# STARTER CULTURES AND DIFFERENT TECHNOLOGICAL APPROACHES IN THE FERMENTATION OF APPLE JUICE

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## INTRODUCTION

In this study, the use of several starter cultures and different technological approaches in the fermentation of apple juice are compared, using innovative microbiological theories that have already been used with success in oenology. The purpose is to define a fermentation protocol that is able to provide us with the best guarantees of success and quality in the production of cider.

Cider is an alcoholic beverage obtained from the fermentation of juice from mainly apples or mixtures of apples and pears. Like wine, cider has antique roots; the purpose was to make use of the abundance of apples at harvest. In the more northern countries of Europe (Normandy, Bretagne, Southern England and the Basque Provinces), where apples grow well due to the cold and rainy climate, the production of cider is part of the tradition, while in the more southern regions (Greece, the Balkans, Italy, Southern France, Spain and Portugal), the cultivation of grapes for wine is more common.

*Saccharomyces cerevisiae* is essential in the alcohol fermentation of apple juice and grape juice: in both cases, the transformation of sugars take place in a non sterile environment, rich in indigenous micro-organisms (lactic and acetic bacteria, apiculate yeasts) that could cause alternations in the final product. The regularity and the good outcome of the fermentation are closely linked to the presence of starter cultures that are able to assure a quick start of the fermentation, the prevalence over undesired micro biotic species, and the accentuation of the characteristics of the fruit, giving the beverage the expected pleasantness.

The two juices, apple and grape, in which *S. cerevisiae* will work, have some substantial differences. Apple juice has a sugar content of 80-120 g/l, often increased by the use of molasses in order to obtain an alcoholic degree hardly over 8.5% vol. Grape juice, on the other hand, has a higher sugar content of up to 240 g/l, giving wines characterised with a degree of alcohol often over 13% vol. In addition, the sugars present in the grape juice are only glucose (G) and fructose (F), with an average proportion F/G of 1.09. Apple juice, on the other hand, presents a higher content of fructose (F/G equals 1.7) and a quantity of saccharose varying from 10-20%, often increased by the use of molasses. Finally, the content of yeast assimilable nitrogen (YAN), a fundamental factor for obtaining a good fermentation course and avoiding extremely slow fermentations, varies substantially in the two types of juice. As a matter of fact, in cider, a large deficiency of nitrogenous substances and vitamins is often caused by the use of water to obtain the desired sugar content when sugar molasses from plants and diluted concentrated juices are used.

Using the experience gained in oenology, an innovative research approach will be applied in this study of cider fermentation. An inoculum technique that guarantees a quick start of the fermentation and the prevalence of the inoculated strains is proposed, allowing a better control of the process and assuring the reproducibility and the homogeneity of fermentations. In addition, different nutritional strategies will be evaluated in order to obtain more stability, security and efficiency in the fermentation process. The experiment, conducted on several strains, also made it possible to evaluate

the performance of different *S.cerevisiae*, identifying significant technological differences between them.
This study has been conducted in collaboration with some of the most important British companies in this field.

## MATERIAL AND METHODS

**Micro-organisms**
Three active dry yeasts available on the market have been used: strain A is a *S.cerevisiae r.f. bayanus*, known for its capacity to adapt even to musts with very low content of nitrogenous substances; yeast B is an old strain, *S.cerevisiae r.f. bayanus*, considered very reliable, which grants regular fermentations and contributes to an elegant and persistent aroma; the third strain used, labelled with the letter C, is a strain with both an outstanding quickness in the fermentation start as well as an excellent resistance to high degrees of alcohol.

**Inoculum technique**
Two different inoculum techniques have been compared. In the first procedure, 'direct', the yeasts have been dissolved in water with a temperature of 35°C and containing 5% of saccharose, with the proportion 1 kilo of yeast in 10 litres of solution. After 20 minutes, the volume was increased by 50% with apple juice diluted in water 1:4, not adding sulphur dioxide. After another twenty minutes, the volume was doubled with more diluted apple juice and it was used after 20 minutes of acclimatisation. This procedure, rather laborious if done manually, allows us to obtain a biomass with excellent gemmation, and this can be obtained industrially with the equipment developed specifically for this purpose (Reactivateur – AEB).
In the second technique, 'step 10', the yeasts have been hydrated as already described, but they were inoculated in 10% of the total mass and transferred in the final volume, after keeping them for 24 hours at 20°C. The 'step 10' technique has been developed with the purpose of guaranteeing both the prevalence of the selected yeast in the primary holder and to inoculate the yeasts in full activity in the exponential phase of the growth.

**Nutritional strategies**
The nutritional strategies in terms of quantity, quality and contact time of the used nutrients vary in the different tests that have been conducted. The correct nutrition is very important ➜

for the reduction of the fermentation time and in order to avoid that the cider remains a long time with small residual sugars that could function as triggers for microbiological infections of bacteria or other yeasts.
In the trials, two types of nutrients have been used: one, based on yeast derivatives, brings amino acids, vitamins and microelements; the other provides vitamin B1 and nitrogen salts that are the main causes of nutrition deficiency of yeasts.

**Fermentation methods**

The experimentation was divided into two parts. In the first part, the more theoretical one, juice concentrate with apples from Trentino was used, the concentrate was diluted in water, and refined beet sugar was added. In the second part, in order to resemble the microbiological situation in industrial conditions, musts given directly from the companies were used.

**Analytical techniques**

The efficiency of the yeast under acclimatisation was controlled using charts of lead acetate: if they darken, it indicates a metabolic alteration that leads to the production of hydrogen sulphide.
The fermentation trials were conducted in thermostat at 24°C, the fermentation course was controlled by the weight loss due to liberation of $CO_2$. The data was then transformed into ethanol, produced to enable a better comparison with the industrial reality.
During the fermentation, cellular counts were made using Coulter Z series of the Beckman Coulter.
The quantity of residual sugars at the end of the fermentation has been determined using the analytical enzymatic kit from Boehringer-Mannheim.

## RESULTS AND DISCUSSION

**Influence of the inoculum technique on the fermentative kinetics**

In the first phase of the study, the objective is to define an inoculum protocol in order to obtain the most of the starter cultures. By doing this, the intention is to satisfy the industrial requirements both in terms of fermentation time as well as in terms of the prevalence of the inoculated yeasts over the undesired microbic species; the cause for fermentative anomalies and relevant sensorial flaws. The three *S.cerevisiae* strains used in the experiment have been inoculated both with the 'direct' method as well as with the 'step 10' technique. For this test, must from concentrate of apple juice diluted with drinking water, which was acidified, was used, this is shown in table 1. The musts were selected for the purpose of evaluating the behaviour of the yeast in the presence of abundant quantity of amino acids and with extreme concentrations of ethanol.

*Table 1: Composition of must in the first test*

| | Must 1 | Must 2 |
|---|---|---|
| Concentrated apple juice | 25% | 10% |
| Expected alcohol degree % | 10.5% | 4.2% |
| pH | 3.4 | 3.4 |
| Acidification with | $H_2SO_4$ | $H_2SO_4$ |
| $SO_2$ (ppm) | 80 | 100 |
| YAN (ppm) of concentrate Yeast Assimilable Nitrogen | 172 | 69 |

Even if the percentage of apple juice used in the preparation of must 1 provided a sufficient quantity of assimilable nitrogen, it was decided to apply a supplementary nutrition to both the musts, according to the strategies shown in table 2.

*Table 2: Nutritional strategies applied in the first test*

| Method of inoculum | Direct inoculum | Direct inoculum | Step 10 | Step 10 |
|---|---|---|---|---|
| Code | (1) | (2) | (3) | (4) |
| The initial step | - | - | 50 ppm FE<br>97 ppm EV | 100 ppm FE |
| At inoculum | 50 ppm FE<br>281 ppm EV | 100 ppm FE | 184 ppm EV | 200 ppm EV |
| The second day | | 200 ppm EV | | |


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With reference to the evaluated variables, the experimental plan was created; the summery is shown in table 3.

*Table 3: Schedule of the performad tests*

| Code | Type of inoculum | Yeast | Must | Nutrition |
|---|---|---|---|---|
| D-A-10-1 | DIRECT | S.cerevisiae - A | 1:10 | 1 |
| D-B-10-1 | DIRECT | S.cerevisiae - B | 1:10 | 1 |
| D-C-10-1 | DIRECT | S.cerevisiae - C | 1:10 | 1 |
| D-A-10-2 | DIRECT | S.cerevisiae - A | 1:10 | 2 |
| D-B-10-2 | DIRECT | S.cerevisiae - B | 1:10 | 2 |
| D-C-10-2 | DIRECT | S.cerevisiae - C | 1:10 | 2 |
| D-A-4-1 | DIRECT | S.cerevisiae - A | 1:4 | 1 |
| D-B-4-1 | DIRECT | S.cerevisiae - B | 1:4 | 1 |
| D-C-4-1 | DIRECT | S.cerevisiae - C | 1:4 | 1 |
| D-A-4-2 | DIRECT | S.cerevisiae - A | 1:4 | 2 |
| D-B-4-2 | DIRECT | S.cerevisiae - B | 1:4 | 2 |
| D-C-4-2 | DIRECT | S.cerevisiae - C | 1:4 | 2 |
| S10-A-10-3 | STEP 10 | S.cerevisiae - A | 1:10 | 3 |
| S10-B-10-3 | STEP 10 | S.cerevisiae - B | 1:10 | 3 |
| S10-C-10-3 | STEP 10 | S.cerevisiae - C | 1:10 | 3 |
| S10-A-10-4 | STEP 10 | S.cerevisiae - A | 1:10 | 4 |
| S10-B-10-4 | STEP 10 | S.cerevisiae - B | 1:10 | 4 |
| S10-C-10-4 | STEP 10 | S.cerevisiae - C | 1:10 | 4 |
| S10-A-4-3 | STEP 10 | S.cerevisiae - A | 1:4 | 3 |
| S10-B-4-3 | STEP 10 | S.cerevisiae - B | 1:4 | 3 |
| S10-C-4-3 | STEP 10 | S.cerevisiae - C | 1:4 | 3 |
| S10-A-4-4 | STEP 10 | S.cerevisiae - A | 1:4 | 4 |
| S10-B-4-4 | STEP 10 | S.cerevisiae - B | 1:4 | 4 |
| S10-C-4-4 | STEP 10 | S.cerevisiae - C | 1:4 | 4 |

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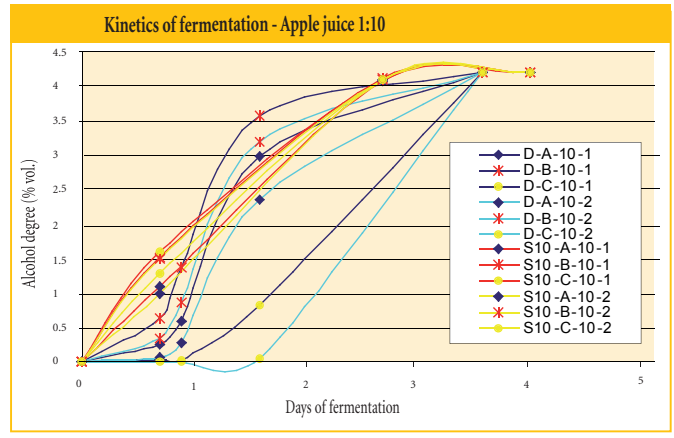
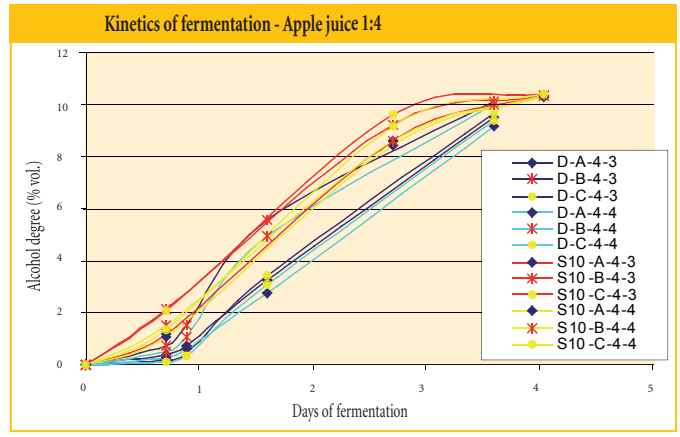
In figure 4, we can see the fermentation course of the must containing 10% of concentrated apple juice. It is clearly visible that the fermentation with 'step 10' inoculum has a quicker start, guaranteeing a greater security over the presence of undesired indigenous micro flora (lactic and acetic bacteria, apiculate yeasts); with this strategy there are, therefore, greater guarantees of avoiding increase of volatile acid, the production of acetaldehyde, and unpleasant aromas.
With the inoculum technique 'step 10', the differences between the yeasts are not significant, even if *S.cerevisiae* 'A' appears slower in the fermentation start. With the direct inoculum of the yeasts, the differences are more noticeable.

*Fig. 4: The fermentation course in the must containing diluted apple juice 1:10*



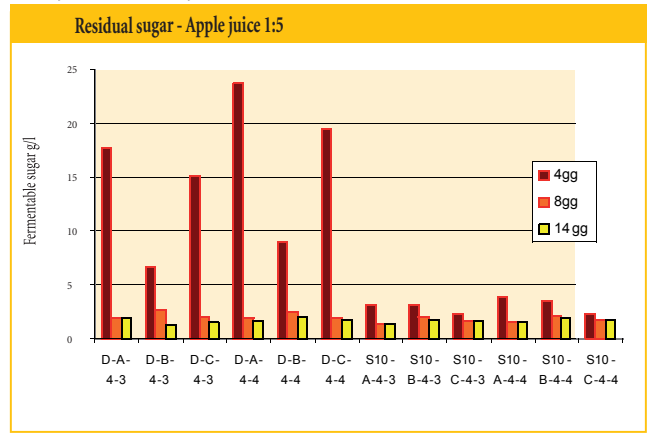

Furthermore, in the test with apple juice diluted 1:4, it is visible that the inoculum method 'step 10' makes it possible to obtain a more certain start, avoiding the proliferation of any possible undesired micro flora. With the direct inoculum, relevant differences between the yeasts and the strain *S.cerevisiae* 'B' appear markedly better; the other two strains have a much slower fermentation start. The nutrition 1 and 3, which are more abundant, guarantee a better kinetics, but it is important to remember that the purpose of a good nutrition is not to increase the speed of the fermentation. In fact, the appropriate nutrition is the one that makes it possible to avoid stuck and slow fermentations in the final phase.

*Fig. 5: Fermentation course in the must containing apple juice diluted 1:4*




After four days of fermentation, the residual sugar in all the samples was measured. The ciders obtained with apple juice diluted 1:10 had completed the fermentation, while the same figures for the samples diluted 1:4 are shown in figure 6. From the obtained information, it is clearly evident that the ciders fermented with the inoculum technique 'step 10' have a better speed in the consumption of the sugar. One interesting point is that in all the fermentation, a small amount of residual fermentable sugar is present, also after 14 days from the end of the fermentation. Even if the quantity is very small, less than 1 g/l, this could be a serious problem during the storage or coming phases of elaboration of the product. Small concentrations of micro flora could develop, consuming the small amounts of residual sugar, and create haze, difficulties in clarification and filtration, or give abnormal odours.

*Fig. 6: Sugar consumption from the 4th day to the 14th day in the must diluted 1:4*



This study has shown that, also for ciders, the fermentation technique 'step 10', which consists of an initial inoculum of 10% of the mass for 24 hours, makes it possible to obtain not only a quicker fermentation start, but also a quicker conclusion. In this way, the day used initially to prepare the inoculum is systematically regained. The complete consumption of the sugar has not been obtained with certainty in the predicted time, on the contrary; in the final stages of the fermentations, there is a visible and excessive slowdown. The experience matured in other areas suggests that this is linked to the nutrition technique.

**Influence of the nutritional technique on the complete consumption of sugar**

After observing that inoculum technique 'step 10' makes it possible to systematically obtain the prevalence over the indigenous micro flora, it was of interest to study what influences the nutritional strategy has on the consumption of the sugars in the final stages of the cider fermentation. Furthermore, concentrated apple juice diluted in drinking water was used in these tests and the sugar level has been increased by using refined beet sucrose. In table 7, the preparation protocol of the must and the inoculum is shown.

*Table 7: Composition of the must used in the nutrition*

| | In the must used for inoculum (10%) |
|---|---|
| Concentrated apple juice | 25% |
| Nutrient FE | 1000 ppm |
| Lactic acid | 2.7 g/l |
| | In the must for the fermentation (90%) |
| Concentrated apple juice | 2% |
| Beet sugar | 170 g/l |
| Lactic acid | 2.7 g/l |
| Metabisulphite | 35 ppm |
| | Final composition |
| Concentrated apple juice | 4.5% (660 g/l of sugar) |
| Sugars | 182.7 g/l |
| Potential degree of alcohol | 10.6% vol. |
| Total actic acid | 2.7 g/l |
| YAN juice | 31 ppm |
| Nutrient FE | 100 ppm |

The potential alcohol degree in the must, 10.6% vol., is higher than the one for most common ciders. This was held to be the easiest way to reproduce the effect of the technological factor that creates problems in the metabolism of the *S.cerevisiae* in the final phases of the fermentation.

In the first test, only the strains *S.cerevisiae* 'A' and *S.cerevisiae* 'C' were taken into consideration. The nutrient EV has been added according to the protocol in table 8.

*Table 8: Nutrition strategies in the first group of nutrition tests*

| Inoculum method | Step 10 | Step 10 | Step 10 |
|---|---|---|---|
| Code | N1 | N2 | N3 |
| 200 ppm EV | 2nd day | 3rd day | 4th day |
| 200 ppm EV | 3rd day | 7th day | 7th day |

As figure 9 shows, the fermentation condition, which is critical with regard to the availability of nitrogenous substance, has pointed out that the performance of strain 'C' is clearly better than strain 'A'.

*Fig. 9: Fermentation kinetics in the first group of nutrition tests*

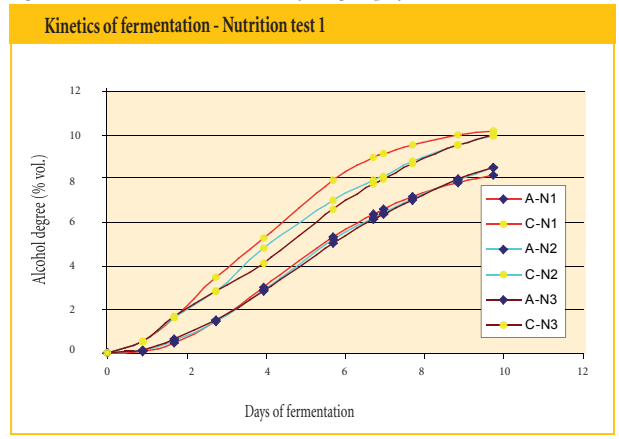
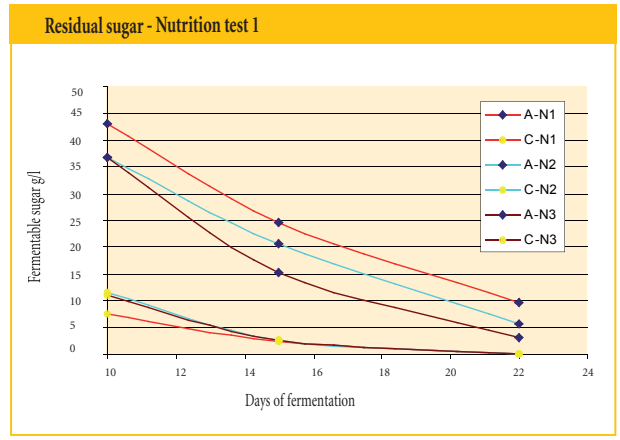



*Fig. 10: Final phases of fermentation in the first group of nutrition tests*

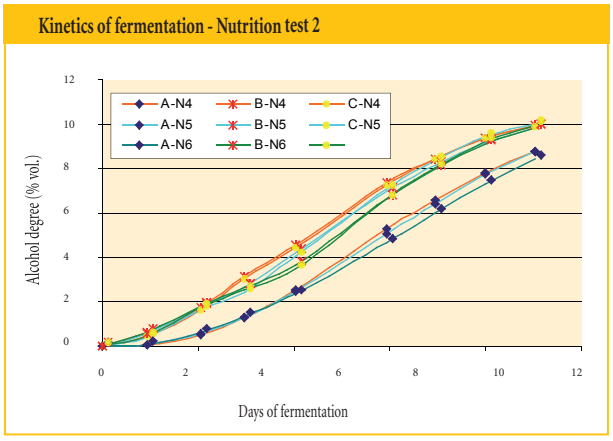


In addition, the information on the residual sugar confirms that strain 'C' performs better than strain 'A'. However, in experimental conditions, the sugars are not completely consumed in the scheduled time: 10 days. This observation points out that the tested nutritional strategies are not suitable and that there is an excessive slowdown in the final phases, exposing the cider to risks of undesired attacks of microbes. For these reasons, more nutrition tests were conducted, this time inoculating the three test yeasts and using larger doses of nitrogenous nutrients, dividing the addition of them according to the schedule in table 11.

*Table 11: Nutritional strategies in the second group of nutrition tests*

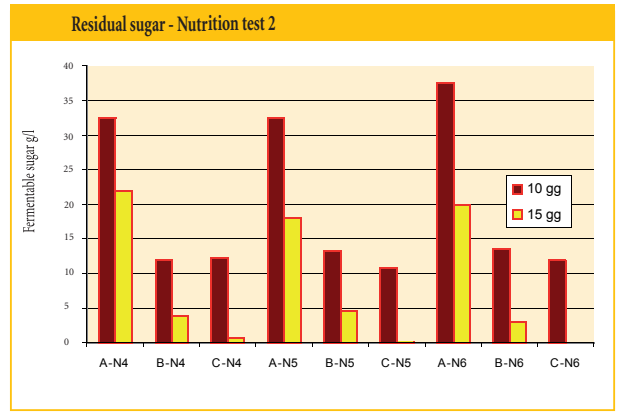
| Inoculum method | Step 10 | Step 10 | Step 10 |
|---|---|---|---|
| Code | N4 | N5 | N6 |
| 200 ppm EV | 2nd day | 3rd day | |
| 300 ppm EV | 4th day | 4th day | |
| 500 ppm EV | | | 4th day |

*Fig. 12: Fermentation kinetics in the second group of nutrition tests*




The strain *S.cerevisiae* 'A' is still the slowest. For the other two yeasts, the positive effect of the nutrition on the fourth day, nutritional strategy N6, is clearly evident, while the nutritional strategies N4 and N5 have fermentation that are more regular. The data for the residual sugar (fig. 13) confirms the observations on the fermentation kinetics and highlights that *S.cerevisiae* 'C' succeeds in using up all the sugars in 15 days from inoculum. Strain 'B' has characteristics similar to yeast 'C', while *S.cerevisiae* 'A', which is *a r.f bayanus*, needs longer time to obtain a complete consumption of residual sugars.

*Fig. 13: Final phases of fermentation in the second group of nutrition tests*




It was considered convenient to conduct another nutrition test, increasing the doses and the division of the nitrogenous substances, in order to verify the effects on the kinetics performance of the strains 'A' and 'C'. The nutritional strategies applied are shown in table 14, and they are based on a division in three and on doses from 500 to 900 ppm of nitrogen salts.

*Table 14: Nutritional strategies of the third group of nutrition tests*

| Inoculum method | Step 10 | Step 10 | Step 10 | Step 10 | Step 10 | Step 10 |
|---|---|---|---|---|---|---|
| Code | N7 | N8 | N9 | N10 | N11 | N12 |
| 3rd day | | | 200 ppm | 200 ppm | 200 ppm | 400 ppm |
| 5th day | 500 ppm | 900 ppm | 200 ppm | 400 ppm | 300 ppm | 500 ppm |
| 7th day | | | 200 ppm | 300 ppm | | |
| TOTAL | 500 ppm | 900 ppm | 600 ppm | 900 ppm | 500 ppm | 900 ppm |

The strain *S.cerevisiae* 'C' confirms a major fermentation speed (fig. 15) and shows a clear slowdown in the tests N7 and N8 from which it quickly restarts after the integration of the nutrient. This phenomenon is most certainly due to a deficiency in nitrogenous substances which the strain shows at four to five days from inoculum.

Strain 'A', on the other hand, is slower and seems almost unable to use the nitrogenous substances added during the fermentation. From the analysis of the conducted nutrition tests (fig. 9, 12 and 15), one can draw the conclusion that the performances of this strain improve when nutrients are added, but never reach a sufficient level. ➜

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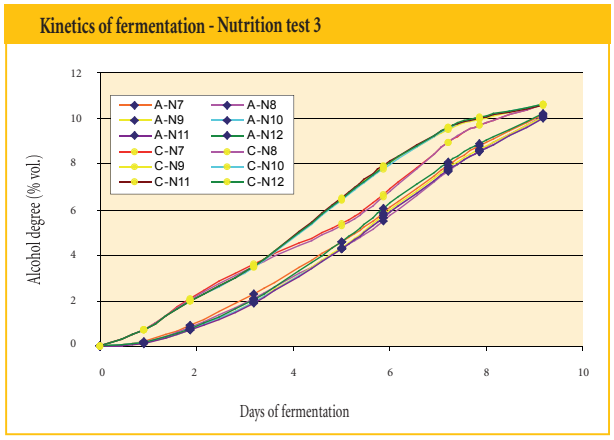
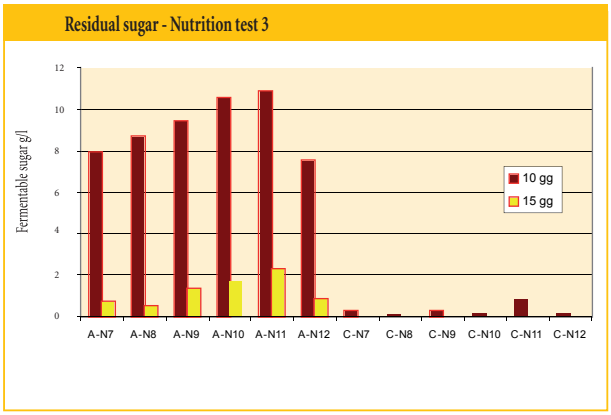
*Fig. 15: Fermentation kinetics of the third group of nutrition tests*




Furthermore, the data on the residual sugar (fig. 16) confirms that the increase and the division of the use of the nutrients lead to an improvement of the technological results. Strain 'C', which has completely consumed the sugars after 21 days with 400 ppm of nutrient (fig. 10), needs 15 days with 500 ppm (fig. 13) and completes the fermentation in 10 days with 900 ppm (fig.16).

Strain 'A', with the addition of 400 or 500 ppm of nitrogenous nutrient, leaves around 20 g/l of residual sugar after 15 days of fermentation. Also, with 900 ppm of nutrient, the fermentation is not completed in the set time (fig. 16).

*Figure 16: Final phase of fermentation in the third group of nutrition tests*




**Fermentation of industrial ciders**

The gathered information permits us to determine that the inoculum technique 'step 10' guarantees a quick fermentation start and the reproducibility of the fermentation process. The trials conducted with different nutritional strategies have shown that with the correct use of nitrogenous substances, it is possible to prevent sluggish/stuck fermentations.

It was then decided to apply the obtained results on the fermentation of two reconstructed musts, using raw material used in industries.

**Cider reconstructed in laboratory**

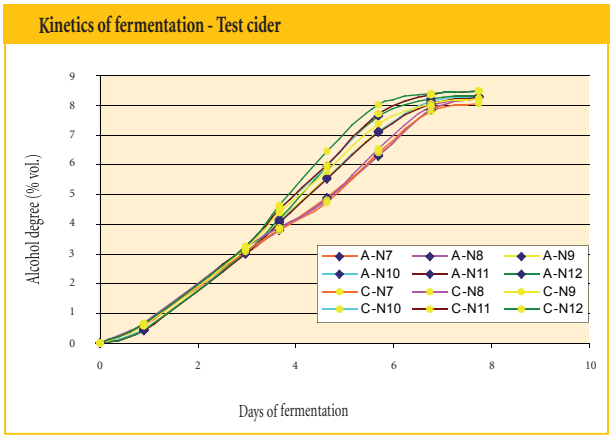
In the first trial, a concentrated apple juice was used, this was diluted with drinking water and the sugar level was increased with sugar syrup. In table 17, the preparation protocol of the must and the inoculum is shown. The nutritional strategy used is the same one as in the third group of nutrition tests, as shown in table 14.

*Table 17: Composition of the must used in the nutrition tests*

| | Concentrated apple juice | Sugar syrup |
|---|---|---|
| Sugars | 560 g/l | 515 g/l |
| YAN | 252 ppm | 80 ppm |
| Total $SO_2$ | 280 ppm | 5 ppm |
| Indigenous yeasts | 1.5 * $10^6$ (plate count) | 4 * $10^6$ (plate count) |
| | In the must used for the inoculum (10%) | |
| Concentrated apple juice | 25% w/v | |
| Nutrient FE | 1000 ppm | |
| Lactic acid | 1 g/l | |
| | In the must for the fermentation (90%) | |
| Concentrated apple juice | 19% w/v | |
| Sugar syrup | | 14.2% w/v |
| Bentonite | 1.2 g/l | |
| Lactic acid | 1 g/l | |
| Metabisulphite | 150 ppm | |
| | Final composition | |
| Concentrated apple juice | 19.6% | |
| Sugar syrup | | 12.8% |
| Sugars | 146 g/l | |
| Potential alcohol degree | 8.5 % vol. | |
| YAN juice | 50 ppm | |
| Total $SO_2$ | 114 ppm | |
| Indigenous yeasts | 6.7 * $10^5$ (plate count) | |

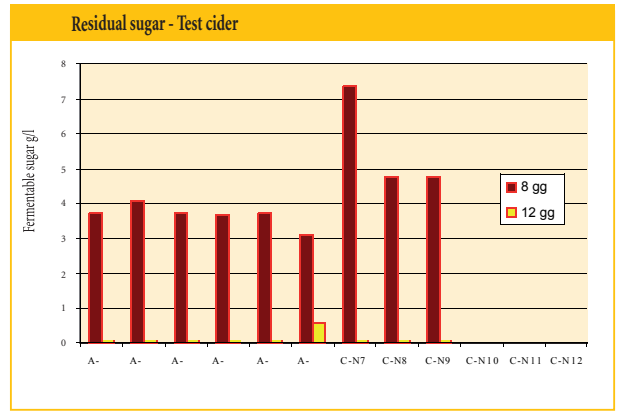
In the preliminary phases of the fermentation (fig. 18), the differences of the two yeasts seem to be less clear compared to the previous tests. This almost certainly depends on the characteristics of the must; having less sugar it is less selective and therefore makes it possible for *S.Cerevisiae* 'A' to compete better with strain 'C'.
In the tests N7 and N8, both the strains show a slowdown of fermentation before the addition of nutrients, which takes place on the fifth day. This result confirms that strain 'A' has metabolic difficulties which makes it impossible to profit from the nutrition, unless under ideal conditions.
Nutrition N12, with 900 ppm of nitrogenous salts divided between the third and the fifth day of fermentation, determines the best kinetic performance for both yeasts. The addition of the same amount, concentrated to the fifth day of fermentation, does not produce the same technological effects, pointing out the importance of the division of the nutrition. In connection with the poorer kinds of nutrition and the late additions of nutrient, the seventh day of fermentation also determines a partial deterioration of the kinetic course in these trials.

*Fig. 18: Fermentation kinetics of the cider*




With regard to the complete consumption of the sugar present in the must (fig. 19), the better behaviour of strain *S.cerevisiae* 'C' is evident once more. This strain is able to complete the fermentation in only eight days in the conducted trials following the nutritional schedules N10-11-12, while four more days are needed in the trials N7-8-9. Also, in this series of trials, strain 'A' has difficulties in completely using up the sugars in a short period of time. This confirms the hypothesis of a difficulty of the strain to use the nitrogenous substances available.

*Fig. 19: Final phase of the cider fermentation*




## Industrially reconstructed cider

In this last series of trials, the behaviour of the yeasts in an industrially reconstructed must filtered in laboratory to reduce the high microbiological contamination over $15*10^6$ UFC/ml, which had developed during transport, was studied.
In this way, it was possible to evaluate the differences between the studied strains under extremely critical conditions, given the fact that the start of the indigenous fermentation has removed all the useful nutritional elements from the must, such as vitamin, assimilable nitrogen and mineral microelements.
The composition of the must after filtration, as given in table 20, shows a microbial level lower than the limits of what is relevant, a very low quantity of assimilable nitrogen, and a pH lower than 3.0. The nutritional strategies used are the same as in the previous tests (table 14) with the exception of test N11, which was not conducted.

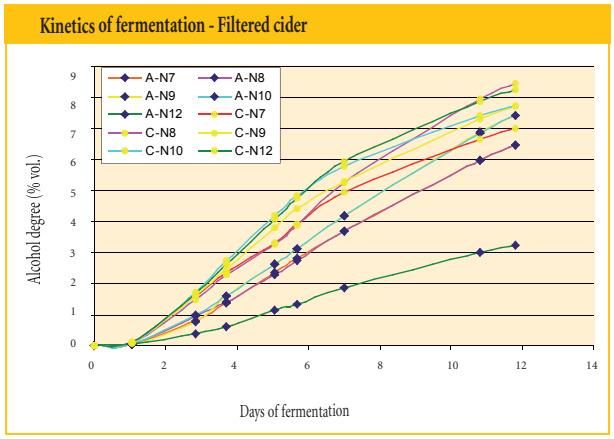
*Table 20: Composition of the must used in the filtration tests*

| | Must for cider |
|---|---|
| Sugar | 151 g/l |
| Haze | 0.61 ntu |
| **YAN** | **24 ppm** |
| **PH** | **2.78** |
| Total acidity | 4.1 g/l |
| Indigenous yeasts after filtration | $<1.25 * 10^5$ |

The filtration of the must determines an abrupt slowdown of the fermentation, in which one can also observe a prolongation of the latency time (fig. 21). Strain 'A', in particular, is affected by the anomalies of the must and has a kinetic course which is very slow. Such behaviour cannot be justified by the low level of YAN alone, but is also due to the lack of other vital factors

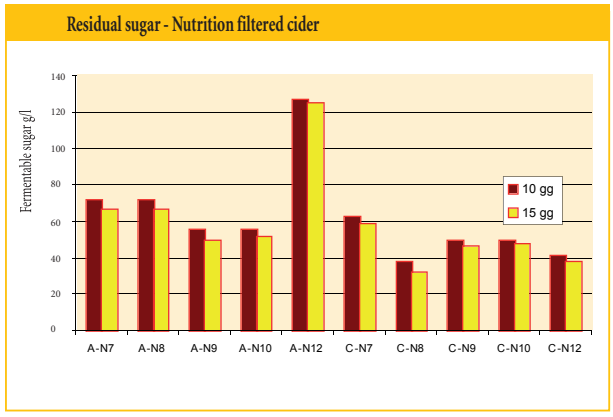
(vitamin, micro elements) that, obviously, are real limiting factors. Strain 'C', even if it is less affected by the filtration, is not able to completely consume all the sugar.

*Fig. 21: Fermentation kinetics of the filtered cider*




Under these very drastic conditions, none of the different kinds of nutrition used are able to complete the fermentation and only in two cases with the strain *S.cerevisiae* 'C', the residual sugar is lower than 40 g/l.
In the nutritional schedules N7 and N8, strain 'C' shows, yet again, a slowdown before the addition of the nutrients, which takes place all at one time on the fifth day of fermentation. N8, which is more abundant than N7, makes it possible to obtain a larger consumption of sugar. These phenomena are not noticeable with strain 'A', which confirms once more that it has a limiting factor (probably due to the need of vitamin) which makes it impossible to use the YAN available.
Furthermore, in the nutritional schedules where the nutrition is divided, strain 'C' profits considerably from the higher doses (N10, N12), while strain 'A' is always slower, and with nutrition N12, presents absolutely abnormal results that confirms its unreliability under critical conditions.

*Fig. 22: Final phases of the fermentation of filtered cider*




## CONCLUSIONS

The main goals of good alcohol fermentation are:
• the speed at start in order to avoid the indigenous micro flora to proliferate.
• the complete consumption of the sugar, in order to avoid bacterial species that could make the clarification or storage more difficult.
• the use of a reliable yeast that can guarantee the reproducibility of the results even when the conditions are far from ideal.

In this experiment, the prevalence on the indigenous micro flora can be obtained systematically with the inoculum technique 'step 10', which is already used with great success in the oenological sector.

The complete consumption of the sugars and the reduction in fermentation time are closely linked to the quality and the division of the used nutrients. The best results are obtained by carrying out two or three additions, so that the yeast can have the substances at its disposal during the whole fermentation.

This study has showed that there are yeast strains, such as *S.cerevisiae* 'A', which are unable to efficiently use the nitrogenous substances made available for them, unless under extremely good conditions. This phenomenon, due to the necessity of availability of high quantities of other nutritional factors, makes the fermentations very long. *S.cerevisiae* 'C' has an ideal behaviour both in terms of speed of the fermentation as well as in terms of the complete consumption of the yeasts. This strain increases its performance according to the nutrients used, showing that there is no other limiting factor other than the quantity of available nitrogen, which it can use with the best efficiency.