



SafOEno™ SPK 05
“Prise de mousse”
with a shortened
1-step yeast starter
preparation

Content

- 01** Introduction

- 02** Selection of Safœno™ SPK 05

- 03** Safœno™ SPK 05's performance during PDM

- 04** Safœno™ SPK 05 key characteristics

- 05** What happens during the starter preparation?

- 06** “Prise de mousse” with a shortened 1-step yeast starter preparation

- 07** Technical comparison of two protocols

01 | Introduction



Introduction

Pied de cuve

The “Prise de mousse (PDM)” or secondary fermentation is the step that allows a base wine to become sparkling or effervescent. It requires the **preparation of a yeast starter** also known as “**pied de cuve (PDC)**”.

Preparation time

The quality of the PDC is therefore essential to a successful PDM.

Traditionally the PDC is prepared in **three days** but this study showed that the preparation time can be reduced by **half (36h)** and to a **single step**.

Protocol

This work is based on the **optimization of the existing protocol** by adjusting the sugar and assimilable nitrogen contents.

02

Selection of Safœno™ SPK 05



Multiple partnerships



Fermentis yeast collection

Isolation and first screenings/microvinifications



Lesaffre industrial division

Dry yeast production through the E2U™ process



Microvinifications with other partners

-IFV (Institut Français de la Vigne et du Vin) - France

- University of Reims Champagne-Ardenne - France



Selection through microvinifications

Evaluation of SafOeno™ SPK 05 on white must:
Blend of Pinot Noir and Pinot Meunier - Champagne

Partners: IFV and University of Reims

Must analysis

Turbidity (NTU)	51.7
Total acidity (g/L H ₂ SO ₄)	6.4
Titration acidity (g/L tartaric acid)	9,8
pH	3.06
Sugars (g/L)	178.8
Malic acid (g/L)	5.5
Density at 20 °C (g/cm ³)	1.0766
Potential alcohol (% vol)	10.63
Tartaric acid (g/L)	6.7
Assimilable nitrogen (mg/L)	259
Ammoniacal nitrogen (mg/L)	136
Alpha amino nitrogen (mg/L)	123



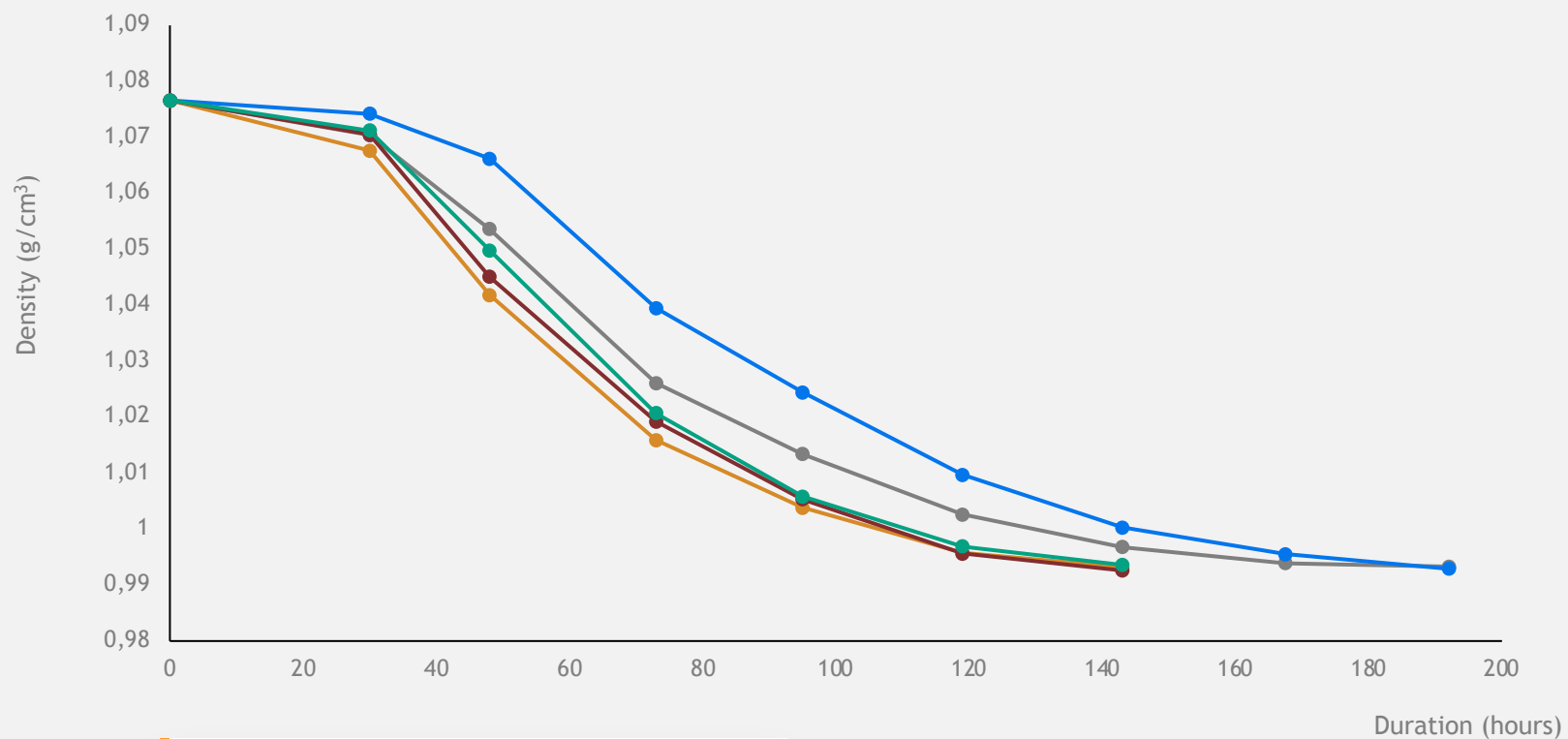
Yeast inoculation
rate:

20 g/hL
1.7 lb/1000 gal

ALF temperature:

18 °C
(64.4 °F)

Fermentation Kinetics



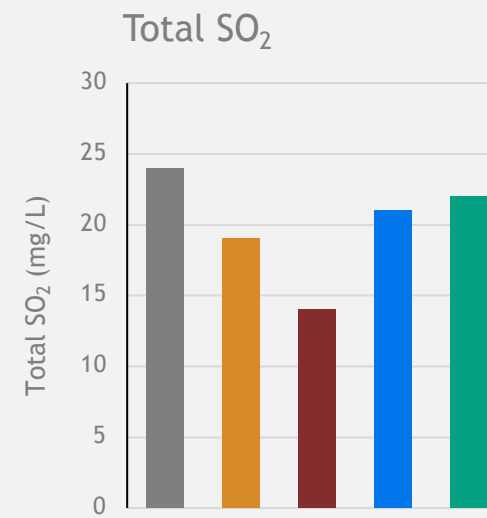
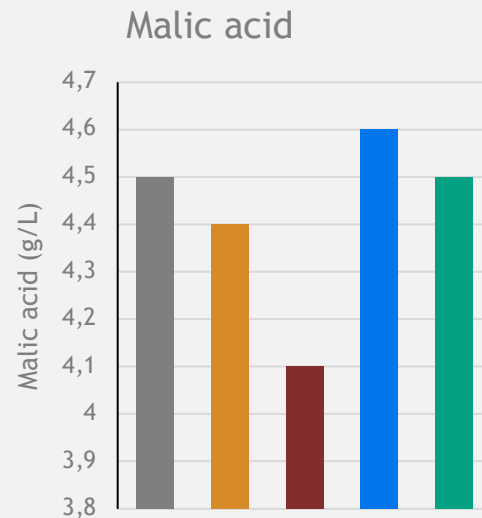
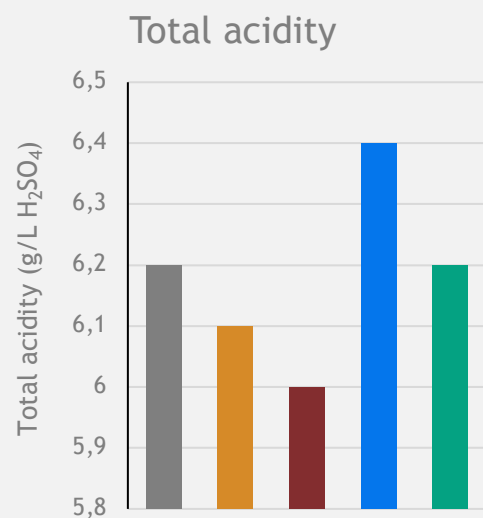
SafOEno™ SPK 05
shows a fast and regular kinetics

Analytical parameters end ALF

	Reference 1	Reference 2	SafCEno™ SPK 05	Reference 3	Reference 4
Alcohol (%Vol)	10.84	10.91	10.91	10.88	10.89
Total acidity (g/L H ₂ SO ₄)	6.2	6.1	6	6.4	6.2
Titrate acidity (g/L tartaric acid)	9.5	9.3	9.2	9.8	9.5
pH	2.96	2.96	2.97	2.94	2.95
Volatile acidity (g/L H ₂ SO ₄)	0.17	0.16	0.15	0.17	0.18
Volatile acidity (g/L acetic acid)	0.20	0.19	0.18	0.20	0.21
Glucose + fructose (g/L)	0.3	0.2	0.1	<LQ (0.1)	0.3
Total SO ₂ (mg/L)	24	19	14	21	22
Free SO ₂ (mg/L)	6	6	6	6	6
Malic acid (g/L)	4.5	4.4	4.1	4.6	4.5
Tartaric acid (g/L)	4	3.9	3.8	3.9	3.8
Calcium (mg/L)	94	92	92	91	94
Ammoniacal nitrogen (mg/L)	2	3	3	2	3
Alpha amino nitrogen (mg/L)	20	20	22	19	20

For most of the oenological parameters, **SafCEno™ SPK 05** behaves similarly to the reference strains but differences appear for malic acid consumption and the SO₂ production.

Analytical parameters end ALF



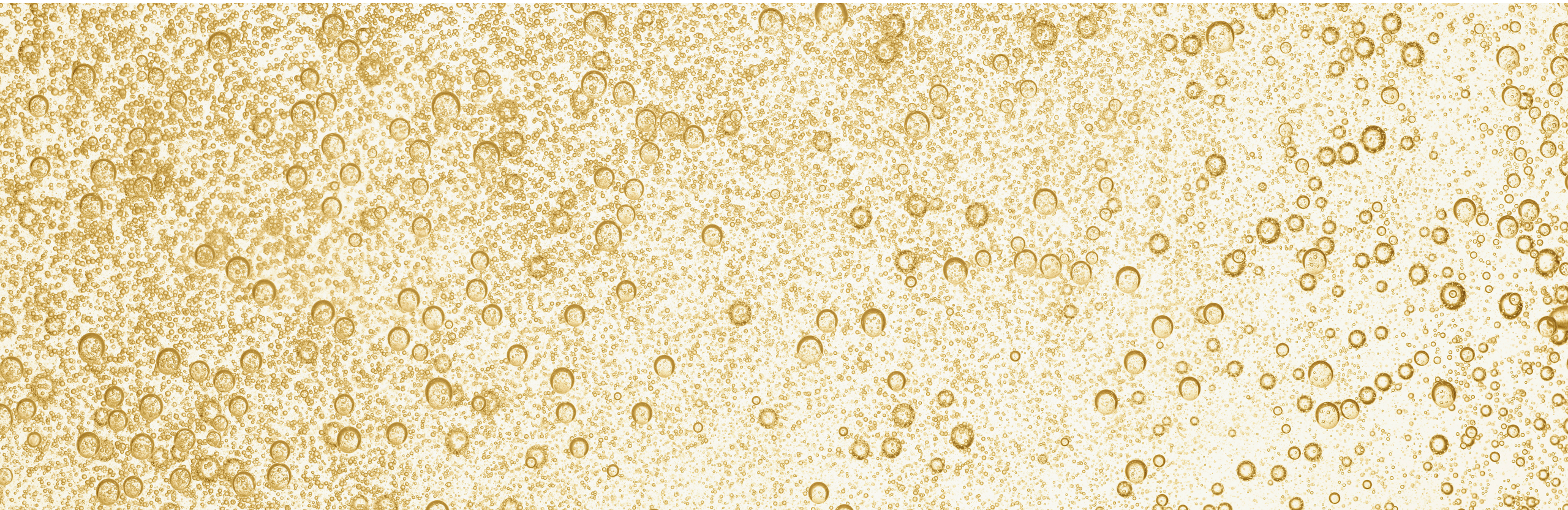
- Reference 1
- Reference 2
- SafOeno™ SPK 05
- Reference 3
- Reference 4

Higher malic acid consumption (9%) compared to the reference strains leading to a lower total acidity level.

Lower production of SO₂ (35%) compared to the reference strains.

03

SafOEno™ SPK 05's performance
during PDM



Selection through microvinifications

Evaluation of SafŒno™ SPK 05 on white must:
Pinot Meunier - Champagne

Partners: IFV and University of Reims

Must analysis

Total acidity (g/L H ₂ SO ₄)	6.5
Titrate acidity (g/L tartaric acid)	9,9
pH	3.03
Sugars (g/L)	186
Malic acid (g/L)	5.4
Potassium (mg/L)	1612
Density at 20 °C (g/cm ³)	1.0794
Potential alcohol (% vol)	11.05
Tartaric acid (g/L)	7.8
Assimilable nitrogen (mg/L)	271
Ammoniacal nitrogen (mg/L)	143
Alpha amino nitrogen (mg/L)	127



Yeast inoculation
rate:

20 g/hL
1.7 lb/1000 gal

ALF temperature:

18 °C
(64.4 °F)

Analytical parameters end ALF

	Reference 2	SafCEno™ SPK 05	Reference 4
Alcohol (%Vol)	11.02	11.1	11.04
Total acidity (g/L H ₂ SO ₄)	5.9	5.7	6
Titration acidity (g/L tartaric acid)	9.0	8.7	9.1
pH	2.99	3	2.95
Volatile acidity (g/L H ₂ SO ₄)	0.17	0.14	0.18
Volatile acidity (g/L acetic acid)	0.20	0.17	0.21
Glucose + fructose (g/L)	1.2	0.7	0.8
Total SO ₂ (mg/L)	98	85	92
Free SO ₂ (mg/L)	43	45	46
Malic acid (g/L)	4.3	4.1	4.5
Tartaric acid (g/L)	3.8	3.8	3.8
Ammoniacal nitrogen (mg/L)	3	4	3
Alpha amino nitrogen (mg/L)	29	38	20

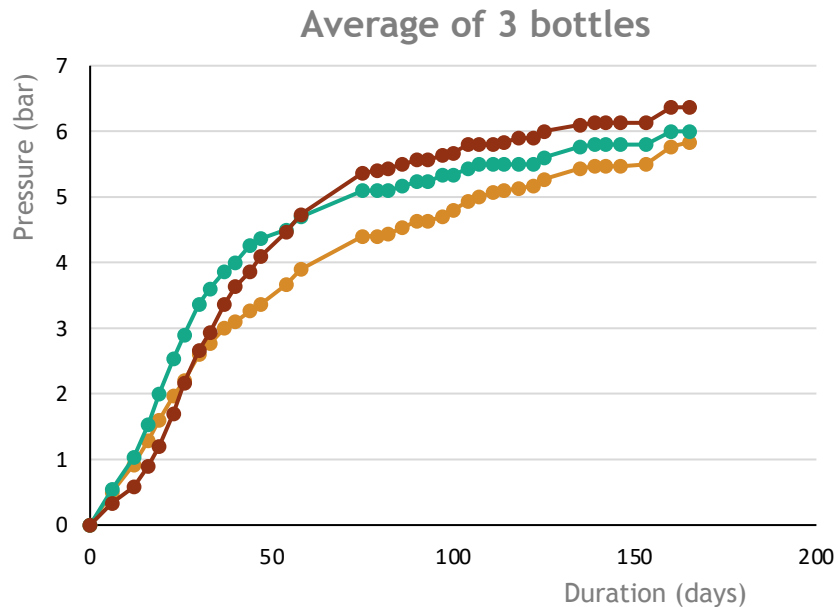
Higher malic acid consumption (7%) compared to the reference strains leading to a lower total acidity level.

Lower production of SO₂ (11%) compared to the reference strains.

No significant differences in the duration of ALF.

Secondary fermentation or « Prise de mousse »

Pressure monitoring with aphrometers



● Reference 2 ● Reference 4 ● SafCEno™ SPK 05

Analytical parameters end PDM

	Reference 2	SafCEno™ SPK 05	Reference 4
Alcohol (%Vol)	12.18	12.24	12.19
Total acidity (g/L H ₂ SO ₄)	4.1	4.2	4.4
Titrate acidity (g/L tartaric acid)	6.2	6.4	6.7
pH	3.09	3.19	3.18
Volatile acidity (g/L H ₂ SO ₄)	0.22	0.19	0.25
Volatile acidity (g/L acetic acid)	0.26	0.23	0.30
Glucose + fructose (g/L)	3.6	1.7	1.1
Total SO ₂ (mg/L)	26	33	47
Malic acid (g/L)	<0.2	<0.2	<0.2
Tartaric acid (g/L)	2.9	3.7	3.5

SafCEno™ SPK 05 shows excellent fermentation kinetics during the PDM, reaching 6 bars of pressure faster than reference yeast 4. In these conditions, reference yeast 2 does not consume all the sugars.

Descriptive tasting results after 13 months post PDM

SafOeno™ SPK 05

Fine bubbles, very nice light foam, straw yellow - **Fresh and elegant nose** - yellow with green reflections - very nice foam - the mouth seems slightly sweet - effervescence in the mouth a little discreet. - **good length, persistent fruitiness** - sweetness - Balanced finish, no fermentative notes.

Reference 2

Finer but important foam - Effervescence in the mouth very present, drying, a little bitter - **Nose rather discreet** but clear. **Less fruity perception in the taste.** Slightly evolved yellow color – **Balanced** wine, satisfactory overall but **short aromatic length.**

Reference 4

Foam : fine bubbles, discreet collar despite the effervescence – **Discreet nose:** more freshness, **less aromas** – Mouth: good balance, good length, **more sweetness**, more aromatic freshness. Very nice balance and length in the mouth, **more freshness.** – Good global balance.

Taking into consideration the lower fermentation performance during the PDM and the tasting notes, Reference 2 is excluded from the study.

Selection through microvinifications

Evaluation of SafOeno™ SPK 05 on white must:
Pinot Meunier - Champagne

Partners: IFV and University of Reims

Wine analysis after ALF

Alcohol (%Vol)	10.79
Total acidity (g/L H ₂ SO ₄)	5.7
Titration acidity (g/L tartaric acid)	8.7
pH	3.03
Volatile acidity (g/L H ₂ SO ₄)	0.15
Volatile acidity (g/L acetic acid)	0.18
Glucose + fructose (g/L)	0.3
Total SO ₂ (mg/L)	34
Free SO ₂ (mg/L)	6
Malic acid (g/L)	4.3
Tartaric acid (g/L)	3.8
Ammoniacal nitrogen (mg/L)	3
Alpha amino nitrogen (mg/L)	31



Yeast inoculation
rate:

20 g/hL
1.7 lb/1000 gal

ALF temperature:

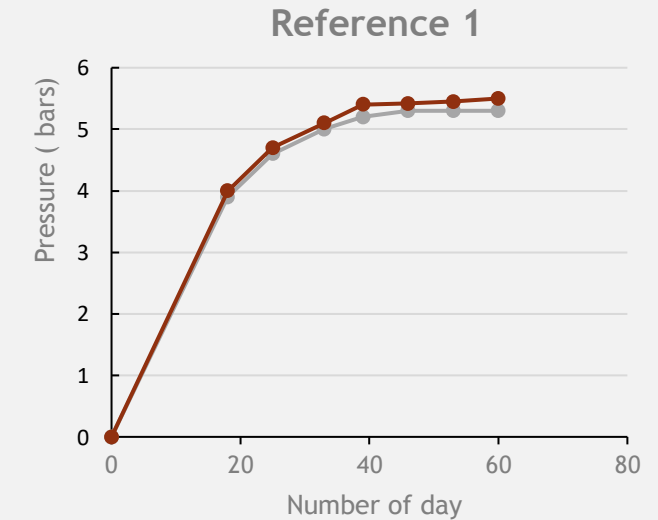
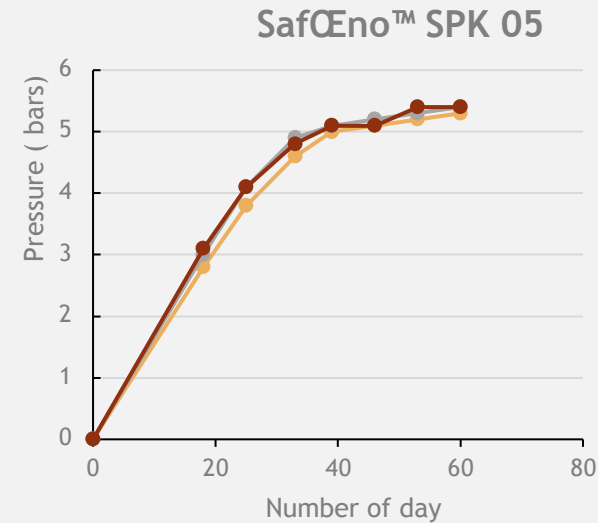
18 °C
(64.4 °F)

Secondary fermentation or « Prise de mousse »



Pressure monitoring with aphrometers

Excellent fermentation performance for Safœno™ SPK 05 during the PDM.



● Bottle A ● Bottle B ● Bottle C

04

SafOEno™ SPK 05 key characteristics



Key characteristics

produces clean and mineral sparkling wines

Thanks to its **great fermentation abilities and aromatic features**, SafŒno™ SPK 05 produces **white wines with clean and fresh profiles**.

For the secondary fermentation (Prise de mousse), SafŒno™ SPK 05 **shows fast and regular kinetics** and allows to quickly produce elegant, fresh and clean sparkling wines.

Technical characteristics

Fermentation abilities

High implantation strength

Recommended maximum alcohol:
up to **14% v/v**

Medium lag phase

Recommended temperature range:
12-20 °C (50-86 °F)

Fast and regular fermentation kinetics (first fermentation and “Prise de Mousse”)

Low to medium YAN requirements
YAN/S = 0.7-0.8

High resistance to **low pH (2.8)**



Metabolic characteristics

Low volatile acidity production

Medium malic acid consumption (25%)

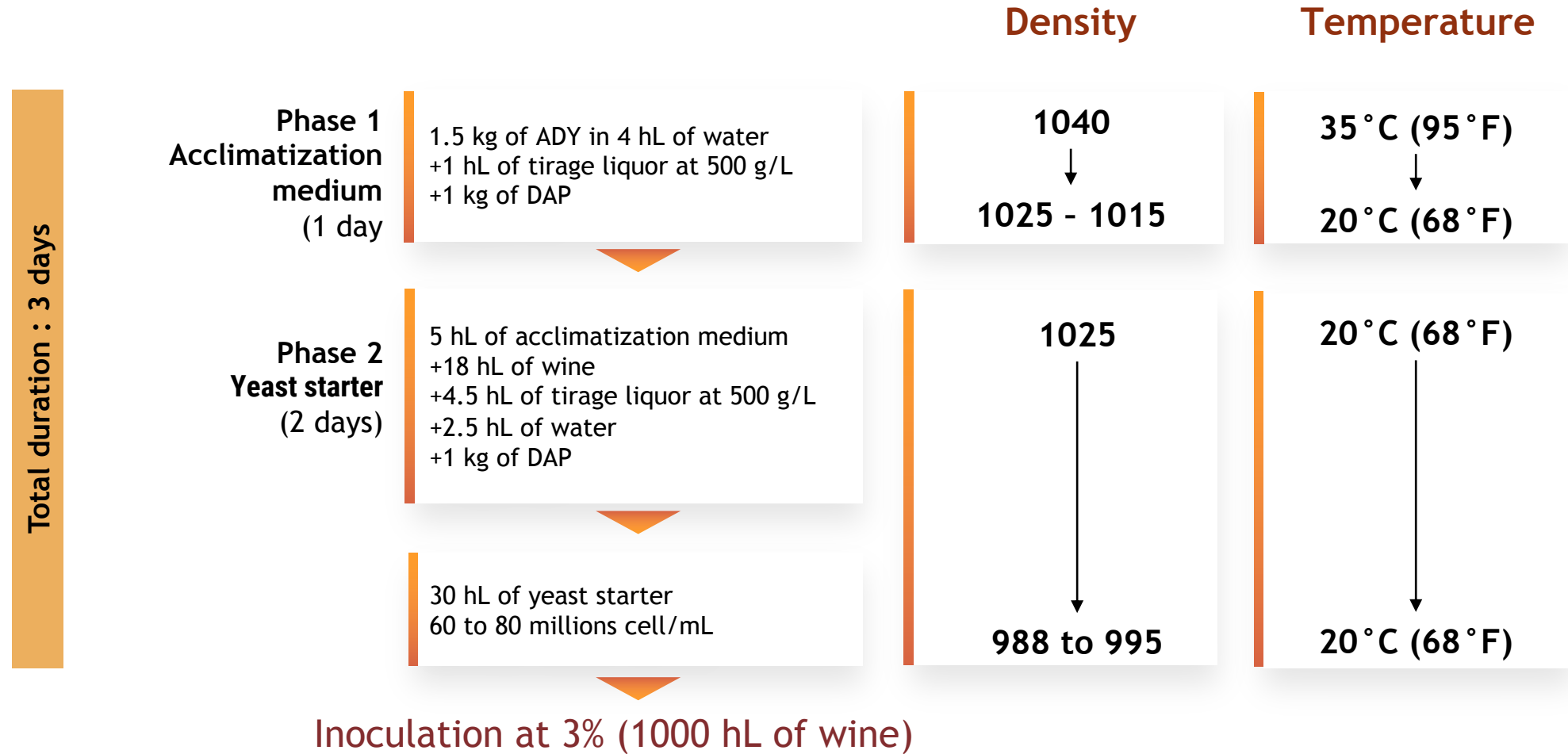
High SO₂ resistance (between 50 and 75 mg/L)

Very low production of SO₂ and H₂S.

05 | What happens during the starter preparation?



Traditional Champagne protocol for yeast starter preparation



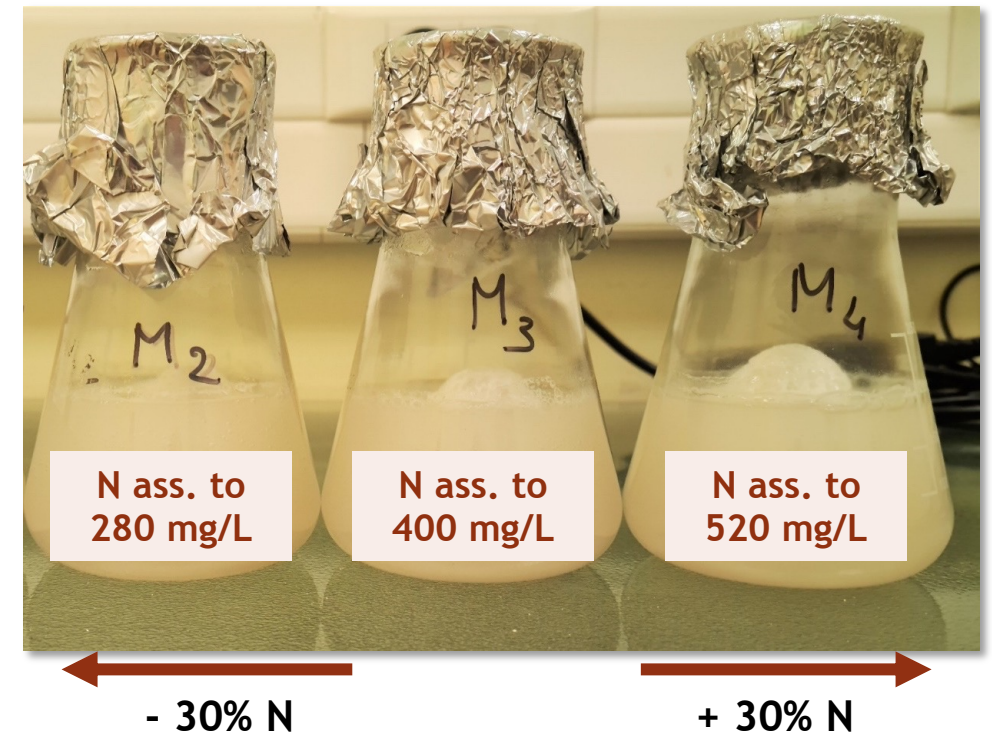
The acclimatization phase during the yeast starter preparation

Technical objective: to study the consumption of nitrogen and sugars by SafCEno™ SPK 05 during phase 1.

Phase 1 Acclimatization medium (1 day)

1.5 kg of ADY in 4 hL of water
+1 hL of tirage liquor at 500 g/L
+1 kg of DAP

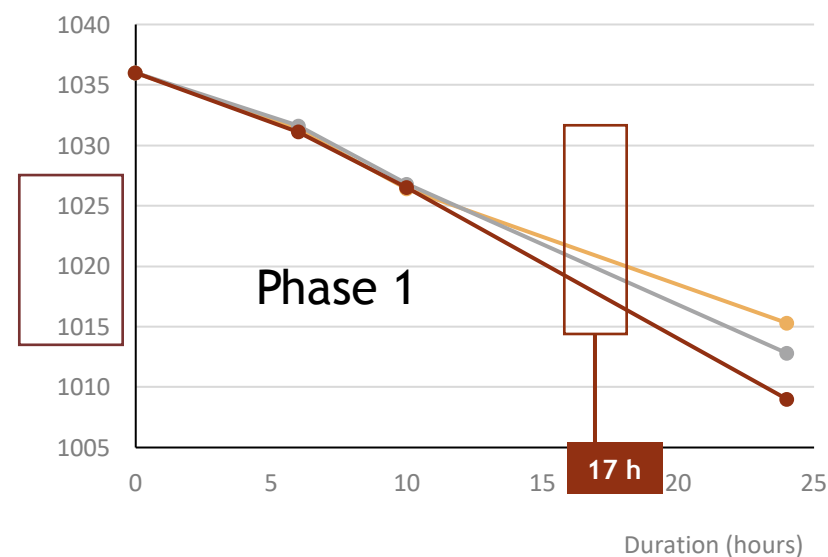
Assimilable nitrogen provided during the phase 1:
1 kg of DAP in 5 hL corresponding to 400 mg/L of nitrogen



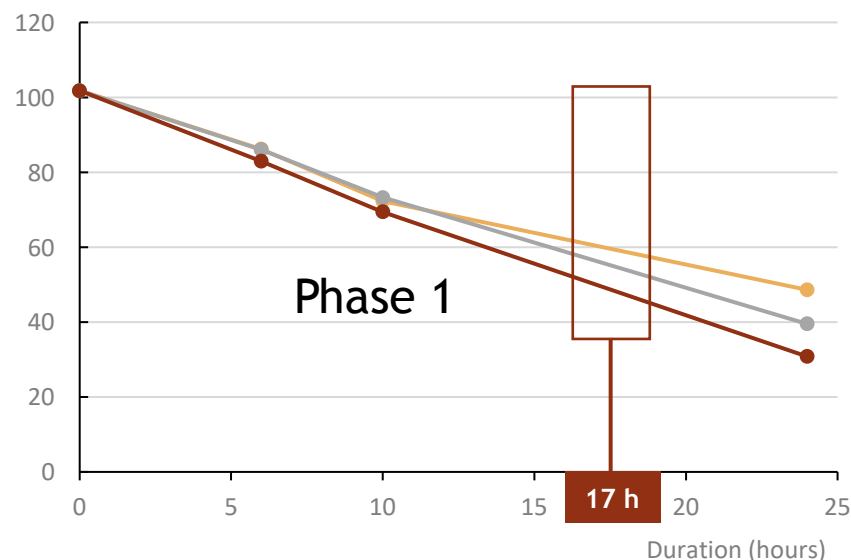
The acclimatization phase during the yeast starter preparation

Effect of the dose of ammoniacal nitrogen on ALF achievement

Density (g/cm³)



Sugars (g/L)



- SafOEno™ SPK 05 N280
- SafOEno™ SPK 05 N400
- SafOEno™ SPK 05 N520

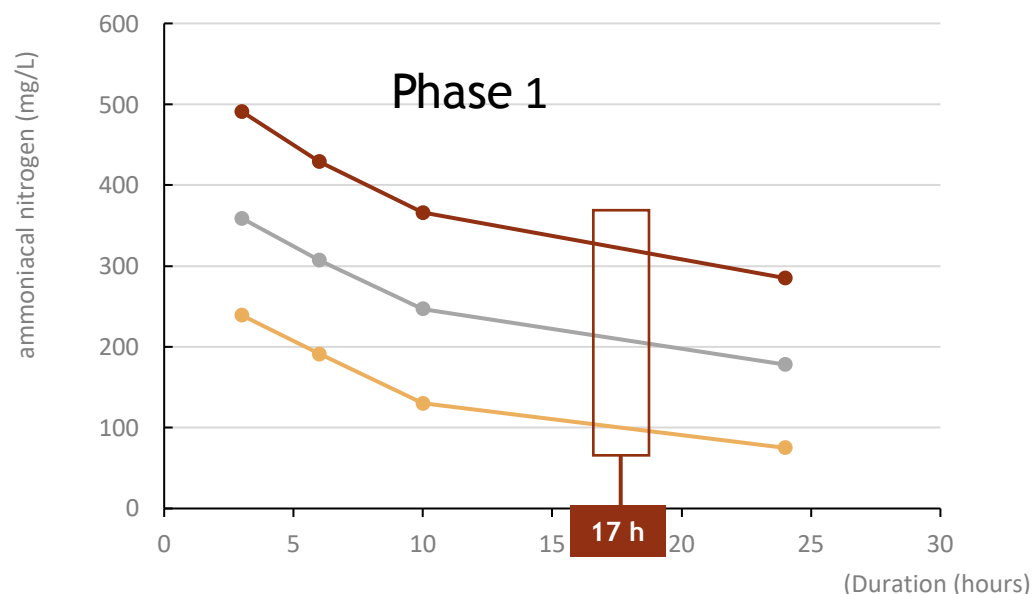
Consumption and sugar content after 17h (Phase 1 end) for N400

- Sugars consumed: 43 g/L
- Remaining sugars: 57 g/L

SafOEno™ SPK 05 consumes approximatively 50% of the sugars initially present.

The acclimatization phase during the yeast starter preparation

Consumption of assimilable nitrogen (provided by DAP) during the phase 1



- SafOEno™ SPK 05 N280
- SafOEno™ SPK 05 N400
- SafOEno™ SPK 05 N520

Consumption and content of N assimilable after 17 h (Phase 1 end) for N400

- N assimilable consumed: 193 mg/L
- Remaining N assimilable: 207 mg/L

= SafOEno™ SPK 05 consumes approximately 50% of the N assimilable initially present.

No nitrogen deficiency for any of the 3 dosages of DAP.

Low needs for nitrogen of SafOEno™ SPK 05 compared to the usual quantity recommended in traditional protocol.

06 | “Prise De Mousse” with a shortened 1-step yeast starter preparation



1-step yeast starter preparation protocol


Phase 1
Acclimatization medium
(1 day)

1.5 kg of ADY in 4 hL of water
+1 hL of tirage liquor at 500 g/L
+1 kg of DAP

Phase 2
Yeast starter
(2 days)

5 hL of acclimatization medium
+18 hL of wine
+4.5 hL of tirage liquor at 500 g/L
+2.5 hL of water
+1 kg of DAP

Taking into consideration :

- Safœno™ SPK 05's great fermentation abilities during the first alcoholic fermentation and the PDM.
- Safœno™ SPK 05's low needs of nitrogen compared to the initial amount recommended by the classical Champagne protocol.
- The interest of reducing tank turnover time.
- The importance of limiting energy costs.

= Idea to shorten the preparation time of the starter protocol

1-step yeast starter preparation protocol

Protocol with two phases (3 days)

Phase 1 Acclimatization medium (1 day)

1.5 kg of ADY in 4 hL of water
+1 hL of tirage liquor at 500 g/L
+1 kg of DAP

Phase 2 Yeast starter (2 days)

5 hL of acclimatization medium
+18 hL of wine
+4.5 hL of tirage liquor at 500 g/L
+2.5 hL of water
+1 kg of DAP

30 hL of yeast starter
60 to 80 millions cell/mL

Inoculation at 3% (1000 hL of wine)

Protocol 1-step (36h)

NO PHASE 1

25 hL of wine
+1,5 kg of SafCEno™ SPK 05
+5 hL of tirage liquor at 500 g/L (83 g/L of sugar)
+1,5 kg of DAP (100 mg/L N ass)

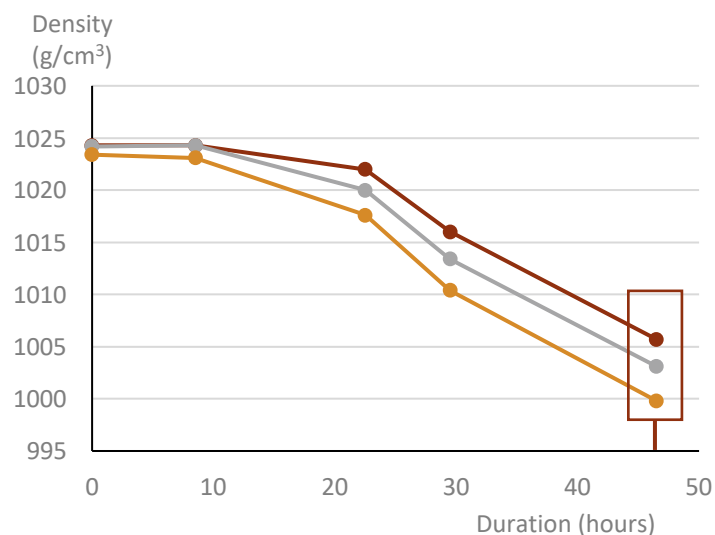
30 hL of yeast starter
60 to 80 millions cell/mL

Inoculation at 3% (1000 hL of wine)

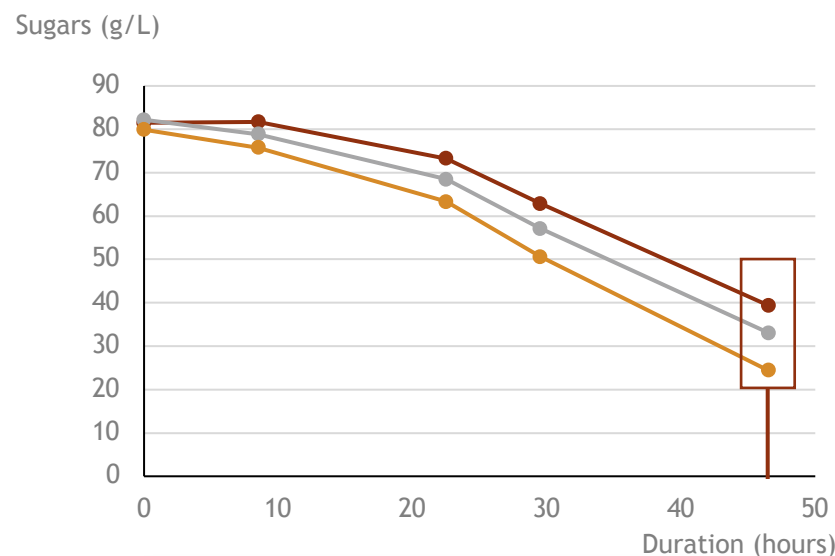
1-step yeast starter preparation protocol

Technical objective: to study the consumption of nitrogen and sugars by SafCEno™ SPK 05 during phase 2.

Effect of the dose of ammoniacal nitrogen on ALF achievement



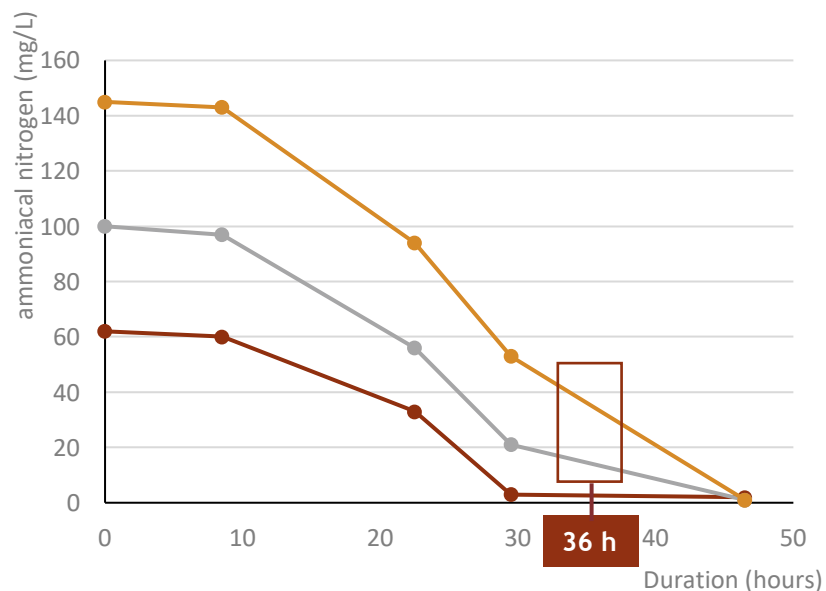
After 48h, SafCEno™ SPK 05 continues to ferment (density = 1000).



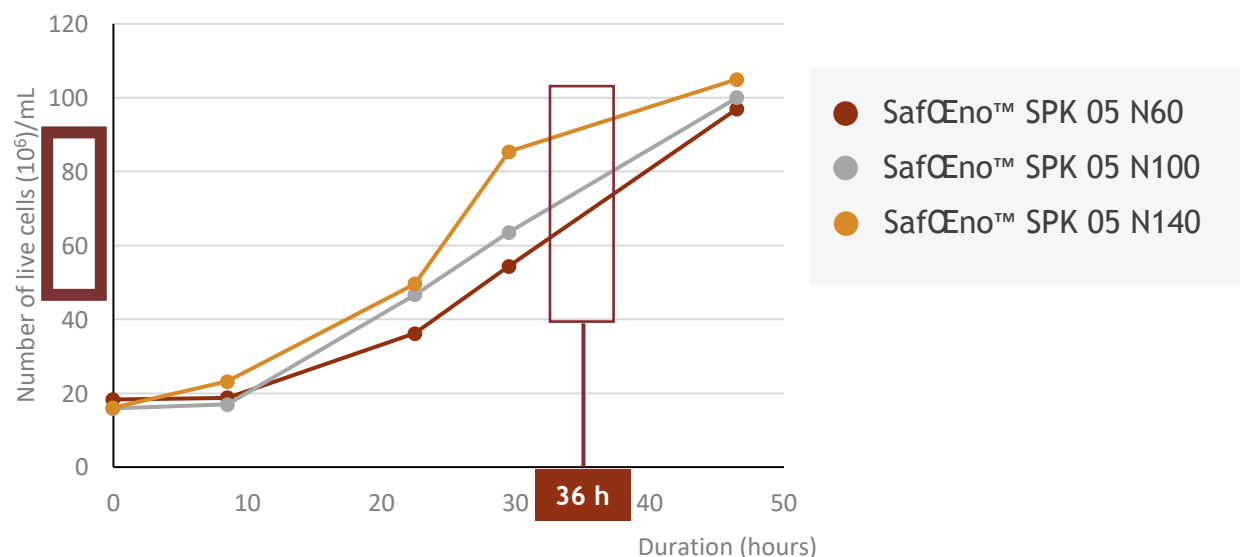
After 48h, SafCEno™ SPK 05 consumes approximately 50% of the sugars initially present.

1-step yeast starter preparation protocol

Technical objective: to study the consumption of nitrogen and sugars by Safœno™ SPK 05 during phase 2.



After 36h, no nitrogen deficiency for higher DAP concentration.

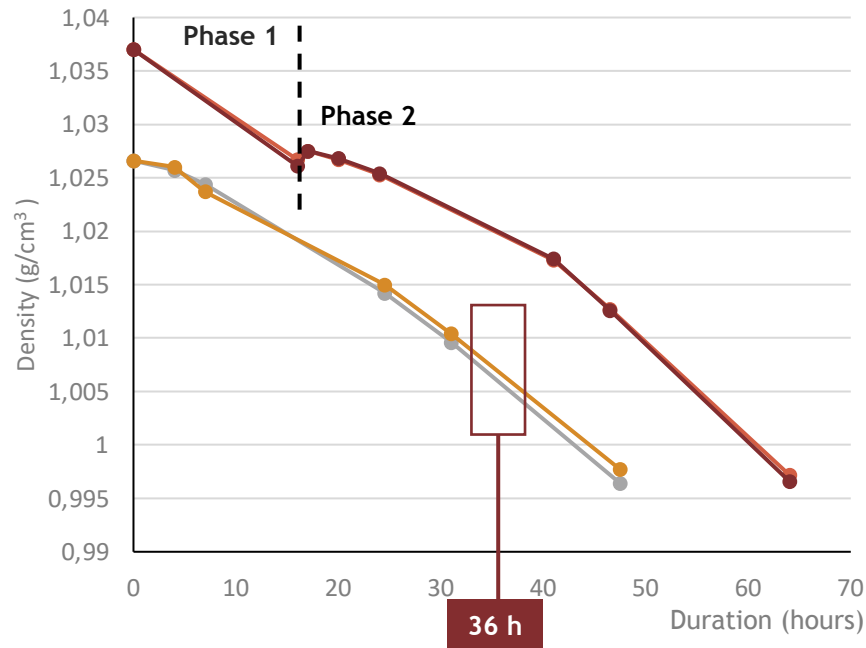


After 36h, the required yeast cell count for tirage is reached.

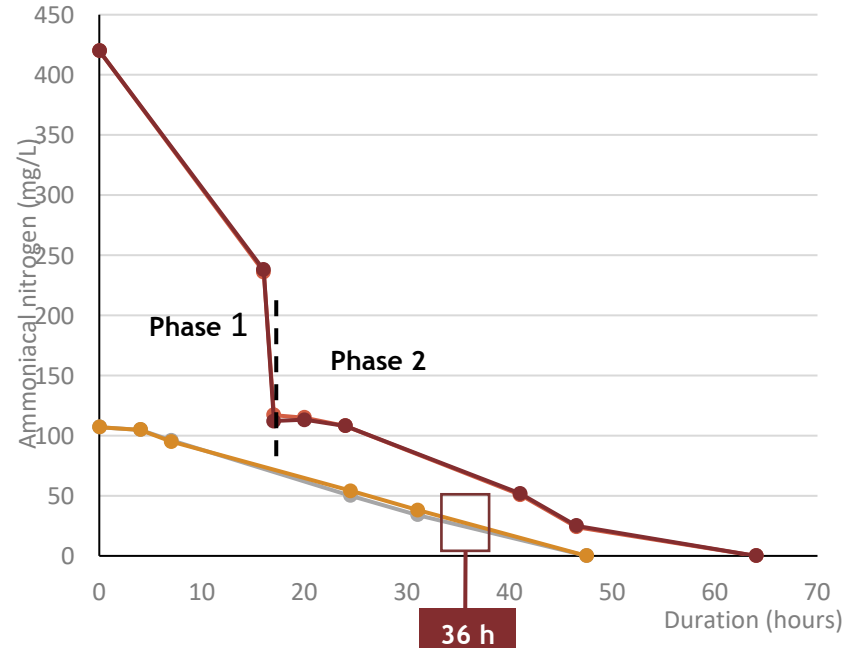
07 | Technical comparison of two protocols



Comparison of the two protocols for SafCEno™ SPK 05



Gain of 17 hours with the one-step protocol.



After 36h, no nitrogen deficiency for SafCEno™ SPK 05 with the protocol 1-step.

A & B : 2 phases protocol

● SafCEno™ SPK 05 A

● SafCEno™ SPK 05 B

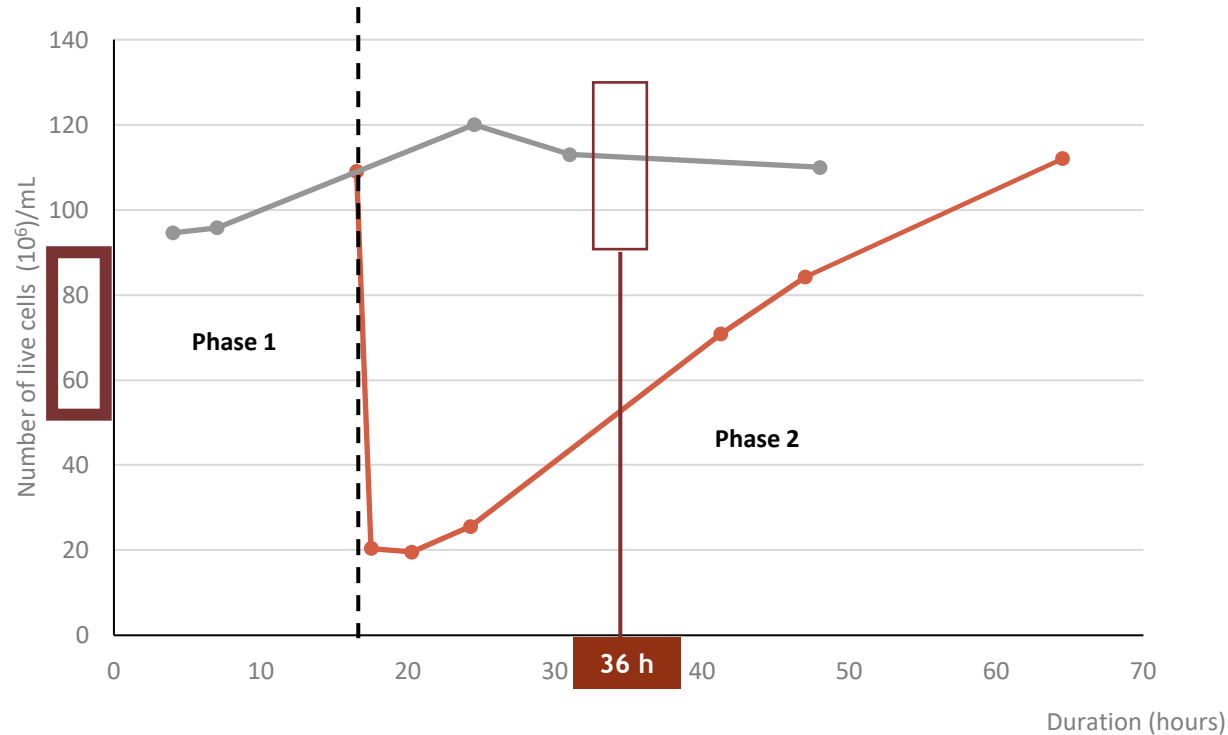
C & D : 1-step protocol

● SafCEno™ SPK 05 C

● SafCEno™ SPK 05 D

Comparison of the two protocols for Safœno™ SPK 05

Goal achieved
before the
inoculation of
the bottles
(tirage).



A : 2 phases protocol

● Safœno™ SPK 05 A

C : 1-step protocol

● Safœno™ SPK 05 C

After 36h, Safœno™ SPK 05 have a cell count superior to 110.10^6 cells/mL

To conclude...



Traditionally the PDC is prepared in three days, but this study showed that the preparation time can be **reduced by half (36h) and to a single step.**



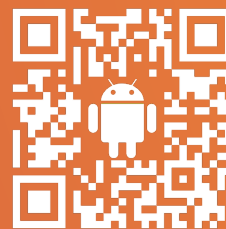
This work is based on the **optimization of the existing protocol** by adjusting the contents of sugars and assimilable nitrogen.



It allows us to propose a new technical solution for the yeast starter preparation able to **perform a PDM in a time equivalent** to the protocol traditionally used.



The tasting results showed **no sensorial differences** between the sparkling wines made from the two “**pied de cuve (PDC)**” preparation methods.



Thank you for
your attention!



www.fermentis.com

