

# ALCOHOLIC FERMENTATION

## CHALLENGES:

- Nutrient deficiency (nitrogen, vitamins, minerals)
- Competition from wild yeast, bacteria
- Lack of survival factors (long-chain fatty acids, sterols)
- Ethanol
- Temperature
- Osmotic shock
- Toxins (medium-chain fatty acids, high volatile acidity, high SO<sub>2</sub>)
- Inadequate yeast population (low inoculation rate)
- Highly clarified must
- Pesticide residue

## 1. Nutrient deficiency

Nitrogen (ammonia and amino acid) deficiency.

Slows protein synthesis.

Diminishes Sugar transport activity.

Becomes limiting at <150 ppm initial level (generalization; depends on mix of amino acids and ammonia plus other growth factors).

N addition not related to kinetics at end of fermentation (may still stick w/late addition).

Above 10% alcohol, yeast will not take up N.

Ammonia N (DAP) vs. amino N (organic N).

Vitamin/mineral deficiency

Thiamin: increases viable yeast population, speeds fermentation; deficiency leads to acetic acid production.

Pantothenic acid: deficiency leads to acetic acid and H<sub>2</sub>S production, even if N is adequate. 50 mg/hL limits acetic acid in stuck fermentations.

Biotin: aids viability at end of fermentation.

Inositol: aids membrane phospholipid synthesis.

Other growth factors: pyridoxine, nicotinamide, riboflavin (found in dry yeast, inactivated yeast).

Minerals: Potassium, magnesium, phosphorus, zinc.

Oxygen

Yeast needs oxygen during growth.

Creates survival factors (sterols and fatty acids) and increases effectiveness of N addition.

**Action:**

Monitor Yeast Available Nitrogen (YAN).

Use complex nutrients after lag phase and during growth phase (1/3 fermentation).

Augment with DAP if N is very low or sugar is high.

Aeration during growth phase.

## 2. Competition (wild yeast, fungi/mold and bacteria)

Depletes nutrients

Wild yeast can deplete thiamin within 3 hours.

Botrytis may decrease amino acid content 7-61%.

**Action:**

Avoid cold soak if microbial load is high.

If cold soak is used, add 50% of the yeast to the must at beginning of the cold soak.

Use sufficient SO<sub>2</sub> to knock down wild microbes.

In whites, if microbial load is high, clarify juice (boost turbidity afterwards with cellulose powder and/or inactivated yeast).

If competition is from lactic acid bacteria, lysozyme can be used to kill them.

If end is sluggish, low SO<sub>2</sub> addition will reduce competition without hurting yeast.

Use enological tannins for antibiotic and antioxidant effects and to augment phenolic structure.

Thermal vinification, flash pasteurization.

### 3. Sterols/fatty acids (survival factors) deficiency

Sterols and long-chain unsaturated FA's maintain membrane integrity.

Enables yeast cell to expel ethanol (EtOH).

EtOH can alter membrane, allowing entry of protons, which acidify cell, causing death.

#### Action:

Use active dry yeast (most are selected for high sterol and FA content).

Rehydrate yeast with rehydration nutrient high in survival factors.

Aeration during growth phase of yeast.

Supplement at 1/3 fermentation with complete nutrient containing survival factors.

### 4. Osmotic shock (high sugar)

High sugar must creates pressure across the yeast membrane from the cell toward the must.

If pressure is high enough, yeast will die.

#### Action:

Use yeast selected to withstand osmotic stress.

Reduce sugar level by addition of water or low Brix must.

### 5. Ethanol

Alters cell membrane, allowing greater entry of protons, acidifying the cells; inhibits nutrient transport.

Toxic effects are exacerbated by high temperatures.

#### Action:

Pick grapes at a reasonable Brix level.

Use yeasts pre-conditioned for high alcohol

One can use weaker yeast at start of fermentation, adding the stronger yeast part way through the fermentation.

Build up sterols and FA's (above) to withstand the high alcohol.

Ferment at lower temperatures.

Increase yeast addition rate to accommodate potential alcohol.

### 6. Temperature

Above 90° F, puts high stress on yeast (stress increases at higher alcohol).

High temperatures (77-90° F, measured under cap) during exponential yeast growth favor acetic acid production.

Too low temperatures, yeast may be overly slow or stop fermenting and may form H<sub>2</sub>S.

#### Action:

Control temperature (~70-75° F during growth), especially in high Brix must.

The higher the potential alcohol, the lower the temperature should be.

For low temperature white fermentations, have sufficient turbidity or stirring/agitation to keep yeast in suspension.

### 7. Toxins (medium chain saturated FA's, volatile acidity, high SO<sub>2</sub>)

Medium chain FA's created during fermentation may slow or stop fermentation.

#### Action:

Fining with yeast hulls or inactivated yeast to adsorb toxins (may need to reinoculate with yeast afterwards).

VA may be from bacteria (acetic and/or lactic acid bacteria) or wild yeasts, or by certain cultured yeasts under stress.

Conditions favoring VA production:

pH < 3.1

pH > 4.0

Amino acid and vitamin deficiency.

High temperature during exponential growth of yeast.

Excessive must clarification.

**Action:**

Careful sorting in vineyard to reduce infected grapes.  
Monitoring sanitary state of grapes in vineyard and winery.  
Clarification of white juice to reduce microbial load (with some turbidity added back).  
SO<sub>2</sub> addition as soon as possible.  
Use lysozyme if problem is from lactic acid bacteria.  
Small amounts of tainted wine can be incorporated into fresh must and the yeast will metabolize the VA.  
Reduce other stresses on the yeast (nutrition, temperature, etc.)

High SO<sub>2</sub> may slow fermentation or kill yeasts.

In general, most cultivated yeasts can withstand moderately high SO<sub>2</sub>.

Higher than 50 ppm SO<sub>2</sub> can deactivate thiamin.

**Action:**

Oxidation of must to reduce free SO<sub>2</sub> (not recommended).  
Dilution with unsulfited juice (recommended).

**8. Inadequate yeast population**

Starting population should be  $\geq 10^6$  cells per mL.

Recommendations of 20-30 g/hL (200-300 ppm) of active dry yeast are based on average European wines.

**Action:**

Increase inoculation rate to offset other stresses, particularly high potential alcohol.

**9. Highly clarified must**

Highly clarifying must can strip it of survival factors; may increase acetic acid.

Makes it harder to keep yeast in suspension.

**Action:**

Utilize rehydration nutrients and complex nutrients with survival factors.  
Add cellulose powder, or cellulose and inactivated yeast.  
Pre-fermentation, pre-settling skin contact can increase fermentability (with clean grapes)

**10. Pesticide Residue**

Certain chemicals, especially fungicides, applied too close to harvest, can interfere with yeast metabolism.

**Action:**

Apply pesticides to grapes only according to directions and extension recommendations.  
Some residues can be lowered by fining with inactivated yeast or yeast hulls.

**GENERAL RECOMMENDATIONS FOR STUCK AND SLUGGISH FERMENTATIONS:**

Determine the source of the problem.

Test glucose/fructose, malic acid, volatile acidity, micro.

Stuck Fermentations (follow restart protocols: supplier websites, Scott Handbook, etc.):

Reds: Press must (helps lower bacteria population).

Light sulfiting to knock down competing microbes.

Fine with yeast hulls or inactivated yeast to remove toxins (20-30 g/hL).

Reinoculation with strong yeast at higher than normal levels per protocols.

Use rehydration nutrients and keep fermenting wine at 20-25° C.

Whites: Light sulfiting (20-30 ppm) when MLF is not desired.

Fine with yeast hulls or inactivated yeast.

Reinoculation with strong yeast at higher than normal levels per protocols.

Pro-Restart (alginate encapsulated yeast): pre-acclimated to alcoholic environment.

Avoid serial inoculations:

After 5 generations, survival factors can be too depleted to complete fermentation.

# LAG PHASE OF VARIOUS YEASTS

AMH- Long lag phase  
BC- Long  
BDX- Short  
BM45- Long  
BRL97-Short  
Clos-Short

D21-Very short  
D47-Very short  
D80-Very short  
D254-Very short  
EC118- Very short  
GRE- Short

QA23- Medium  
Rhone 4600- Short  
R-HST- Short  
RC212- Medium  
RP15-Short  
Syrah- Very Short

## TEMPERATURE, YAN, DOSAGE

Yeast dose and maximum temperature/Brix level/YAN

<u>Brix</u>	<u>Yeast Dosage</u>	<u>Max. Temp.</u>	<u>YAN (Butzke)</u>
20-21	2.0-2.5 lb./1000 gal.	90 F	200
22	2.5-3.0 lb./1000 gal.	85 F	
23	2.5-3.0 lb./1000 gal.	80 F	250
24	2.5-3.0 lb./1000 gal.	75 F	
25+	3.0 lb./1000 gal.	70 F	300-350
35-50	4.0+ lb./1000 gal.	65 F	

## FERMENTATION PHASES

Lag Phase:

Up until first signs of fermentation, usually within 24 hours of inoculation.

Growth Phase:

Increasing yeast biomass; EtOH production starts during late growth.

Stationary Phase:

Starts at approximately 5% alcohol; if problems start, still possible to revive cells and move back to growth phase.

Death Phase:

Only 25% vital cells; controlled degradation of yeast. Approximately last half of fermentation.