

LYSOVIN

WINE

LYSOVIN

Application of Lysozyme in winemaking



Overview

Fermentation

Wine Spoilage

SO₂ Comparison

Advantages

Usage

Activity

Certifications

LYSOVIN

KEY INGREDIENT: LYSOZYME¹
NATURAL ANTIMICROBIAL
MOST ABUNDANT IN HEN EGG WHITE

LYSOVIN ALLOWS:

Management of LAB

Reduction of volatile acidity

Reduction of SO₂ dosage

ACHIEVE CLEAN FERMENTATION

Manage Sluggish Fermentation

Manage Stuck Fermentation

Achieve Post Malolactic Fermentation MLF Stabilization

1. Found in milk, mammalian secretions, oysters, plants, etc.

OVERVIEW

In the wine industry, **LYSOVIN** is used to prevent spoilage through the growth of lactic acid bacteria such as: (e.g., *Lactobacillus* spp. and *Pediococcus* spp.), which are associated with sluggish/stuck fermentations, increases in volatile acids and the production of undesirable compounds such as biogenic amines, most notably histamine. Bioseutica's **LYSOVIN** can also control the onset of **malolactic fermentation (MLF)** and can contribute to the microbiological stability of wines, once alcoholic fermentation and/or MLF are completed.

LYSOVIN is a natural and flexible ingredient which allows winemakers to achieve different quality-enhancing objectives, depending on the dosage and the timing of its addition. In 1995, following tests approved by both the Italian and French authorities, Bioseutica obtained the final authorization from the **TTB (Alcohol and Tobacco Tax and Trade Bureau)** to utilize **LYSOVIN** in winemaking. Bioseutica's efforts also led to the filing of an international patent claiming, among other things, that the use of different types of **LYSOVIN** salts prevents bacterial contamination of wine and enables winemaking without the use of sulfites. Over ten years of experience in the wine industry has proven **LYSOVIN** to be effective not only as a cure but also as a preventative during the winemaking process.



OVERVIEW

- **LYSOVIN** helps to maintain clean fermentation and to avoid sluggish or stuck fermentation without SO₂ addition;
- **LYSOVIN** prevents production of undesirable compounds in wine¹;
- **LYSOVIN** can control the onset of malolactic fermentation (MLF) and ensures the microbiological stability of wines²;
- **LYSOVIN** works effectively at higher pH levels where SO₂ is no longer effective;
- **LYSOVIN** has a proven track record in the wine industry as approved food grade ingredient.

1. Such as volatile acids and biogenic amines, most notably histamine.

2. Once alcoholic fermentation and/or MLF are completed.



LYSOVIN



LIQUID

OR



GRANULAR

The lysozyme component applied in both products has a high purity of at least 95%, Lysozyme is a natural ingredient, extracted from hen egg white



JECFA

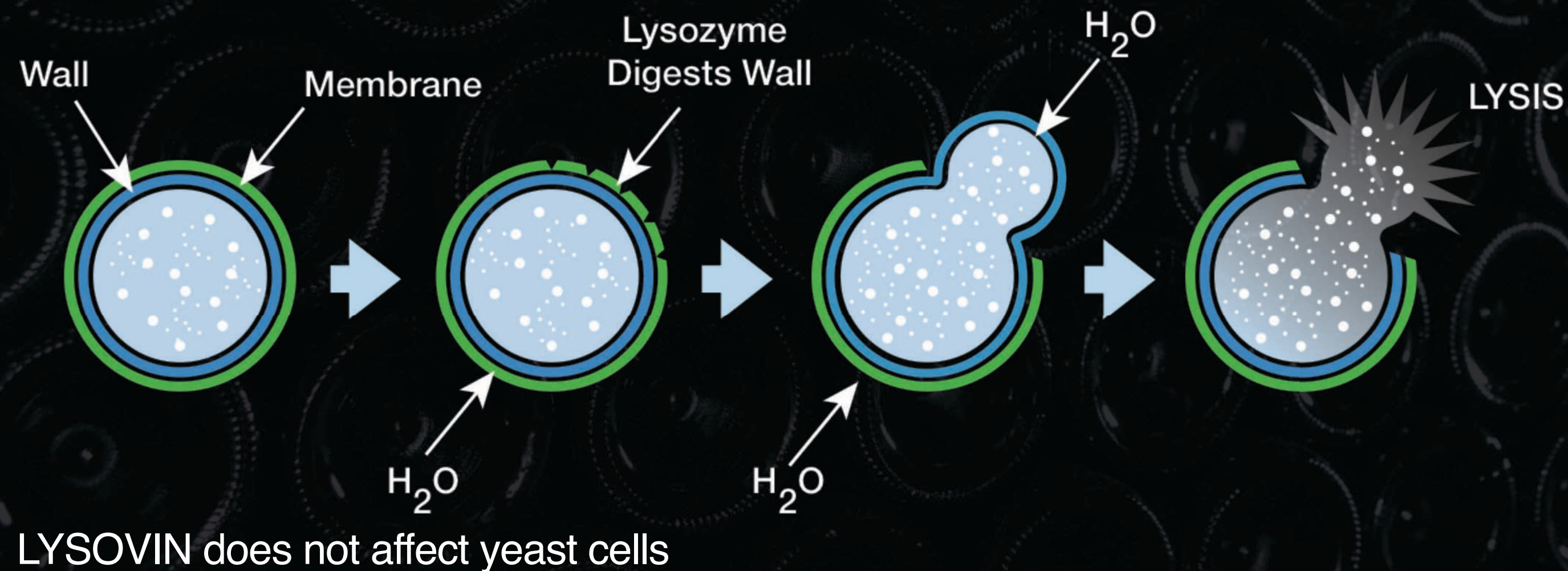


LYSOVIN is: Certified GRAS by the FDA; Approved for use in Organic foods; Certified Kosher; Accepted Whole Foods ingredient; Classified as food by JEFCA-Joint FAO/WHO expert Committee on Food Additives; Produced under FSSC22000 certification;



LYSOVIN ENZYMATIC FUNCTION

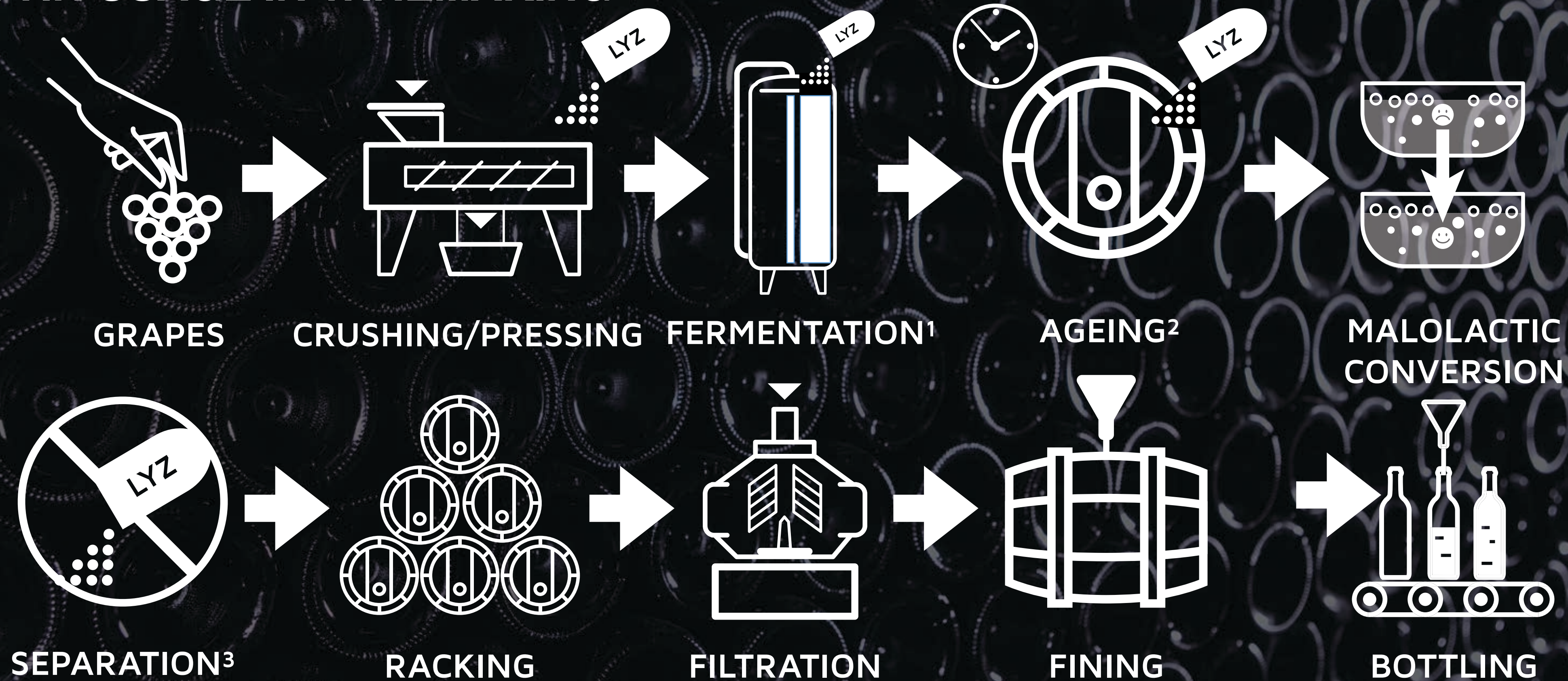
LYSOVIN kills unwanted lactic acid bacteria (LAB) in wine such as: *Lactobacillus* spp. and *Pediococcus* spp. The active ingredient in LYSOVIN is Lysozyme, a natural enzyme extracted from hen egg white. Lysozyme hydrolyses the peptidoglycan layer in the bacterial cell wall.



LYSOZYME IN HEN EGG WHITE

- 1 egg = 60 g (liquid)
- Egg white = 34 g (liquid)
- Egg white solids = 3.4 g
- Egg white solids = 3.4% lysozyme
- Lysozyme = 3.4g x 3.4% = 0.11 g/egg
- 1 kg lysozyme: 9,100 eggs

LYSOVIN USAGE IN WINEMAKING

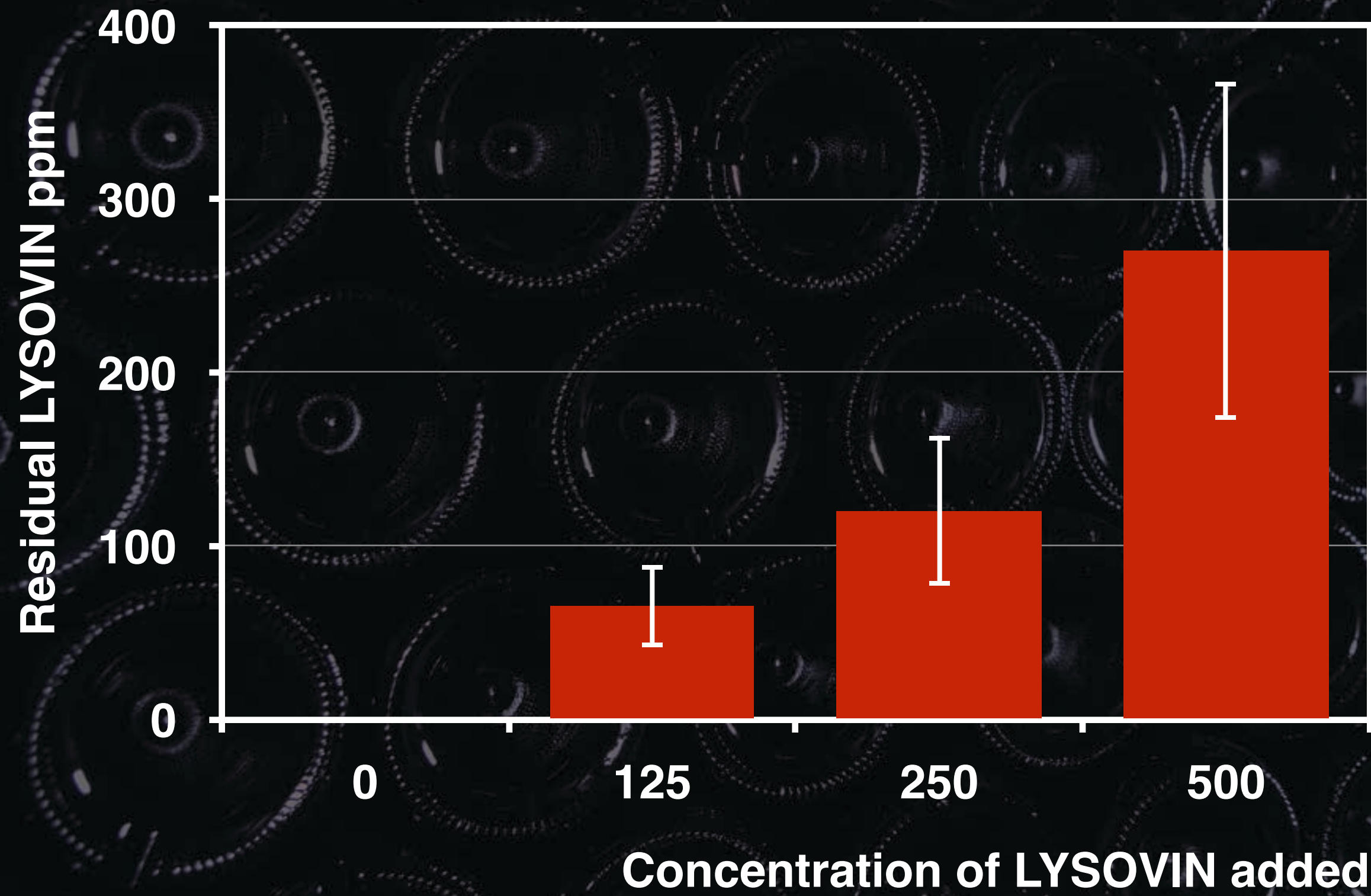


1. Alcoholic fermentation - Sugar + Yeast >> Alcohol + CO2. First addition of LYSOVIN to prevent stuck/sluggish fermentation
 2. LYSOVIN to control MLF and prevent spoilage
 3. LYSOVIN is removed

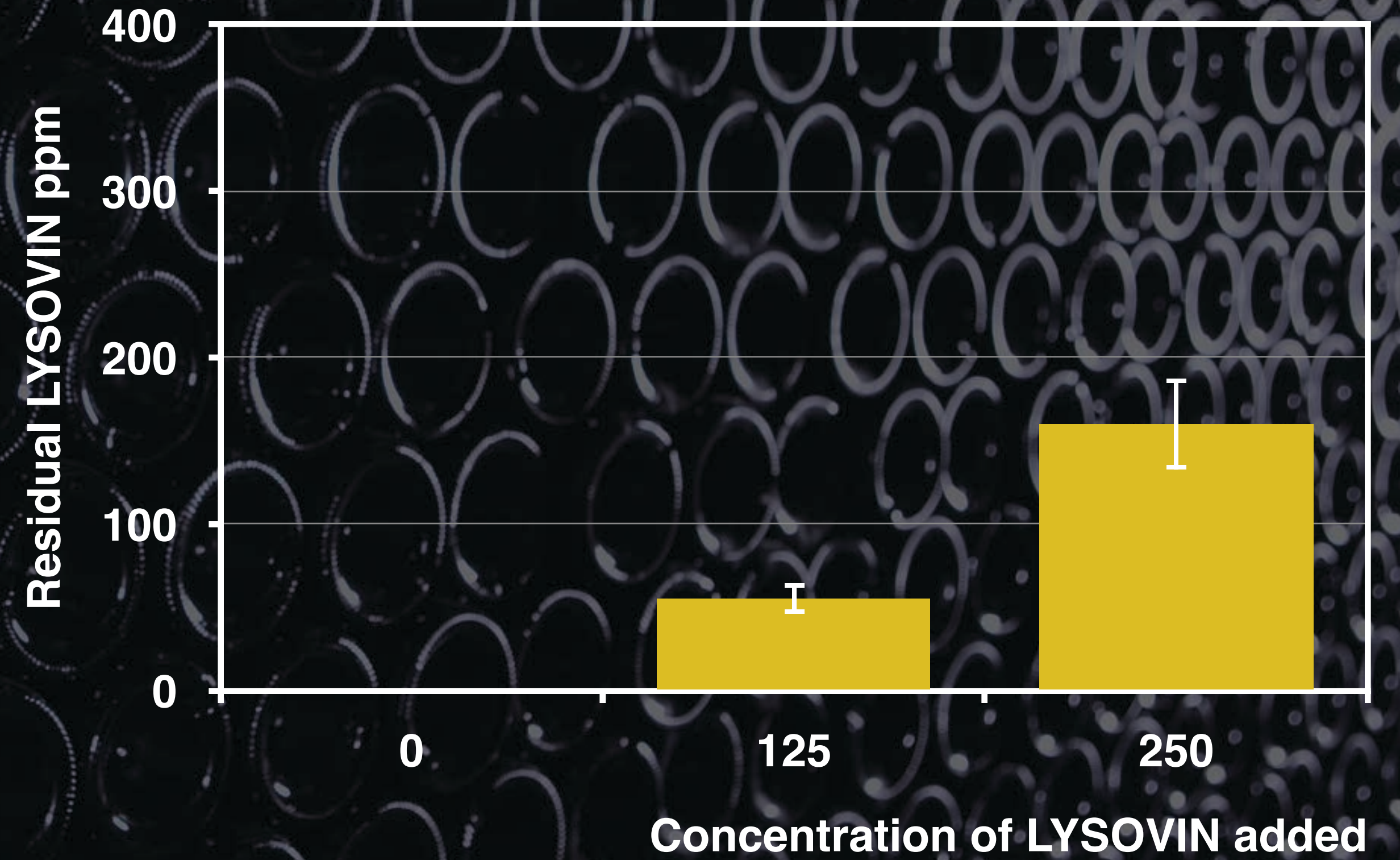


RESIDUAL LYSOVIN IN RED AND WHITE WINES

Red Wines



White Wines



WINE SPOILAGE PROBLEM CAUSED BY LAB LACTIC ACID BACTERIA

- **HIGH CONCENTRATION OF ACETIC ACID**

High volatile acidity
Unmarketable wine
Stuck/sluggish fermentation

- **BIOGENIC AMINES**

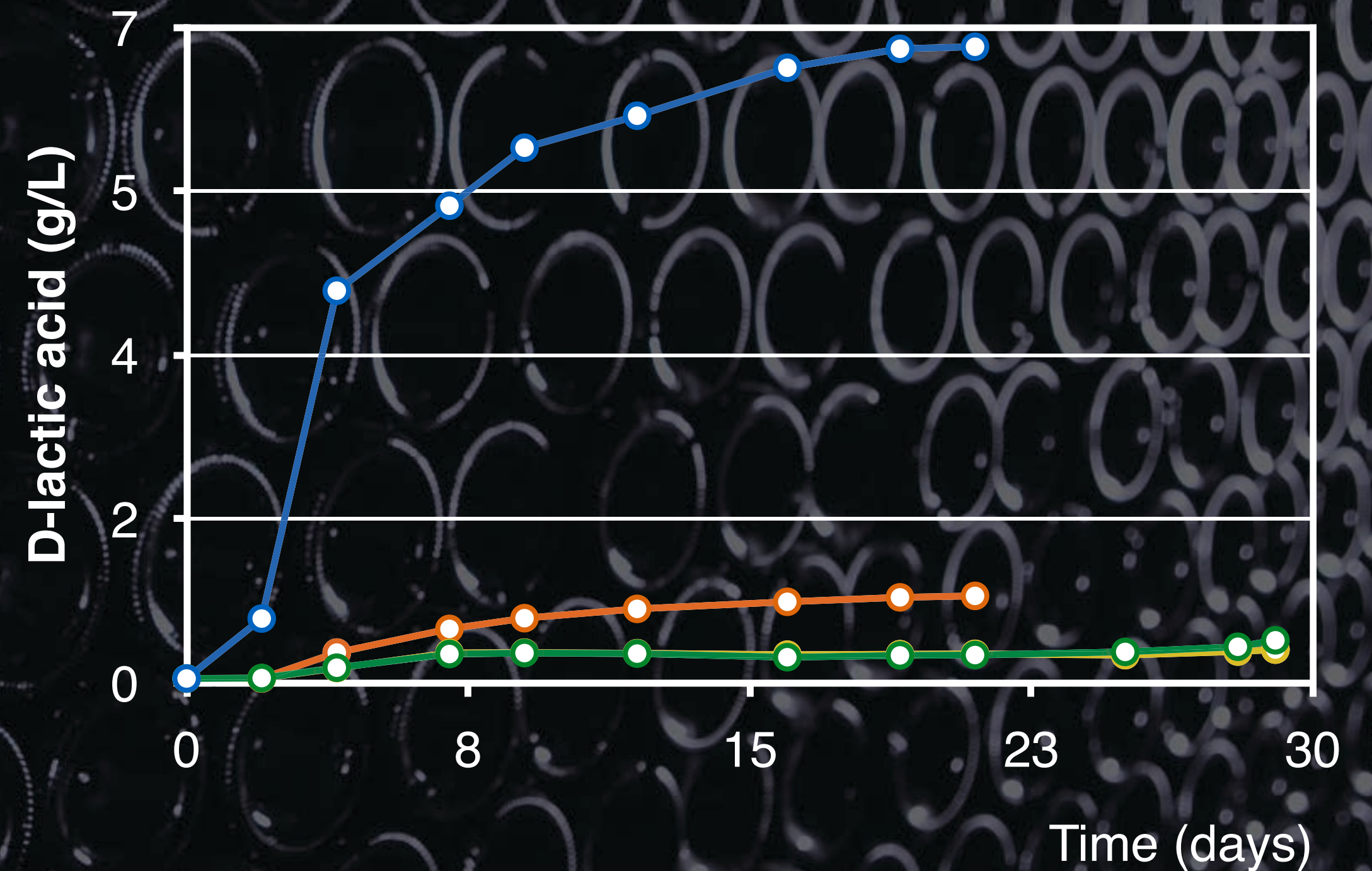
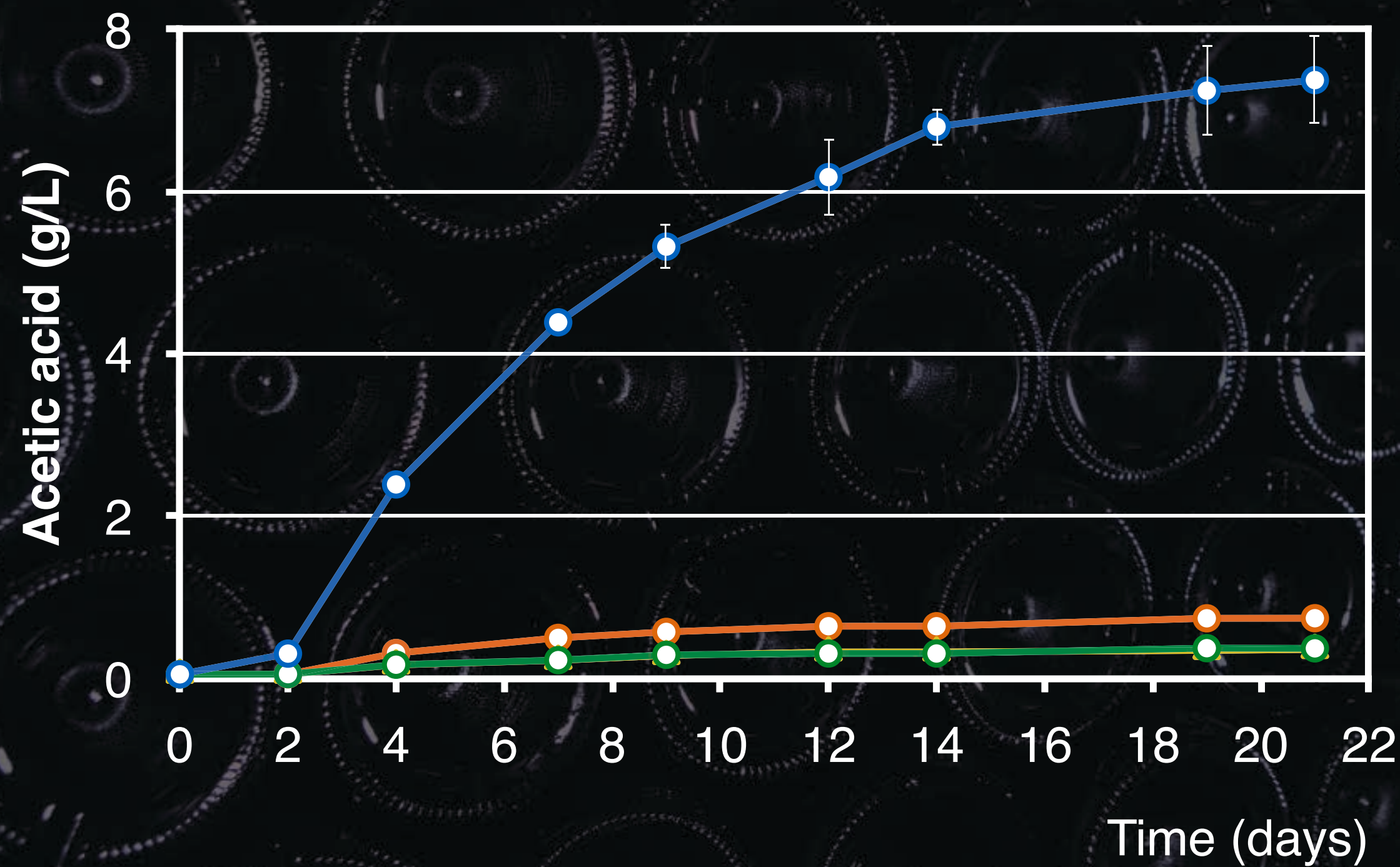
Intolerance for some consumer
Max. of 10 ppm imposed by some countries

- **POLYSACCHARIDES: ROPINESS** (HIGH VISCOSITY)

Acrolein: bitterness
Diacetyl: buttery flavor

LAB IMPACT ON D-LACTIC AND ACETIC ACID

In addition to the production of acetic acid and converting the L-malic acid in the grape juice to L-lactic acid, some LAB also consume sugar and produce D-lactic acid.

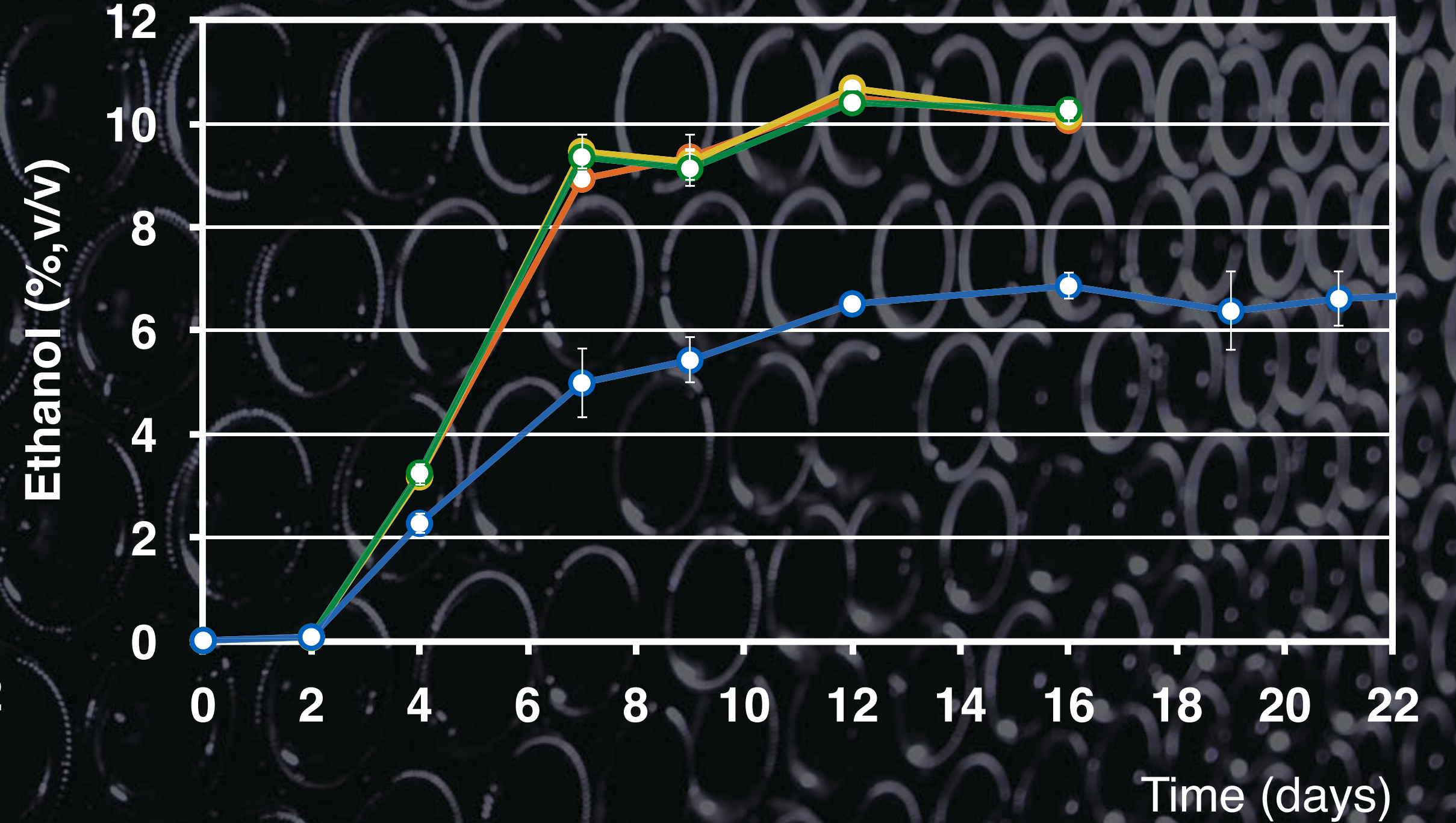
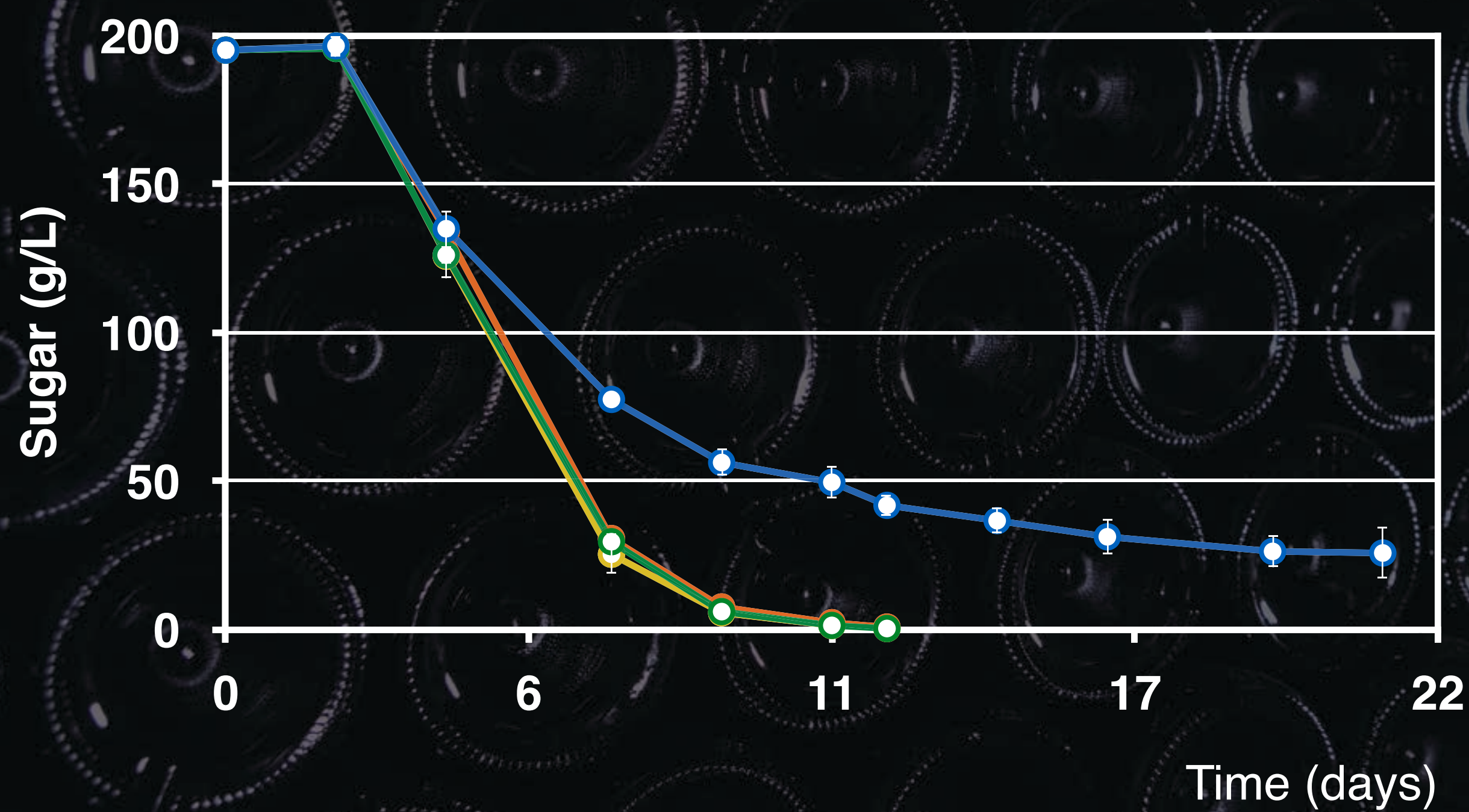


● Control ● 125 ppm Lysovin ▲ 250 ppm Lysovin ● 50 ppm SO2



LAB IMPACT ON SUGAR AND ETHANOL

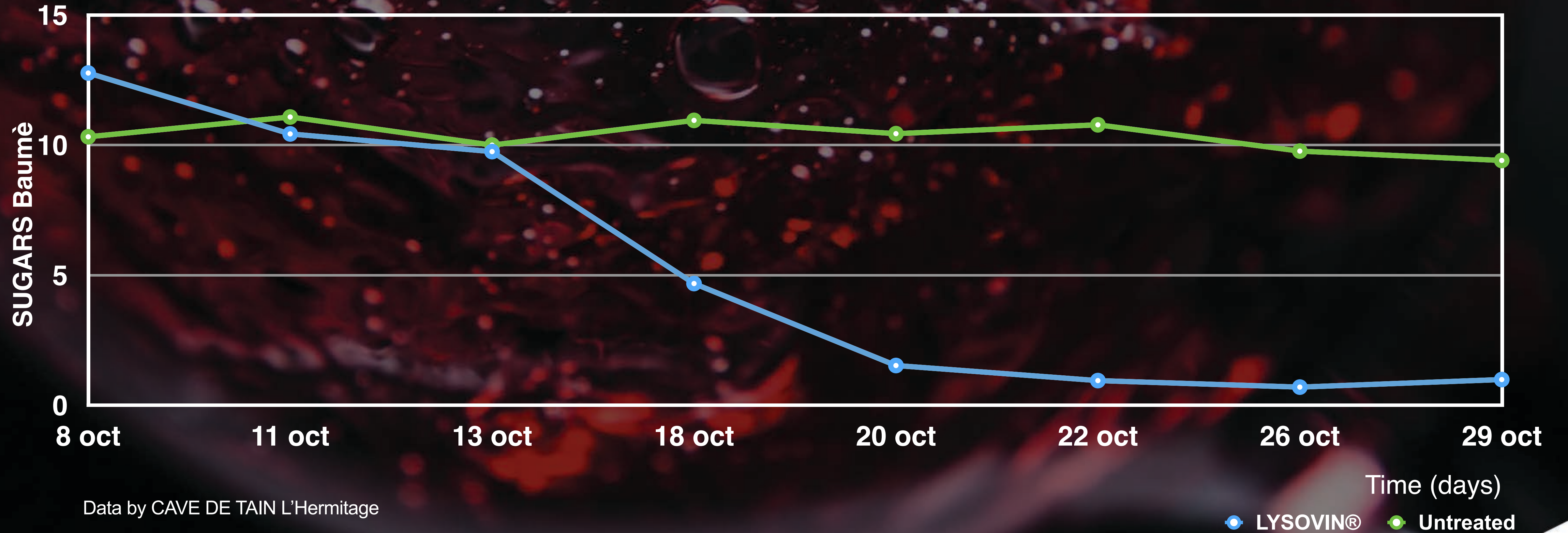
As LAB consume sugar it unavailable for the yeast to produce alcohol. In combination with the stuck fermentation, the ethanol content in the control lowers.



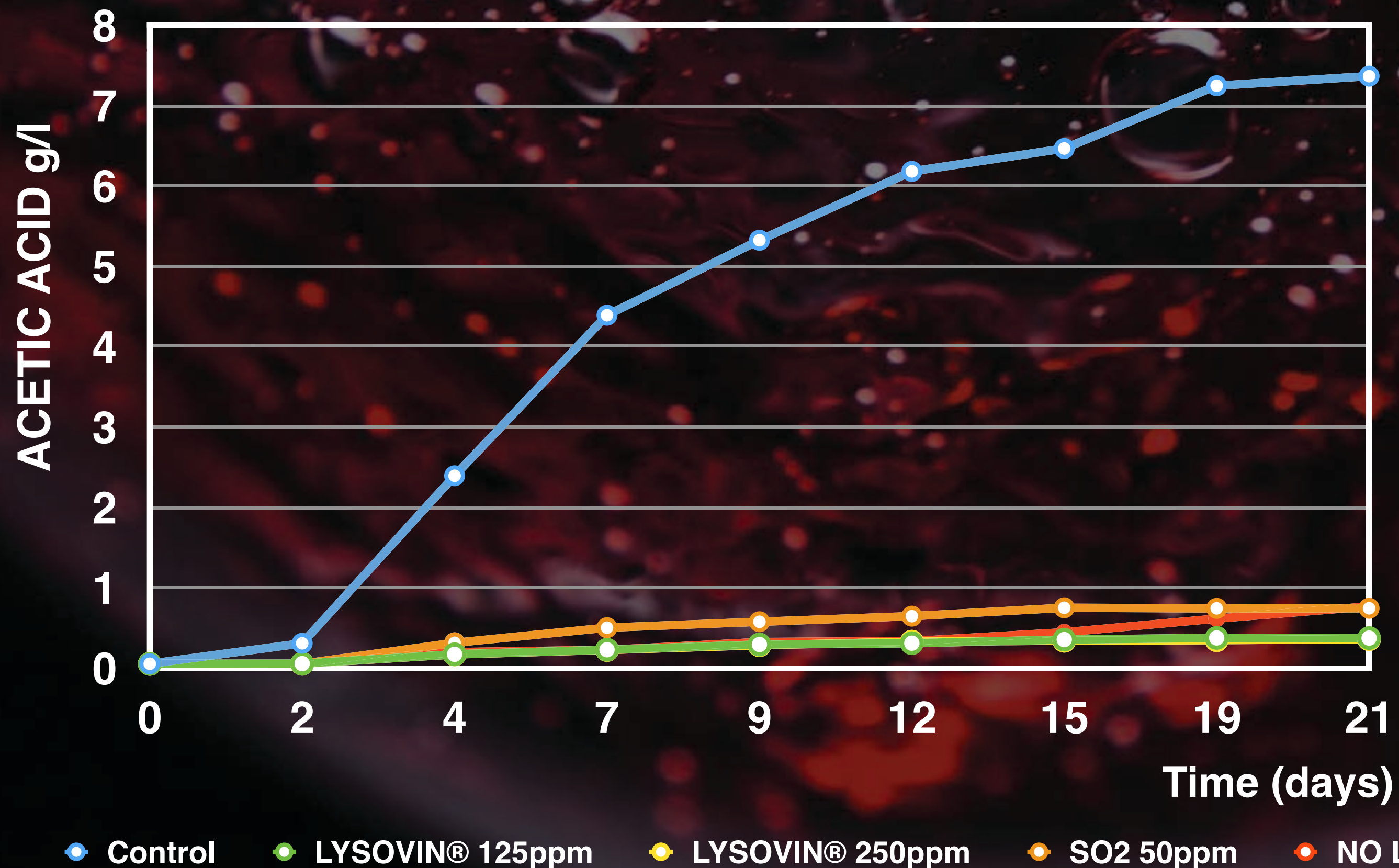
Control 125 ppm Lysovin 250 ppm Lysovin 50 ppm SO2

SLUGGISH FERMENTATION - CROZES HERMITAGE 1999 - 20000 L

LYSOZYME TREATMENT IMPACT ON THE END OF ALCOHOLIC FERMENTATION



LYSOVIN PREVENTS ACETIC ACID PRODUCTION



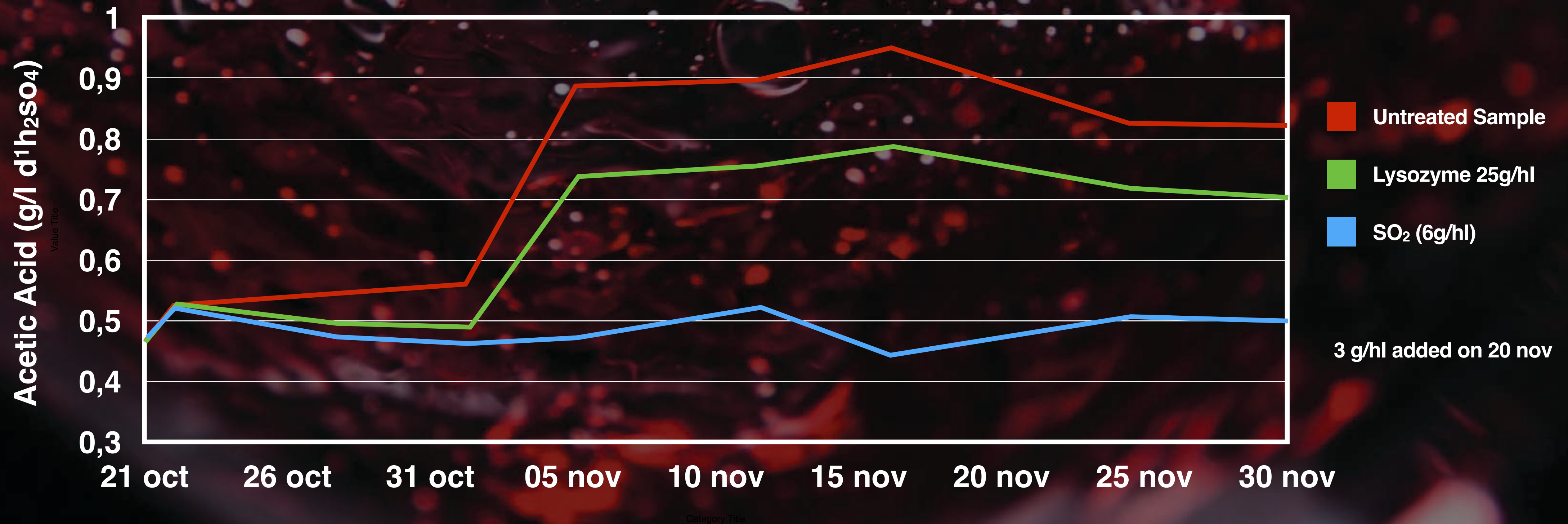
Sterile Cabernet Sauvignon juice inoculated with a strain of wine spoilage lactic acid bacteria, *Lactobacillus hilgardii*. The treatments included were: Control (with LAB inoculation without LYSOVIN or SO₂, 125 and 250 ppm lysozyme, 50 ppm SO₂, Control (no LAB or lyso or SO₂).

YEAST INOCULATED IN EACH TREATMENT WITH WINE PRODUCTION

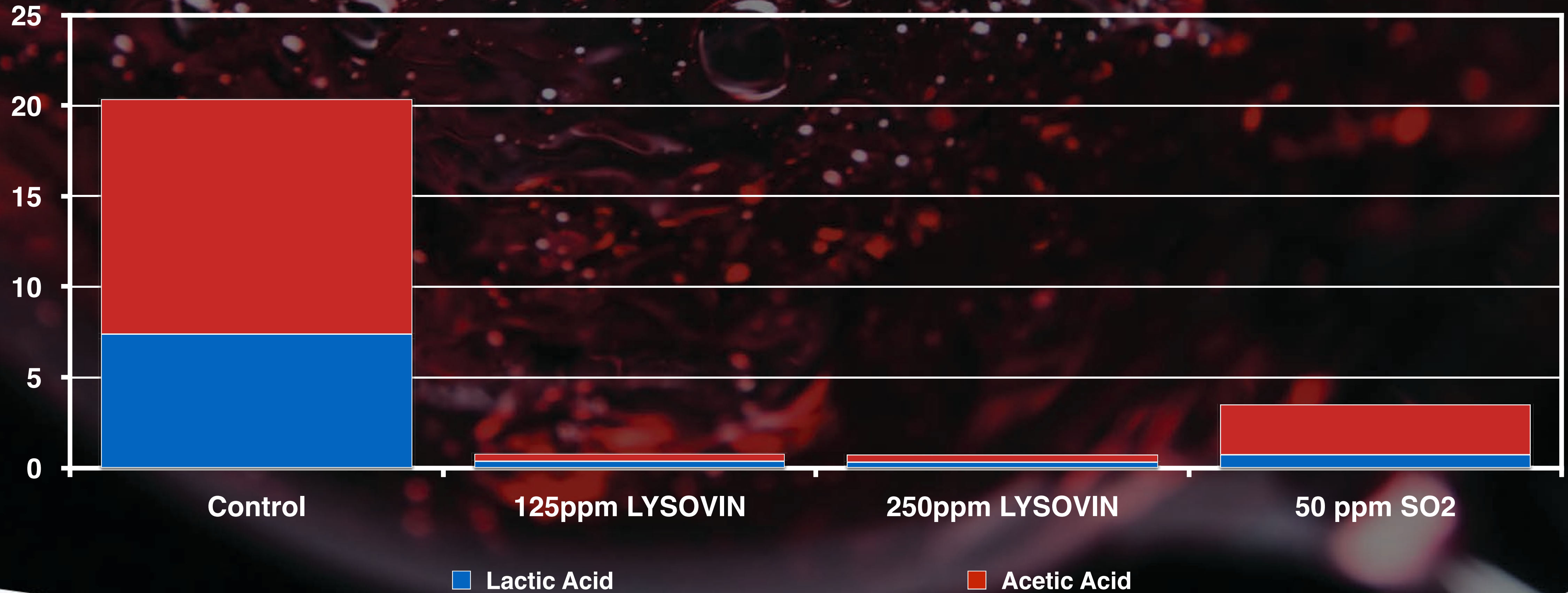
TESTS CONDUCTED DURING THE FERMENTATION:

- LAB and yeast cell counts
- Acetic acid
- Sugar
- Ethanol

ACETIC ACID VALUE - EVOLUTION AFTER TREATMENT

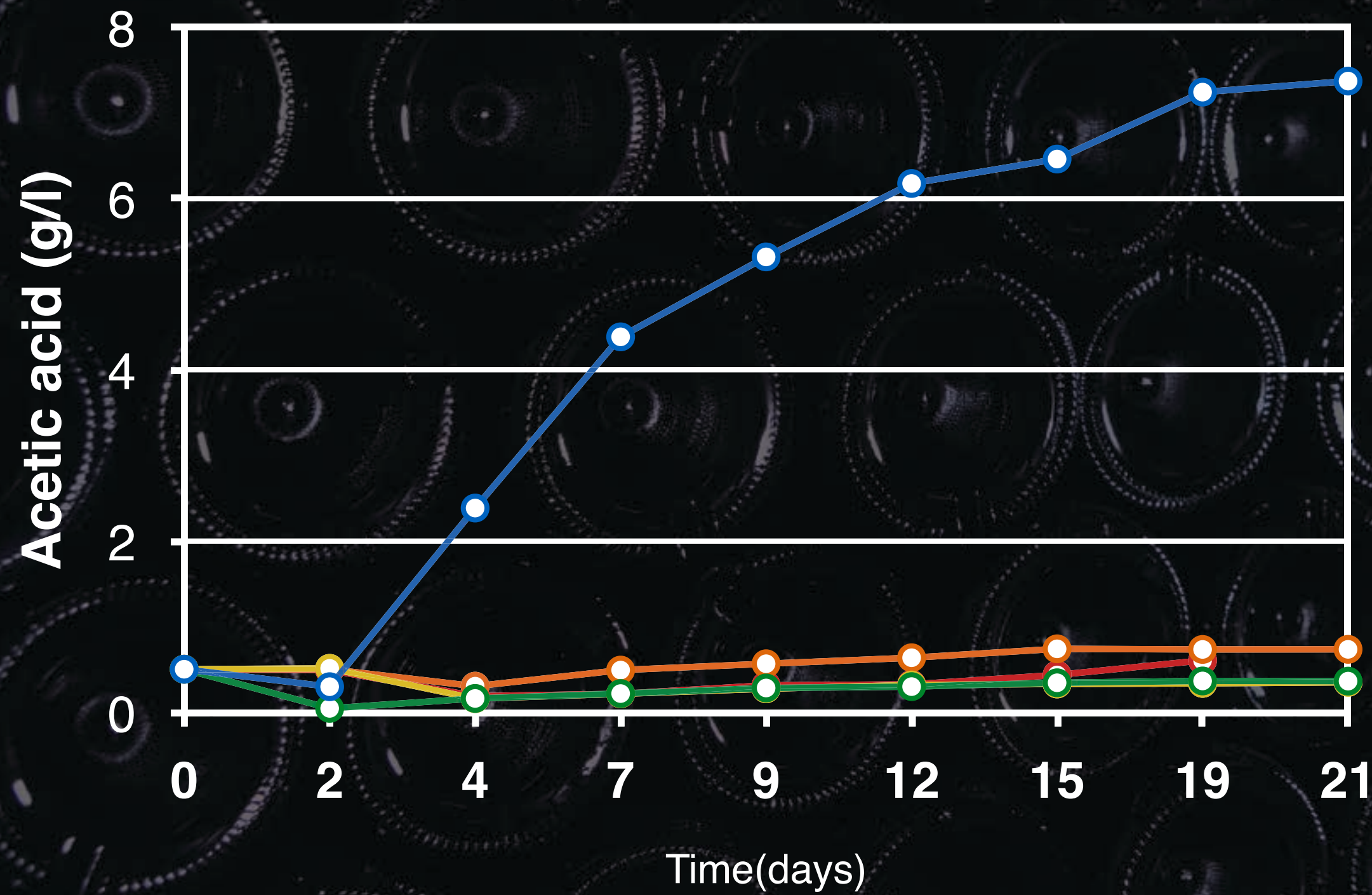


ACETIC ACID VALUE - EVOLUTION AFTER TREATMENT

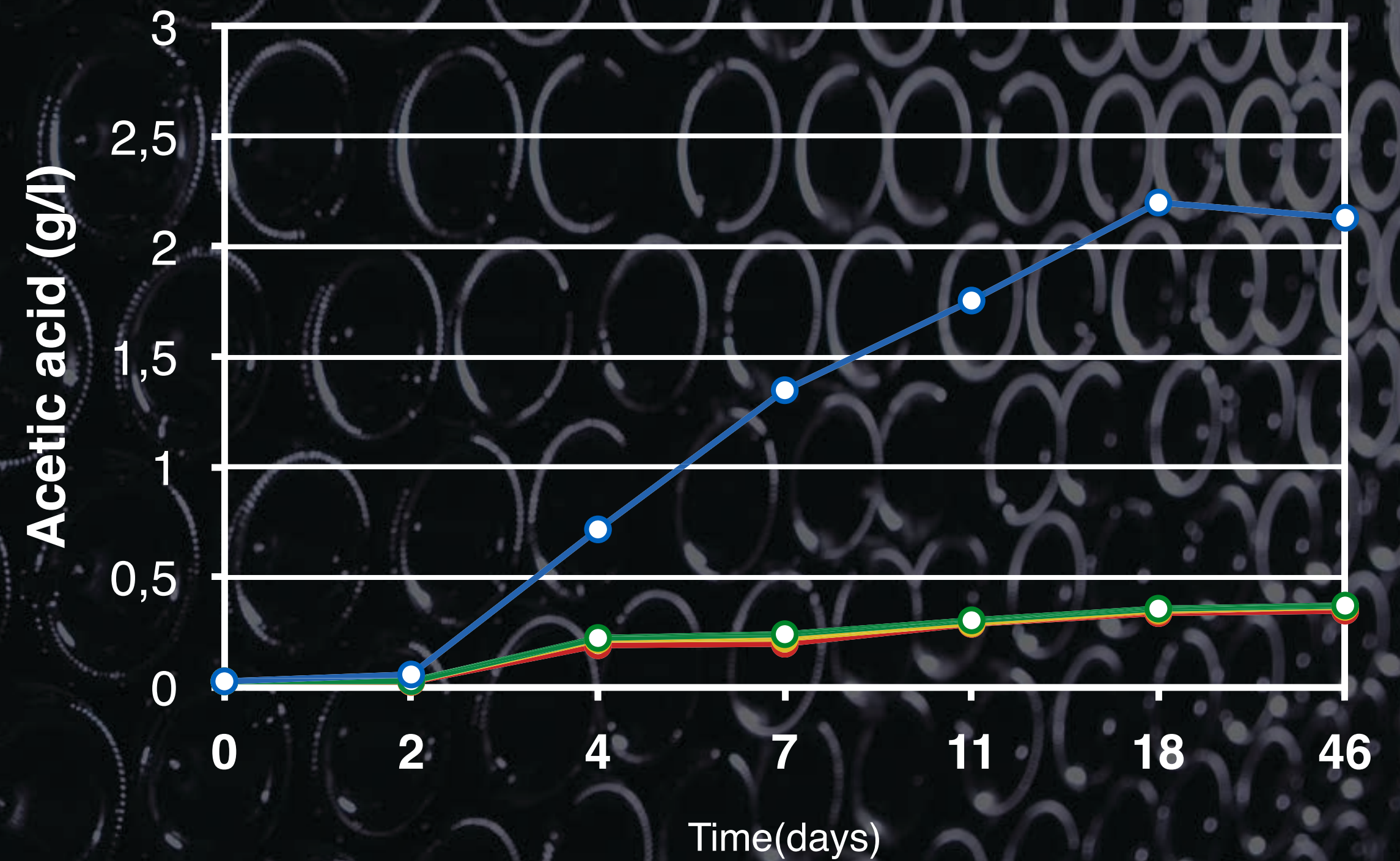


LYSOVIN LOWERS CONCENTRATION OF ACETIC ACID

L. hilgardii



L. collinoides



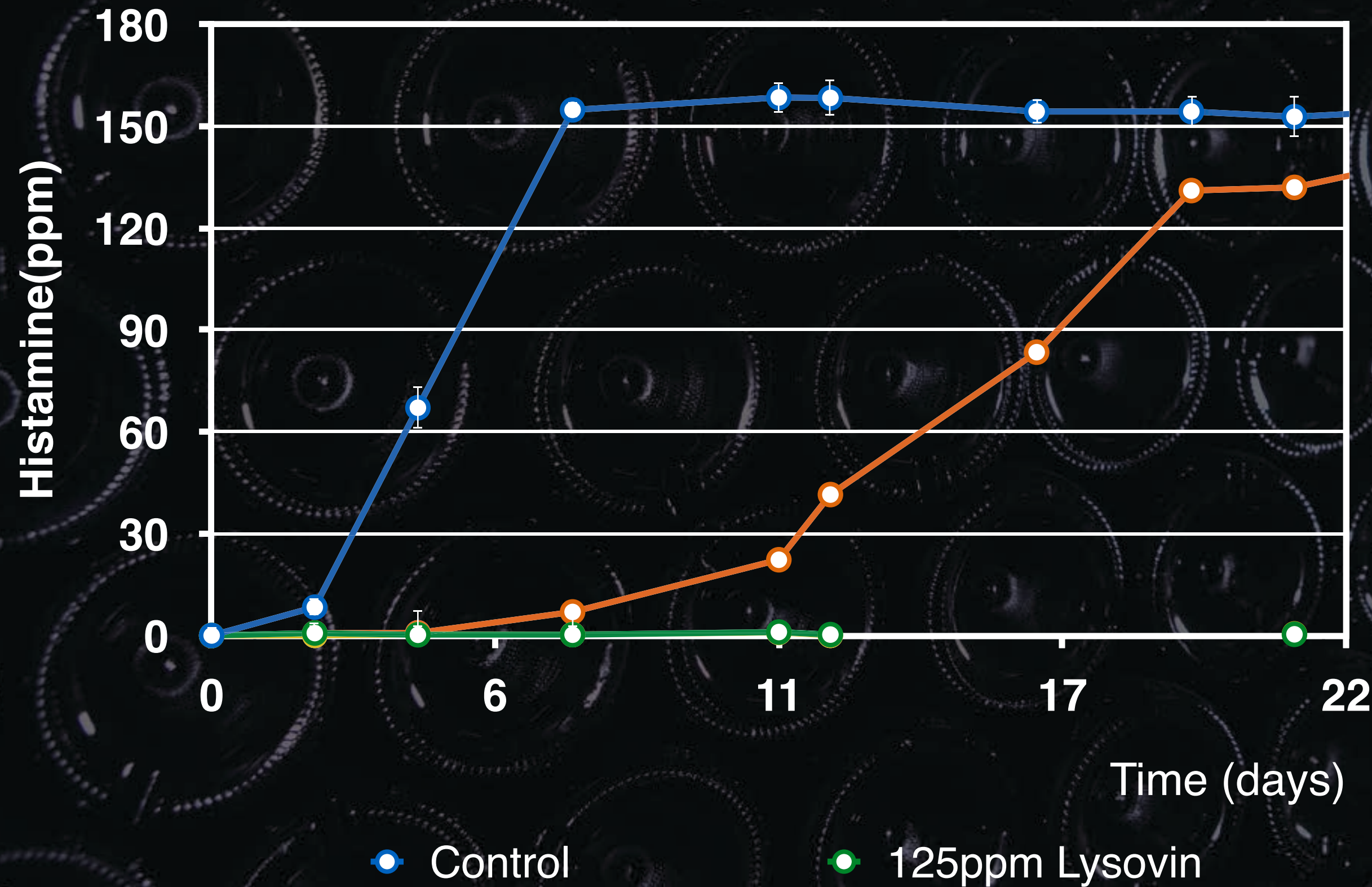
● Control ● 125ppm LYSOVIN ● 250ppm LYSOVIN ● 50ppm SO2 ● NO LAB Control



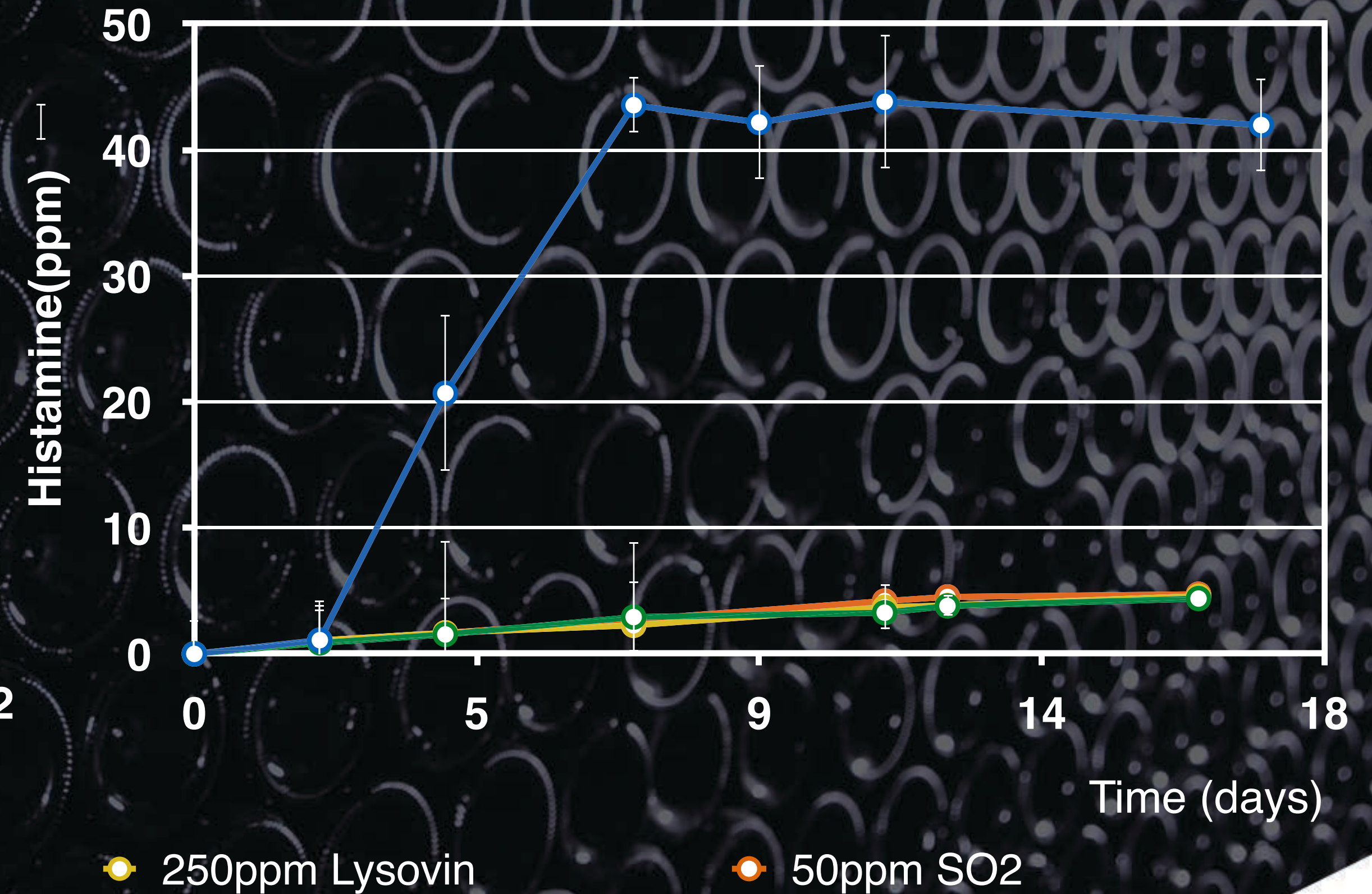
LYSOVIN CONTROLS THE PRODUCTION OF BIOGENIC AMINES BY SPOILAGE LAB

STUDY WITH *L. HILGARDII*

200 ppm histidine was added into Pinot Noir juice



Chardonnay juice, no histidine added

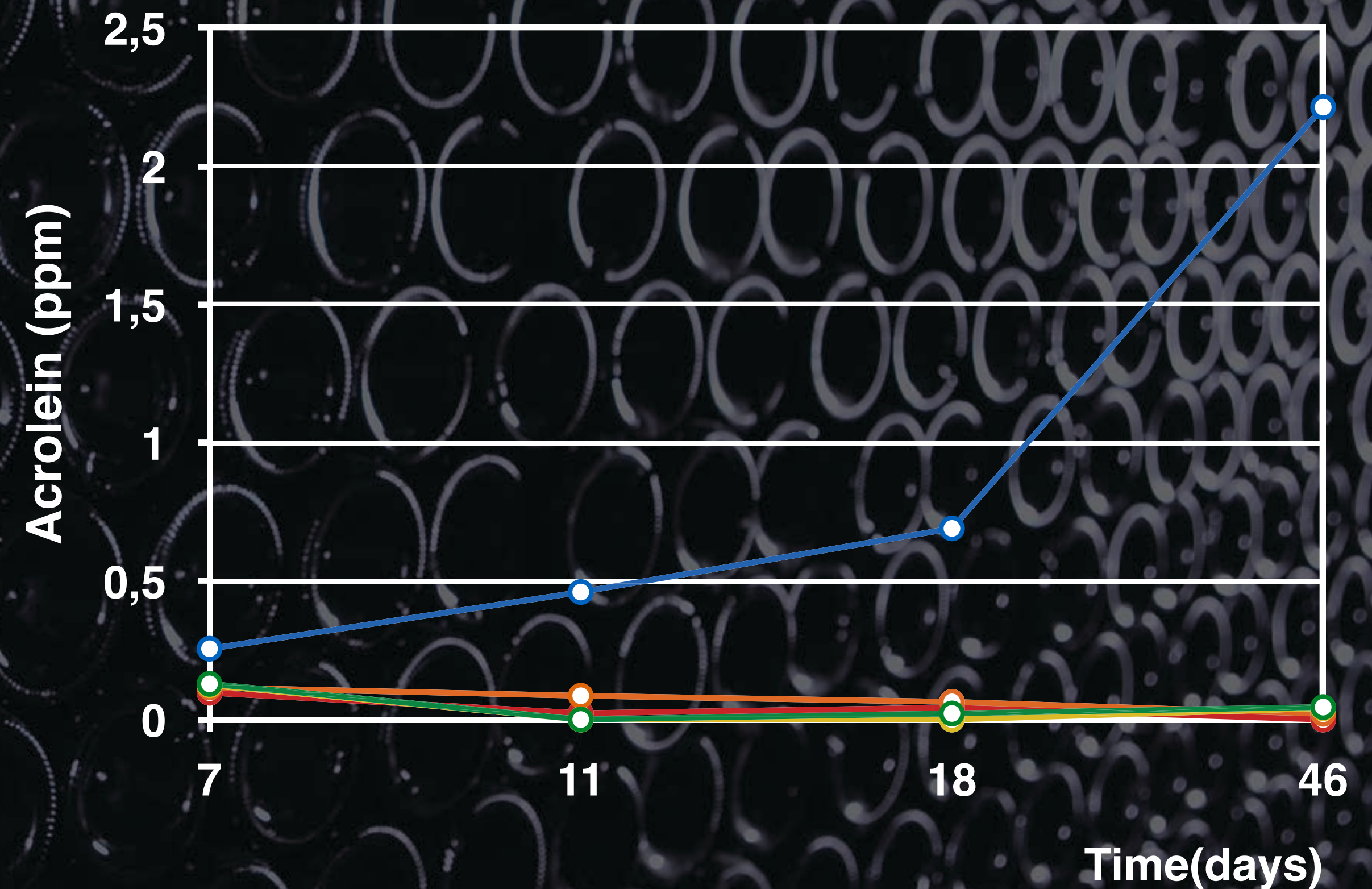


LYSOVIN REDUCED THE PRODUCTION OF ACROLEIN

STUDY WITH *L. HILGARDII*

Acrolein: Bitterness

Wine quality may be compromised by the presence of 3-HPA due to the potential for spontaneous conversion into acrolein under winemaking conditions. Acrolein is highly toxic and has been implicated in the development of bitterness in wine. Interconversion between 3-HPA derivatives and acrolein is a complex and highly dynamic process driven by hydration and dehydration reactions. Acrolein is furthermore highly reactive and its steady-state concentration in complex systems very low. As a result, analytical detection and quantification in solution is problematic.



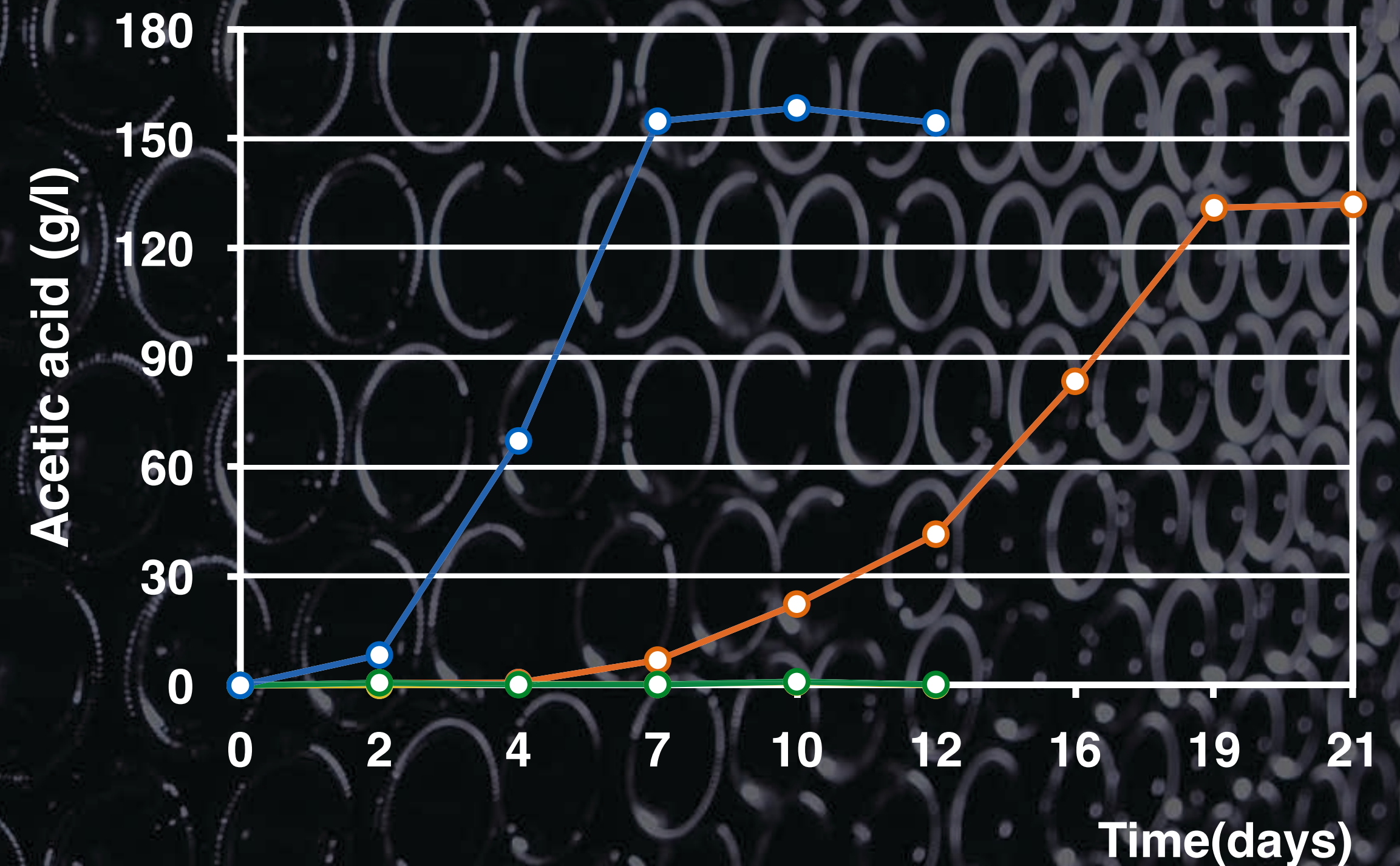
● Control ● 125ppm LYSOVIN ● 250ppm LYSOVIN ● 50ppm SO2 ● NO LAB Control

LYSOVIN CONTROLS HISTAMINE LEVEL

Histamine: Intolerance

Histamine is a biogenic amine which is naturally present in many foodstuffs – also in wine. Some people react to high histamine foods with headaches or other symptoms. More and more winemakers are therefore offering low histamine or histamine-free wine. Here's what to look out for when producing such wines.

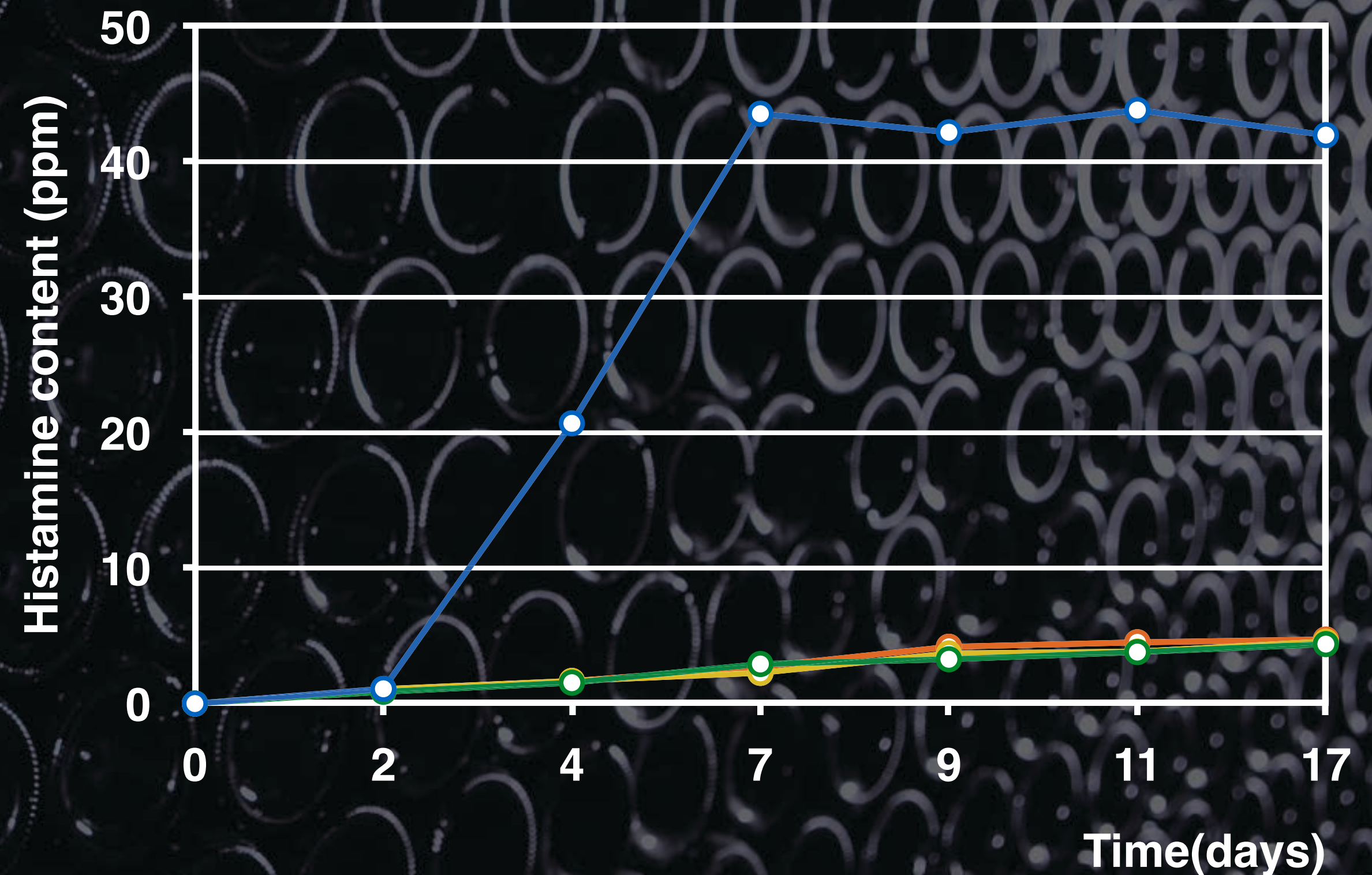
- Control
- 125ppm LYSOVIN
- 250ppm LYSOVIN
- 50ppm SO₂



LYSOVIN PREVENTS THE PRODUCTION OF HISTAMINE

Histamine: Intolerance

Histamine is present in a variety of fermented products such as wine, aged cheeses, and sauerkraut. Red wine has 20–200% more histamine than white wine, and those who react to it may be deficient in the enzyme diamine oxidase. Experts believe that in some individuals, alcohol consumption may lead to elevated plasma histamine levels even in the absence of histamines in the beverage consumed. A study of 16 people with an intolerance to red wine found no difference in reactions to low and high histamine wines. Other biogenic amines may also have an effect.



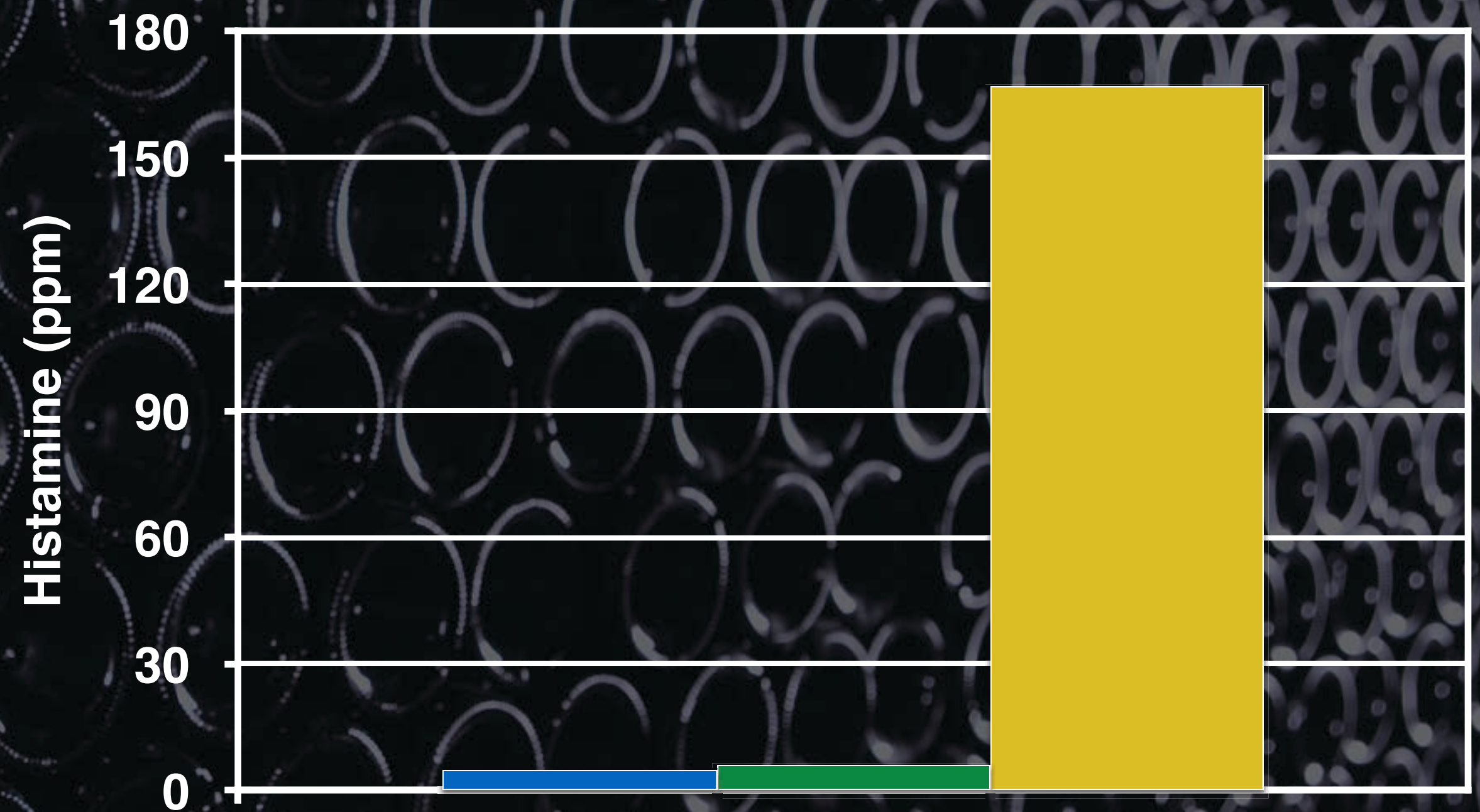
- Control
- 125ppm LYSOVIN
- 250ppm LYSOVIN
- 500ppm LYSOVIN

LYSOVIN PREVENTS THE PRODUCTION OF HISTAMINE

Histamine: Intolerance

Graphs is from a trial in which the commercial yeast for the alcoholic fermentation was contaminated. The control here has no spoilage LAB inoculated, no lysozyme or SO2 was added either. Only yeast was inoculated into the sterile juice. The contamination level was about 500-1000 CFU of LAB per gram of yeast.

- Lysovin 125 ppm
- Lysovin 250 ppm
- Control



SO₂ ANTIMICROBIAL LIMITATIONS

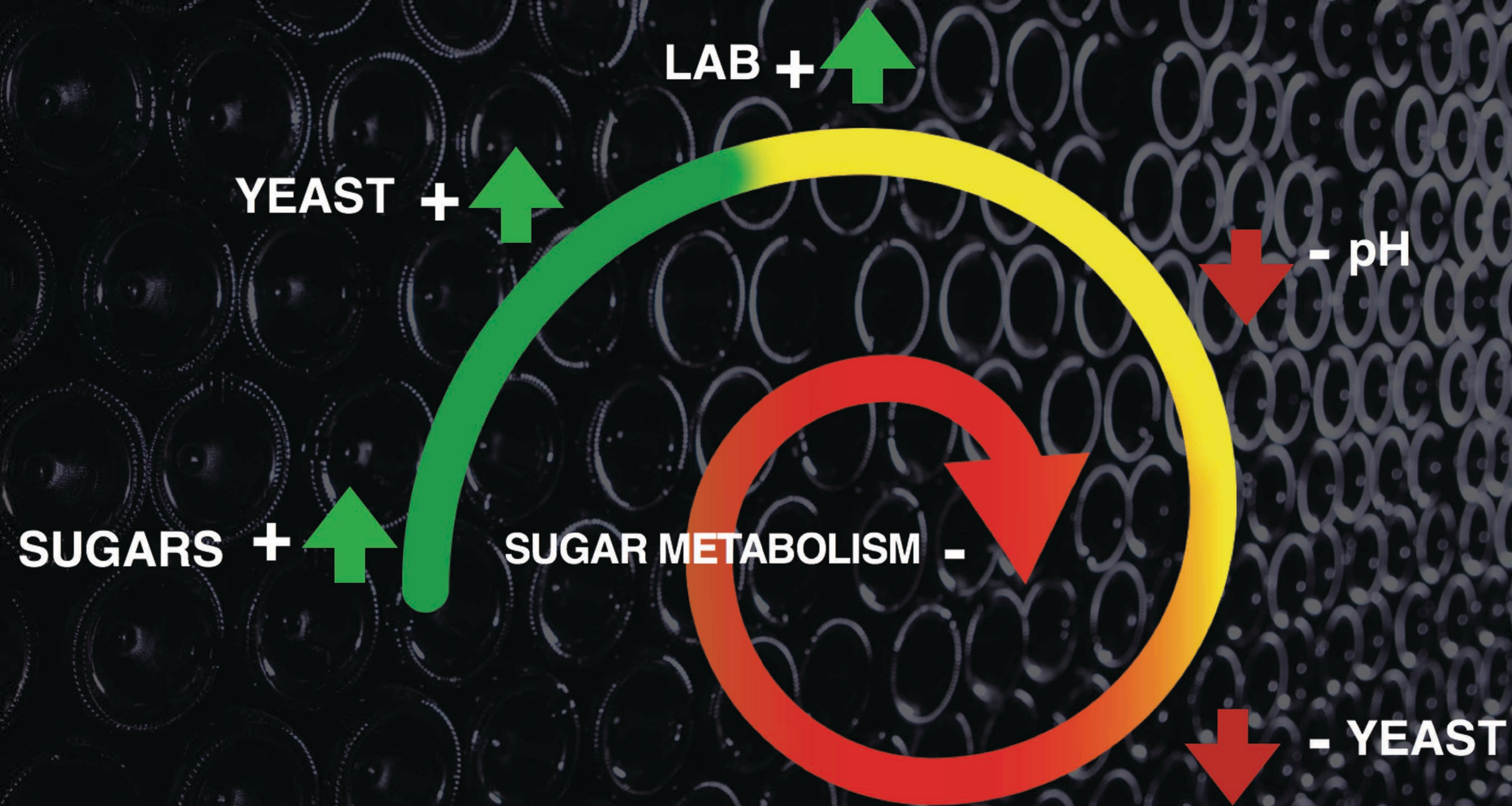
The efficacy of sulphite is affected by the pH of wine. The higher the pH of wine, the lower the concentration of the active form.

Forms: SO₂, HSO₃⁻, SO₃⁼ Antimicrobial active form:
molecular SO₂

- pH 3: 5-10%
- pH 4: <1%

Efficacy of sulphur dioxide is low in high pH wines
Increasing health concerns
LYSOVIN provides a good alternative to SO₂.

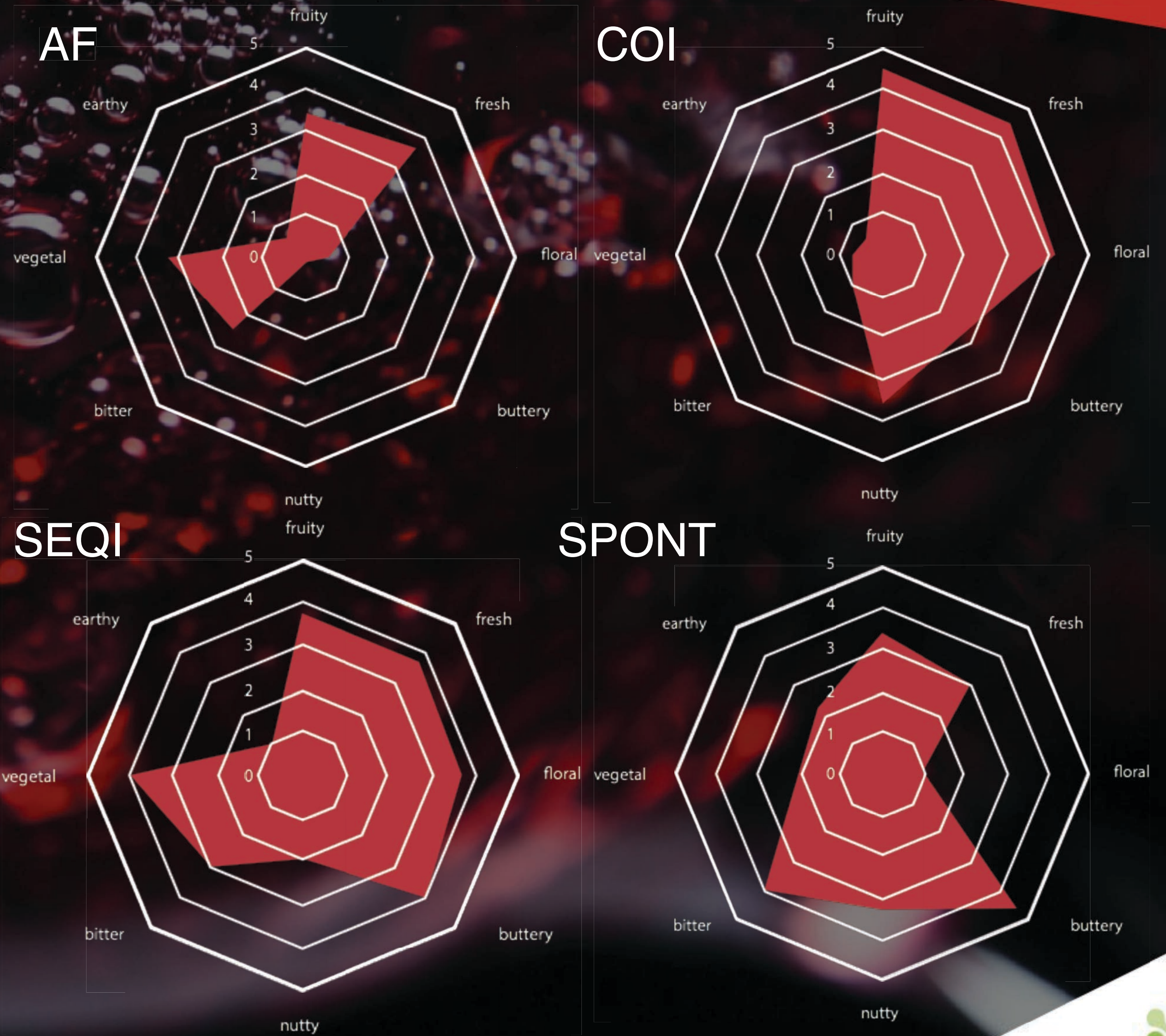
MLF AND YEAST



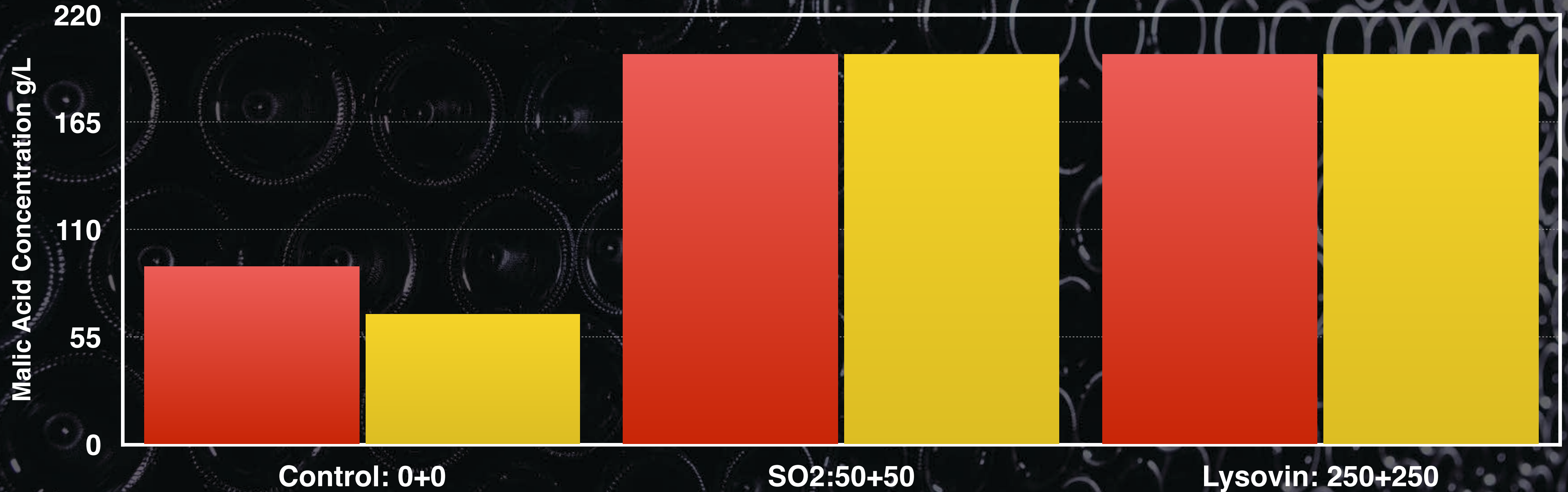
LYSOVIN AND SENSORY EVALUATION

The sensory evaluation indicated that the timing and method of MLB inoculation significantly affected the taste and aroma of the wines. In general, malolactic fermentation diversified the wine aroma profile (Figure 1). The coinoculated wines were noted to be higher in fruity, fresh, and floral sensations than the wines which had used sequential MLF. The spontaneous process was perceived as producing wines with more buttery and bitter notes. Pleasant balanced buttery and nutty aromas were also found in the coinoculated wines. The sequential and spontaneous regimes had no nutty aromas but instead strong buttery aromas were noted.

Figure 1. Descriptive sensory evaluation of the red and white wines produced with different inoculation regimes. Scale 0 – 5: 0-lack, 1-very light, 2- light, 3- noticeably, 4- intensive, 5-very intensive sensibility (value 5 is the most desirable for fruity, fresh and floral aromas, but undesirable and not acceptable for buttery, nutty, bitter, vegetal, earthy aromas)



COMPARISON BETWEEN LYSOVIN AND SO₂ ADDITION TO INHIBIT MLF* SAUVIGNON BLANC

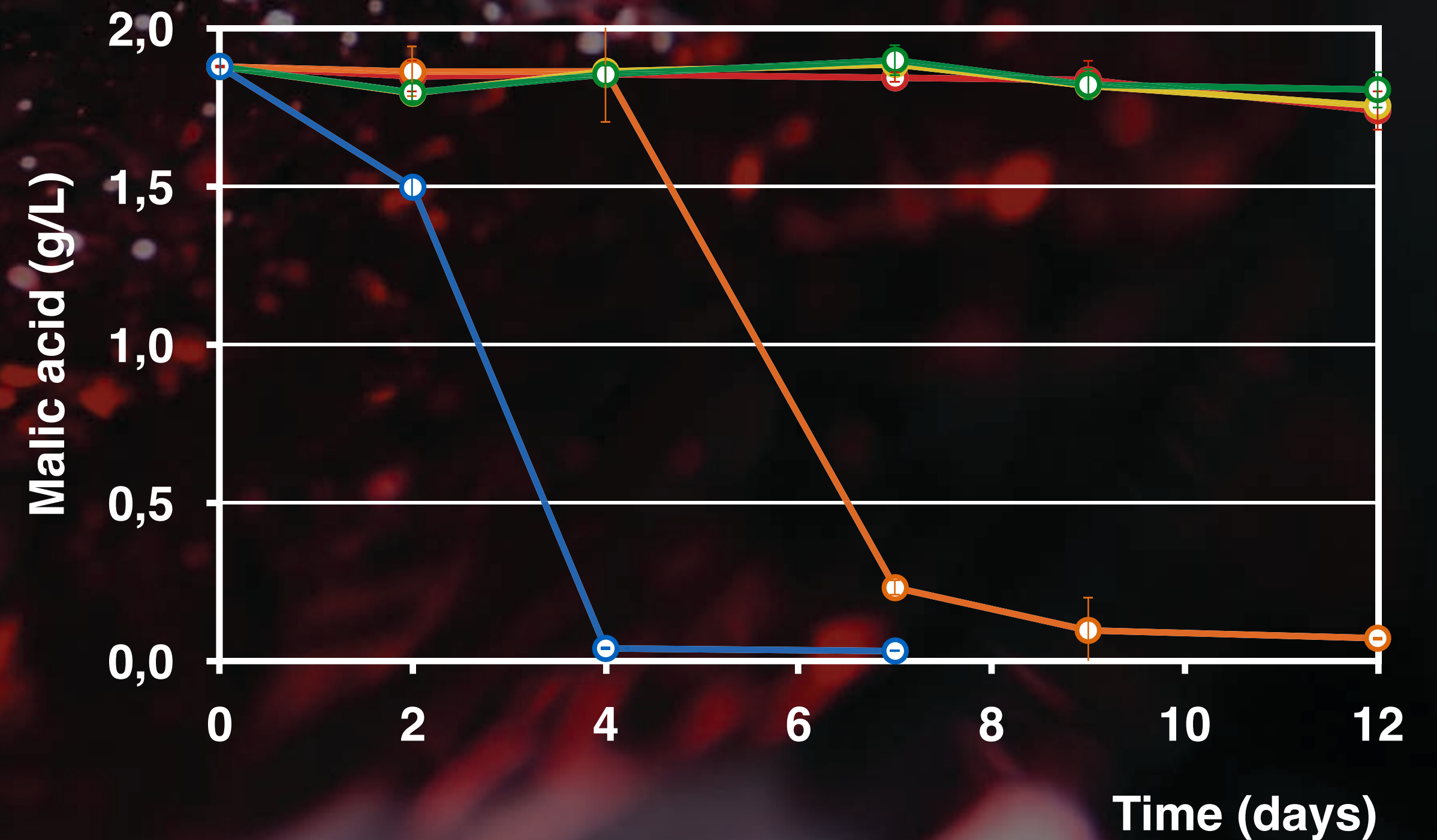
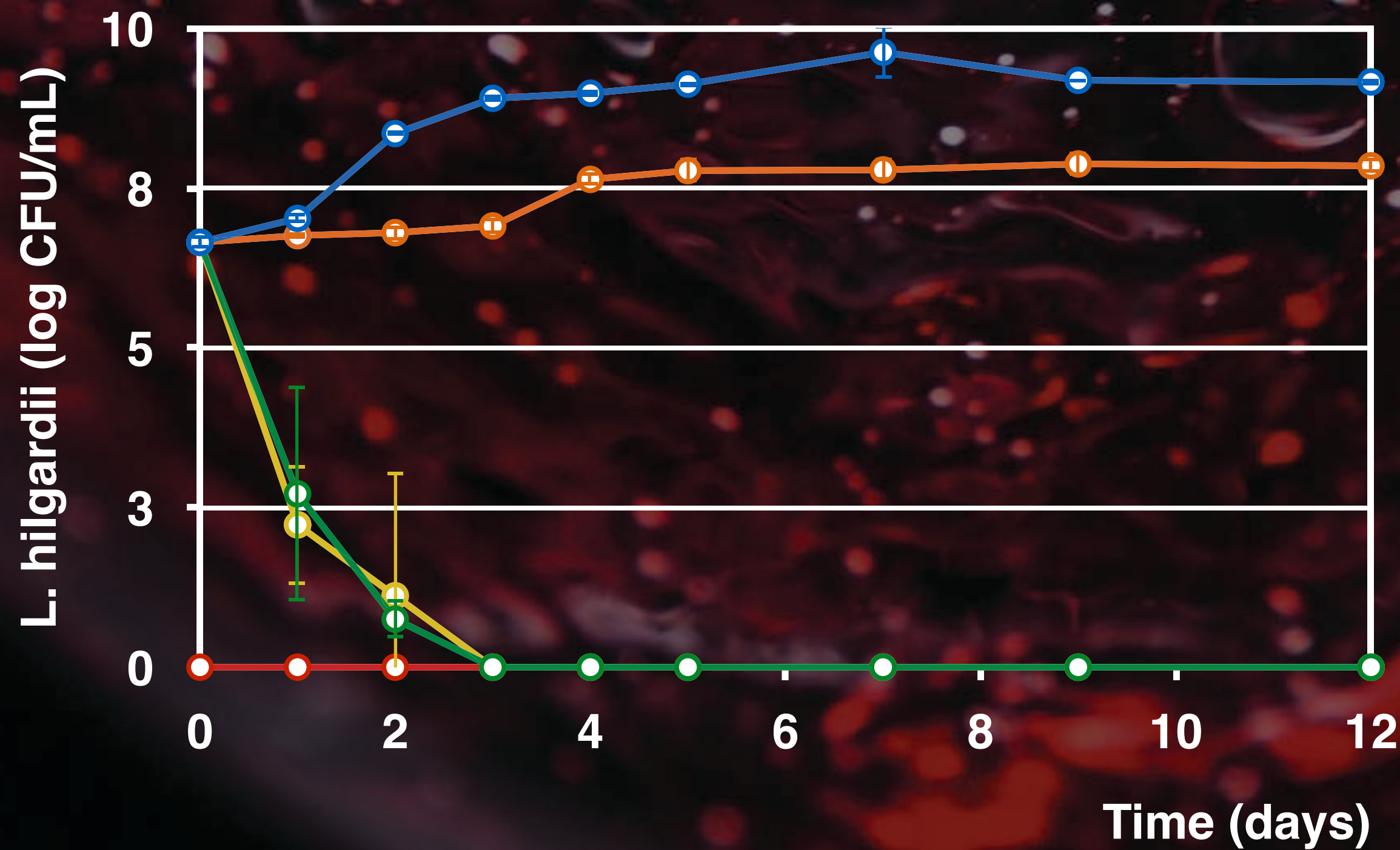


*Malolactic Fermentation

■ Val de Loire ■ Bordeaux



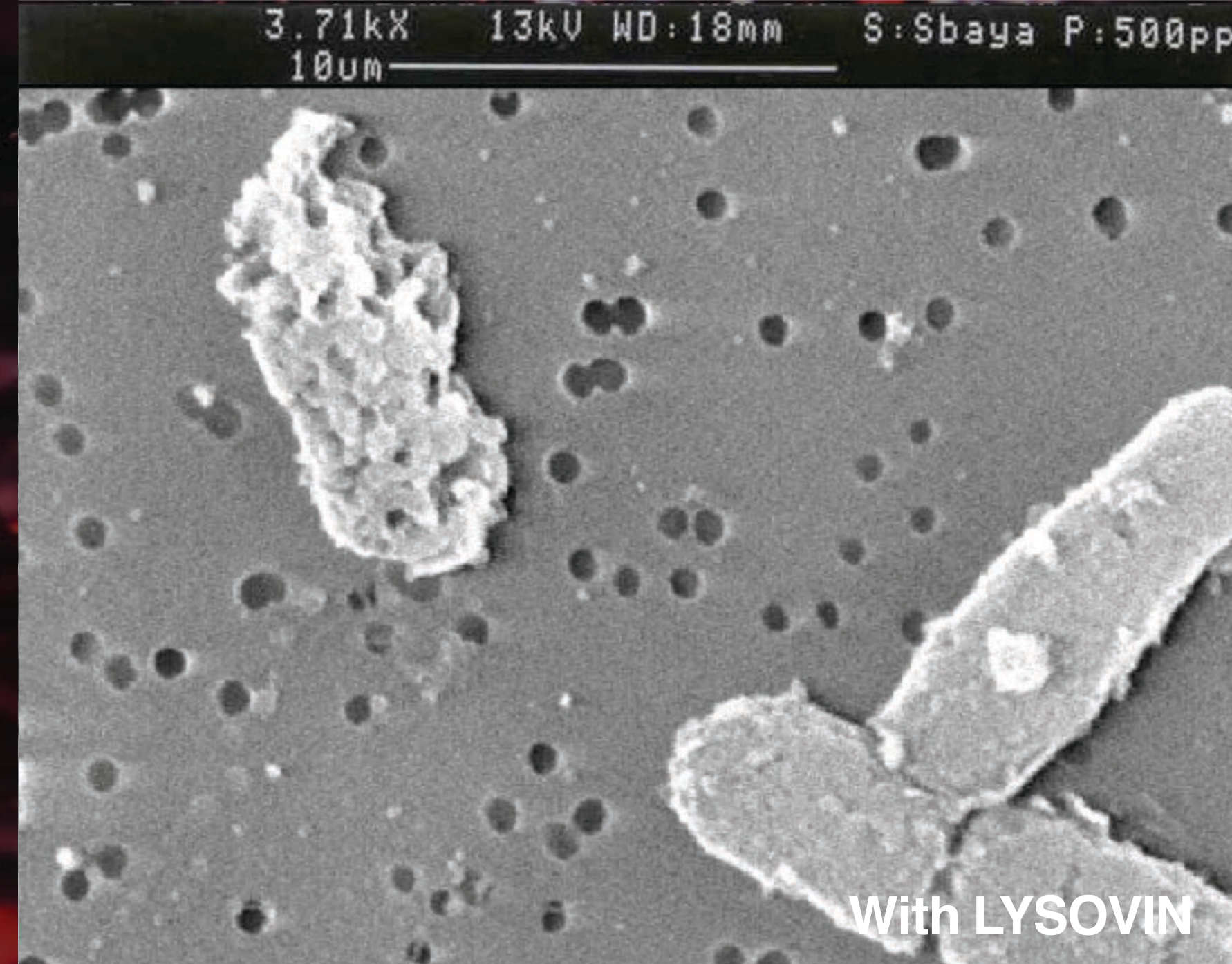
COMPARISON BETWEEN LYSOVIN AND SO₂ ADDITION TO INHIBIT MLF* SAUVIGNON BLANC



*Malolactic Fermentation ● Control ● 125 ppm Lysovin ● 250 ppm Lysovin ● 50 ppm SO₂ ● No LAB

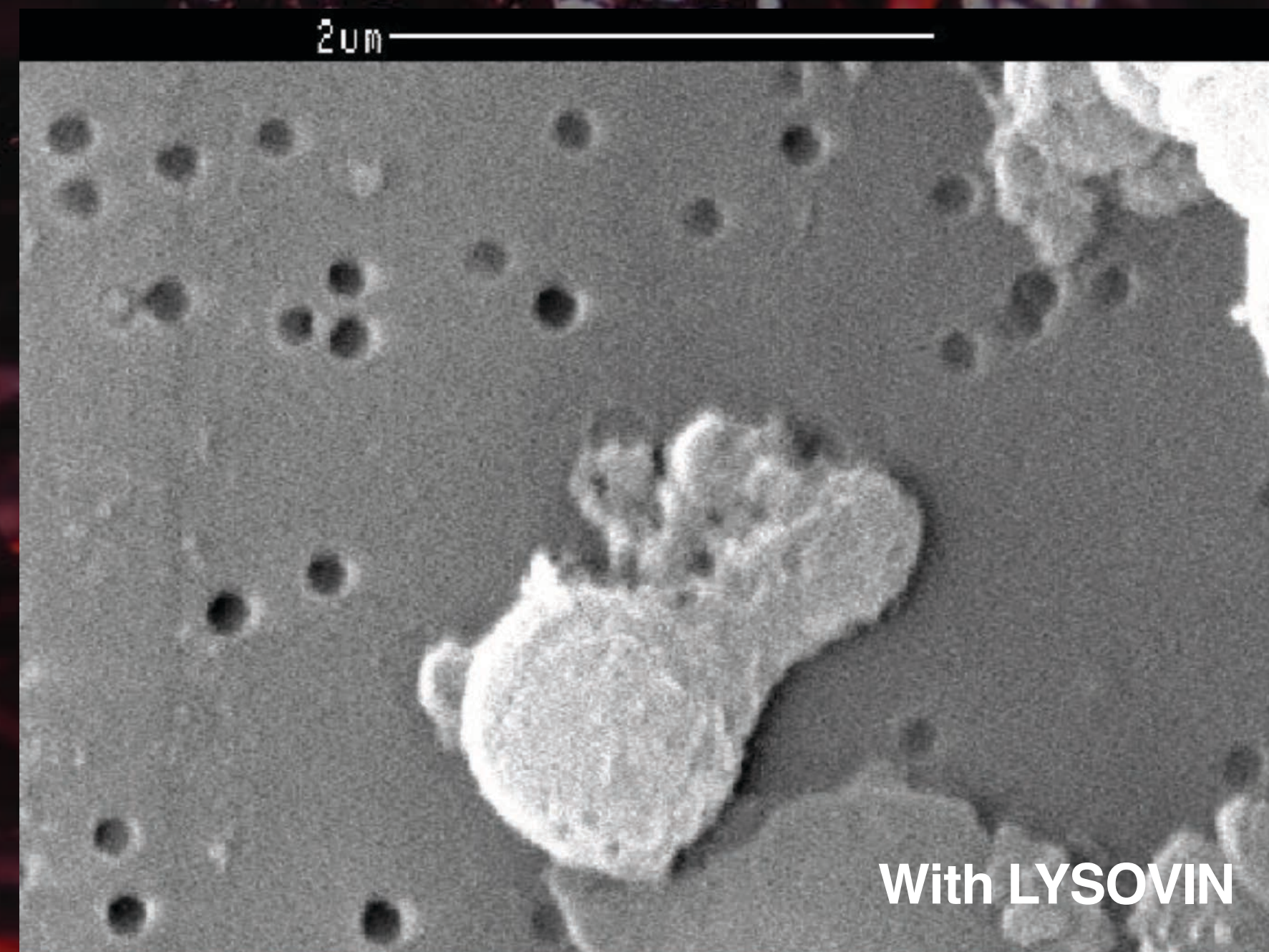
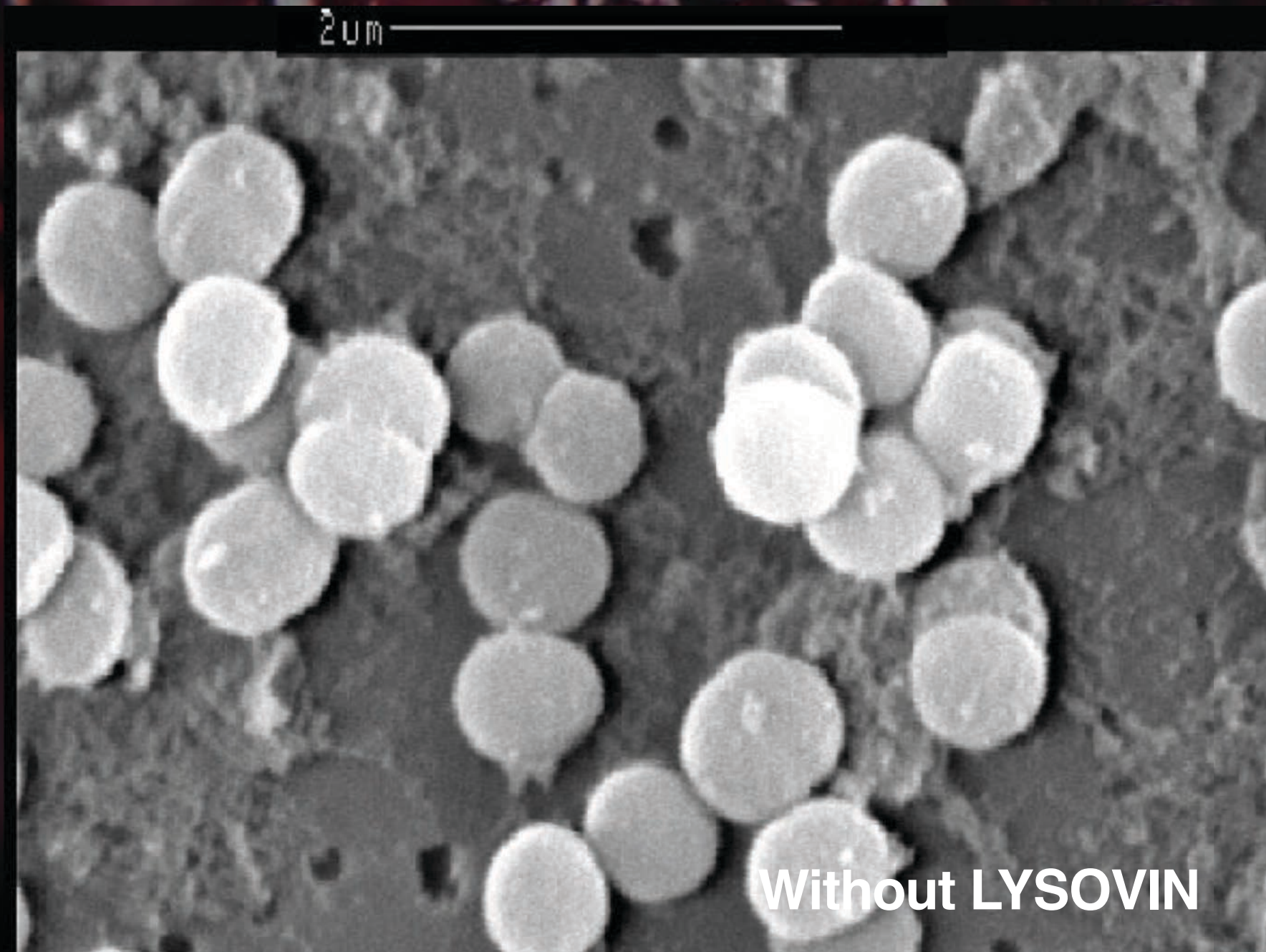


EFFECT OF LYSOVIN ON *LACTOBACILLUS* SP.



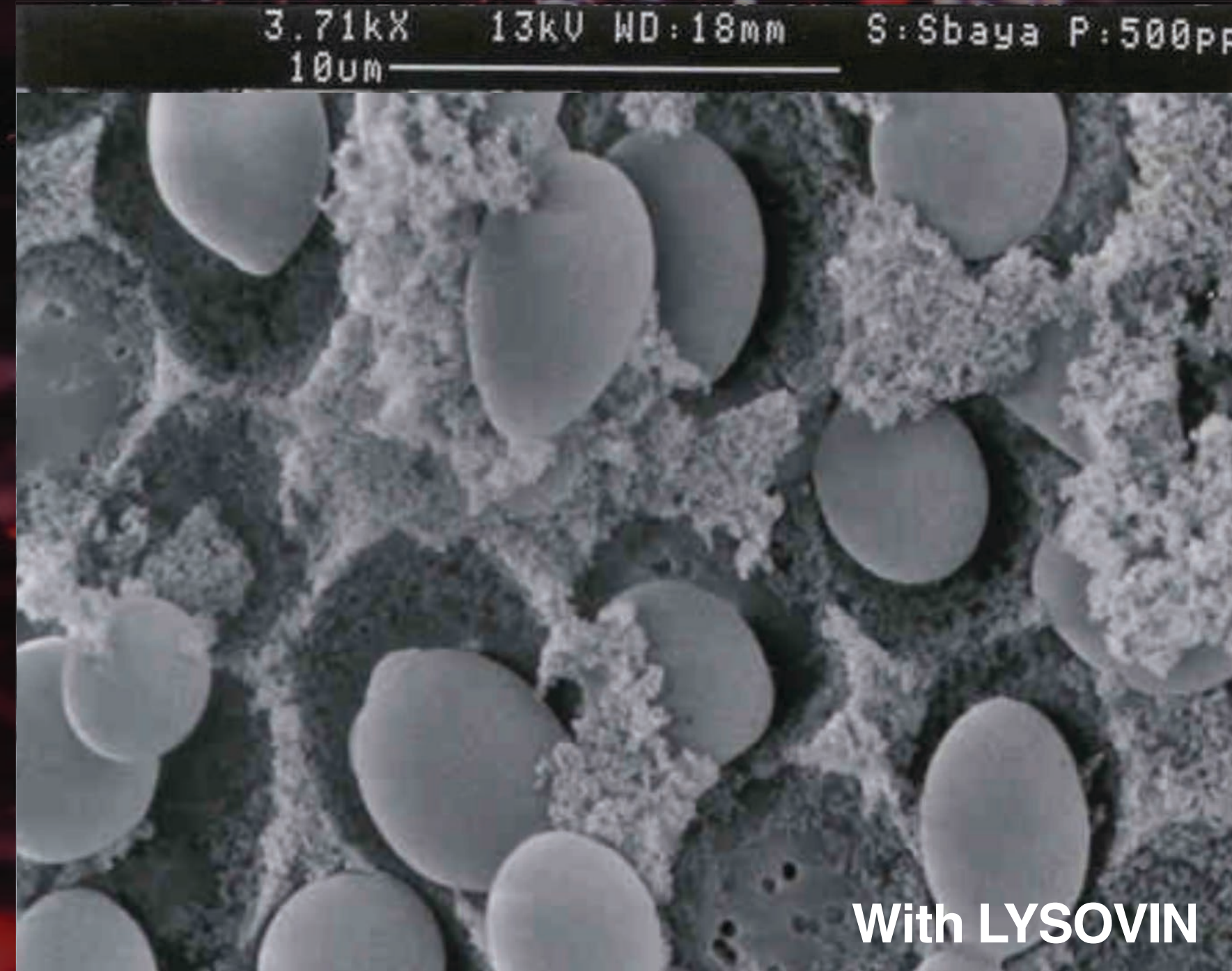
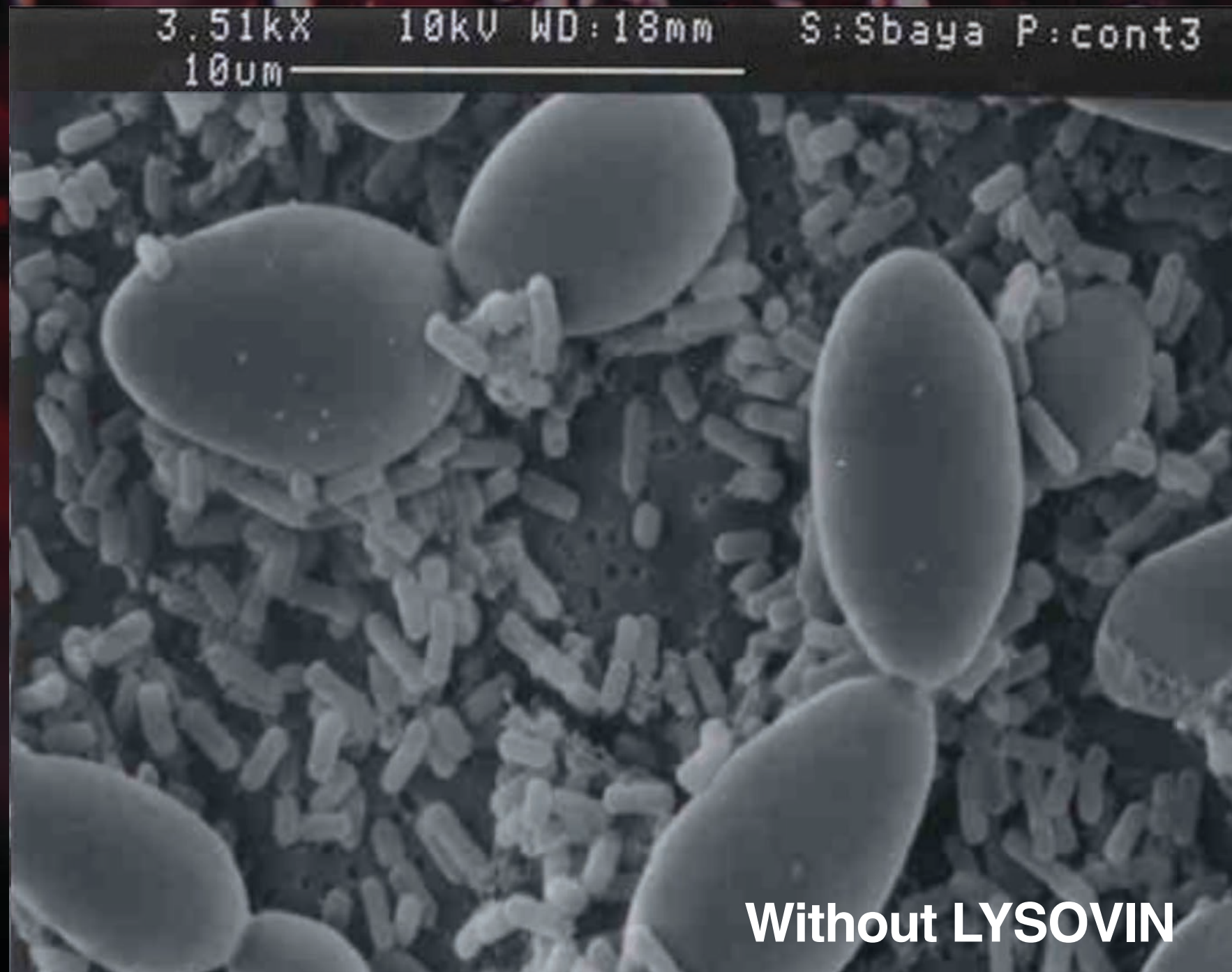
Data by Neova Technologies Inc. Canada

EFFECT OF LYSOVIN ON *PEDIOCOCCUS DAMNOSUS*



Data by Neova Technologies Inc. Canada

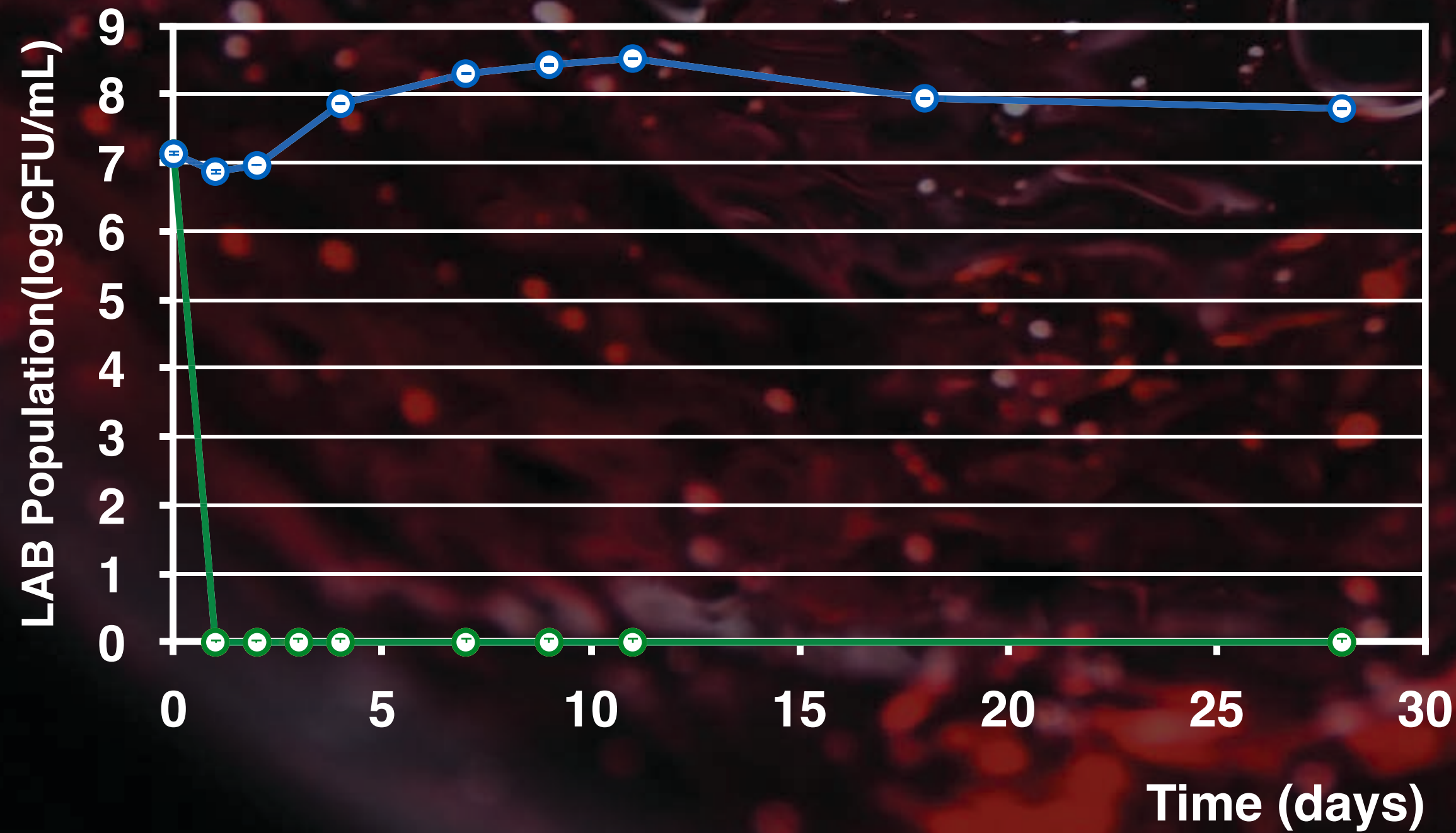
LYSOVIN HAS NOT EFFECT ON FERMENTING YEAST



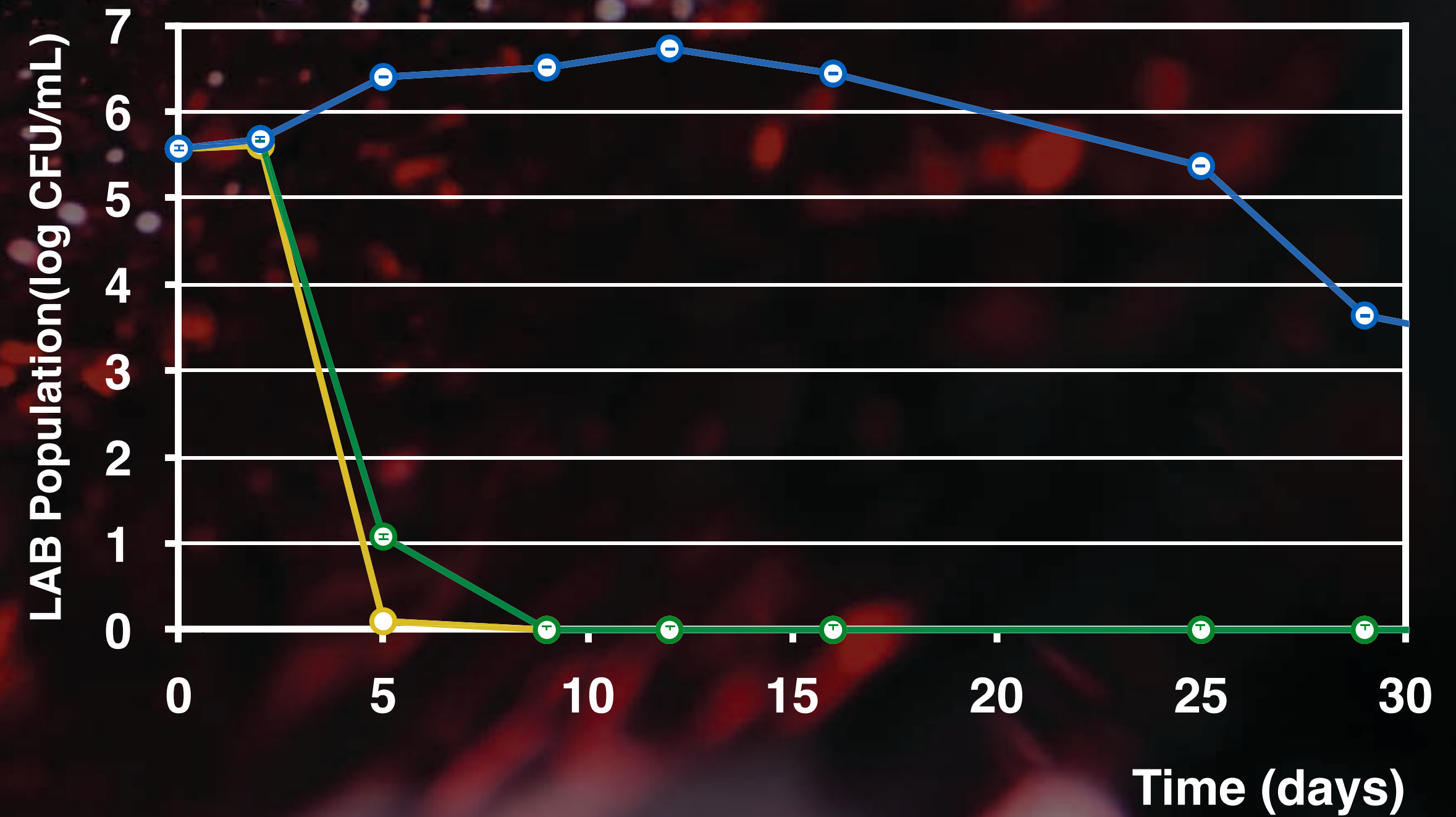
Data by Neova Technologies Inc. Canada

LYSOVIN INHIBITS SPOILAGE LAB GROWTH

L. collinoids



P. damnosus

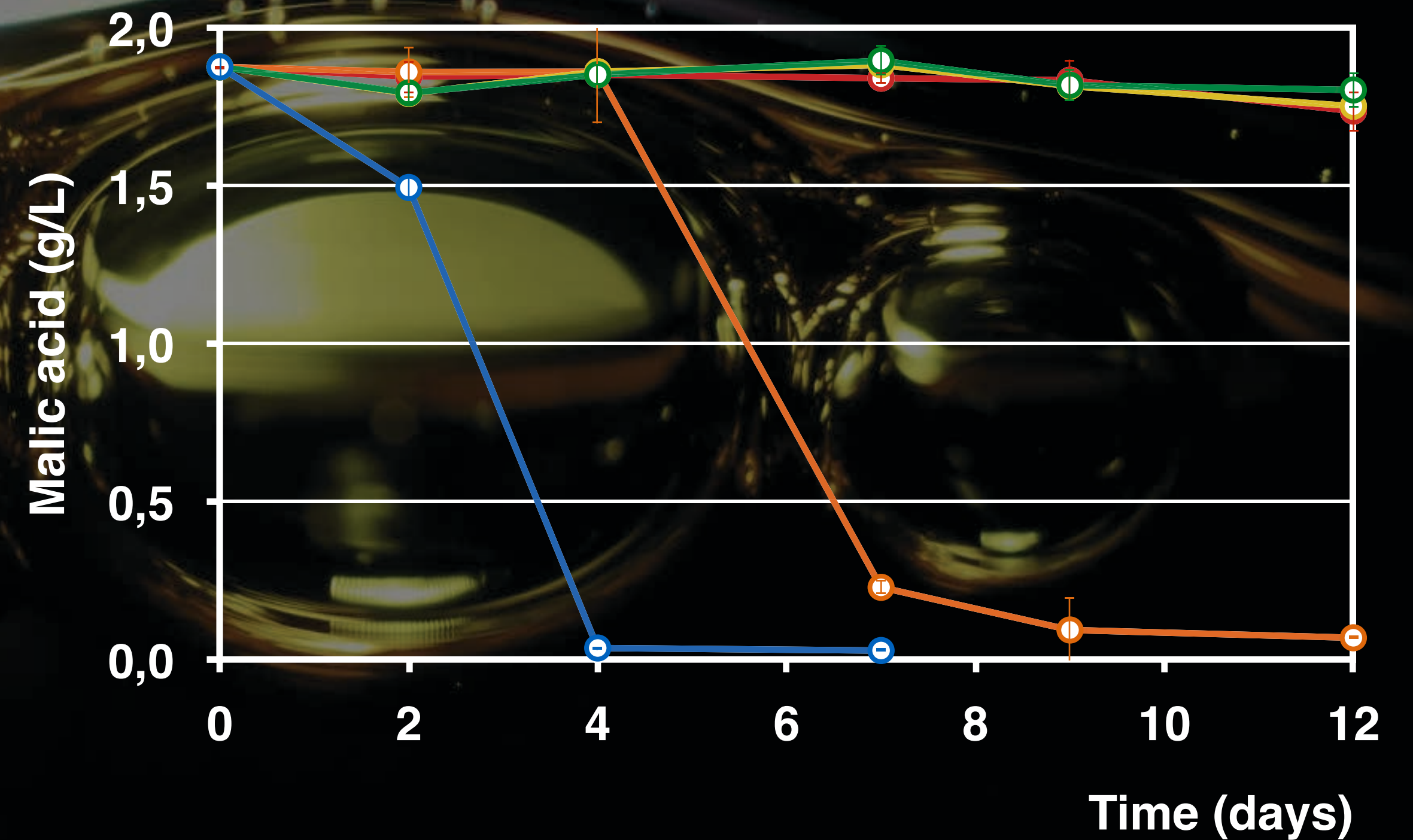
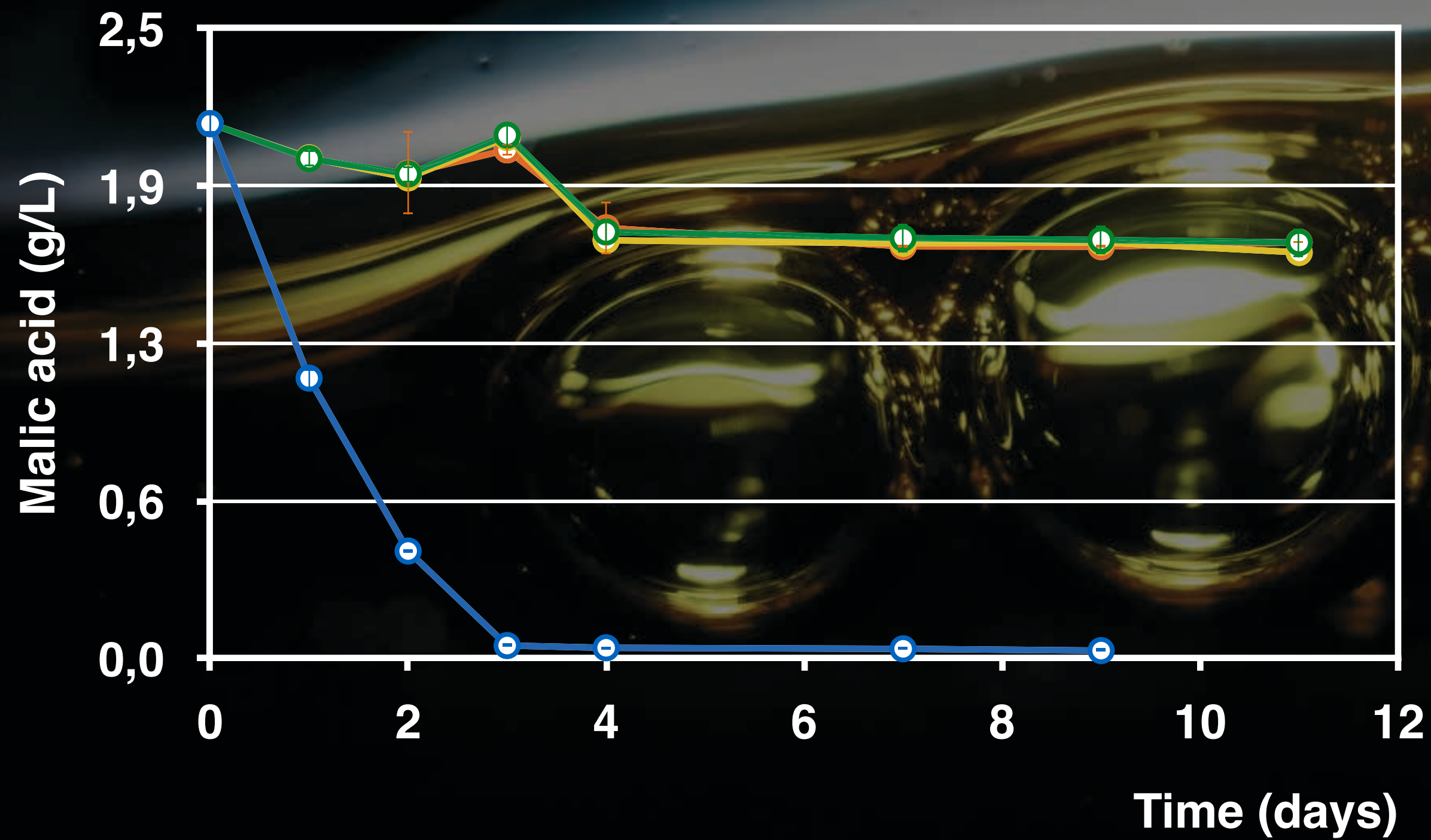


Data by Neova Technologies Inc. Canada

● control ● 125 ppm Lysovin ● 250 ppm Lysovin

LYSOVIN CONTROL LAB SPOILAGE AND HELPS MLF MANAGEMENT

STUDY WITH *L. hilgardii*

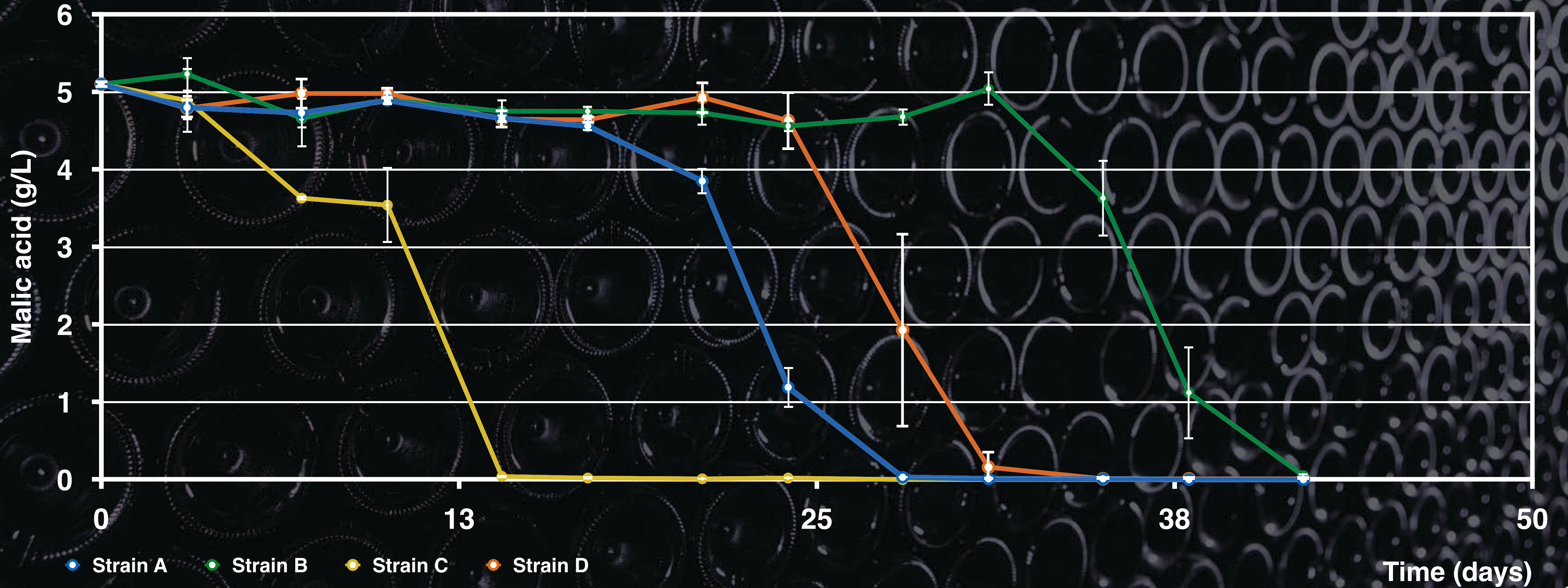


● control ● 125 ppm Lysovin ● 250 ppm Lysovin ● 50 ppm SO2

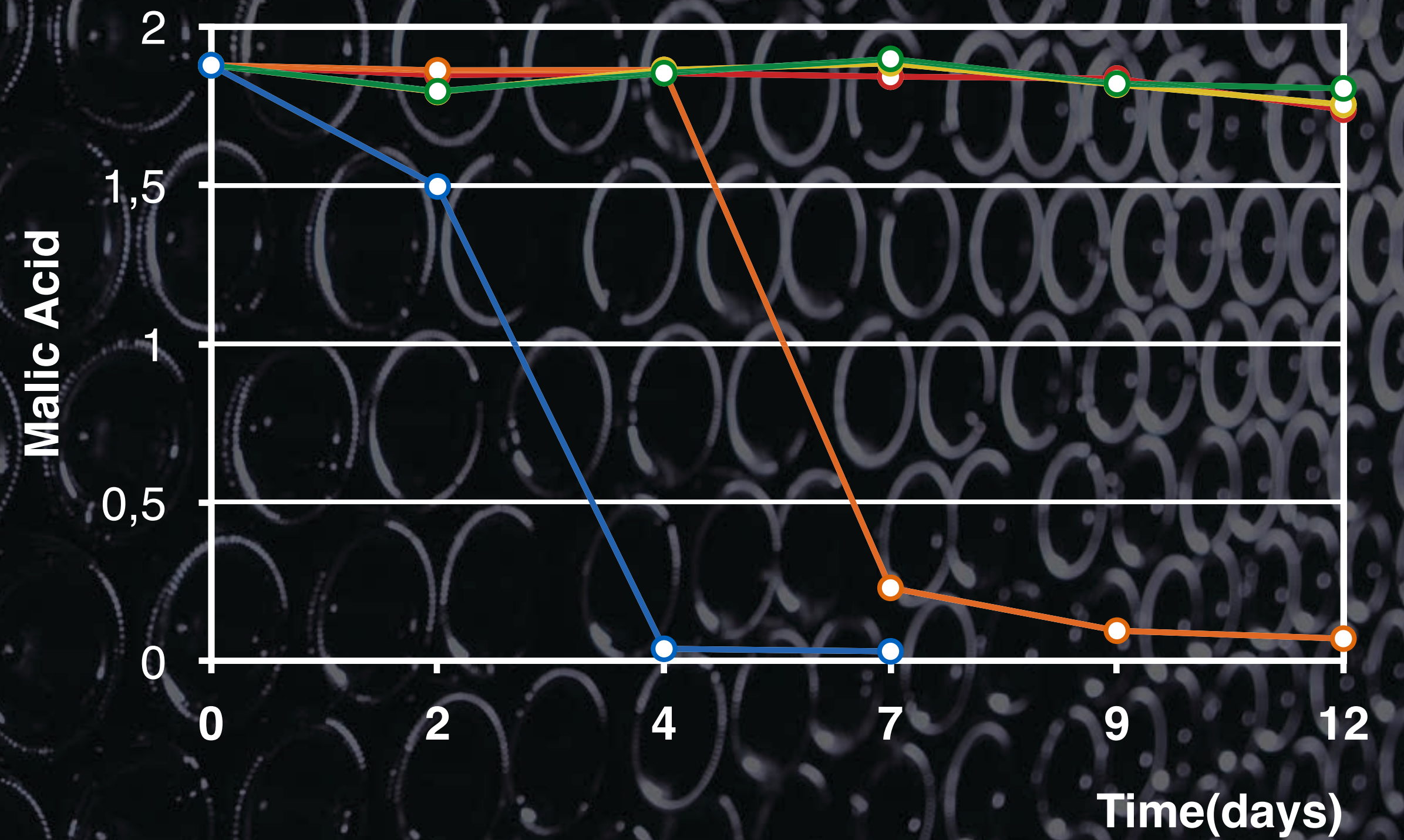
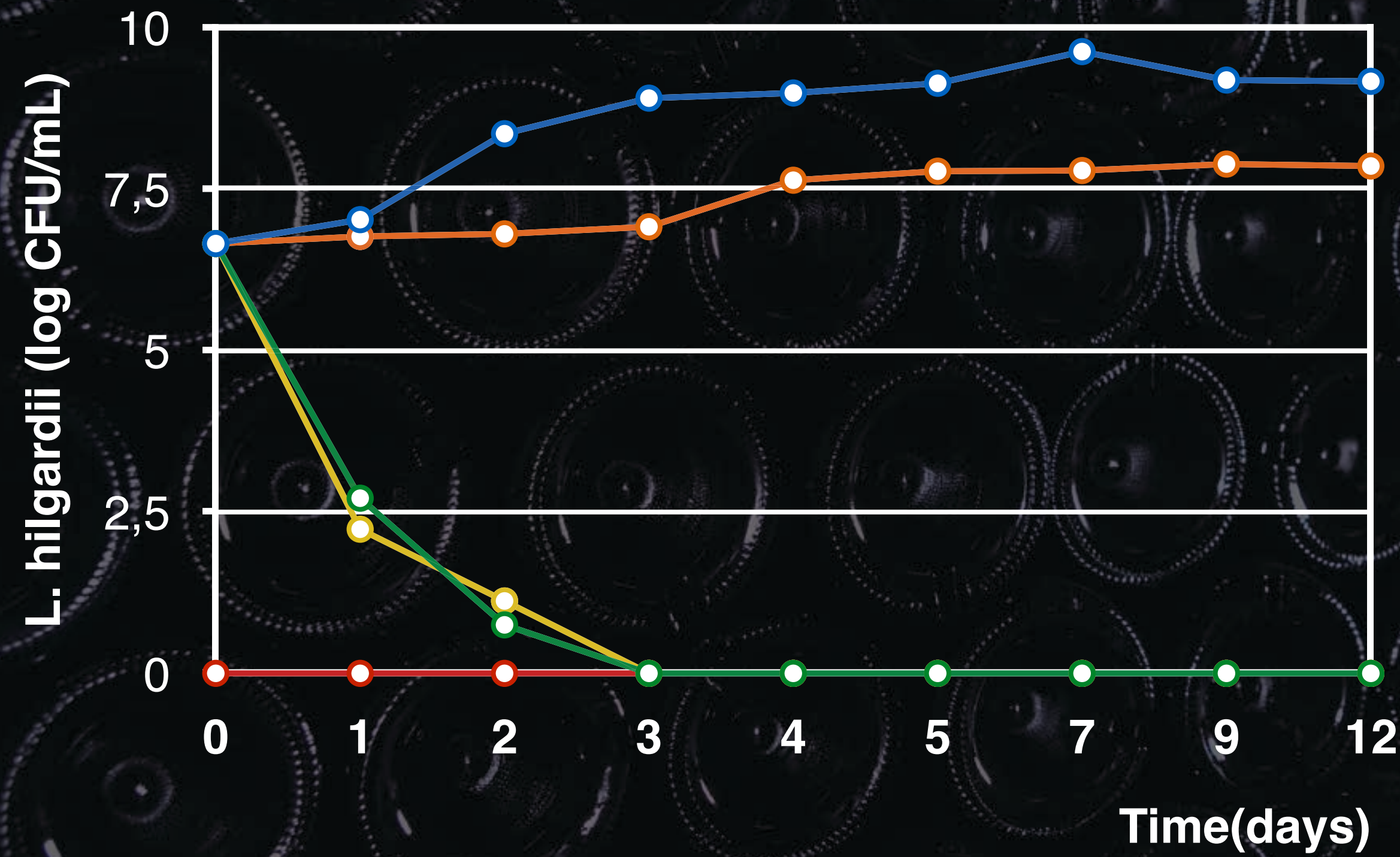


SENSITIVITY OF MLF CULTURES TO LYSOVIN

Chart title



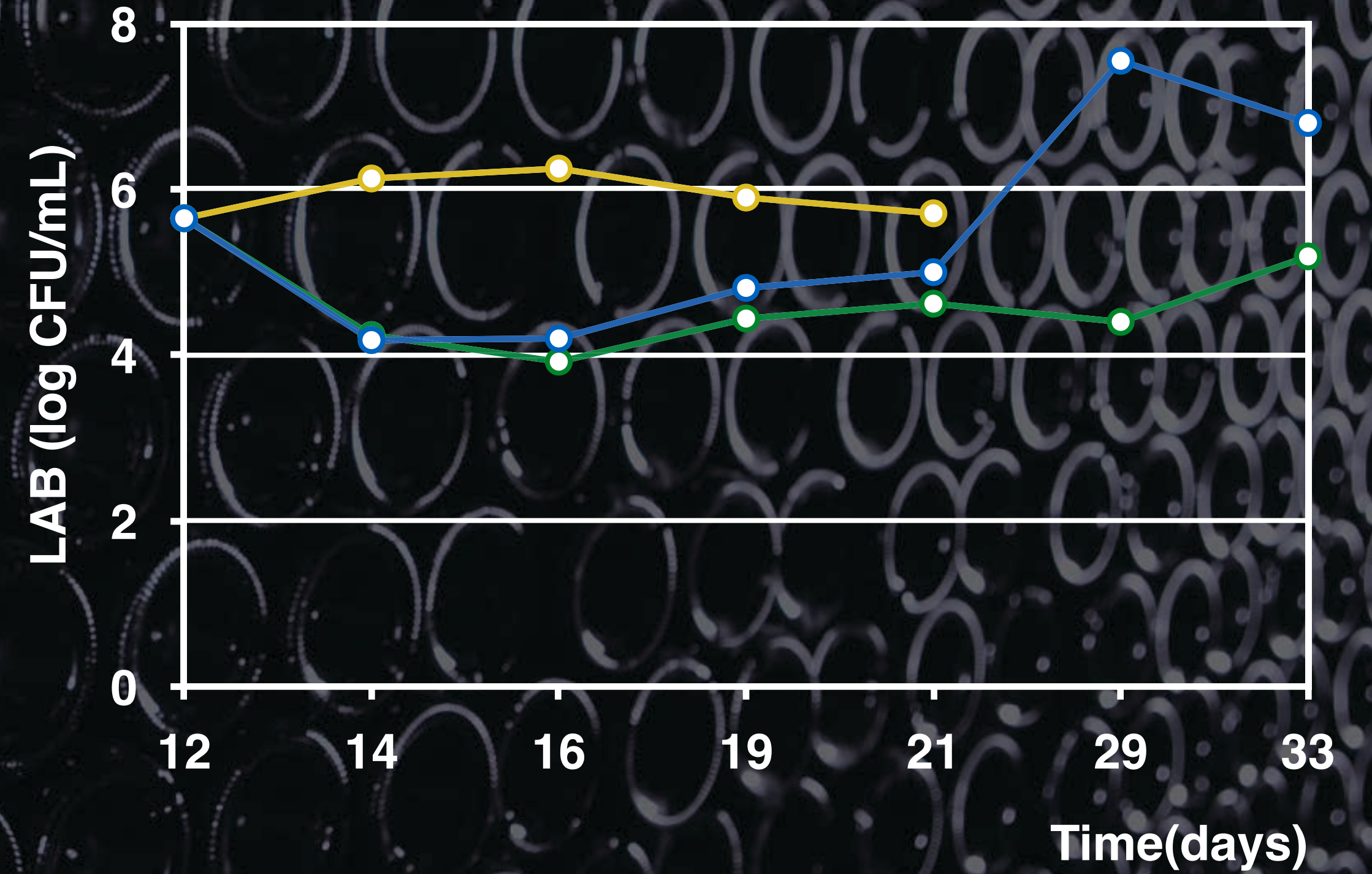
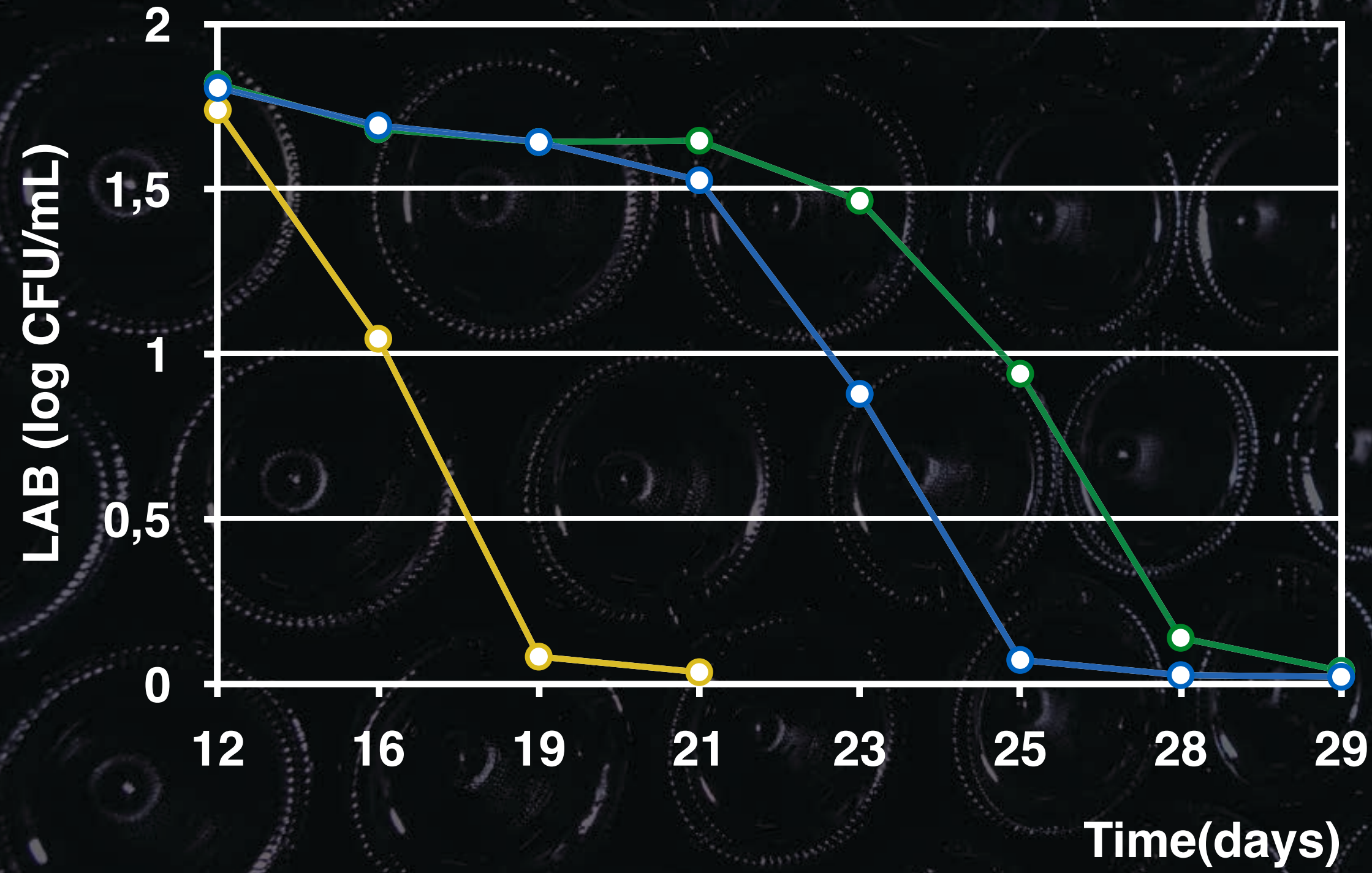
LYSOVIN PREVENTS UNDESIRABLE MLF



● Control
 ● 125ppm LYSOVIN
 ● 250ppm LYSOVIN
 ● 50ppm SO2
 ● No LAB



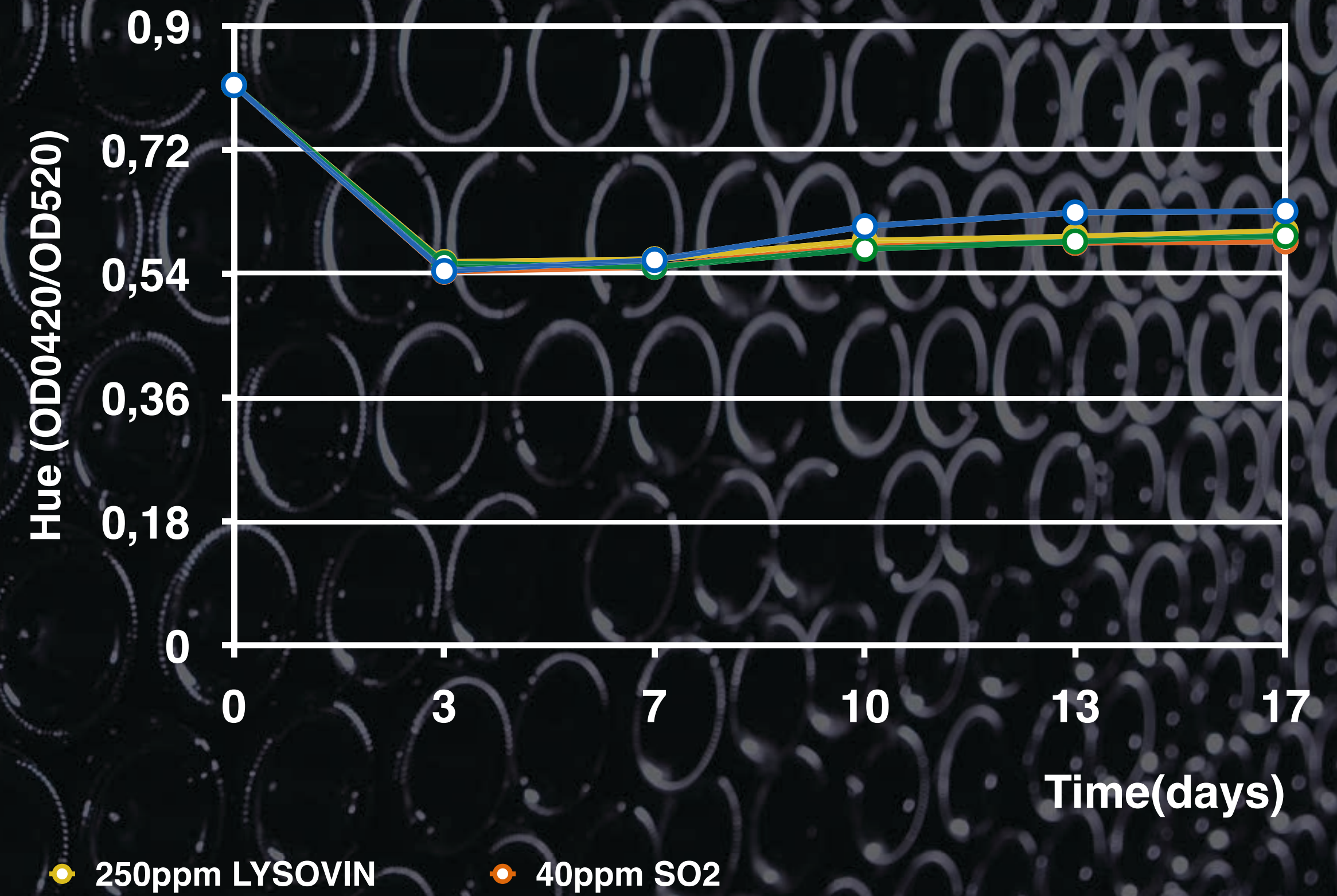
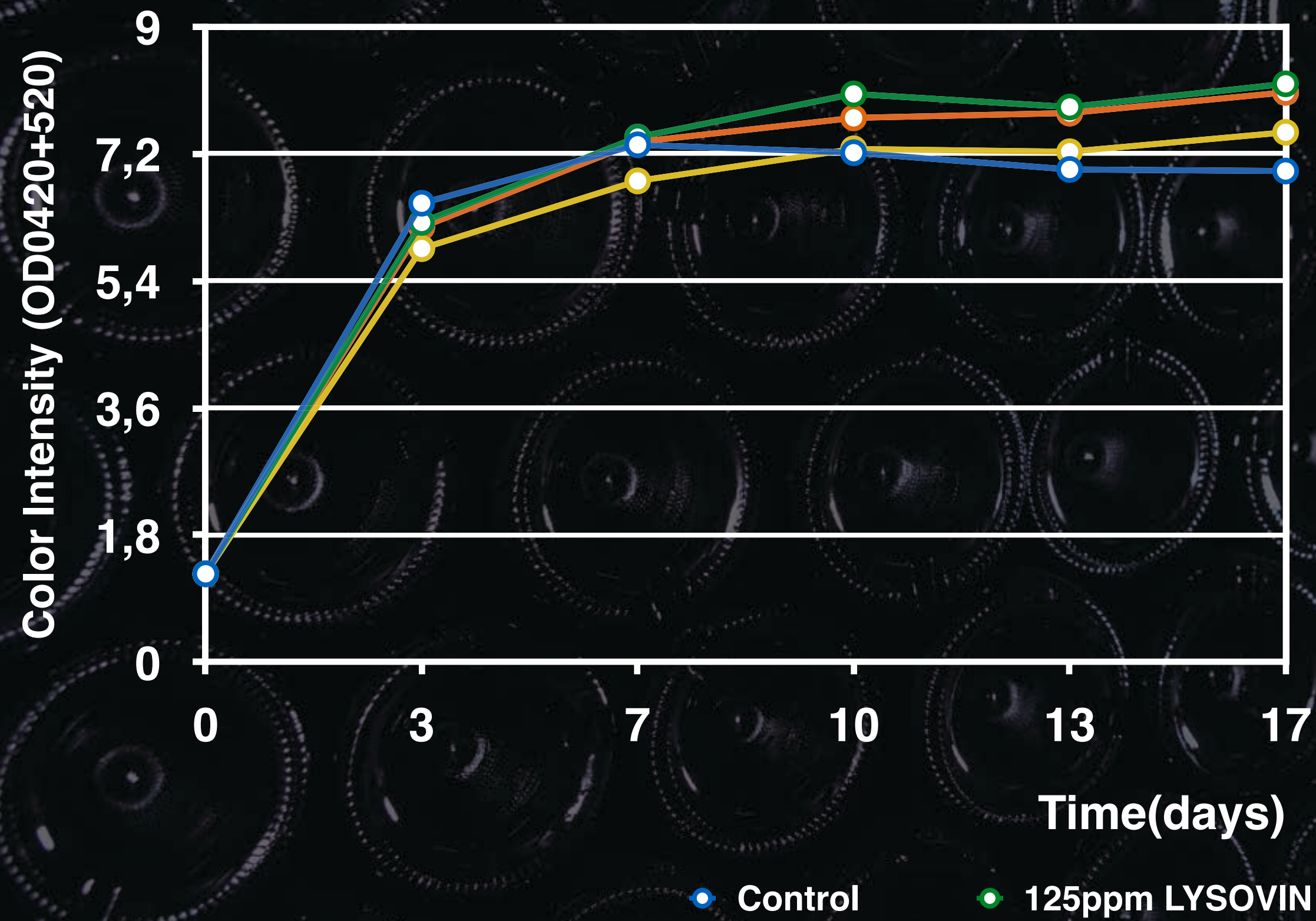
LYSOVIN ALLOWS THE COMPLETION OF DESIRABLE MLF



● 125ppm LYSOVIN ● 250ppm LYSOVIN ● No LAB



LYSOVIN AND WINE COLOR



LYSOVIN - ADVANTAGES FOR WINEMAKERS

- Controls wine spoilage
- Inhibit growth of LAB
- Prevents the production of acetic acid
- Helps manage stuck/sluggish fermentation
- Controls the production of compounds causing organoleptic defects
- Preserves the intensity of the color
- Improves the stability and shelf life of wine
- Reduces or delays use of SO₂
- Avoids production of biogenic amines
- Optimize desirable MLF levels
- Does not interfere with fermentation yeast

CERTIFICATIONS

Certified GRAS by the FDA.

Approve for use in Organic foods.

Certified Kosher and Halal.

Accepted Whole Foods ingredient.

Classified as food by JECFA-Joint FAO/WHO expert Committee on Food Additives.



JECFA



Our extraction methods are the only ones validated for their capability to inactivate the Avian Influenza virus (Texcell-Pasteur Institute).



LYSOVIN INFORMATION & DOCUMENTS

DOCUMENT	1	2	3
SAFETY DATA SHEET	✓	✓	✓
TECHNICAL PRODUCT DATA SHEET	✓	✓	✓
DECLARATION OF ALLERGEN STATUS	✓	✓	✓
DECLARATION ON GMO STATUS	✓	✓	✓
KOSHER CERTIFICATES	✓	✓	✓
DECLARATION OF IONISING TREATMENT			✓
DECLARATION ON USE OF NANOTECHNOLOGY/NANOPARTICLES AND OR BIOSOLIDS			✓
CERTIFICATE/DECLARATION OF ANIMAL ORIGIN STATUS			✓



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