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LEBANON INDUSTRY VALUE CHAIN DEVELOPMENT (LIVCD) PROJECT

December 2016

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Assignment objectives

The Lebanon Industry Value Chain Development Project is designed to assist the Lebanese private sector and selected NGOs and Foundations to improve the competitiveness of selected value chains in rural areas of Lebanon.

The LIVCD team has conducted a series of assessments on promising value chains which are included in the LIVCD portfolio.

Following the assessment, the team developed an upgrading work plan which includes activities, targets, outputs and outcomes; the purpose is to engage the private sector, farmers and other types of organizations in interventions to improve the competitiveness of the sector and create impact.

The purpose of the assignment was to in the transfer of knowledge of vinegar making to Lebanese processors that will lead to:

1- Improved processing efficiency by procuring specific yeasts and vinegar producing bacteria that will aid in improving vinegar production

2- Improve product quality

3- Increased procurement from local farmers of vegetables, especially apples and grapes as a result of increased demand for improved quality finished products.

Assignment action Plan

A. Activity outline

In previous visits by the consultant numerous problems in vinegar production were identified. In order to maximize the utilization of resources and time it was decided that the project would focus on the following activities:

1. Sourcing and procuring yeasts and non-cellulose forming vinegar producing bacteria
2. Setting up and running trials of the procured yeasts in Lebanese apple and grape juice in different locations.
3. Setting up and running demonstrations of the Acetobacter to show they can make vinegar with a minimum of cellulose production
4. Run parallel controls in laboratory conditions in order to closely monitor microbial activity.
5. Use the opportunity to demonstrate and discuss vinegar making

Sourcing microorganisms

1. Yeast were secured from
    a. Fermentis
    Kevin Lane
    Kevin.Lane@lsaf.com

2. Acetobacter cultures were secured from
    a. Carolina Biological
    2700 York Road,
    Burlington, NC 27215-3398 •
    800.334.5551

More information on the cultures is included in the addenda.

Lebanon Vinegar Production Experiment

Objective: The objective of these trials is to determine the effects of aggressive yeasts and pure Acetobacter aceti on vinegar production in Lebanon.

Problem: It is a working hypothesis that deficiencies in vinegar production in Lebanon is caused in large part by the competition of undesirable wild yeasts and cellulose producing Acetobacter strains. More specifically wild yeast produce wildly varying results, many of which are undesirable. The value of the usual practice of chemically treating the substrates is counter balanced and sometimes out weighed by the potential untoward effects on the Acetobacter.

A method is needed to maximize the alcohol production from the substrates while creating an optimum environment for the production of vinegar by the Acetobacter.

Hypothesis: It is believed that the introduction of aggressive, fast acting, yeasts will quickly dominate the working culture, pushing out the wild yeasts. The high tolerances to alcohol of these new yeasts suggests the complete utilization of sugars while rendering the substrate toxic to most unwanted organisms. The absence of sugar will also reduce the tendency toward the creation of cellulose producing strains of Acetobacter.

Such an environment is highly advantageous to strains of Acetobacter which do not produce cellulose. It is expected that a pure strain of non-cellulose forming Acetobacter, Acetobacter aceti, in an optimum environment will render a superior product, free of cellulose. This has been the experience of the consultant

Rationale of the procedure

Setting up the trials as directed by the method in this trial aims to compare the production of the specific new, aggressive yeasts to the wild cultures found at each of the producers facilities. By charting the rate of sugar consumption against that of the control we will be able to compare how fast and complete sugar is consumed compared to the wild yeasts. By microscopically observing samples will be able to see the degree to which the specific yeast to push out the wild yeast. By testing under various conditions in various facilities in different parts of the country with different substrates, we expect to have data which will allow us to compare how these various conditions affect the production of alcohol, the substrate used by the Acetobacter aceti bacteria to make vinegar.

The trials of the Acetobacter was meant more as a demonstration than a test. The goal was to demonstrate that the domination of a culture with a non-cellulose forming

strain of the bacteria would increase the chances of producing a cellulose free product.

The Acetobacter aceti are defined by their ability to produce acetic acid. They have been identified and utilized around the world for centuries to produce acetic acid. They are hardy and produce vinegar in nature without human intervention. The efforts of wine makers are largely directed at keeping these organisms from spontaneously turning their wine to acetic acid, also-know-as vinegar. So if, as in the case of our trial, they fail to make acetic acid, the cause must be investigated and the trial repeated.

Method:

Supplies

Each site will need

- 8 ± 20 liter clean/new food grade plastic containers with tops that seal tight

- These tops will need to have a ±1.4cm hole drilled near the center
- 8 -1 meter X 1 meter pieces of muslin or cotton cloth
- Some means of filtering the juice [fine netting]
- 40 liters of crushed apples
- 40 liters of crushed grapes
- Clean room with running water, light and power
- 6 ± 1 liter jars with plastic tops
- Triple scale hydrometer/set
- Thermometer
- 6 silicone Fermenting Grommets for Mason Jar, Ball Jar, Kerr Jar CM Reusable BPA-free Food Grade Grommets 3/8" inch
- 6 Air locks

Grommets	Air Lock	Hydrometer
		

- Yeast
  - SAF Spirit HG 1
  - Pasteur Champagne
  - Premiere Cuvee
- 3 viles/tubes of Acetobacter aceti [vinegar bacteria]

Prepare Yeast Cultures

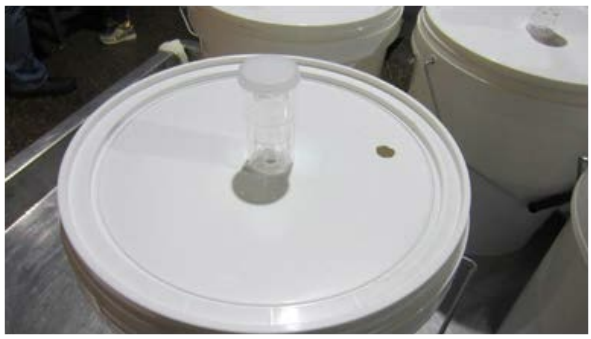
Weigh out .5 gram packets of each yeast for each container.

The three yeast to be tested have been obtained from Fermentis, a commercial yeast manufacturer and distributor. They are:

- Saf-Spirit HG-1
- Premier Cuvee
- Pasteur Blanc/Champagne

These yeasts have been chosen for their aggressiveness and high tolerances to alcohol. High tolerance to alcohol allows for the most complete consumption of alcohol. The goal is to convert all of the sugar into alcohol.

Prepare Containers



Make holes and insert grommets and airlocks in tops

Clean containers

Prepare Juice

Crush and lightly strain fruit to make enough clear juice to make 10 liters for each sample. The procedure will be repeated for each sample of yeasts (3) and one control of wild yeast for apple and grape substrate. This will total eight samples total.

Prepare Pitch [Pitching is a term denoting aerating the yeast culture to encourage the multiplication of yeast cells.]

- Remove 1 liter of juice
- Bring the liter to 38-40 degrees C
- Add .5 gram of yeast
- Stir vigorously or pour back and forth to mix and aerate for minute. Leave for about 20 minutes.
- Make observations for about one hour and make notes.

When yeasts appear active, pour into juice

Test, Observe and make Notes

- Test the juice to know its baseline sugar and potential alcohol content aroma, color and other parameters as outlined on data recording sheets.
- Observe and describe what you see in notes.

Start Fermentation

- Add airlock, observe and note time of first observation of production of carbon dioxide.
- Place in cool dark place out of traffic and away from insects.

Test and Observe Fermentation

- Test for change in sugar level at least every six hours.
- Make observations and note changes in log.

Fermentation End Point Observation

- When tests indicate sugar is depleted, note time first observed and any other observations.
- ***Hint***: when the airlock stops moving it is probably done.

Inoculation of Acetobacter

Prepare Acetobacter aceti inoculate by gently extracting acetobacter from tubes and adding them to 1 liter of sterile water with 2% ethanol.

- (Methanol or isopropyl alcohol CANNOT be substituted.)
- Shake vigorously to mix well.
- Divide into equal portions of 125 ml.
- This procedure is meant o equal distribute the contents of Acetobacter.

- Remove Top with Airlock
- Add Acetobacter aceti
- Test for baseline ph, observe and make notes.
- Cover container with cloth and bind tightly so that no insects may enter but air can.

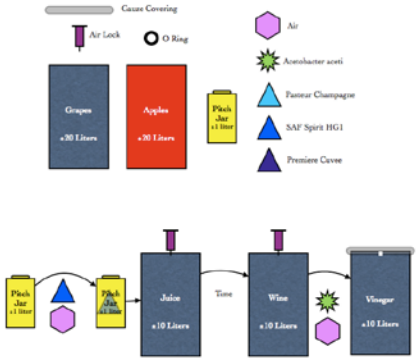
Test, Observe and Note

- Test the ph every six hours until change is noted then every two hours.
- Note changes in ph and log in the notes.

Tests: The following test should be performed and noted in the flow chart.

- Sugar using a triple scale hydrometer
- Ph using the best Ph meter available
- Aroma by nose with best descriptive terms (in language which provide the most accurate description to the tester)
- Taste by mouth with best descriptive terms (in language which provide the most accurate description to the tester)

Vinegar Flow Chart



Observations: It will be critical to make as many observations as possible in order to evaluate the results and provide a baseline for finding solutions to future problems. These observations will also provide opportunities for efficiently modifying the processes to meet the needs of individual producers.

Observations should at minimum include color, turbidity, aroma, and taste changes over time. When possible inspection of cultures under a microscope should be made. Accurate notes, complete with photographs, should be taken.

Sugar Alcohol Observation Note Sheet

Company			
Date/Time		Location	
Substrate		Yeast	
Start Sugar		End Sugar	

For each substrate/yeast please record the time the reading was taken and the reading in each time block.

Entry	1	2	3	4	5	6	7	8	9
Time									
Sugar									
Alcohol									

Entry	10	11	12	13	14	15	16	17	18
Time									
Sugar									
Alcohol									

Notes:

Lebanon Vinegar Production Experiment

Acetic Acid Observation Note Sheet

Company			
Date/Time		Location	
Substrate		Yeast	
Start ph		End ph	

For each substrate/yeast please record the time the reading was taken and the reading in each time block.

Entry	1	2	3	4	5	6	7	8	9
Time									
ph									
%									
Alcohol									

Entry	10	11	12	13	14	15	16	17	18
Time									
ph									
%									
Alcohol									

Notes

The trials were performed with four partners at their facilities.

A. **LIVCD partners**

a. Sonaco Alrabih represented by Mr Fadi Abi Nader
b. Msallem represented by Mr Wissam Msallem
c. Mechaalany representeer by Mr Bernard Mechaalany
d. Judi Lebanon represented by Mr Hatem Kaadan
e.

Additionally parallel studies were conducted by PhD students under the supervision of Dr. Mireille Kalsassy at University of St. Joseph. These studies were valuable to compare a laboratory performance with performance in places where the production will actually take place. The use of lab facilities help to provide unique information to our partners which they would not normally have access to.
For example we were able to take images and videos of the organisms that make their product. We were able to show visual evidence that the new yeasts had pushed out the wild yeasts. Apparently many of them had never seen the key players in their production.

Zouhair Abi Nader is a producer using an acetator, that produces vinegar rapidly, which he built himself. His design uses the drip method in which the alcohol substrate is pumped up and gravity dripped over beechwood chips. Air is counter forced up through the chips. This hyper oxygenated atmosphere speeds the metabolism of the Acetobacter thus increasing the rate of production.

The other producers are using variations of the Orleans method. These methods take more time but to not require large capital expenditures, special expertise and extremely close monitoring.

Zouhair Abi Nader did not do the yeast trial since his alcohol productions methods are mature and optimized for his operation. He chose to follow the acetobacter portion of the trial in order to look for potential solutions for a major problem he has with Acetobacter xylinum. It is hoped that he can be helped with this problem in the future.

B. **Trial/demonstration facts &challenges by the partners**

The conditions and substrates varied more than expected but those are the conditions, which the vinegar will be produced. The trial revealed some of the unique challenges the individual partners faced that would only appear when observing them make vinegar under more easily controlled conditions. Working

closer with the partners allowed the consultant to discover problems which shorter visits had not revealed.

Major challenges:

Challenge 1:

- Test protocols were weak and rendered inconsistent Though they have staff that could perform these tests, they often lack adequate testing kits and protocols.

Recommendation 1:

- Processors were informed on the required test kits and laboratory tests required to properly produce vinegar.
- LIVCD team offered and linked many processors to vendors in order to buy the needed equipment.

Challenge 2:

- Significant temperature variation at each site. Temperature is a key element for vinegar fermentation; however processors were performing the trials in their routine vinegar area, which was subjected to temperature variation and affected the second fermentation results. These problems only became apparent during discussions about the failure of the Acetobacter to perform as expected.

Recommendation 2:
Since the trials were trying to mimic conditions close to the actual conditions, the trials need to be repeated with close attention paid to the temperature.

Refractometers can only be counted on to measure sugar at the very beginning of the fermentation process. Once alcohol is introduced, the refractometers is know to be incorrect.

Alcometer can only measure alcohol in grain neutral spirits which contain no sugar. Small amounts of sugar will render the readings incorrect.

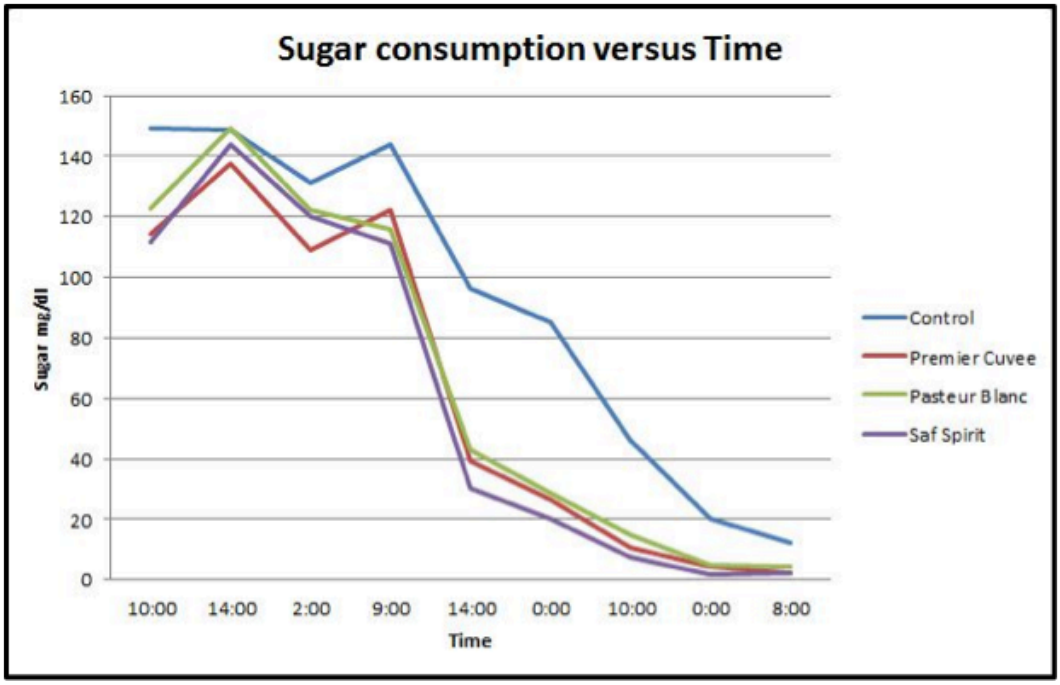
The ability to ascertain whether there are pesticides or other agricultural chemicals in the substrates can be useful. Many chemicals, even in small amounts, can interfere with the metabolism of micro-organisms and even cause them to mutate.

In addition to the tests which can and should be done often on site, utilization of third party labs to make new findings, corroborate or call into question onsite tests, or serve as independent documentation of the products would be valuable to the partners.

C. Results and discussion

a. Yeast Demonstration

- The specific yeast out performed the wild yeast in all cases where records were available. They were faster and consumed all measurable sugar. Some questionable aromas were noted but they also existed in the controls so it is assumed that these aromas are products of the substrate. This needs further investigation.
- The experiences with the specific yeasts, where the protocols were followed, showed clear advantages over the use of wild yeasts. The specific yeast started quickly and quickly dominated the wild yeasts. The data collected from the trials done at the various vinegar making facilities and the university shows the specific yeasts started earlier and consumed all measurable sugar faster than the control cultures.
  The domination of the specific yeast could also be verified by the near disappearance of the wild yeasts when samples were subjected to microscopic examination.
- The Saf Spirit, Premier Cuvee and Pasteur Blanc/Champagne yeasts were robust and aggressive. They began to consume the available sugar much quicker than the wild yeast as demonstrated in this graph created from the data.



Scale up: The trials were designed for 10 liter batches to make the math easier for scale up. One needs to begin with direct multiplication to scale up. The dosage is that which is recommended by the developers of the yeasts. That said, experience and production needs will suggest adjustments. Good testing and record keeping will prove quite valuable here.

In these trials we did not filter the substrate between the production of alcohol and the conversion to vinegar. This will be an important step to add to the scale up.

Some risks to consider are agricultural chemicals, unexpected infection by unwanted organisms, and inaccurate testing. http://www.ajevonline.org/content/34/4/278

Large fluctuations in ambient temperatures can have unexpected impacts on these organisms. The consultant does not have enough experience with these newly introduced organisms to be able to anticipate the precise impact on these organisms but, general knowledge of the organisms in these trials suggests an impact is to be expected.

Investigations into the cause of the occasional undesirable odors should be investigated to ascertain their origin. The specific yeasts are represented by their developer to be flavor

and aroma neutral. That said this should be watched for to see if they are indeed neutral.

- It is essential that a means of consistently and accurately testing and keeping records be developed and utilized. Absent this it will be difficult to impossible to make real improvements in vinegar making.

  This article from the wine growers website might be a starting point for considering the options available for measuring sugar/alcohol in the sugar/alcohol phase of the vinegar production.

Ways to Measure Alcohol

From
http://www.winegrowers.info/wine_making/Alcohol.htm

There are several methods of measuring the alcohol content of a liquid, all require a representative, homogenous sample. The principal methods are:-

Density and Refractive Index method:
The refractive index of alcohol is different from water, so can be used as an indicator of alcohol content. It is the quickest method, taking only a few minutes. It is prone to interference by other compounds in the wine, but the accuracy is dependent mainly on correcting for the temperature of the wine, a sophisticated refractometer will have a temperature compensation facility, the accuracy can be at best +-0.5 %vol.

Distillation followed by hydrometry/refractometry method:
The alcohol must first be separated from the wine by distillation with special equipment. The specific gravity of the distilled alcohol is then determined with a hydrometer which is calibrated directly in %vol at 20 °C. If the distillate temperature varies from 20 °C then a correction needs to be applied, since the volume of the liquid changes with temperature. 0.1 %vol is added for each 0.5 °C below 20 °C, or subtracted for each 0.5 °C above 20 °C, so this is very significant.
This is a complex and time consuming procedure, the accuracy can be affected by the work of the technician as there are various possible causes of error, such as interference from co-distilled compounds, when there are ethyl esters (cleaved to ethanol during the analysis) and other alkali-stable steam volatile compounds present.

The MISCO Palm Abbe VINO5 Digital Wine-Alcohol Refractometer
A much superior alternative to the hydrometer: The MISCO Palm Abbe VINO5 Digital Wine-Alcohol Refractometer from USA ($525, www.misco.com) is engineered specifically for testing the potential alcohol content of grape must, as well as the actual Alcohol by Volume in the finished wine. Measurements are made with precision comparable to mid-range bench-top refractometers costing thousands of dollars more. The actual Alcohol by Volume of a particular finished wine can be established following procedures and methods recognized by international bodies. After distilling a small volume of wine, the ethanol distillate is measured on the Palm Abbe VINO5 Digital Wine/Alcohol Refractometer and the actual alcoholic strength (%vol) or specific gravity is displayed. Protection against inaccurate readings due to temperature differences (a major concern in refractive index measurement) is assured with non-linear temperature compensation specific to grape juices. Temperature compensation is automatic for fluids read between 0 and 50°C. The accuracy is claimed to be +-0.25 %vol, twice the accuracy of any competing instrument.

Ebulliometer method:
The ebulliometer is a measuring device that is designed to evaluate the boiling point of different types of liquids. It's use in the wine industry is based on the fact that alcohol boils at 78.4 °C, a lower temperature than water, so the boiling point of alcohol-water mixtures changes as a function of their concentration. A precision thermometer is involved, to determine the boiling temperature of the wine within 0.02 °C. The boiling point of any liquid

depends on the atmospheric pressure, so the zero point has to be set against the boiling point of pure water prior to starting each test.

The method is fairly quick and sufficiently accurate (+-0.5 %vol) for general purposes. It is affected by atmospheric pressure changes, a barometric change of 4 mm (during a test) would cause an error of 0.5 %vol. A simple ebulliometer is less accurate than the Distillation method.

There are two modern types of the device that are currently in use. The Swietoslawski ebulliometer relies on an isobaric method. This form of ebulliometer contains a boiler, Cotterell pumps, a condenser, and a thermowell.

The measurements obtained with this type of ebulliometer are considered to be very exact. An isobaric ebulliometer provides measurements of such factors as the exact temperature needed to reach a boiling point, solvent purities within the properties of the wine sample, and the molecular weight of the substance. The use of a resistance temperature device helps to create the accurate readings on the vapor-liquid equilibrium of the wine, which is one factor that makes the isobaric approach the most accurate reading possible with this type of device.

An isothermal ebulliometer contains similar components as the isobaric type, but usually involves the presence of a stirring mechanism that is operated by a small pump. The stirring takes place during the process of boiling the mixture and is thought to increase the chances for a more accurate reading of such gases within the mixture as methanol. While not as prominently in use as the isobaric type, there are a some wineries that prefer this method.

[**Note that some providers in Lebanon have discontinued sales of this piece of equipment due to its association with drug manufacture in the country. That said apparently it is still possible to get it in Lebanon**.]

Gas chromatography (GC) method:
The equipment is expensive to buy so the analysis is normally carried out by contract analytical chemists. It is considered to be the most accurate means of ascertaining the alcohol content of wines and is used by HM Customs & Excise.

Essentially, the gas chromatograph works by analyzing the mixture of organic compounds found in the wine. The gas chromatograph has a series of filters made from porous materials. Samples taken from the wine batch are gathered into a syringe and then injected into an ejector port on the device. The temperature of the injector port must be in excess of the boiling point for the sample in order accurate readings to occur. This allows the components of the wine to convert into gas, which then is then pushed into the filters by way of helium or a similar carrier gas.

As the wine gases pass through the filters, the compounds are identified by electronic equipment and the alcohol content is determined. While some models of the gas chromatograph feature a printer that creates a graph of the progress of the wine sample, new models utilize a terminal display, making it possible to view the results without having to decipher a graph.

Modern gas chromatographs are smaller and less expensive to purchase and maintain compared to equipment from years ago. Using a gas chromatograph for alcohol measurement has the extra benefit of providing the winemaker with additional information on the nature of the wine.

There are a number of manufacturers around the world that provide state of the art gas chromatograph equipment to the wine industry. Research and development departments at these manufacturers routinely find ways to fine tune the efficiency of their products, which makes it possible for the process of accurately gauging the alcohol content of wine more accurate every year.

Spectroscopy (infra red) method:
The Alcolyzer Wine from Anton Paar which uses a patented method based on Near Infrared spectroscopy to determine the alcohol content in a highly alcohol-specific range. For this reason, the other constituents of the beverage do not influence the result. This means, the determination of white or red wine, sweet or dry wine, can all be done with one adjustment.

A highly alcohol-specific range of the spectrum was identified between 1150 and 1200 nm. The evaluation method uses the significant alcohol peak in this area and two spectral points very close to it for defining the baseline. Extensive investigations showed that the alcohol results based on this type of evaluation are virtually free of influences from other known wine constituents. This allows adjustments to be done simply with water for the zero point and one binary ethanol/water mixture.The Alcolyzer Wine utilizes an optical set-up without any moving parts. The instrument consists of a Near Infrared Light Emitting Diode, a condenser lens, a sample cell, a collimator lens to focus the parallel beam and a grating spectrometer with a detector array. The absorption information read by the detector array is used to determine the alcohol content of the sample. A measurement is carried out in less than one minute. The Alcolyzer Wine has a built-in Peltier thermostat to ensure accurate and automatic control of the temperature in best time. Consequently, there is no need for manual temperature adjustment and correction.

Methods such as the combined density and refractive index method or Ebulliometer determination tend to be inaccurate because the underlying measuring properties are non-specific to alcohol. Traditional analysis methods such as distillation are time consuming and require experienced operators. The accuracy of the above mentioned methods can be difficult to maintain and repeatability is often at unacceptable levels. Compared to distillation, an acknowledged reference method for alcohol determination, the Alcolyzer Wine achieves accuracies of ± 0.1 %vol and repeatabilities of ± 0.01 %vol.

More recently a Mid Infrared wine analyzer has been released that has similar accuracies to the Anton Parr Near Infrared instrument with a cheaper price tag. If you want information about it contact: Wilks Enterprise, Inc.
www.wilksIR.com

Not recommended for sugar measurement

Blood glucose meters
Current methods for accurately measuring residual sugar (RS) concentrations in wine can be time-consuming, requiring multiple preparation steps and careful training. Sugar test pills and strips are rapid and easy to use for

monitoring RS concentrations from 1 to 20 g/L but only give an approximate RS concentration. Recently, hand-held, digital glucose testing monitors have become available for use by diabetics to accurately and rapidly monitor blood glucose concentrations. We evaluated one of these monitors for its sensitivity and accuracy in model wine solutions. Wines of varying RS concentrations were tested and the results compared to standard procedures. The influence of pH and ethanol and SO_2 concentrations on the assay were investigated. The monitor showed promise for measuring glucose concentrations of 1 g/L or less in wines. In model wine solutions, a nonlinear response was obtained with glucose concentrations of 2 to 10 g/L. The evaluated type of system is not suitable for routine monitoring of fermentations in a winery.

http://www.ajevonline.org/content/49/2/225

Alcometer
This device is designed for grain neutral spirits and alcohol that has no sugar. Small quantities of sugar will cause inaccuracies with this device. If the liquid is distilled, the distilled alcohol can be measured.

Others
If an instrument is not in common world wide use for winemaking, inquiries should be made to the manufacturer to see if it can be recommended for the proposed use.

b. **Acetobacter demonstration:**

- **The Acetobacter we used failed to produce acetic acid**. To compound and complex the problem it, or something, also consumed all of the alcohol, in all of the trials. This was a wholly unexpected and so far still an unexplained result. It is still under investigation.

  The fact that all of the trials from all of the individual cultures, even those under lab controlled conditions conducted at Saint Joseph's University, rendered the same unexplained results suggest a mutation. The hypothetical mutation could be from various, even if unlikely, causes. There is a working hypothesis that low temperature at the initial inoculation from the tubes could have caused it. While anything is possible, these organisms commonly live in various temperatures and will usually go dormant rather than mutate. nothing has been found in the literature so far that points to a clear etiology.

  Though every effort was made to avoid radiation from Xradiation during transport, it is possible that even low levels of ambient radiation while waiting for one of the many "hand" inspections may have been enough to cause mutations. The literature is not supportive of this hypothesis but these particular organism were not the ones tested.

  The key points in the failure is that;

  1. All or most of the alcohol was consumed
  2. Little to no acetic acid was produced
  3. There was no evidence of any acetic acid being

produced. This means that the usual attribution of "over acidification can not be supported without additional data and explanation.

Queries to Carolina Biologicals, the company that sells the organisms as well as the American Type Culture Collection, the origin of the cultures, have been started and and an investigation ongoing. They have found nothing in the literature so far to explain our results. No customers have reported experiencing anything like this. The consultant has never encountered anything like this.

- **Information on the Acetobacter** can be found here. https://en.wikipedia.org/wiki/Acetobacter_aceti
- **Scaling up** of Acetobacter should be done by growing up a culture using the technology in use on site. Once the culture has reached scale the production will be continuous. That is to say the producer will draw off a given quantity (typically 50%) of properly acidified (>5%) vinegar and then replace it with an equal amount of alcoholic substrate. This exchange never stops as long as problems do not arise.

  If problems do arise a new culture should be obtained. The new culture can be a dormant culture maintained in a freeze dried state or can be one maintained in a carefully monitored active state. When needed, the culture would be grown up in laboratory conditions to 10 liters. The facility should be able to grow the culture to the desired volume from there.

  It should be clarified here that yeasts typically die and are filtered out once they have served their purpose of converting sugar into alcohol. They are replaced in each cycle.

  Acetobacter culture on the other hand continue indefinitely. They should be reinoculated if and when undesirable strains become a problem. The introduction of undesirable strains is not an unusual problem and is usually solved in this way. This is why the innoculation of the pure culture of Acetobacter aceti phase of the trial was very important and its failure disappointing.

  Acetobacter are hardy organisms. That said they are prone to infection by undesirable strains like the Acetobacter xylinum, a cellulose producing strain. The production of cellulose reduces the amount of acetic acid produced, clog machinery and creates cometic issues that hurt sales of the product. It is therefore important to be able to remedy this whenever it becomes a problem. Re-inoculation is a straight forward solution.
- It will be necessary to set up an arrangement with a third party to maintain pure cultures of Acetobacter aceti for re-inoculation . These cultures can be isolated from local sources and maintained by someone with experience in close care and monitoring of micro-organisms.

- Measuring acetic acid in vinegar is critical for production and final sales. Without this the producer is likely to lose money or even go out of business. The producer is, in the final sales analysis, in the business of selling acetic acid. If the quantity of acetic acid is not accurately known, the producer can not know the value of the production. Just as important, the efficient operation of the plant is not possible without ongoing knowledge of the amount of acetic acid being produced.This comes from careful monitoring of the amount of acetic acid produced.

  The usual method of measurement is titration. This method is quite tricky and requires doing the test a number of times and then averaging the results. Use of an accurate ph meter to replace phenolphthalein colorant used in this test can streamline the process and improve accuracy. The challenge is to identify someone at each plant who will be fanatical about these measurements. Lacking that, it has been the consultant's experience that the results are unreliable.

  At any rate the industry could benefit from a third party testing relationship to verify their final results before sending product to market.

D. Recommendations:

a. Optimal growth conditions for Acetobacter is between 25 and 30 degrees Celsius in a pH that ranging from 5.4 to 6.3. While prefer they alcohol, they can also metabolize sugar. An obligate aerobic organism, this organism requires oxygen to carry out its life processes. The role of the producer is to manipulate the Acetobacter's environment to the producers production requirements.

   Using oxygen availability, temperature and substrate control the rate of vinegar can be manipulates. The control of available of "free oxygen" is the most usual, direct and effective mechanism of production control. The more oxygen the faster the rate of production. The down side of the fast production is things can go wrong really fast as well. If producers have not mastered slow production, it is difficult to see the adaption of faster methods will be advantageous.

   That said, eventually producers will need to adapt those faster

production methods in order to compete in commodity market vinegar sales. This is typically a low margin high volume business secured only by good and tight customer relations. Markets won on price are often lost on price in the race to the price/profit bottom.

b. Unlike the specialized yeast the needed, Acetobacter can quite likely be cultured, grown and maintained from Acetobacter cultured locally. At the time of designing these trials no such person or business was known so cultures had to be brought in order to perform the trials. If someone in Lebanon could provide such a service, it could create a new business that could have far reaching implications for the agricultural community at large. This service could be combined with testing services alluded to above as well as a consultancy for other food products. In this way it should be a sustainable business model.

c. Since the trials were trying to mimic conditions close to the actual conditions, the trials need to be repeated with close attention paid to the temperature.

d. It is recommended that the trials be repeated under the direction of Dr. Mireille Kallassy. The consultant could be available to her if required. However the consultant believes she is more than capable of setting up and providing valuable feedback to the partners.

E. Next steps:

a. Investigations into the cause of the occasional undesirable odors should be investigated to ascertain their origin.

b. Since the trials were trying to mimic conditions close to the actual conditions, the trials need to be repeated with close attention paid to the temperature.

c. Under the supervision of Dr Kallasy :

- Acetobacter trials needs to be repeated using the live culture under optimal growth temperature to demonstrate the minimal formation of Acetobacter xylinum
- Inoculation and locally isolated Acetobacter to be used in trial versus the ATCC acetobacter in order to determine if the locally isolated Acetbacter will give the same result and will be easier for Lebanese processor to obtain and replicate.
- The consultant believes that Dr Kallasy is more than capable of setting up and providing valuable feedback to the partners especially on the local Acetobacter isolation. The consultant could be available to her if required.

At the conclusion of the experiments a meeting was held with Dr. Mireille Kalassy to discuss the experiments, analyze findings and explore potential collaborations between her and the partners. It was confirmed that she has the baseline expertise and resources to help partners improve their vinegar.

The following day a workshop was held with the participating producers to go over finding, and answer questions. The participants were attentive and had excellent questions. They had reasonably clear and aggressive plans for expansion.

The PowerPoint presentations done by Dr, Kallassy and the consultant are attached in the addenda.

A number of LIVCD staff and university professionals participated in the trials. Their reports are also attached. I found this team to be unusually competent and attentive to details. They made the work unexpectedly smooth despite the obvious obstacles that lay all about. They were creative. They were enthusiastic. They asked questions, took notes and engaged thoroughly in their work. Most of all they cared. My gratitude to them