APPLICATION REPORT

Spontaneous perfection!

From spontaneous fermentation to active dry yeast

Author:
Dr. Ilona Schneider, Oenologist

Team Leader Product Management
Beverage Treatment and R&D

Development Engineer –
Food & Beverage

Eaton Technologies GmbH,
Langenlonsheim, Germany

IlonaSchneider@eaton.com

Unique characteristics of grapes, such as types and varieties are important features that have a decisive effect on the quality of wine. Other than the type of grape, the cellar technology and the enological process as a whole, particularly the yeasts involved in fermentation, play important roles. In wine preparation, the selection of yeast strains and the use of active dry yeasts are the entry point for the targeted microbiological control of alcoholic fermentation[1].

In the selection of wine yeast strains, a multitude of different technological and qualitative features must be observed. The important technological features include, a quick start to fermentation, yeast activity during fermentation, the final fermentation level and the formation of alcohol. Qualitative features include the formation of positive aromas and their stable ester bonds, a low level of acetic acid formation (volatile acidity), ethyl acetate and sulfurous substances[1,2].

In the 1980s and 90s, the technological selection features dominated for the most part. With the development of modern oenology, which brought with it changes in the technological processes in the cellar, qualitative selection features are becoming increasingly important.

Selection management

Spontaneous fermentation, the natural microbiological process of alcoholic fermentation by yeasts prevalent in vineyards and the cellar, occurs at the beginning of selection. If this process has a positive outcome, the wine yeast species *Saccharomycetes spp.* will be isolated. The isolated yeasts are removed from the bond with the other fermenting yeasts they have joined with during spontaneous fermentation. Within this bond, yeasts can partially compensate for any weaknesses, such as poor yeast activity. If the isolated yeasts no longer perform as well as they had in bonds, they are not suitable for active dry yeasts. In order to verify their suitability as an active dry yeast, selected Saccharomyces spp. isolates from a spontaneous fermentation are placed in 0.26 gallon (1 liter) flasks with must for fermentation. After micro-scale wine formation (microvinification), the performance of the isolates, can be checked using laboratory and sensory analysis.

If an isolate passes this test, industrial production of the active dry yeast follows. An isolate is cultivated in various intermediary stages using raw materials such as molasses, various nitrogen- and phosphorus-based nutrients, and a multitude of different yeast nutrient supplements. During subsequent propagation in large fermenters, fermentation management is of particular importance. Controlled air intake is just as important as carefully dosed additions of molasses, so that the yeast cells remain in the propagation cycle (aerobic metabolism) and do not transition to alcoholic fermentation (anaerobic metabolism).

This ensures a high rate of biomass formation. The next step, cell harvesting, is done using separators. The resulting 'yeast cream' is concentrated using a vacuum rotary filter and the set yeast mass is led through a sieve pore plate for spray drying. After drying, the active dry yeast is packaged in standardized units for sale.

A successful selection project

In cooperation with the winery Zlatan otok on the Hvar island in Croatia, five *Saccharomyces bayanus* and four *Saccharomyces cerevisiae* isolates were selected from a spontaneous fermentation sample of an autochthonous grape variety (Pošip) and tested after microvinification for technological and qualitative selection criteria, such as the formation of volatile acidity, development of fruit aromas (citrus, gooseberry, exotic fruit notes) and the preservation of the natural acid structure.

Of nine isolates, one isolate (HR selection) of the species Saccharomyces bayanus met the selection criteria (see table 1). Characteristic of HR selection is low acetic acid formation (acetic acid ethyl ester and acetic acid-3-methyl butyl ester) and the marked formation of caproic acid, which provides information about the potential for fruit aroma formation.

After laboratory analysis of the mini-samples, two tastings were performed, each with 11 participants and each repeated three times.

The first tasting was based on the DLG 5 Point Testing Scheme (German Agricultural Society).

In this test, 0 to 6 points were awarded for the senses, whereby 6 points is the best score. The tasting confirmed the results of the analysis. The wines that were formed with HR selection were evaluated as clean-tasting (3.67 points) and acid-preserving (4.5 points) (see fig. 1).

In the second tasting, the following aroma attributes were tested: green pepper, grassy green, honey melon, black currants, exotic fruits, gooseberry and citrus. The HR selection was convincing in the honey melon and exotic fruit attributes. Gooseberries and grassy green were perceived very weakly.

Aroma	HR selection	Selection C2-1	Selection C2-5	Selection C2-10	Selection C2-11
Acetic acid ethyl ester [mg/l]	12	23	25	20	24
l-butanol [mg/l]	44	43	41	35	47
2-methyl-butanol [mg/l]	62	73	76	63	72
3-methyl-butanol [mg/l]	12	12	12	11	14
i-ethyl butyrate [mg/l]	257	228	227	265	215
Ethyl lactate [mg/l]	15	14	11	11	12
Hexanol [µg/l]	1091	774	906	893	951
Acetic acid-3-methyl butyl ester	767	924	1023	1167	753
Acetic acid-2-methyl butyl ester	48	42	46	64	41
Caproic acid [mg/l]	834	538	541	659	489
Acetic acid-hexyl ester	243	282	330	406	277

Table 1: Laboratory analysis of aroma formation of various yeast isolates

The first practice fermentation with HR selection under the trading name SIHA® Active Yeast 9 took place in 2015. For this fermentation, 16 Croatian winery operations carried out small-scale (up to 132 gallons/500 liters) and mid-scale (up to 793 gallons/3,000 liters) fermentations. Of the 16 practice fermentations analyzed, three representative wines were selected that reflect the collective result (see table 2).

The technical selection criterion 'good final fermentation', i.e. low total sugar concentrations, could be confirmed with 1.8 to 2.1 g/l. According to the qualitative selection criteria, such as low acetic acid formation, in practice, values of 0.2 and 0.3 g/l were observed.

The influence of the yeast strain on malolactic fermentation (MLF) has not yet been fully analyzed. The acid structure of the wines can only be maintained if a deposit on the fine yeast with stable SO_2 concentrations is possible. If this is not the case, spontaneous MLF begins, as the L-malic acid and L-lactic acid concentrations in wine number 7 show. Or, MLF takes place only partially, which can be seen in the values of wines 9 and 13.

The other parameters show no abnormalities.

Wine number	7	9	13
Total alcohol [g/l]	96.2	92	92.3
Total sugar [g/l]	1.8	1.5	2.1
Glucose [g/l]	0.4	0.7	1.2
Fructose [g/l]	1.2	0.9	0.8
Total acid [g/l]	5.4	5.2	5.3
pH value	3.3	3.4	3.3
Wine acid [g/l]	2.9	2.9	3.4
L-malic acid [g/l]	0.8	0.6	0.5
L-lactic acid [g/l]	0.6	1.2	1.3
Volatile acid [g/l]	0.3	0.2	0.3
Glycerin [g/l]	5.7	7.2	4.9

Table 2: Results of the practice fermentation in 2015, grape variety Pošip from different Croatian wine growing regions.

Observations:
Fermentation temperature: 64 – 68 °F (18 – 20 °C);
Yeast nutrients: SIHA fermentation salt combined with SIHA PROFERM® H+^{2} or SIHA PROFERM Fit

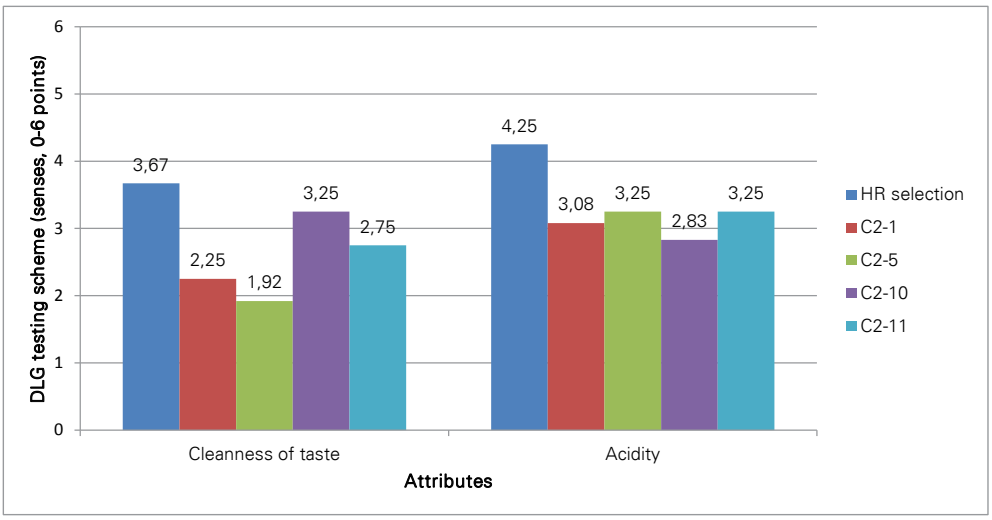


Fig. 1: Sensory evaluation results of different yeast isolates (1st Tasting) according to the DLG 5 Point Testing Scheme

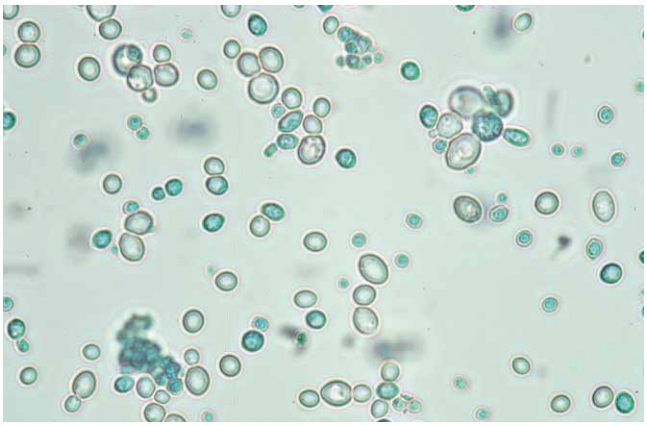
Conclusion

It is a long journey from spontaneous fermentation to an active dry yeast. But the targeted selection of technological and qualitative selection criteria allows an active dry yeast profile to be created and actively selected. In the case of HR selection, this shows that the yeast strain *Saccharomyces bayanus* is marked by good yeast activity, which results in a high final level of fermentation with corresponding alcohol yield for the autochthonous grape variety, Pošip.

The yeast strain is specialized to produce exotic fruit and honey melon aromas. The low formation of acetic acid also supports the sensory perception of the wine. To achieve the formation of aroma, a fermentation temperature of 64 – 68 °F (18 – 20 °C) is necessary, supported by the addition of yeast nutrients. A successful yeast project - spontaneous perfection!

Typical Hvar Island vinyard off the Dalmation Coast of Croatia

References:
[1] H. H. Dittrich, M. Großmann, 2005, Mikrobiologie des Weines, 3rd newly edited edition, Ulmer Verlag, S. 104 – 107
[2] C. Zambonelli, 2003, Microbiologia e biotechnologia dei vini,[i processi biologicie le tecnologie della vinificazione], 3. Ed. Bologna: Edagricole



SIHA active dry yeast 9 under the microscope

SIHA yeast nutrients

North America
44 Apple Street
Tinton Falls, NJ 07724
Toll Free: 800 656-3344
(North America only)
Tel: +1 732 212-4700

Europe/Africa/Middle East
Auf der Heide 2
53947 Nettersheim, Germany
Tel: +49 2486 809-0

Friedensstraße 41
68804 Altlußheim, Germany
Tel: +49 6205 2094-0

An den Nahewiesen 24
55450 Langenlonsheim, Germany
Tel: +49 6704 204-0

China
No. 3, Lane 280,
Linhong Road
Changning District, 200335
Shanghai, P.R. China
Tel: +86 21 5200-0099

Singapore
4 Loyang Lane #04-01/02
Singapore 508914
Tel: +65 6825-1668

Brazil
Rua Clark, 2061 - Macuco
13279-400 - Valinhos, Brazil
Tel: +55 11 3616-8400

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