is limited, or the sample is unique and it is undesirable to conduct destructive analysis (e.g., distillation. In this case, instrumental (non-destructive) methods of analyzing the composition of products based on various principles have come to the fore [1,2,16,17]. However, there are some problems of ensuring repeatability, accuracy and reproducibility of the results, as well as questions of compliance between the results obtained and those found using the method for determining the strength of alcohol by volume OIV-MA-AS312-01A [5]. In addition, the use of some techniques such as infrared spectroscopy (IR spectrometry), nuclear magnetic resonance spectroscopy (NMR spectroscopy), and gas and liquid chromatography, is not practical for many winery laboratories, due to the high equipment costs and ongoing operating expenses.

Refractometry and densimetry—measurement of refractive index and density of matter, are among the oldest methods of analysis of binary mixtures. The combination of refractometric methods with the measurement of other physical properties (density), makes it possible to determine the composition of products and biological objects. Despite the rapid development of analytical equipment, methods for determining the concentration of substances based on the measurement of their density and refractive index are still in demand, due to their simplicity and reliability. The methods of refractometry and densimetry adopted in winemaking and given in Haynes [18], due to the presence of dissolved substances in wine, require certain sample preparation in order to use them in the direct determination of ethanol concentration.

Although there are no particular difficulties when measuring the density of the initial wort, during fermentation the wort is largely saturated with carbon dioxide, which causes a number of problems related to the exact determination of its density. These include the presence of gas bubbles in the liquid phase, the adsorption of gas bubbles on the surface of the hydrometer (which can cause an apparent decrease in the density of the liquid), foaming, and interference from suspended material or yeast cells. To neutralize these effects, it is possible to partially degas the sample before determining its density, remove gas bubbles by rotating the hydrometer, or use larger hydrometers. However, these processes can complicate the operational monitoring of the fermentation process.

An alternative to the densimetric method for determining the concentration of sugars in the wort before fermentation is the refractometric method [1], which can be applied to samples of around 0.1 mL volume. Studies of the possibilities of refractometry for monitoring the fermentation process of wort were conducted by A.S. Vecherom as early as 1958 [19]; however, despite the rather detailed studies conducted, this problem is far from being fully resolved in terms of its application in enochemical practice. According to the concepts underlying the refractometric methods of analysis, in ideal systems (formed without changing the volume and polarizability of components), the dependence of the refractive index (n) of the mixture on the composition is close to rectilinear if the composition is expressed in volume fractions (percentages) [16]. This fact underlies refractometric methods for determining dissolved substances in vegetable and fruit processing products [17], as well as assessing the mass concentration of extract and sugars in grape must before fermentation, along with the densimetric method [20]. The volume occupied by a unit of mass of most soluble substances in solution including the substances in grape must, does not depend on its concentration, which explains its linear relationship with the solution density.

3.2. Determination of the Dynamics of Changes in the Readings of the Refractometer Scale during Alcoholic Fermentation

From the studies carried out, it was found that in the process of alcoholic fermentation of grape must, the refractometer readings on the Brix scale decreased in proportion to the decrease in its density (Figure 1).

Regression analysis performed on the experimental data gave the following empirical formula for expressing the relationship between these values:

$$r = (0.0342 \times B_0 + 6.049) \times B + 969.72 + 0.66 \times B_0 - 0.086 \times {B_0}^2 \quad (2)$$

where r is the wort density, kg/m^3; B_0 is the initial (before fermentation) indication of the refractometer reading, B is the refractometer reading during fermentation.

At $B = B_0$, we obtain an expression for the wort density (kg/m^3) before the start of alcoholic fermentation based on the Brix readings of the refractometer:

$$\mathrm{r}_0 = 969.72 + 6.709 \times B_0 - 0.05158 \times {B_0}^2 \quad (3)$$

Then for the mass concentration of the extract before fermentation, g/dm^3, the following expression can be written:

$$E_0 = \frac{p_0 \times 10 \times B_0}{1000} = \frac{969.72 \times B_0 + 6.709 \times B_0^2 - 0.05158 \times B_0^3}{100} \tag{4}$$

These expressions (Formulas (2)–(4) for the density and concentration of the extract are determined for B_0 in the range of (10–30) °B. Thus, knowing the Brix readings of the refractometer before fermentation and, consequently, the sugar content in the initial wort, for example, using special tables given in dos Santos et al. [1] and Pretorius et al. [20], as well as the density of the wort before and during fermentation, calculated by Formulas (2) and (3), respectively, it is possible to control the sugar content according to the indications of the refractometer readings using Formula (1).

It should be noted that when measuring the refractive index, the wort sample should be filtered through a syringe filter. Without this, foreign inclusions (suspensions, yeast cells) in the wort can settle on the refractometer prism and blur the boundary between light and shadow when taking readings. It is possible to dispense with pre-filtration of the sample, but in this case, the refractometer prism should be rotated so that foreign particles do not settle on it under the influence of gravity, if technically possible.

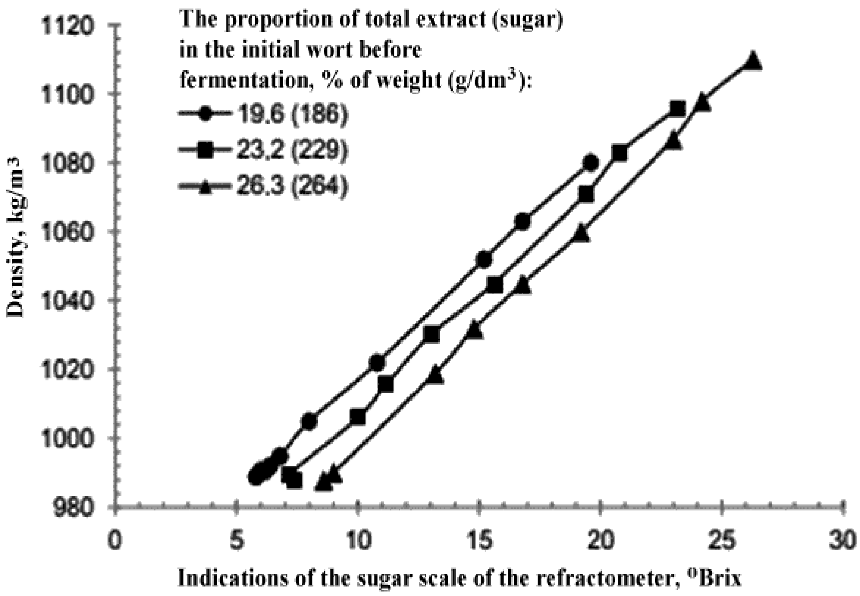

Figure 1. The relationship between grape must density and Brix readings during the process of alcoholic fermentation for different initial sugar content in the must.

Thus, as a result of control in the process of fermentation of grape must, the degree of fermentation of sugars, the values of the coefficients of the yield of alcohol from a unit of fermented sugars (cm^3/g) were determined by the refractometric method. These data are presented in Figure 2.

At the first stage of fermentation, the alcohol yield coefficient increases to a certain local maximum of 0.6, then decreases slightly and increases again towards the end of fermentation. This change in the alcohol yield coefficient is a cumulative characteristic, since it summarizes the yield that was obtained at the previous stages of the fermentation process.

To achieve a value of 0.6 and higher (see, for example, the curve at the initial sugar concentration of 264 g/dm^3 in Figure 2) with an amount of fermented sugars between 50–100 g/dm^3, the rate of ethanol formation in the fermentation interval of grape must sugars from 30–50 g/dm^3 should significantly exceed the rate of sugar assimilation (Figure 2). The accumulation of ethanol is caused by the consumption of metabolites accumulated by yeast cells, following the well-documented biochemical processes in the cell.

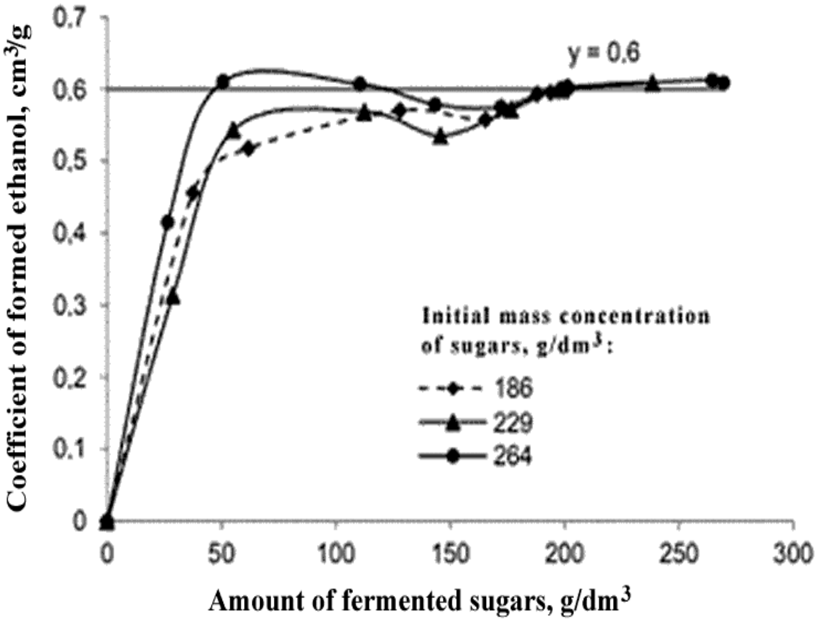

Figure 2. The change in the alcohol yield ratio from a unit of sugars depending on the amount of fermented sugars for musts with varying initial sugar concentrations.

The analysis of experimental data also showed that, despite the significantly smaller amount of alcohol formed at the first stage of fermentation, the decrease in density was proportional to the decrease in the mass concentration of sugars determined according to Magwaza and Opara [4]. Furthermore, the increments were equal in the middle and at the end of fermentation, which indicates that the nature of the resulting fermentation products at the stage of the fermentation and accumulation of yeast biomass is somewhat different than in the middle and end of fermentation. It also suggests that these fermentation products do not contribute to the change in the distillation density when determining the volume fraction of ethanol according to Iland et al. [5].

The average value and 95% confidence interval for the yield of alcohol from the unit mass of sugars in the process of alcoholic fermentation of clarified wort using yeast strain 47-K, obtained on the basis of processing experimental data, is given in Table 1.

In the processing of grape wines, there may be a need to calculate the Brix readings of the refractometer, when a certain mass concentration of sugars is reached, for example, in order to prepare wines with an interrupted process of alcoholic fermentation (table semi-dry, semi-sweet, as well as liqueur wines).

Table 1. The dependence of the alcohol yield ratio on the amount of fermented sugars for grape must with an initial mass concentration of sugars between 160–270 g/dm^3.

Amount of Discarded Sugars, g/dm^3	Alcohol Yield Coefficient, cm^3/g (Mean ± 95% Confidence Interval)
30	0.40 ± 0.12
50	0.54 ± 0.10
80	0.58 ± 0.04
150	0.56 ± 0.03
>200	0.60 ± 0.01

Analysis of the structure of Formula (2) showed that it can be represented as a linear function of the form:

$$\mathrm{r} = \mathrm{Kx}(B_0) \times B + \mathrm{Lx}(B_0) \tag{5}$$

i.e., for a given initial value of B_0, the density dependence on the Brix readings of the refractometer is linear. On the other hand, there is a linear relationship between the decrease in wort density during fermentation and the mass concentration of fermented sugars according to Formula (1).

Based on this, we can write:

$$\frac{p - p_0}{0.453} = \frac{B_0 - B}{X} \tag{6}$$

where X is the change in the refractometer readings with a decrease in the mass concentration of sugars by 1 g/dm^3.

Then, for the mass concentration of sugars (extract) during fermentation, we can write:

$$C = C_0 - \frac{B - B}{X(B_0)} = C_0 - (B_0 - B) \times \frac{1}{X(B_0)} \tag{7}$$

where C and C_0 are the mass concentration of sugars (extract) during fermentation and before fermentation, respectively (in gdm^3); B_0 and B are the Brix readings of the refractometer before and during fermentation (in °Brix); and $1/X(B_0)$ is the proportionality coefficient between the decrease in the mass concentration of sugars and the change in the refractometer readings.

3.3. Example Calculation

We will now demonstrate the obtained regularities for technological calculations. As one example, the initial Brix reading for a particular wort was 25.2 Brix (25.2% by weight). After stopping fermentation by chilling, followed by filtration, the refractometer reading was 10 Brix. From this, we can determine the concentration of the total extract and sugars before fermentation, after stopping fermentation, and the amount of ethanol formed.

We determine the concentration of the extract E_0 before fermentation according to the Formula (4).

$$E_0 = \frac{969.72 \times 25.2 + 6.709 \times 25.2^2 - 0.05158 \times 25.2^3}{100} 278 \text{ g/dm}^3 \tag{8}$$

The mass concentration of sugars in the wort before fermentation is determined from Table 2, which will be 252 g/dm^3. The concentration of the total extract and sugars is determined from the Formula (7), which for our case, at α = 15.56 (Table 2), has the form for the mass concentration of extract:

$$\text{E} = 278.7 - (25.2 - 10) \times 15.56 = 278.7 - 236.5 = 42.2 \text{ g/dm}^3 \tag{9}$$

And the mass concentration of sugars:

$$C = 252 - (25.2 - 10) \times 15.56 = 252 - 236.5 = 15.5 \text{ g/dm}^3 \tag{10}$$

The alcohol concentration, taking into account the variability of the alcohol yield coefficient from the sugar unit, will be:

$$(C_0 - C) \times 0.6 = (252 - 15.5) \times (0.06 + 0.001) = (14.2 + 0.3) = 14.5 \text{ \% vol.} \tag{11}$$

Table 2. The dependence of the initial mass concentration of sugars in the grape must (C_0) and the value of the coefficient α (B_0) on the initial mass fraction of dry substances in the grape must B_0.

B_0	C_0	α	B_0	C_0	α	B_0	C_0	α
10.0	82	14.16	16.8	155	14.76	23.6	233	15.41
10.2	84	14.17	17.0	158	14.78	23.8	235	15.43
10.4	86	14.19	17.2	160	14.80	24.0	238	15.44
10.6	88	14.21	17.4	162	14.81	24.2	240	15.46
10.8	90	14.23	17.6	164	14.83	24.4	242	15.48
11.0	92	14.24	17.8	167	14.85	24.6	245	15.50
11.2	94	14.26	18.0	169	14.87	24.8	247	15.52
11.4	97	14.28	18.2	171	14.89	25.0	249	15.54
11.6	99	14.29	18.4	173	14.91	25.2	252	15.56
11.8	101	14.31	18.6	176	14.93	25.4	254	15.58
12.0	103	14.33	18.8	178	14.94	25.6	256	15.60
12.2	105	14.35	19.0	180	14.96	25.8	259	15.62
12.4	107	14.36	19.2	182	14.98	26.0	261	15.64
12.6	109	14.38	19.4	185	15.00	26.2	263	15.66
12.8	112	14.40	19.6	187	15.02	26.4	266	15.68
13.0	114	14.42	19.8	189	15.04	26.6	268	15.71
13.2	116	14.43	20.0	192	15.06	26.8	270	15.73
13.4	118	14.45	20.2	194	15.08	27.0	273	15.75
13.6	120	14.47	20.4	196	15.10	27.2	275	15.77
13.8	122	14.49	20.6	198	15.11	27.4	277	15.79
14.0	125	14.51	20.8	201	15.13	27.6	280	15.81
14.2	127	14.52	21.0	203	15.15	27.8	282	15.83
14.4	129	14.54	21.2	205	15.17	28.0	284	15.85
14.6	131	14.56	21.4	208	15.19	28.2	287	15.87
14.8	133	14.58	21.6	210	15.21	28.4	289	15.89
15.0	135	14.59	21.8	212	15.23	28.6	292	15.91
15.2	138	14.61	22.0	215	15.25	28.8	294	15.93
15.4	140	14.63	22.2	217	15.27	29.0	296	15.95
15.6	142	14.65	22.4	219	15.29	29.2	299	15.97
15.8	144	14.67	22.6	221	15.31	29.4	301	15.99
16.0	147	14.69	22.8	224	15.33	29.6	303	16.01
16.2	149	14.70	23.0	226	15.35	29.8	306	16.03
16.4	151	14.72	23.2	228	15.37	30.0	308	16.06
16.6	153	14.74	23.4	231	15.39			

3.4. Determination of the Volume Fraction of Ethanol

As another outcome of the experimental work, calculated values of the volume fraction of ethanol were obtained depending on the density of the product in the range of 970 kg/m^3 to 1130 kg/m^3 in increments of 10 kg/m^3 and the Brix readings of the refractometer in the range from 4.0 to 31.0% by weight, in increments of 1.0. The results of these studies are shown in Table 3. The dashes in the table show cells with a negative value for the volume fraction of ethanol or for the mass concentration of the extract, which are devoid of physical meaning.

We have established the relationship between the mass concentration of the extract and the mass concentration of sugars in the wort. This allowed us to apply our approaches to determining the mass concentration of the total extract by the refracto–densimetric method to assess the mass concentration of residual sugars and the volume fraction of ethanol. Based on mathematical modeling of the composition (volume fraction of ethanol and mass concentration of sugars as part of the extract) and physical properties (refractive index and density) of the wort, tabular functions of the values of the volume fraction of ethyl alcohol in the fermenting wort on the density and readings of the sugar scale of the refractometer were developed. The results of these studies are shown in Table 3 and Figure 3.

Table 3. The volume fraction of ethanol depending on the density of the product ρ and the Brix readings of the refractometer B at 20° C.

	Density, kg/m^3																
***B*, % mass**	**970**	**980**	**990**	**1000**	**1010**	**1020**	**1030**	**1040**	**1050**	**1060**	**1070**	**1080**	**1090**	**1100**	**1110**	**1120**	**1130**
4.0	-	12.48	8.79	5.11	1.43	−2.25	-	-	-	-	-	-	-	-	-	-	-
5.0	-	13.96	10.27	6.59	2.91	−0.77	-	-	-	-	-	-	-	-	-	-	-
6.0	-	15.44	11.75	8.07	4.39	0.71	−2.96	-	-	-	-	-	-	-	-	-	-
7.0	20.61	16.92	13.23	9.55	5.87	2.19	−1.48	-	-	-	-	-	-	-	-	-	-
8.0	22.09	18.40	14.71	11.03	7.35	3.67	0.00	-	-	-	-	-	-	-	-	-	-
9.0	23.56	19.88	16.19	12.51	8.83	5.15	1.48	−2.19	-	-	-	-	-	-	-	-	-
10.0	25.04	21.35	17.67	13.98	10.31	6.63	2.96	−0.71	-	-	-	-	-	-	-	-	-
11.0	26.61	22.92	19.24	15.56	11.88	8.20	4.53	0.86	−2.81	-	-	-	-	-	-	-	-
12.0	28.08	24.40	20.71	17.03	13.36	9.68	6.01	2.34	−1.33	-	-	-	-	-	-	-	-
13.0	29.65	25.97	22.28	18.60	14.93	11.25	7.58	3.91	0.24	-	-	-	-	-	-	-	-
14.0	31.22	27.54	23.85	20.17	16.50	12.82	9.15	5.48	1.82	−1.85	-	-	-	-	-	-	-
15.0	32	29.10	25.42	21.74	18.07	14.39	10.72	7.05	3.39	−0.28	-	-	-	-	-	-	-
16.0	-	30.67	26.99	23.31	19.04	15.96	12.29	8.62	4.96	1.29	−2.37	-	-	-	-	-	-
17.0	-	32.23	28.55	24.88	21.20	17.53	13.86	10.19	6.53	2.86	−0.80	-	-	-	-	-	-
18.0	-	-	30.12	26.44	22.77	19.10	15.43	11.76	8.09	4.43	0.77	−2.89	-	-	-	-	-
19.0	-	-	31.78	28.10	24.43	20.76	17.09	13.42	9.76	6.10	2.44	−1.22	-	-	-	-	-
20.0	-	-	-	29.66	25.99	22.32	18.65	14.99	11.32	7.66	4.00	0.34	−3.31	-	-	-	-
21.0	-	-	-	31.32	27.65	23.98	20.31	16.65	12.98	9.32	5.66	2.01	−1.65	-	-	-	-
22.0	-	-	-	32.98	29.31	25.64	21.97	18.31	14.64	10.98	7.32	3.67	0.01	-	-	-	-
23.0	-	-	-	-	30.96	27.29	23.63	19.96	16.30	12.64	8.98	5.33	1.67	-	-	-	-
24.0	-	-	-	-	32.61	28.95	25.28	21.62	17.96	14.30	10.64	6.99	3.33	−0.32	-	-	-
25.0	-	-	-	-	-	30.60	26.94	23.27	19.61	15.95	12.30	8.64	4.99	1.34	−2.31	-	-
26.0	-	-	-	-	-	32.25	28.59	24.93	21.27	17.61	13.95	10.30	6.65	2.99	−0.65	-	-
27.0	-	-	-	-	-	-	30.29	26.62	22.97	19.31	15.65	12.00	8.35	4.70	1.05	-	-
28.0	-	-	-	-	-	-	31.98	28.32	24.66	21.01	17.35	13.70	10.05	6.40	2.75	−0.90	-
29.0	-	-	-	-	-	-	-	30.07	26.41	22.75	19.10	15.45	11.80	8.15	4.50	0.85	−2.79
30.0	-	-	-	-	-	-	-	31.81	28.15	24.50	20.84	17.19	13.54	9.89	6.25	2.60	−1.04
31.0	-	-	-	-	-	-	-	-	29.89	26.24	22.59	18.94	15.29	11.64	7.99	4.35	0.70

To calculate the intermediate data of the table, it is advisable to use a bilinear interpolation formula for a function given in equidistant nodes, which has the form:

$$F(p,B) = b_1 + b_2 \times (p - p_0) + b_3 \times (B - B_0) + b_4 \times (p - p_0) \times (B - B_0) \quad (12)$$

where:

$b_1 = \alpha_{00}$,
$b_2 = (\alpha_{10} - \alpha_{00})/(p_1 - p_0)$,
$b_3 = (\alpha_{01} - \alpha_{00})/(B_1 - B_0)$,
$b_4 = (\alpha_{00} - \alpha_{10} - \alpha_{01} + \alpha_{11})/(p_1 - p_0) \times (B_1 - B_0)$

Thus, in order to find the volume fraction of ethanol by the density of the product and the Brix readings of the refractometer, it is necessary to measure the density of the product and take refractometer readings of the product at 20 °C, and then use Formula (12), in combination with the data from Table 3.

3.5. Example Calculation

Let us illustrate this with a practical example (Table 4).

Table 4. The data position in Table 3 for calculating alcohol according to Formula (12) (practical example).

		P_0 = 990	**P_1 = 1000**	
...	...	...	...	...
B_0 = 7.0	...	α_{00} = 18.60	α_{10} = 14.93	...
B_0 = 8.0	...	α_{01} = 20.17	α_{11} = 16.50	...
...	...	...	...	...

To calculate the coefficients b_1, b_2, b_3 and b_4:

$$b_1 = \alpha_{00} = 18.6$$

$$b_2 = \frac{\alpha_{10}-\alpha_{00}}{p_1-p_0} = \frac{14.93-18.6}{1010-1000} = -0.367$$

$$b_3 = \frac{\alpha_{01}-\alpha_{00}}{B_1-B_0} = \frac{20.17-18.6}{14-13} = 1.57$$

$$b_4 = \frac{\alpha_{00}-\alpha_{10}-\alpha_{01}-\alpha_{11}}{(p_1-p_0)\times(B_1-B_0)} = \frac{18.6-14.93-20.17+16.5}{(1010-1000)\times(14-13)} = 0.0$$

The numerical values of the coefficients b_1, b_2, b_3, b_4 are substituted in, as well as the experimentally obtained values of the density p and the Brix readings of the refractometer B. From Formula (12), the desired value of the volume fraction of ethanol can then be calculated:

$$\begin{aligned} F(p,B) &= b_1 + b_2 \times (p - p_0) + b_3 \times (B - B_0) + b_4 \times (p - p_0) \times (B - B_0) \\ &= 18.6 + 0.367 \times (1004.5 - 1000) + 1.57 \times (13.8 - 13.0) \\ &= 18.2045\%\ \text{vol.} \end{aligned} \quad (13)$$

A plot of two variables C_0 and density from Table 3 with bivariate and univariate graphs is shown in Figure 3.

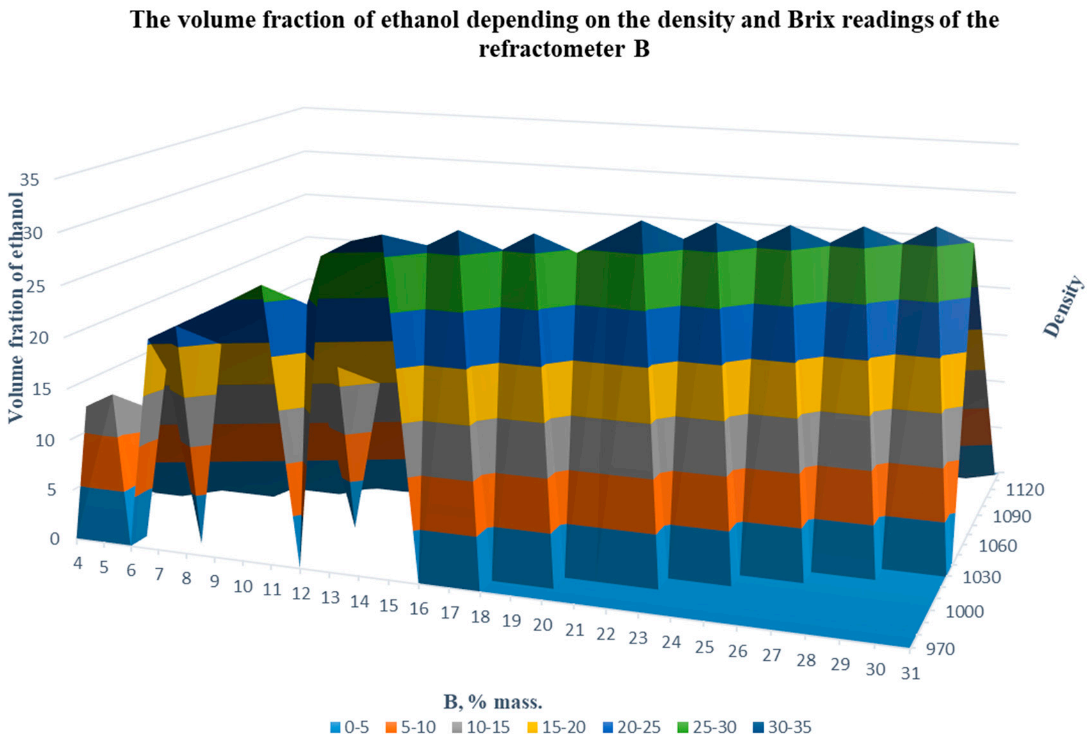

Figure 3. The volume fraction of ethanol depending on the density (kg/m^3) of the product p and the Brix readings of the refractometer B at 20 °C.

The boxplot (or box-and-whisker plot) shows the distribution of the quantitative data C_0 and density from Table 3 in a way that facilitates comparisons between variables or between levels of a variable (Figure 4). The box shows the quartiles of the dataset, with the whiskers expanding to show the rest of the distribution.

As a result of the work done, about 30 samples of wine products with a volume fraction of ethyl alcohol in the range of 0–20% vol. were measured. An experimentally conducted verification of the conformity of the volume fraction of ethanol obtained by the proposed method in comparison with the method of determination certified in winemaking according to Iland et al. [5] showed that in 95% of cases the discrepancy between the methods does not exceed 0.1% vol. in the case of using a pycnometric method or a glass alcohol meter type ASP-1 for measuring density, followed by finding the desired densities according to the density table of water-alcohol solutions [6]. The use of general-purpose hydrometers of the AON-2 type increases the discrepancy between the methods to 0.3% vol. The use of a glass alcohol meter is especially important for determining the density of table wines with a completed cycle of alcoholic fermentation, the density of which is usually lower than the density of water.

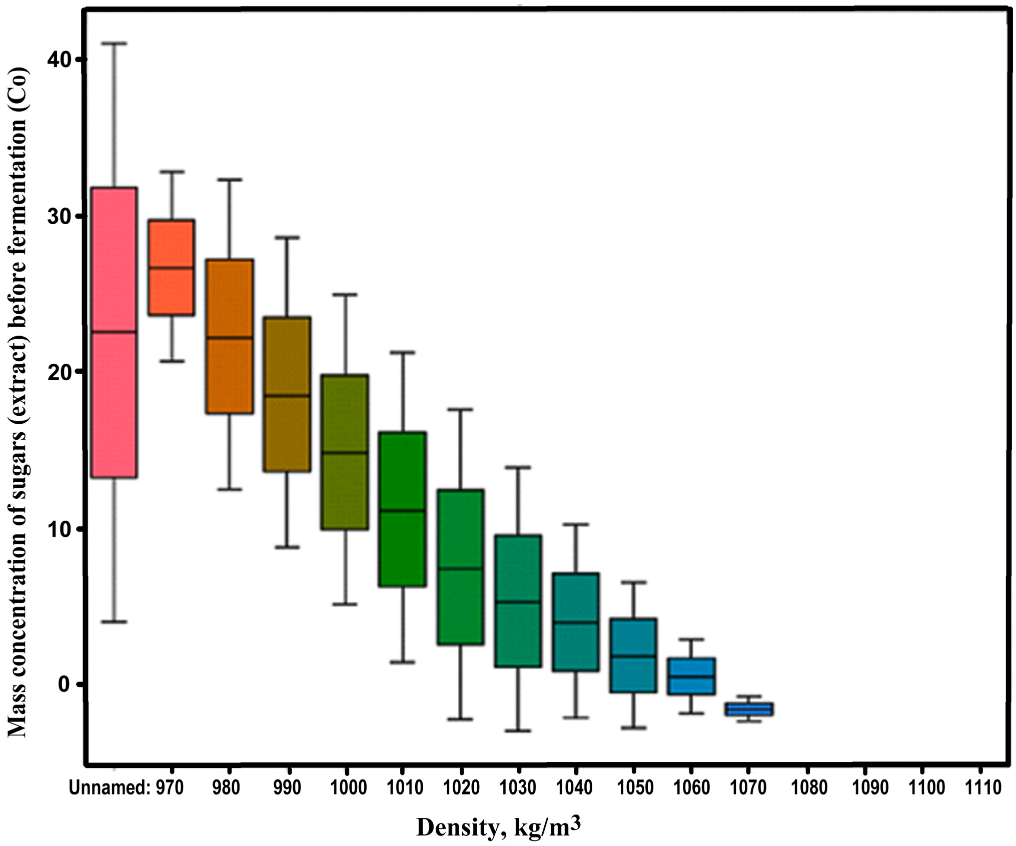

Figure 4. The box-and-whisker plot of the distribution of quantitative C_0 data and density for comparison between variables or between levels of a variable. The bar colours are a visual aid for distinguishing between different densities.

4. Conclusions

As a result of this study, we can conclude that the decrease in the Brix readings of the refractometer during alcoholic fermentation of grape wort is proportional to the amount of fermented reducing sugars defined according to Magwaza and Opara [4], with increments that depend on the initial content of extractives before fermentation. Hence refractometry can be used to monitor the ethanol concentration as an alternative to the densimetric method.

An experimental verification of the compliance of the value of the volume fraction of ethyl alcohol obtained by the proposed method in comparison with the method certified in winemaking showed that in 95% of cases the discrepancy between the methods does not exceed 0.1% vol. in the case of using the pycnometric method or the readings of a glass alcohol meter to measure the density, followed by finding the desired density according to the density table of water-alcohol solutions.

From the experimental results, a rapid, non-destructive method was presented for determining the volume fraction of ethanol in liquid wine products the technological process and in finished products. The method can be the basis for the development of an appropriate regulatory document regulating its use in the wine industry, as well as for the development of a technical specification for the creation of a portable device for determining the concentration of ethanol in wine products, based on the simultaneous measurement of density and refractive index of liquid media.

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