Advances in Enzymatic Analysis Technology

Small-format instruments now available to maximize accuracy and reliability for the wine industry

By Richard Carey

I last wrote about several laboratory analyses testing for important wine and grape constituents using enzymatic protocols measured spectroscopically in April 2014. (See "Ramping Up Your Winery's Lab.") Since that time, Megazyme introduced a new spectrophotometer called the MegaQuant Wave.

In addition to this new entrant designed for small to medium-sized wineries, two other instrument producers are competing in this segment of the market. The instruments are discussed in the order of price of market entry; check with each manufacturer for their exact costs.

Prior to the introduction of purpose-built instruments, incidental users of enzymatic analysis had to use basic spectrophotometers. These instruments had significant limitations, not the least of which was that the user had to do the calculations by hand and use equations that many in the wine industry did not feel comfortable computing.

The harbinger of the effort to supply a purpose-built, small-format enzymatic analysis instrument for smaller wineries was the Monza analyzer from Randox. This instrument was featured in the April 2014 *Wines & Vines* article mentioned above. Astoria-Pacific, another company in this market area, has offered a larger format, purpose-built system for many years that is a step up in sophistication from the Randox Monza. It has an auto sampling system and is useful for wineries that have a higher volume of analyses on a regular basis.

In the past, the most cost-effective means of analyzing wine constituents using enzymes was to buy a simple spectrophotometer such as the Spec 20, originally produced by Bausch & Lomb but now sold by Thermo-Scientific. The key to this instrument's operation was a diffraction grating that split the incoming light source into discrete narrow bands of light (10 nm or less) and measured by a photometer. This instrument was designed to study between 940 nm and 340 nm wavelengths of light. For many enzymatic analyses, the most

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KEY POINTS

For many years, small to medium-sized wineries that wanted to analyze wine components using enzymes had to work with spectrophotometers. Now, small-format enzymatic-analysis instruments are available to maximize accuracy and reliability at the appropriate wavelength for analyses needed by the wine industry.

Megazyme's MegaQuant Wave includes a series of filters that allow only a narrow band of light for six standard wavelengths, which accommodate all of the available tests for wine analysis. This instrument also has the ability to automate the results needed to calculate the concentration of the sample accurately.

The Randox Monza is similar to the MegaQuant Wave, but with some differences in the number of analyses and equipment included and price. The Astoria from Astoria-Pacific uses auto sampling and is capable of running 200 tests or more per hour.

important wavelength to measure was 340 nm, right at the hairy edge of its ability. This wavelength, which falls at the transition to the UV portion of the spectrum, has the best absorbance of light that can uniquely identify the degree of the enzymatic reactions used by the wine industry.

Unfortunately for the less expensive spectrophotometers, this wavelength is a difficult one to measure because it falls at this transition point. There is not a lot of intensity of that wavelength in the less expensive light sources used in this type of instrument. Couple this fact with the need for a precision diffraction grating to single out only the wavelength needed, and the cost of an instrument dramatically increases to do the job.

As a result, the lower cost instruments were a constant fiddle for winemakers to get good consistent answers. During the past several years, however, new technology has provided solutions that have solved the analysis of the wavelength measurement by designing special purpose-built instruments that maximize accuracy and reliability for those wavelengths.

Another factor that is as important as wavelength is enzymatic purity. The higher the quality of the enzymatic materials, the more stable and accurate the results, and the longer the shelf life will be for the enzymatic kits used in the analyses. This latter fact is an important consideration when a winery is choosing a company to be their reagent supplier, as is the formulation in which the kit components are supplied. In some cases, the reagents must be used within a few days to a week of preparation. This can be extremely wasteful for a small laboratory analyzing just a few samples each day or week. The Megazyme kits are formulated so the reagents are stable and can be used for at least a year after preparation.

Megazyme MegaQuant Wave

In order to lessen instrument cost and increase reliability and accuracy, Megazyme changed the diffraction grating to a series of filters in the MegaQuant Wave. These filters allow only a narrow band of light centered on the target wavelength. In the case of the MegaQuant Wave, there are six standard wavelengths included (see table "Standard Wavelengths") to accommodate all of the available tests for wine analysis, with an option of adding two more.

The filters are important because they provide a simple mechanism for the instrument's software to measure two wavelengths at the same time. As a result, this permits an internal standardization of the light intensity transmitted through the sample, and this standardization helps to determine a true sample absorbance more accurately. It also accounts for the slight perturbations due to imperfections in the borosilicate glass tubes used for testing. In the past, the alternative was to use quartz crystal cuvettes at hundreds of times the cost of "regular" glass tubes.

Another important addition to this instrument, which spectrophotometers do not have, is the ability to automate the results analyses needed to calculate the concentration of the target analyte accurately. These analyses are calculated by recording the change in the absorbance of the light passing through the reaction solution. This change in absorbance (increasing or decreasing) is a direct function of a specific enzyme reaction acting on the analyte being measured and is proportional to the concentration of this analyte. At low concentrations, up to a given upper concentration, the reaction is linear and it is important to determine when the reaction starts to deviate from linearity.

It is at this point where the quality of the assay reagents and the quality of the light signature interacts with the software of the instrument to give the widest measuring range. The MegaQuant Wave software increases the range of linearity of the absorbance curve from a normal 1.5 absorbance units to 3.0 units. In the first half of the range, the linearity is within 1%; in the second half, it is 2%. In combination with high quality assay reagents, MegaQuant Wave accommodates a wider linear dynamic range, meaning fewer dilutions are needed to analyze a single sample accurately.

The next criterion is an instrument's lowest detectable absorbance. The lower limit of detection varies depending on the assay. If lower limits are important to a winery, the buyer should be sure that the instrument being considered measures at or below the lowest concentration expected in a sample. The basic concept of detection limits applies across all enzymatic testing procedures.

For the first example, I will use the analysis of sulfites in wine, since that is one of the enzymatic analyses not usually discussed when talking about enzymatic analytical protocols. This analysis is useful to the winery and should be an important factor in evaluating whether to purchase this type of instrument.

The smallest differentiating absorbance is defined as the lowest detectable absorbance change. However, this is dependent on the quality of the spectrophotometer, quality of assay reagents and skill level of the analyst and, for improved accuracy, a higher absorbance change may be recommended. For the sulfite reaction, the smallest differentiating absorbance is 0.01 abs units. Therefore, if the test is performed with a standard sample size of 0.1 ml, the smallest differentiating absorbance corresponds to 3.4 mg/L, and the recommended lower limit of detection is 6.8 mg/L using an absorbance difference of 0.02. However, if the maximum recommended sample size of 2 ml is used, then the lower limit of detection is reduced to 0.34 mg/L using an

STANDARD WAVELENGTHS

Monza	Megaquant Wave	ChemWell (rAPID-T)	Astoria and Astoria2
340	340	340	410 – 1000 nm Interference Filters
415	405	405	
510	505	420	
546	545	480	
578	580	505	
600	630	540	
660		570	
700		660	
		Customer Selected	

Different wavelengths allow wineries to perform a variety of tests.

absorbance difference of 0.02. This indicates the ability of this reaction to detect the presence of sulfites down to 6.8 ppm with a 0.1 ml sample or down as low as 0.34 ppm by simply changing the sample size to 2 ml.

Lower limits of detection for two other assays of major wine analytes are: 1) Acetic acid, which has a lower limit of detection of 0.254 mg/L using an absorbance difference of 0.02 and the maximum sample volume of 2 ml. 2) L-malic acid, which has a lower limit of detection of 0.25 mg/L using an absorbance difference of 0.01 and the maximum sample volume of 2 ml. From these data, one can anticipate an analytical range for just about any foreseeable amount of target analyte a wine will contain. At these levels, sample-to-sample variance will be within the range of reasonable expectations of accuracy.

In preparing the reaction mixture for these enzymatic analyses, one uses the cuvette to introduce the components of the reaction mixture. A standard sample size for most tests is 0.1 ml, and the total volume is usually between 2 ml and 3 ml. The other components consist of buffers and supporting reagents that allow the enzyme to function. Other than possibly the buffer, these reagents, including the enzyme, hardly ever total more than a few tenths of a milliliter. The rest of the volume for the reaction is water. In the case where the quantity of the analyte being measured is lower than the lower limit of detection for the assay and therefore too low to register a minimum change in absorbance, the only way to get that change is to increase the volume of the sample size. This increase in sample size must have a compensatory maintenance of the total volume of the test (usually by reduction in the amount of water). In this manner, the calculated amount of analyte can be quantitatively measured. It follows that precision in the addition of all the reagents is critical. All calculations are based on a very specific volume.

A key parameter required to obtain the optimum efficiency of an enzyme reaction is temperature. To assure that the test reactions reach full completion in the times suggested, it is best to have an incubation temperature set as recommended for the test being performed. For this purpose, the MegaQuant Wave has an integrated temperature block on the instrument that will accept the round 12 mm tubes. This block's temperature can be set by the control panel's utilities function, which allows the test's incubation temperature to be the same from test to test for each sample.

The above discussion shows the depth of information these new instruments can provide the winery so they can understand the wines' components and make informed decisions about how to produce better wines. The software installed on the MegaQuant Wave stores 120 test settings in the instrument. An additional program called SFCapture is available for download and allows

ANALYSIS KITS FOR SPECTROMETERS

Test Kits**

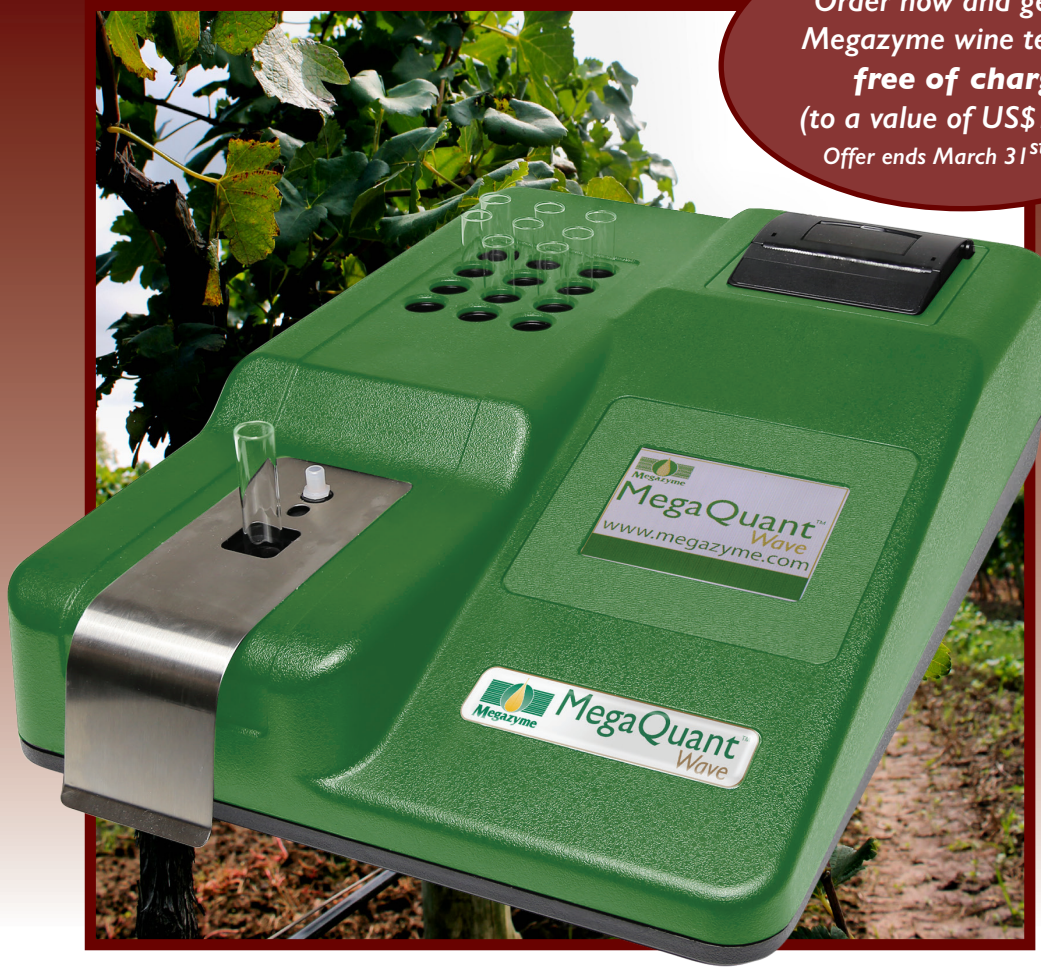
Megazyme	Randox	Chemwell*	Astoria-Pacific
Acetaldehyde	Acetic acid	Acetic acid	Alkalinity
Acetic acid	Ammonia	Acetaldehyde	Alpha Amylase
Ammonia	Calcium	Amylase	Ammonia
α-Amylase	CO_2 Total	Ascorbic acid	Bitterness
β-Amylase	Chloride	Alkalinity	Boron
Amylose/Amylopectin	Copper	Ammonia	Bromide
Amyloglucosidase	Fructose + Glucose	L-Arginine	Calcium
Arabinan	Glucose	Cellulase	Chloride
L-Arabinose/D Glactose	Glycerol	Chloride	Color
L-Arginine/Urea/Ammonia	Iron	Chlorine	Cyanide
L-Ascorbic acid	L-Lactic acid	Cyanide	Ethanol
L-Asparagine/ L-Glutamine/Ammonia	Malic acid	Ethanol	Free Amino Nitrogen
Aspartame	Magnesium	Fructose + Glucose	Glucose
Citric acid	Potassium	Glucose	β-Glucan
Ethanol	Sodium	Lactose/ D-Galactose	Iodine
Formic acid	Total antioxidant status	Lactic acid	Iron, Total
D-Fructose		L-Malic acid	Lithium
L-Fructose		Nitrite	Magnesium
Glucomannan		Nitrate	Niacin
Malt β-Glucanase/ Lichenase Assay		Nitrogen by OPA	Nitrite
β-Glucanase		Nitrogen, TK	Nitrate
D-Glucose		Orthophosphate	Nitrogen, Total (Per.)
Glucose oxidase		Phenol	Nitrogen, TK
α-Glucuonidase		Phosphorus, Total (Per.)	Orthophosphate
Glycerol		PANOPA	Phenol
D-Lactic acid		Silica	Phosphorus (TK)
L-Lactic acid		Sulfite, Free	Potassium
D-Malic acid		Sulfite, Total	P_2O_5
L-Malic acid		Tartaric acid	Protein
PANOPA			Reducing Sugars
D-Sorbitol			Reducing Sugars, Total
Succinic acid			Riboflavin
Sucrose			Selenium
Sulfite			Silicate
Tartaric acid			Sodium
Urea			Sulfate
			Sulfide
			Sulfite, Free
			Sulfite, Total
			Thiamin
			Total hardness
			Urea
			Vitamin C

*Chemwell is distributed by more than one company. Not all kits listed are available from one supplier.
**Each supplier has additional kits available that target other industries, Check with your representative for other kits that might be useful for your analytical purposes.

The Perfect Match!

Megazyme Wine Test Kits and the MegaQuant Wave™ Spectrophotometer

And more !!!
Order now and get 3-4 Megazyme wine test kits ***free of charge*** *(to a value of US$1,000).*
Offer ends March 31st 2017



Low Cost! The least expensive, most reliable spectrophotometer available today.

Versatile! Pre-programmed to run the full range of Megazyme advanced wine test kits
– allowing up to 4 absorbance readings per sample for sequential analyses e.g. ammonia/urea/arginine, glucose/fructose.
– installed test settings for automated results analysis.

Cost effective! Get twice as many assays per kit with the full range of Megazyme test kits by simply halving all sample and reagent volumes.

Compact! Small benchtop footprint with built-in incubation block, printer and pump (for flow-through applications).

Fully supported by Megazyme's full range of advanced wine test kits

- cost effective.
- superior stability of reagents
- rapid determinations

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users to transfer data to a computer through a USB connection.

Features of the MegaQuant Wave

Many of the enzymatic test kits produced by Megazyme can be performed using the MegaQuant Wave, and for many of these, the test settings are preinstalled and permit automated results analyses. The software used by the MegaQuant Wave was developed exclusively for this instrument. As required by many tests used for wine analysis, a major feature is the capability to perform up to four absorbance readings per sample. For tests that are unable to be programmed, the MegaQuant Wave can be used as a standard spectrophotometer.

Another exclusive feature is that the dilution factor of each individual sample can be input while performing that sample test, and the automated results analysis will account for that dilution factor. The instrument also comes with the other elements required to run flow-through analyses.

On start-up, the main LCD screen shows the four primary menu choices: run tests, manage tests, settings and utilities. The MegaQuant Wave comes with many of the Megazyme test kit procedures preinstalled, including all of the major analytes used for wine analysis. The touchscreen directs the user to the list of tests from which to select an analysis. In order to be accurate, a stylus should be used to scroll through the choices on the screen and select one from the various options.

Once a test has been selected, a number of parameters need to be entered prior to starting a test. The system prompts the user to enter the number of samples and usually a blank test for reference. Once the number of samples is entered, the system will prompt the user to insert the first tube. At this stage the user must also enter the dilution factor of each sample, and this is accounted for in the final calculation of results. The user then prepares the first round of reagent additions to the series of tubes scheduled for analysis and waits the appropriate amount of time for the enzyme to incubate.

After the initial incubation period, the tubes are inserted sequentially into the instrument. When the first absorbance readings are completed, a second round of reagent additions is required. The user adds the appropriate reagents to the sample tubes and waits for the second incubation period to complete. The tubes are then inserted into the instrument to record the second round of absorbance readings; results are calculated, stored and printed or output to a computer using SFCapture so that no results are lost.

To conserve the system lamp life, there is an automatic lamp shut off if too long a period of time elapses between the initial setup and the last activity. The delay to restart the lamp is only about 40 seconds.

The additional equipment needed to run the enzymatic tests includes some automatic pipets and tips for injecting the reagents and samples. Today there is a wide range of automatic pipets from which to choose, ranging from simple pipets from Dynalon for less than $30 for a single volume dispenser to options well above $100. There are also pipets that are multi-volume dispensers that cost several hundred dollars. Be sure to check the lower priced pipets for their accuracy, as some are very affordable and can be accurate for up to 5,000 injections. The downside of these less expensive pipets is that they are not repairable, whereas the better quality pipets are. To perform the majority of the enzymatic tests used in wine analysis, only two or three multi-volume pipets are required (1 ml, 200 microL and 20 microL).

Megazyme recommends using the disposable 12 mm borosilicate tubes with the MegaQuant Wave. These tubes are very inexpensive and, with care, can be reused several times before too many scratches make them not useful. Another consideration is the time it takes to clean between uses, which may be the best reason to just dispose of them.

Depending on the Megazyme test, the standard full-size test volume is about 3 ml. However, a major advantage of the MegaQuant Wave is that the cost per test can be reduced by half, if half the volumes of all reagents are used in the test, thereby obtaining twice as many tests per kit. Performing the tests with these lower volumes is easily achieved using the recommended 12 mm borosilicate tubes. When evaluating whether to purchase an instrument that has an important component of the total cost, it is important to factor in the cost per analysis for each manufacturer. When making that comparison, be sure that the evaluation is based on the same criteria. Megazyme, Randox and Astoria-Pacific have different volumes of reagents that are used, and a winery's cost should be evaluated based on the amount of reagent that will be used in that winery. Don't compare the cost of one company's full volume analysis to another's half volume analysis cost or the cost of even smaller volumes of test reagents.

Another important consideration is test accuracy. When one is adding 20 μl and the volume is reduced to 10 μl, there is a greater chance that a larger error will be introduced in just the natural variance of the reagent added to the sample. The advice in this case is: Don't try to save on the cost of the analysis until you know your average error.

In my laboratory I have run acetic acid, L-malic acid, glucose, fructose, SO_2 free and total tests on this instrument. Every one of the test results came well within the standards expected for any wine analysis. The precision of the tests was excellent. In the malic acid test, for example, it is not unusual in a triplicate test to have results that varied by no more than 3 ppm on a 50 ppm sample.

It should be noted that Unitech Scientific has what appears as a similar instrument to Megazyme. It is slightly more expensive but, more importantly, does not contain the exclusive software of MegaQuant Wave and therefore does not offer the same functionality.

Randox Monza

The Randox Monza has many of the same functional abilities and procedural steps as the MegaQuant Wave. The table "Analysis

ENZYMATIC ANALYSIS EQUIPMENT SUPPLIERS

Company	Phone	Website
Advantage Bundling SP	(866) 286-3546	onestoplabsupplies.com
Alpine Scientific	(530) 756-6082	alpinescientific.com
Astoria-Pacific International	(800) 536-3111	astoria-pacific.com
Beckman Coulter Inc.	(714) 993-5321	beckmancoulter.com
Bruker Optics–Bruker AXS Inc.	(608) 276-3000	brukeroptics.com
Cynmar Corp.	(800) 223-3517	cynmar.com
Enartis USA	(707) 838-6312	enartis.com
Forston Labs	(800) 301-1259	forstonlabs.com
Hach Co.	(800) 227-4224	hach.com
Hanna Instruments	(800) 426-6287	hannainst.com/usa
Optek-Danulat Inc.	(888) 837-4288	optek.com
Megazyme	(312) 212-4361	megazyme.com
Randox Food Diagnostics	(304) 728-2890	randoxfood.com
Shimadzu Scientific Instruments	(800) 477-1227	shimadzu.com
Thermo Fisher Scientific	(800) 225-1480	thermoscientific.com/water
Unico	(732) 274-1155	unicosci.com
Unitech Scientific LLC	(562) 924-5150	unitechscientific.com
Veris Technologies Inc.	(785) 825-1978	veristech.com
Vinotec Napa	(707) 953-7072	vinotecnapa.com
Vintessential Laboratories	(613) 598-72242	vintessential.com.au
VWR International	(951) 303-4553	vwr.com

For more information about the suppliers listed above, visit winesandvines.com/buyersguide or see *Wines & Vines*' 2016 Buyer's Guide.

Kits for Spectrometers" gives a list of the analyses performed by each company's device.

Randox and Megazyme are primarily enzyme and reagents producers, and both companies got into the equipment business to help sell their enzymes and provide a full service to their customers. There are enough similarities between the addition rates and the functional operation that a winery can convert one company's enzyme kits for use in the other's instrument.

Be aware that modifications in test protocols will likely need to be made. There are differences in the analyses that each company has available and for which their instruments are designed to run. The analyses may be able to be performed, but not in automated mode. Randox can analyze cations using their test kits. Megazyme does not sell cation test kits. If a winery wants to run those tests, they should consider the choice of adding the protocol to a Megazyme unit or purchasing a Randox Monza. Astoria-Pacific has a more open-architecture software, and I believe they would be able to include cation analysis, if needed.

The wine laboratory is as important to the success of a winery as any other element in the process of setting up that winery.

The Monza has 64 direct access keys for 64 analyses and has a greater number of programmable channels for analysis (192). It comes with two more standard filters than the MegaQuant Wave. The Monza has a free-standing heating block that does not come as part of the unit and must be plugged in separately. This Monza has fewer places than the MegaQuant Wave, and it accepts only cuvettes, not round tubes. At 12 kg (26 pounds), the Monza is heftier in both weight and price than the MegaQuant Wave.

Astoria Pacific

I first saw the Astoria Pacific instrument in Europe many years ago. At that time, Astoria Pacific was a fledgling company, and it has grown over the years. The Astoria-Pacific line brings in several interesting elements but at a significantly increased price above the two previously discussed systems.

Instruments at this level use auto-sampling. Auto-sampling requires a much more complicated means of delivery of the test components, including the sample wines. Each reagent must be installed in the instrument for a run prior to a test. For example, L-malic acid has four different components for the test plus the wine sample and water. Bottles of test reagents and water are placed into the instrument, each in its specific holder. The wine samples are transferred into empty sample bottles in trays that are fed into the instrument. In sequence, reagent from each of the four reagent bottles enters the reaction chamber of the instrument through a series of tubes. A probe then sucks a sample of wine out of each sample bottle in the tray. The instrument then analyzes the reaction mixture in essentially the same manner as the other two instruments discussed above.

The reaction chamber is small, on the order of 250 μl to 500 μl, so very small amounts of test components are needed. Once the reaction is completed, the reaction chamber is evacuated, rinsed and prepared for the next sample.

This overly simplified description varies somewhat within the range of companies that service this sector of analytical instruments. The differentiating factor is both the software and the automation of the systems. Astoria-Pacific has a robust software

system that shows many graphical ways to look at the sample data.

The flagship instrument for Astoria-Pacific is their Astoria, followed by their Astoria 2. These are samplers that run 200-plus tests per hour. Both Randox and Megazyme have larger instruments than the Monza and MegaQuant Wave, and those should also be evaluated when making a decision on this level of equipment purchase.

There is, however, one interesting factor at play here. There is no question that with a five-figure cost for instruments that can handle hundreds of samples per hour, there is a market opportunity for companies with an instrument that can handle 30 to 100 tests per hour at a lower price. Both Megazyme and Astoria-Pacific now supply the ChemWell-T auto-analyzer manufactured by Awareness Technology. The ChemWell-T auto-analyzer is more affordable and comes with open-system software. From Megazyme, optimized test settings are available for many of the company's test kits. These instruments use a much-reduced assay volume compared the manual assay formats (about 10 times less) and therefore the cost per test is reduced accordingly. It should be noted that the reagents of many of the Megazyme test kits can be easily prepared as two or three reagent assays for use with any auto-analyzer.

Summary

The wine laboratory is as important to the success of a winery as any other element in the process of setting up that winery. Historically, wineries have often decided to do basic tests including total acidity, SO_2 and pH while sending out for tests that are more technologically challenging. For those simple tests, hydrometers, a refractometer and basic labware are the essential equipment.

A winery should factor in the time that each test takes to run when calculating lab expenses.

Whether these functions are performed using wet chemistry or some of the simpler laboratory instruments, the cost of these basics will be $2,000 to $3,000 to perform the four or five most basic tests a winery needs virtually every day. A winery also should factor in the time that each test takes to run when calculating lab expenses.

Adding a MegaQuant Wave, its associated pipets and other lab gear adds some costs but removes others. The net comes down to a total cost of about $5,000. The costs of the test kits are considered to be consumables. The best news is the significant increase in the types of analytes the winery can add as "in-house" tests and procedures. A winery will be able to measure primary amino nitrogen, ammonia, L-lactic acid, L-malic acid, acetic acid, glucose, fructose, ethanol, acetaldehyde, ascorbic acid and citric acid.

Finally, in speaking with Megazyme, I learned that they have the ability to add two additional filters to their unit at a marginal cost increase. The company is testing the ability to add filters for measuring hue, color intensity and density of wines using the MegaQuant Wave instrument. If there are enough requests from wineries, the 420 nm and 520 nm filters could be included with the instrument instead of the other normal enzymatic filters. This would give the instrument the ability to run wine color balance equations, virtually eliminating any need for a spectrometer and possibly eliminating the need for a nephelometer that is used in determining heat stability.

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