Scientific Papers, Series B, Horticulture, Vol. LVI, 2012

ISSN Online 2286-1580, ISSN-L 2285-5653

A NEW REFRACTOMETRIC METHODOLOGY USED TO MONITOR FERMENTATIONS

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Abstract

A new refractometric methodology for monitoring fermentations was developed. Refractive index is influenced by two facts that occur simultaneously during fermentations, the decrease of sugars from wine (leading to a decrease of refractive index) and the increase of alcohol concentration, due to fermentation of sugars (leading to an increase of refractive index). This deviation of refractive index induced by the presence of alcohol in solution can be corrected by applying some mathematical equations derived by polynomial regressions. By this methodology a good prediction model can be obtained. The methodology allows the winemaker to follow the fermentations and decide when to add nutrients or cool down the wine to slow the fermentation. If the fermentation rate is too slow, real Brix values determined refractometrically decreases by 0.2-0.4 units/day and a decision is rapidly required. Also, the decision of pressing red-wine at a certain sugar concentration or the establishment of the fermentation ending can also be based on accurate refractometric determinations.

Keywords: *refractometry, fermentation, brix, refractive index*

INTRODUCTION

Monitoring alcoholic fermentation evolution is absolutely necessary for many reasons, but especially in order to prevent the stuck fermentations. Many methodologies are applied worldwide for monitoring the progress of alcoholic fermentations [2, 5, 10]. This paper proposes a new methodology of fermentative process monitoring by using a handheld refractometer with automatic temperature compensation.

The novelty of this methodology is that includes mathematical calculations that corrects the direct readings of Brix units (actual Brix) in order to compensate the effect of alcohol continuous increase on the refractive index.

To make an accurate methodology, it is absolutely necessary to determine the initial refractive index (initial Brix) in grape juice (before yeast inoculation or fermentative process begins), and then to record the evolution of this index during fermentation, under the form of a fermentation graphic.

As well known, when the fermentative process evolves, the refractometer readings change due to the presence of alcohol in the solution, as shown in figure 1 as an exemple.

Multiple polynomial regression equations are able to correct these values and the calculated refractometric units can be used to monitor the residual sugar evolution.

In a sugar solution a direct relationship exists between Brix degrees and refractive index that has been determined using conversion tables [4, 6, 7, 12]. Also, a direct relationship between alcoholic concentrations in solutions and the measured refractive index of that solution [14].

MATERIALS AND METHODS

Starting from the facts exemplified in figure 1 and the simplification that the fermenting wine is a solution of sugar, water and alcohol the following equation can be derived:

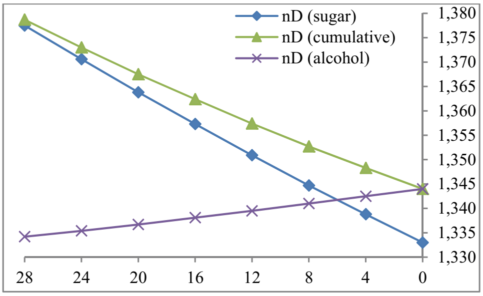

Fig. 1. Relative measurements of refractive indices in solutions of sugar and alcohol [13]

$n_D = n_{D\ wat.} + \Delta n_{D\ alc.-wat.} + \Delta n_{D\ sug.-wat.}$
(Eq 1)

where:
n_D – uncorrected refractive index of the wine measured during fermentation;
$n_{D\ wat.}$ – refractive index of distilled water;
$\Delta n_{D\ alc.-wat.}$ – difference between the refractive index of an alcohol solution of known concentration and the refractive index of distilled water;
$\Delta n_{D\ sug.-wat.}$ - difference between the refractive index of a sugar solution of known concentration and the refractive index of distilled water;

The mathematical model was developed taking into account the maximum values of sugars and alcohol which can be found in wines. Regression analyses were by using the tabulated values for alcohol and sugar solutions, measured in the range of 0 to 28 Brix and from 0-16% vol./vol., respectively. The goal was to find the relationships between refractive index and degrees Brix, in the case of sugar solutions and the relationships between refractive index and alcoholic strength by volume, in the case of alcohol solutions.
Based on mathematical regression a complex mathematical model was created so that to replace hydrometers for monitoring of alcoholic fermentation.
Commonly used in winemaking, Brix refractometer, with automatic temperature compensation is used to measure the concentration of sugars in musts. Used on wines, due to the presence of alcohol, they give higher values on the Brix scale than the normal values directly correlated with the existing amount of sugar.
When alcohol starts to accumulate in fermenting must, to find out the correct alcoholic concentration during fermentation by refractometery, the difference between initial and real Brix degrees should be calculated.
Do to these contrary influences of sugar and alcohol on the refractive index the actual Brix values read on the refractometer is misleading and the directly measured value unusable as such. Theoretically, on the Brix scale the value of 0 signifies that the fermentation is finished. Practically, by calculating the differences between initial Brix value and real Brix value, the amount of probable alcohol from wine can be determined by the following equation:

$c\%\ v/v = (a - d) \times b;$
(Eq 2)

where:
$c\%\ v/v$ – probable amount of alcohol that was formed into the fermentative process;
a - initial Brix value (in grapejuice);
b - conversion of Brix value to alcohol concentration, for values ranging from 0.55 and 0.63 [1, 3, 8].
d - real Brix value;

By using equation 2, knowing the amount of alcohol present in the fermentation tank at a certain time, the real Brix value (d) can be calculated using adjusted refractive indices.

RESULTS AND DISCUSSION

From the tabulated Brix values for the various sugar solutions we can derive Equation 3 and use it to calculate the refractive indices corresponding to the initial and real Brix values:

$n_D = 6e - 6 \times °B^2 + 0.0014 \times °B + 1.333;$
$(r^2 = 1)$
(Eq 3)

where:
n_D - refractive index of a sugar solution;
$°B$ - Brix degrees of sugar solution;

Equation 4 is derived in a similar way from the tabulated Brix values for the various sugar solutions [4, 6, 7, 12] and is used to calculate the corresponding Brix values for a refractive index:

$$°B = -1504.2 \times {n_D}^2 + 4705.3 \times n_D - 3599.4;$$
$(r^2 = 1)$
(Eq 4)

where:
n_D- refractive index of a sugar solution;
$°B$- Brix degrees of sugar solution;

From the tabulated values for the various alcohol solutions with concentrations expressed as mass percent (% w./w.) we can derive Equation 5 and use it to calculate the refractive indices corresponding to a solution with a certain alcohol concentration:

$$n_D = -2e - 7 \times c\%^3 + 1e - 5 \times c\%^2 + 0.0006 \times c\% + 1.333;$$
$(r^2 = 1)$
(Eq 5)

where:
n_D- refractive index of an alcoholic solution;
$c\%$- alcoholic concentration in mass percentage (%w./w.);

For the case of alcoholic solution with concentrations reported as volume percentages (%v./v.) as in tables [9, 11, 14] the equation 5 becomes equation 6:

$$n_D = 5e - 6 \times c\%^2 + 0.0005 \times c\% + 1.333;$$
$(r^2 = 0.9999)$
(Eq 6)

where:
n_D- refractive index of an alcoholic solution;
$c\%$ - alcoholic concentration in volume percentage (%v./v.);

The transformation of volume percentage in mass percentage for the alcoholic solutions is easily achieved by using equation 7:

$$c\%\, w/w = \frac{c\%\, v/v \times 0.79074}{d_{20}^{20}};$$
(Eq 7)

where:
$c\%\, w/w$ – alcoholic concentration in mass percentage (%w./w.);
$c\%\, v/v$ – alcoholic concentration in volume percentage (%v./v.);
0.79074 – relative density of ethanol at 20°C;
d_{20}^{20}- relative density of a solution of alcohol with c% v/v concentration;

This type of transformation can also be expressed under the form of a table (Table 1).

Table 1. Relation between ethanol concentration and relative density at 20°C [9, 11].

Ethanol % w./w.	Ethanol % v./v.	d_{20}^{20} of alcohol solutions
0.00	0.00	1.00000
1.00	1.26	0.99813
2.00	2.52	0.99629
3.00	3.77	0.99451
4.00	5.02	0.99279
5.00	6.27	0.99113
6.00	7.51	0.98955
7.00	8.75	0.98802
8.00	9.98	0.98653
9.00	11.21	0.98505
10.00	12.44	0.98361
11.00	13.66	0.98221
12.00	14.88	0.98084
13.00	16.10	0.97948
14.00	17.32	0.97816
15.00	18.53	0.97687
16.00	19.74	0.97560

In order to validate the model, several simulations were performed.
For example, we took the case of a grape juice which fermented 12 real Brix degrees. Assuming that the initial value for the grapejuice before the start of fermentation was 24, in accordance with the equation 3, the refractive calculated index was 1.37006.
Real Brix value was the difference between the initial value and the fermented Brix value, that is 24 - 12 = 12, to which the corresponding refractive index calculated by using equation 3 is 1.35066. Then, by using equation 2 and the conversion of Brix values into alcohol concentrations (in the range 0.55 and 0.63) we

can calculate the amount of alcohol formed in the fermentation process.

We disregarded here the fact that the conversion factor is slightly influenced by the temperature of fermentation, the yeast strain, the variety and so on. Therefore, by using an average Brix-alcohol conversion factor of 0.59 we obtain an alcohol concentration of 7.08% expressed as v./v. This alcohol concentration of 7.08% v./v. is equal to 5.66% w./w. alcohol according to equation 8. According to equation 6, the refractive index corresponding to a solution of alcohol 5.66% w./w. alcohol can be calculated. Then, by using equation 1 it is calculated the uncorrected refractive index (corresponding to the actual Brix value), that is the value one would actually measure by using a refractometer on the fermenting grapejuice at that certain time.

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By solving similar equations for all other possible cases that can be found in the process of turning grape juice into wines, a matrix of results was built and analysed so that to derive an equation based on which the real Brix values be obtained form the actually measured Brix values.

For the mathematical data processing the following software packages were used: Origin Pro 8.0, SPSS 17.0, Statistica 10.0.

Origin Pro 8.0 software was used to calculate manual repeated regressions until they led to equation 8, that calculates real Brix degrees (d) with high precision, taking into account initial Brix degrees (a), conversion factor Brix-alcohol (b) and current Brix degrees (c).

$d = (k_1) \times c^2 + (k_2) \times c + (k_3)$
(Eq 8)

where:
$k1 = (ka1) \times a + (ka2);$
$k2 = (kb1) \times a^2 + (kb2) \times a + (kb3);$
$k3 = (kc1) \times a^2 + (kc2) \times a + (kc3);$

where:
$ka1 = 4.0385e - 4 \times b - 1.21202e - 4;$
$(r^2 = 0.98091)$
$ka2 = -0.03113 \times b + 0.00942;$
$(r^2 = 0.99999)$
$kb1 = -6.63638e - 4 \times b + 2.60639e - 4;$
$(r^2 = 0.99159;)$
$kb2 = 0.0522 \times b - 0.01953;$
$(r^2 = 0.9956)$
$kb3 = 0.60913 \times b + 0.97331;$
$(r^2 = 0.99985)$
$kc1 = -0.00325 \times b + 7.20833e - 4;$
$(r^2 = 0.98434)$
$kc2 = -0.96363 \times b + 0.23933;$
$(r^2 = 0.99741)$
$kc3 = 1.25163 \times b - 3.02807;$
$(r^2 = 0.95666)$

where:
a – initial Brix value, measured before the strat of fermentation;
b – Brix-alcohol conversion factor (from 0.55 to 0.63) [1, 3, 8];
c – actual Brix value, measured with then refractometer in the process of fermentation (two times per day);
d – real Brix value, calculated by manually repeated regressions (calculus that exclude the effect of alcohol on the refractive index);
k1, k2, k3 – coefficients of primary polynomial regression;
ka1, ka2 – coefficients of k1 coefficient polynomial regression;
kb1, kb2, kb3 – coefficients of k2 coefficient polynomial regression;
kc1, kc2, kc3 – coefficients of k3 coefficient polynomial regression;

The data processing with Statistica 10.0 and by using multiple polynomial regression analysis resulted in the following equation:

$d = -0.00268 \times c^2 + 1.41448 \times c - 0.40253 \times b^2 - 5.35318 \times b + 0.00115 \times a^2 - 0.33467 \times a + 0.61759;$
$(r^2 = 0.999357);$
(Eq 9)

where a, b, c, d have the same meaning as in equation 8.

SPSS 17.0 was used to calculate the matrix with intermediary data. Multiple linear regression was preformed and lead to the following equation:

$d = -0.28699 \times a - 5.73434 \times b + 1.33082 \times c + 0.78605$;
$(r^2 = 0.99919)$;
(Eq 10)

where a, b, c, d have the same meaning as in equation 8.

Statistica 10.0 was used to compare the results, by calculating the correlation between theoretical real Brix values and calculated by using the 3 equations previously determined (Eq. 8, 9 and 10).
The correlation coefficients are presented in Table 2, showing a very good correlation between all the derived equations, meaning that all the equations provide reliable results.

Table 2. The correlation between 3 equations derived with 3 different software packages for the real Brix value calculation

Real Brix degrees (d)	Theoretical	Origin Pro 8.0	SPSS 17.0	Statistica 10.0
Theoretical	1.000000	0.999998	0.999595	0.999678
OriginPro 8.0	0.999998	1.000000	0.999597	0.999681
SPSS 17.0	0.999595	0.999597	1.000000	0.999917
Statistica 10.0	0.999678	0.999681	0.999917	1.000000

*Correlations significant at $p < 0.05$;
N=987 (Casewise deletion of missing data);

From correlation analysis that was preformed for all calculated equations against theoretical real Brix degrees, it can be said that the most accurate results was obtained using the program OriginPro 8.0 with manually repeated regressions, that lead to Equation 9, which has an r = 0.999998 for statistical assurance level of 99.5% (risk failure 0.5%).

The results of this study allowed us to obtain equations with which we can estimate the relative density, alcohol concentration and sugar content during fermentation.

The same equations can be modified so that the measurements should be done not only with a refractometer, but also with a hydrometer with Brix scale or any other scale correlated with Brix.

Estimation of sugar content from must
This estimation can be obtained with the equation 11.

$$Sugar\ g/l = \frac{a}{1.75} \times 16,83; \qquad \text{(Eq 11)}$$

where:
a – initial Brix value, measured before the strat of fermentation;
1.75 – approximate Brix value for a sugar solution form which 1% v./v. alcohol results [1];
16.83 – grams of suchrose that produce through fermentation 1% v./v. alcohol [1];

Estimation of residual sugar content from fermenting wine

This estimation can be obtained with the equation 12.

$$Sugar\ g/l = \frac{a-d}{1.75} \times 16.83; \qquad \text{(Eq 12)}$$

a – initial Brix value, measured before the strat of fermentation;
d – real Brix value, calculated by multiple regressions (calculus that exclude the influence of alcohol on the refractive index);
1.75 – approximate Brix value for a sugar solution form which 1% v./v. alcohol results [1];
16.83 – grams of sucrose that produce through fermentation 1% v./v. alcohol [1];

CONCLUSIONS

The methodology proposed allows for sugar and alcohol estimation in a grape juice undergoing fermentation, by using a simple measurement method, such as refractometry.
The equations derived for sugar and alcohol estimation at a certain moment of fermentation can easily be used by any winemaker. Also, the equations can be used to plot with a common software, such as Excel, the evolution of sugar and alcohol concentration during fermentation.
Monitoring the fermentations with this refractometric methodology has a series of advantages, as follows:

- as opposed to the measurement with the hydrometer the readings are not influenced by carbon dioxide bubbles that are released into the fermentation;
- although the determination of Brix values before fermentation is a prerequisite, the determination of potential alcoholic strength and the sugar concentration of sugars in the grape juice is simple;
- the graphical display of the kinetics of fermentation allows to intervene in time to control the fermentation rate and, to some extent, allows the selection of an appropriate yeast strain for a certain type of wine.

REFERENCES

[1] Antoce Oana Arina, 2007. *Oenologie - Chimie și analiză senzorială.* Editura Universitaria, Craiova.

[2] Bisson Linda F., 2002. VEN 124 LAB MANUAL. An Introduction to Wine Production, Department of Viticulture and Enology University of California, Davis, p.36-41;

[3] Boulton R., Singleton V. L., Bisson L. F. şi Kunkee R. E., 1998. *Principles and practices of winemaking. Aspen Publishers*. Gaithersburg, Maryland, p.137-138.

[4] Bureau Of Sugar Experiment Stations, 1970. *Laboratory Manual For Queensland Sugar Mills*. 5th Edition. Watson Ferguson And Company, Brisbane, Australia;

[5] Cotea Valeriu D., 1985. *Tratat de oenologie vol. I. Vinificaţia şi biochimia vinului*. Editura Ceres, Bucureşti.

[6] Frederick J. Bates and associates, 1942. *National Bureau of Standards. U.S. Department Of Commerce. Circular 440 Table 114 - Polarimetry, Saccharimetry and the Sugars*. By United States Government Printing Office Washington.

[7] International Organisation of Legal Metrology, 1993. *Refractometers for the measurement of the sugar content of fruit juices*.

[8] Jackisch Philip, 1985. *Modern Winemaking*. Cornell University Press, New York, p.43-45.

[9] Norbert Lange (editor), 1961. *Handbook of Chemistry, 10th edition.* Mcgraw Hill Publishers, New York.

[10] Ribéreau-Gayon P., Dubordieu D., Donèche B., Lonvaud A., , 2006. *Handbook of Enology (volume 1.) - The Microbiology of Wine and Vinifications*. 2nd Edition, John Wiley & Sons Ltd.

[11] Technology Reports Centre, Department of Industry for the Department of Prices and Consumer Protection (Orpington), 1975. *Alcoholometry* "International alcoholometric tables". Fourth International Conference on Legal Metrology, English Translation by BIML.

[12] United States Department of Agriculture, 1981. *Technical Procedures Manual*. Sucrose table conversion, 135-A-50.

[13] Wilkes Eric, 2005. *Surviving Baume, Brix and Vintage*. Fosters Wine Estates IWAG Seminar Mildura.

[14] http://www.refractometer.pl/ refraction-datasheet-ethanol, accessed on 15th march 2012. *Refractive indices of ethanol solutions at 20°C*. 589.29 n;