The beauty of self destruction: yeast autolysis in sparkling wines

New Zealand consultant **Russell Moss** looks at sparkling wines and the essential process of autolysis – and explores ways it can be fast-tracked while further improving the quality of the outcome.

THE process of in-bottle ageing after secondary fermentation (*prise de mousse* – or setting the foam) is essential to sparkling wine production while employing the traditional method.

After this fermentation has come to completion, the wine ages on the yeast lees in the bottle.

The yeast consume themselves enzymatically through a process known as autolysis, which literally means "self-destruction".

The cells then release their breakdown products, known as autolysates, which impart flavours, aromas and surfactant properties which, in turn, affect bubble production and foam stability (Dharmadhikari, n.d.).

This process is the hallmark of high quality sparkling wine.

However, this also requires considerable resources on behalf of the producer, and few options have been explored to expedite the process and alleviate this financial burden on a winery.

THE PHYSICAL PROCESS

The second fermentation is generally undertaken with a strain of *Saccharomyces bayanus.*

The yeast chosen for this task must be able to withstand a low pH, low temperature and high alcohol content.

The yeast are also chosen for their ability to flocculate and "fall out" as well as their ability to undergo autolysis quickly, as the process represents a substantial spatial commitment on behalf of the producer (Alexandre and Benatier, 2006).

The *cuvee* is inoculated with about 3-4X10^6 cells/ml of yeast, which will go through about three or four generations before the end of the secondary fermentation.

AT A GLANCE:

- Yeast autolysis is the hallmark of high quality sparkling wine. However, it also requires considerable resources on behalf of the producer.
- The yeast chosen for the second fermentation must be able to withstand a low pH, low temperature and high alcohol content.
- A way in which you can speed up the autolytic process is to introduce a killer yeast strain in conjunction with a sensitive strain upon the second inoculation of the base wine.
- Research on methods to increase the rate of autolysishas yet to produce a commercially viable alternative to the age-old methods used by the Champenois.

This will give a final concentration of yeast of approximately 1-1.5X10^7 yeast cells/ml.

After about two months the number of fermenting cells will have dramatically decreased to about 10^6 cells/ml.

Once all of the usable sugars present in the wine have been consumed, the cells show signs of autophagy and will begin to consume their own energy reserves (e.g. glycogen). The cell then begins to swell and expand prior to undergoing autolysis.

Autolysis occurs upon the death of the yeast cells when the conditions of the medium in which the yeast cells must operate are such that they can no longer perform their normal functions (Castor and Vosti, 1950).

This process occurs over a long period of time due to the low temperature and pH of the wine.

Like any enzymatic reaction, autolysis occurs at faster rates with increasing temperatures until the temperature reaches a point at which the proteins begin to denature.

The optimal temperature for yeast autolysis is approximately 60C. However, it must occur at temperatures around 10-12C, as the wine is stored at a low temperature.

Also, a low pH, as found in sparkling wine, can act as an inhibitor of autolysis (Alexandre and Benatier, 2006).

Another rate limiting factor is CO^2. Autolysis occurs at a slower rate when yeast is in a liquid saturated by carbon dioxide, such as in sparkling wine (Castor and Vosti, 1950).

However, Markides (1987) notes longer times on yeast lees improves wine flavour and aroma and the low temperature of sparkling wine aging improves the flavours brought forth through autolysis.

Bayon et. al (2003) found the specific yeast strain does not necessarily have a significant impact on the volatile compounds found in sparkling wine.

However, the time the wine spends on yeast lees does appreciably influence the volatile composition of a sparkling wine.

Lees aging is necessary in order to integrate the maximum amount of autolytic breakdown products into the wine.

However, it may also be beneficial for the winemaker to devise methods to speed up the process, as in-bottle autolysis can directly consume time and space and, indirectly, increase cost.

THE BIOCHEMICAL PROCESS

After the yeast cell dies, the cellular constituents, including membrane bound organelles, become disorganised and release enzymes into the cytoplasm which begin to interact with the other components of the cell.

In the beginning, the cell contains enzyme inhibitors which hinder the enzymatic reactions.

However, these inhibitors are soon degraded and the enzymes are then free to break down the various components of the cells.

When the enzymes come into contact with other cellular components, reactions occur and the cellular constituents begin to degrade.

The enzymes released include proteases, which are responsible for the degradation of proteins into smaller units such as polypeptides, peptides and amino acids (Dharmadhikari, n.d.).

However, passive release of amino acids from the yeast cell also occurs soon after the secondary ferment has come to completion.

Therefore, the release of amino acids is not a signal that autolysis has begun (Leroy et. al, 1990).

After cellular death, there is a lag phase between death and autolysis. Autolysis begins around 4-6 months after the beginning of the secondary fermentation.

During autolysis, the main enzyme involved in the release of amino acids is protease A. About 60 per cent of the nitrogen released during autolysis comes from this enzyme (Alexandre and Benatier, 2006).

STRUCTURAL SUPPORT

The yeast cell wall, a rigid and semi-permeable barrier, consists primarily of ~60 per cent glucans and ~30 per cent mannoproteins.

These compounds contribute to the shape and strength of the yeast cell.

Besides aiding the structural support of the cell, the mannans are also responsible for acting as a kind of "landing pad" for proteins which are connected to the polysaccharide.

Glucans and mannoproteins are broken down during autolysis by the hydrolytic enzymes, glucanases and proteases.

Glucanases are responsible for hydrolysing the glycosidic links of the glucan chains present in the cell wall, releasing glucose, oligosaccharides and the mannoproteins which are intricately linked with the glucans.

After the release of mannoproteins from the glucans of the cell wall, the protein portion of the mannoproteins are then broken down into polypeptides, peptides and amino acids through proteolysis.

Mannoproteins contribute to the reduction of haze formation and they also prevent the precipitation of tartaric salt, as they block the growth of tartaric crystals.

The mannoproteins present in the wine may also positively contribute to mouth-feel, as well as the length and intensity of aroma (Alexandre and Benatier, 2006).

The mannoproteins released also contribute to quality of bubble production, primarily the size and persistence of said bubbles (more mannoproteins = smaller bubbles and more persistent mousse) (Dharmadhikari, n.d.).

CELLULAR DEGRADATION

As the cell wall is broken down, the perimeter of the cell becomes porous.

After the wall has become riddled with openings, the autolysates (products of autolysis) can pass freely from the cell into the wine.

Not only are the products of degradation passed into the wine, but the enzymes responsible for this breakdown also find their way into the medium.

Therefore, degradation of cellular components can also occur within the wine, as is true of the polypeptides and peptides, which are later further broken down into their individual components, notably amino acids, if the wine is left on lees for long enough.

The pores created by this process are very small; therefore the autolysates present within the cell must be sufficiently broken down to a size that will allow them to pass through the cell wall.

As the cell continues through autolysis and its various components are degraded through enzymatic reactions, the vacuoles begin to shrink and the cell wall appears wrinkly rather than spherical or ovular as it had when it was thriving in the medium during fermentation.

This change in shape is caused by plasmolysis (Alexandre and Benatier, 2006).

AMINO ACIDS

The amino acids and oligopeptides released during this process may contribute to the characteristically "toasty" flavour found in sparkling wines.

Also, this enrichment of amino acids to wine may actually improve the aromas of sparkling wine as they contribute to reactions which are responsible for the creation of certain aromatic compounds, such as lactones which are responsible for a green-nut or curry aroma.

Alexandre and Benatier (2006) hypothesise the monophosphates released during autolysis by nuclease activity on RNA and DNA are responsible

for contributing flavours to wine.

However more research is needed as to understand the true nature of the relationship between these monophosphates and their contribution to flavour.

Further, through the release of fatty acids (lipids), this may give rise to esters and aldehydes.

The ester Farsenol is of particular interest, as it increases the intensity of perfume like aromas.

Aldehydes have also been found to be a product of autolysis. These are sometimes perceived as being grassy. However, these Aldehydes disappear after ageing.

Also, various peptides may contribute to a perceived sweetness or bitterness in sparkling wine.

The sensory evaluation of the base wine may not be an effective means of predicting how a wine will taste after a long period of ageing on yeast lees due to the nature of autolysis.

It has been shown one cannot actually discern between varieties or colour after aging on yeast lees because of the dramatic changes which occur to the wine during yeast autolysis (Alexandre and Benatier, 2006).

POWER OF SURFACTANTS

Fatty acids and lipids can also have surface effects upon foam quality (Jordan & Napper, 2006).

Surfactants (e.g. proteins, glycoproteins) are brought up to the top of the wine by the production of bubbles.

The surfactants present upon the surface and in contact with the bubbles interlock, which prolongs the "life" of a bubble, allowing it to drain more slowly than without these forces being exacted upon it.

However, the products of autolysis are not always a beneficial factor in the production of bubbles.

Fatty acids and lipids also accumulate at the surface which can, upon coming into contact with a bubble, attract the liquid surrounding the bubble.

This force of attraction thins out the "skin" of the bubble, making it able to rupture more easily.

Thus, if there is a high amount of fatty acids and lipids in the wine, the foam will not persist as long as those wines which are low in fatty acids and lipids (Belair, 2004).

The tables illustrate the autolysate compounds which originate from various parts of the yeast cell, the content of the cytoplasm and the cell wall, and the impacts which they have on sparkling wine.

TABLE 1: Autolysates originating from within the yeast cell (Alexandre and Benatier, 2006)

Compound	Contribution to wine
Lipids	Foam quality
proteins	Foam quality and flavour
peptides	Aroma, flavour and foam quality
Amino acids	Aroma, flavour and foam quality
Nucleotides	Flavour
Nucleosides	Flavour

TABLE 2: Autolysates originating from the yeast cell wall (Alexandre and Benatier, 2006)

Compound	Contribution to wine
glucans	Foam quality
mannoproteins	Mouthfeel

POSSIBLE METHODS TO INCREASE THE RATE OF AUTOLYSIS

Gonzalez et al. (2003) have employed UV mutagenesis on yeast cells in order to increase the quantity of polysaccharides and nitrogenous compounds released into a wine during autolysis, as well as increase the rate of this transference.

This use of yeast mutants may provide winemakers with the ability to decrease the time the wine spends on yeast lees.

However, more research needs to be conducted before this process becomes a viable commercial option.

There is a long period of time between the completion of secondary fermentation and autolysis (approximately 2-3 months).

This could be sped up by incorporating dead yeast cells upon the completion of secondary ferment.

A way in which you can speed up the autolytic process is to introduce a killer yeast strain in conjunction with a sensitive strain upon the second inoculation of the base wine.

The killer strain will carry out secondary ferment, whereas the sensitive strain of yeast will die and begin autolysis almost immediately, thereby reducing the time required for aging on yeast lees (Todd et. al, 2000).

More recent work on rapid yeast autolysis has led to investigation into the possibility of using genetic modification to increase the speed at which autolysis occurs.

However, this has yet to produce a viable product that is available on a commercial scale (Cebollero et. al, 2008).

CONCLUSION

The process of autolysis which occurs during the bottle aging of sparkling wines is crucial to the sensorial impact of this fine effervescent delight.

However, this process presents many challenges to the vigneron.

The most formidable challenge is that of time and space required for bottle aging.

This issue can cause considerable financial strife for a commercial enterprise.

Research has been conducted on methods to increase the rate of autolysis; however it has yet to produce a commercially viable alternative to the age old methods used by the Champenois.

It is only through autolysis the vigneron may achieve the small pearl necklace of bubbles, persistent foam and complex nutty, floral and perfumed aromas that are the hallmark of fine sparkling wines.

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