Influence of Yeast Strains in the Production of Quality Tropical Fruit Wines

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INTRODUCTION

- Wine making is one of the first biotechnological products known to man yet still considered as product of all seasons.
- The interest in making fruit wines is fun and have not faded, instead there still growing interest from many persons who have fallen under the spell of this fascinating art.

Today's presentation highlights:

- Protocols in the production of quality fruit wines
- Types of yeast and their functions
- Stages of fruit wine starter culture developments
- Comparison of different types of wine yeast starter
- Development of local active dry yeast wines starter
- Advantages of active dry yeast starter culture
- Mixed pure culture of *S. cerevisiae* for wine production
- Innovator: Adoptor: Policymaker: Regulator Partnership

what is WINE?

- Wine refers to the alcoholic beverage produced by naturally fermenting ripe grape juice in the early days.
- Today, fruit juices as well as juices from vegetable portions or sap of plants are also made into wines and differentiated from grape wine by specifying the fruits/raw materials from which they are manufactured.
- Wines from tropical fruits have varied characters, flavors, aromas and colors that are contributed by the kind of fruit, the method of manufacture and most importantly the wine yeast strains.

Numerous researches by workers from different part of the world contributed to the progressive changes within the wine industry that led to the development of new techniques, consistent quality and distinctive characters of the wines.

PROTOCOLS IN THE PRODUCTION OF QUALITY FRUIT WINE

- Selection of right equipment
- Preparation of the selected yeast strain
- Extraction and adjustments of fruit juices for fermentation
- Management of fermentation process
- Harvesting, racking, aging, filtration, fining and bottling:
- Quality assurance/control of the wine

TYPES OF YEAST AND THEIR FUNCTIONS

Yeast that belongs to *Saccharomyces cerevisiae* Hansen consists of several strains with varying technological behavior. They are generally considered fermentative organisms that produce alcohol and carbon dioxide from sugars thus playing important role in the production of various types of wines, bread, beer, distilled spirits and industrial ethanol for biofuel.

Industrially important strains of *Saccharomyces cerevisiae* belong to three main groups:

1) production of alcoholic beverages, distillery and industrial ethanol using wide range of sugar-containing material and fruit extracts;
2) production of bakery products with main function is to leaven the dough; and
3) manufacture of beer.

TYPES OF YEAST AND THEIR FUNCTIONS

Baker's Yeast:

The function of yeast in bread-making is four-fold:

a. **Increase dough volume by the evolution of gas during fermentation of the available carbohydrates in the flour;**
b. **Develop structure and texture in the dough by the stretching effect of the expansion due to gas production;**
c. **Impart a distinctive flavor; and**
d. **Enhance he nutritive value of the bread.**

TYPES OF YEAST AND THEIR FUNCTIONS

Brewer's yeast: **Produce alcoholic beverage with limited amount of ethanol (8%), taste like beer hence with bitterness and off-flavors. Brewer's yeast is classified into two main groups according to their behavior at the end of the fermentation process. The bottom-fermenting used to produce lager type beer and the top-fermenting for the production of ale beer.**

TYPES OF YEAST AND THEIR FUNCTIONS

Distillery Yeast: Special type of yeast strain use in the preparation of potable spirits (rum, whiskey, gin, etc) and for the production of industrial ethanol for bio-ethanol fuel and other applications. The strains should have sugar, temperature and alcohol tolerance due to the complexity of the raw materials, fermentation, and maturation process.

Wine Yeast: Strains of wine yeast varied in their metabolic activities defending on the type of fruit subjected to alcoholic fermentation. They are more subjected to technological innovation thus creates fresh demands on the strains of yeast employed.

SELECTION OF *Saccharomyces cerevisiae* STRAIN FOR WINE PRODUCTION

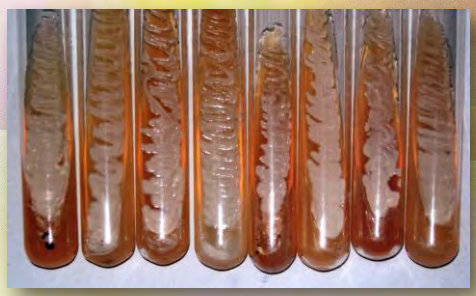
Sources of Yeast Strains:

The yeast *Saccharomyces cerevisiae* was isolated from the environment and fermenting substrates such as fruits, vegetables, rice, traditional alcoholic beverages and starter cultures and others sources over the span of more than 2 decades.

SELECTION OF *Saccharomyces cerevisiae* STRAIN FOR WINE PRODUCTION

Characterization of Yeast Strains:

Exhaustive characterization studies on several wine yeasts yielded information that the isolates have morphological, cultural, physiological genetic distinctions between them thus considered as strains.



Selected yeast strains for fruit wine preparation

In the development of wines from 24 tropical fruits, we noted that the strains differed in metabolic behavior and in the composition of the wines produced by them from different types of fruit. Due to differences in chemical composition of tropical fruits, specific yeast strain or combination of yeast strains must be used in order to produce prize-winning wines.

- More than one yeast strain are needed to bring balance of ethanol, acid, sugar, flavor compounds and impart the fruits' own character in wine.
- For instance, yeast strains that produce excellent balanced character in bignay, lipote and duhat wines differed from the yeast strains good for pineapple, passionfruit, guayabano, langka, papaya and others.
- The high polyphenol-containing fruits require yeast strains that are resistant to tannin while those with high acid content needs acid resistant strains.
- Strains variability were noted in the formation of fixed acids, esters, higher alcohols, hydrogen sulfide, glycerol, pyruvic acid and 2,3-butyolene glycol.
- A strain produced more volatile acidity and others produced an extra exceptionally adherent deposit which resulted in brilliantly clear wines.
- Still other strains have faster onset of fermentation, color stability of the wine and improved flavor formation.

Criteria in Selection of Wine Yeast Strain

Traditionally, selection of wine yeast strain is done using morphological, physiological or biochemical means of yeast characterization. However, these methods are not often able to differentiate between strains of the same species.

Wine yeast, *S. cerevisiae* consists of different types or strains represents varied metabolic activities. Therefore, it is important to have complete understanding of the metabolic mechanisms of the biochemical pathways of catabolism of sugars to ethanol and carbon dioxide.

Good wine yeast is one which will impart a vinous or fruit-like flavor, will ferment sugar to a low content producing 14-18% ethanol, resistant to the antiseptic compounds normally added to the fruit "must" and is characterized by remaining in suspension during fermentation and then agglomerating to yield coarse granular sediment that settles quickly and is not easily disturbed in racking.

Technological Behavior of Wine Yeast Strain

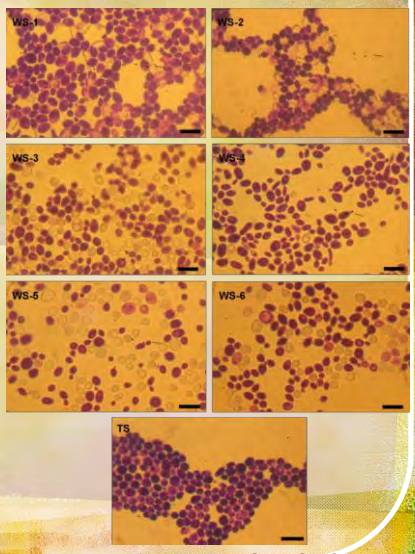
1. ETHANOL TOLERANCE: The yield of ethanol varies with strain of yeast, its adaptability to ethanol and phase of growth when it is added to the fermenting mixture.

- The maximum ethanol concentration that can be produced by yeasts varies from 0 to about 19% (v/v). Those strains that tolerate high concentrations of ethanol store less lipid and carbohydrate compared with less tolerant strain.

The molecular mechanisms of ethanol tolerance have been continuously sought by identifying the genes involved in yeast response to alcohol stress. It has been reported that 250 genes have been implicated in ethanol tolerance, suggesting that the trait is under polygenic control (Hu, et al, 2007).

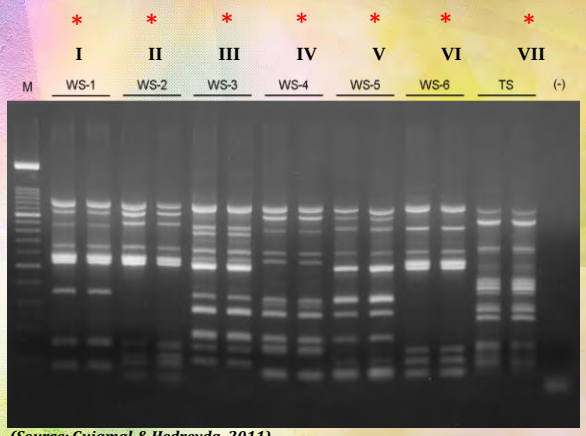
Technological Behavior of Wine Yeast Strain

- Application of modern DNA–based techniques on local six local wine strains of *S. cerevisiae* obtained strain-specific markers for ethanol tolerance (Guiamal & Hedreyda, 2011).
- Using microsatellite typing and RAPD PCR, DNA profiles were generated to distinguish the ethanol tolerance of 6 local wine strains from one another and from the control non-wine strain.



(Source: Guiamal & Hedreyda, 2011).

Technological Behavior of Wine Yeast Strain



(Source: Guiamal & Hedreyda, 2011).

The genetic markers provide an accurate means of strain characterization to ensure the use of correct strains of yeast in the production process and ultimately ensure consistency in the quality of the final product (Espinar, et al., 1999)

2. HIGH TEMPERATURE TOLERANCE

The temperature during fermentation has a direct effect on the yeast activity. The optimum for growth and multiplication of ordinary yeast is 30°C and usually inhibited at temperature higher than 32°C. Some strains of yeast are capable to maintain its metabolic activity when temperature increased (>40°C) during fermentation.

3. HIGH SUGAR CONCENTRATION TOLERANCE

As a principle, when sugar concentration is raised, the rate of fermentation and the maximum amount of ethanol produced decreased. However, special type of yeast strains can be selected and conditioned to grow at higher sugar concentration (>30% sugar).

4. ACID TOLERANCE

Many fruits contain high concentration of acids so that correction of the acid content during the formulation stage is important. This is normally done by diluting the fruit juice or adding calcium carbonate to have a fermentation mixture with about pH 3.5-4.5. In this type of substrate, acid tolerance strain must be used as fermenting yeast.

5. PRODUCTION OF DESIRABLE FLAVOR COMPOUNDS

Desirable qualities conferred by a yeast are attributable to the ability of the strain or mixture of strains of *S. cerevisieae* to excrete in sufficient quantity certain organic compounds that affects the taste, bouquet and smell of the alcoholic beverage or spirit.

WHAT IS STARTER CULTURE OF INOCULUM?

Starter culture is the major key factor in successful fermentation process. The starter culture must be active and made from pure culture of yeasts that were selected for specific type of substrate/fruit.

STAGES IN THE DEVELOPMENTS:

Period 1: In 1878 started the use of starter culture in wine making where sound grapes were crushed several days before the main harvest and the juice was allowed to ferment naturally in a warm room. Bulk starter culture was prepared by increment transfers of the fermented juice until desired volume of starter culture was achieved.

Period 2: In 1880 to 1890, the development of isolating microorganisms and the use of pure culture inoculation, pave way to discontinue the use of chance inoculation.This led to the first distribution of pure yeast cultures (in agar slant) for wine making.

Pure culture inoculation generally yields more consistent results. Some wine makers use a mixture of pure cultures to give the wine a distinctive bouquet. An important reason for the use of pure cultures is to produce wine with most desirable flavors and odors.

Period 3: The biochemical pathways of breakdown of sugars by yeast were elucidated and ecology of yeasts (natural microflora), their natural distribution and nature of the fermentation were studied providing information of the role of mixed yeast strains in flavor formation inherent in specific fruit.

Period 4: The technology of production of active wine yeast pressed-cake using single pure culture strain was developed. Due to the low stability and fast lost of viability of the wine yeast pressed-cake pure, the active dry yeast technology was developed also using single wine yeast strain that is specific for the type of wine to be produced.

COMPARISON OF THE DIFFERENT TYPES OF WINE YEAST STARTER CULTURE

Agar Slant Culture: Generally, the yeast culture is supplied on agar slants, and the yeast should be propagated in sterile medium by the wine-maker. The cost of aseptic facilities, agar medium needed for propagation, highly trained person in handling the culture and the need for constant renewal to maintain its viability are only some of the disadvantages of using yeast in agar slant culture.

COMPARISON OF THE DIFFERENT TYPES OF WINE YEAST STARTER CULTURE

Liquid Culture: Handling of liquid yeast culture is a problem of wine makers. Like the agar slant culture, it needs also trained personnel to mass produce the required amount needed for efficient fermentation. Usually 1-3% by volume of a liquid starter is required. It is also of the concern of the wine maker to maintain the culture in active stage of growth when required hence it is a tedious and expensive process.

Compressed cake-type: Frozen yeast starters, as moist cakes (compressed) or powders/granules have largely replaced liquid starters. Objection to liquid or agar slant yeast cultures is the time taken to prepare them during the busy fruiting season and the great chance of contamination in handing. It will require trained technician to handle the pure culture of yeast.

COMPARISON OF THE DIFFERENT TYPES OF WINE YEAST STARTER CULTURE

Active Dried Yeast Pellets/Powder: Active yeast in dry form is an innovation that will limit the use of yeast propagation as well as yeast recycling thus avoiding contamination with wild yeasts and bacteria and achieving the highest efficiency of sugar to ethanol conversion. Yeast strains varied in their resistance to desiccation. Optimal drying temperature range was 25° to 35°C.

DEVELOPMENT OF LOCAL ACTIVE DRY YEAST STARTER CONTAINING COMBINATION OF STRAINS OF *Saccharomyces cerevisiae*

- Development of biological catalyst or starter culture in active dry form was the accomplishment of the project supported by the Department of Agriculture Biotechnology Program.
- The project developed biological catalysts (termed BIO-NOL) containing selected distillery strains of *Saccharomyces cerevisisae* for the industrial production of ethanol from sugarcane, sweet sorghum and cassava for biofuel application.

DEVELOPMENT OF LOCAL ACTIVE DRY YEAST STARTER CONTAINING COMBINATION OF STRAINS OF *Saccharomyces cerevisiae*

FOUR TYPES OF BIO-NOL CATALYSTS SPECIFIC FOR SUGARCANE JUICE, SWEET SORGHUM STALK JUICE, SORGHUM GRAINS FLOUR AND CASSAVA FLOUR.

ADVANTAGES OF ACTIVE DRY YEAST STARTER CULTURE

1. **Avoid contamination during propagation.**
2. **Higher efficiency of conversion of sugars to ethanol.**
3. **Storage of yeast in the dry state is also convenient.**
4. **Ease in controlling yeast count**
   - The number of viable yeast cells is approximately constant for a given weight. The wine maker thus can easily control the amount of yeast.
   - Too much yeast should not be used since it will accelerate the rate of fermentation thus forming much heat or energy that will be harmful to the yeast cells.
   - Ideally, 10^6 cells/ml must be present in the mixture to have normal fermentation rate. Lower cell count slows down fermentation while higher cell count will encourage formation of by-products affecting the quality of wine.
5. **Stability of dried yeast starter culture:** Unstable yeast strains have tendency to lose viability after several transfers and used. The ability to produce in low concentration one of more compounds that contribute to the flavor of wine or spirit is also loss

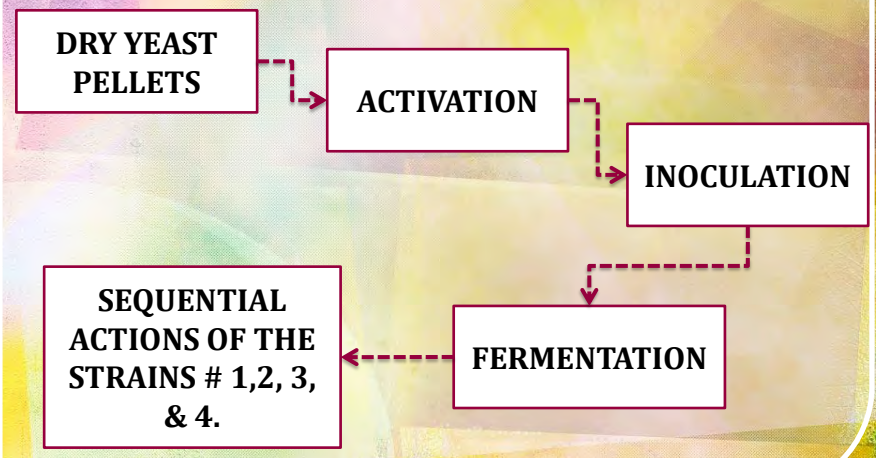
MIXED PURE CULTURE STRAINS OF *S. cerevisiae* FOR WINE PRODUCTION

Under natural conditions, alcoholic fermentation of various substrates (coconut sap, rice, sugarcane, etc.) proceeded through succession of organisms. One strain creates favorable environment for next strain to complete the fermentation process. Therefore, the use of right strains of wine yeast strains in proper proportion is beneficial.

Sequential or parallel fermentation occurs where a strain of wine yeast starts the fermentation of sugar in the substrate until it reached its ethanol tolerant limit (normally 8 to 10%,v/v) then slows down its activity due to the inhibitory effect of ethanol.

Another special type of wine yeast strain contained in the starter culture will continue converting the remaining sugar at higher concentration of ethanol since presence of 8-10%v/v is favorable for their active growth. Lower than 8% ethanol, this special wine yeast do not function efficiently in conversion of sugar to ethanol.

Sequential mode of action of *S. cerevisiae* strains is illustrated as follows:



Sequential mode of action of *S. cerevisiae* strains is illustrated as follows:

Strain 1 (Osmophilic) --→ As the sugar concentration is raised, the rate of fermentation and the maximum amount of alcohol produced decreases, depending on the presence of osmotolerant yeast strains. This special yeast strain convert the initial high sugar concentration (>25°Brix) in the fermenting must to 8-10%,v/v ethanol; then weaken and die. Ethanol tolerant yeast continue the fermentation process.

Strain 2 (Ethanol tolerant) --→ Ethanol tolerant yeast strain only become active in the presence of 8-10%v/v ethanol in the fermenting mixture. Once active, the ethanol tolerant strain start the conversion of residual sugars to produce ethanol up to 15-18%v/v.

Sequential mode of action of *S. cerevisiae* strains is illustrated as follows:

Strain 3 (thermotolerant) --→ **Fermentation process evolved energy (heat) so that temperature increases resulting to the weakening of the yeast activity. "Stuck" fermentations usually occur, depending on strain of yeast, when temperature reaches 32ºC or higher.**

Strain 4 (Flocculating) ------→ **Strains that have the tendency in the later stages of a batch fermentation to agglomerate and to form flocs which settle to the bottom of the fermentation vessel. Flocculating yeast strain achieved maximum conversion of sugar into ethanol and carbon dioxide. Also, aids in the removal of the yeast and other suspended fruit pulp making the wine easily clarified.**

ACTIVATION PROCESS FOR DRIED YEAST STARTER CULTURE

Preparation of yeast starter consisted of activating the 100 g. dried pellet in one liter of pasteurized, cooled and sweetened fruit juice, when bubbling occurred transfer it to 10 liters of newly prepared fruit mixture (fruit: water: sugar), then to 100 liters of fruit mixture. Each stage, allow 24-48-hr fermentation period.

INNOVATOR:ADOPTOR:POLICYMAKER: REGULATOR PARTNERSHIPS

The basic question we have to ask ourselves is what can we do in order that the fruit wine industry prosper in the country? Probably, harnessing the innovations in science and technology is one of the answers.

- Need to establish formal mutual relationship between the innovator and adoptor of the technology rather than just sourcing the information through interviews or other types of communication. In such system, the whole picture of the developed technology is not completely transferred thus failure normally occurs.
- It is a common practice of in developed countries like Japan to have factories that produce only starter cultures for fermentation process. The company is provided with the strict quality control systems, right equipment and expensive facilities.
- The scientist/researcher who isolated, characterized and evaluated the technological behavior of the strain have a mutual agreement with the company/adoptor to do the commercial production of the starter culture.

Examples:

1. Starter culture for yakult production was isolated, characterized and thoroughly studied by Professor Shirota. The yakult factories all over the world used same organism for yakult manufacture which is known as *Lactobacillus casei* Shirota strain. This is the reason why the taste and quality of yakult are the same even you buy it in different parts of the world.

2. Kikkoman soy sauce factory commissioned a starter culture company to mass produce their specially selected soy sauce Kikkoman strains that was isolated and studied over the years by Professor Yokotsuka. The agreement is that the starter culture is for exclusive use by Kikkoman soy sauce factory.

❖ Government guidelines, regulations, standards and laws of Japan were strictly followed by innovator and adoptors. With this arrangement, the innovator/researcher/scientist is acknowledged for the painstaking efforts for the technology innovation while the adoptor of the technology is assured of high quality product that will generate more revenues to the investment.

❖ To make this system operational in our tropical fruit wine industry it is does not only concern the innovator and adoptorbut also our policy makers and regulators have equal important responsibilities

❖ Many studies have been done and published on producing wines from tropical fruits that abounds the country.

Just to cite some:

- Prospects of Fruit Wine Production in the Philippines, Phillip. J. Crop Sci, 1979 vol. 4(4): 183-190
- Manual on Fruit wine Production in the Philippines, UPLB Publication
- 1999 Quality and acceptability of wine produced by strains of *Saccharomyces cerevisiae* Phillip. J. Crop Sci. 1999, vol. 88(2):158-165 and many other earlier publications.

❖ Does this endeavors contributed to the development of our fruit wine industry? Did our concerned government agencies contributed to the growth of this very viable industry in the form of formulating administrative orders, guidelines and others so that the product in the market will be of consistent high quality?

- If so, do our regulating agencies like Food and Drug Administration (FDA) and Department of Trade and Industry (DTI) vigilant enough to make sure that the regulations are strictly enforced by fruit wine manufacturing company? It seems that we do not pay much attention to regulating the quality and labeling of our product.

- Just to prove my point, I wish to pose these questions. Have you tasted *lambanog* made from distilled spirit from molasses or formulated or fortified fruit wines which are currently available in the country? To wine connoisseurs these fake alcoholic beverages can be easily discerned.

- Let the lessons learnt in the nata de coco industry serve as a reminder to us that strict regulation on the product quality is deem very important.

RECOMMENDATION

- We have been testing fruit wines that are locally produced and it seems that there are still lots of improvement that can be done. To be able to truly accomplish the objectives of the techno-demo activity that we are doing now, I suggest that honest-to-goodness hands-on training workshops be done by concerned government agencies and private cooperatives/foundations in collaboration with the technology innovators.

- The activity will cater on current and future fruit wine producers and focus on the new developments in fruit wine technology, like use of active dry yeast starter containing combinations of selected yeast strains.


"Wine is proof that God loves us and wants us to be happy"
Enjoy making wines as you enjoy drinking them.
Thank you