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Short Communication

Malolactic fermentation of Grape Wine by *Lactococcus lactis* var *cremoris* NCIM 2402

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Abstract

Malolactic fermentation of wine is an important process by which the wine gets a slight acid taste and the corresponding esters gives pleasant flavor. This is important in normal and sweet wine. In case of dry wine it can be considered as very insignificant. In this investigation it has been observed that, the wine which are not very expensive, and affordable to any consumer, this fermentation has been stopped and as a result these wine are inferior in flavor and aroma. Here attempt have been made to induce such fermentation during the short bottle ageing periods of the products. The fermentation is carried out by *Lactococcus lactis* var *cremoris* NCIM 2402.The product acceptability has been found to improve significantly without losing other component like the alcohol content.

Introduction

Malolactic fermentation is commonly referred to as "MLF", or as winemaker's call it - malo. MLF is an intricate process that usually follows after the completion of alcoholic fermentation (AF) by yeasts. It is usually used in winemaking where tart tasting malic acid, naturally present in grape must, is converted to softer tasting lactic acid. Malic acid, along with tartaric acid, is one of the principal organic acids found in wine grapes. Its name comes from the Latin malum meaning "apple" (Robinson, 2006 a) and hence it is the acid mostly found in apple. A much milder acid than tartaric and malic is the lactic acid is often associated with "milky" flavors in wine and is markedly less sour than malic acid (Robinson, 2006 b).

MLF is thought to generally enhance the body and depth of wine, producing wines with better palate softness and roundness. Wines, that typically undergo MLF, and are considered "improved" by the process, are full-bodied dry whites whereas medium to full bodied are dry reds. Winemakers generally wait until the end of primary fermentation before adding the malolactic bacteria culture. Ongoing fermentation will be visible in the form of tiny carbon-dioxide bubbles. When the bubbles stop, the fermentation will be complete. The time required for malo-lactic fermentation can be varying depending upon the temperatures.Although MLF is regarded as a secondary fermentation process (post fermentation), it plays an integral role in the production of the majority of red wines, as well as some white cultivars including Chardonnay and some sparkling wines. There are three main reasons for conducting MLF in wine. Firstly, the deacidification of the wine with a concomitant increase in pH, secondly, to contribute to the microbial stability by the removal of malic acid as a possible carbon substrate and thirdly, the modification of the wine aroma profile (Davis *et al*., 1988; Kunkee, 1991; Maicas *et al*., 1999; Liu, 2002; Ugliano *et al*., 2003). Most malolactic culture cause a slight increase in volatile acidity by 0.05-0.2 g/l. A controlled MLF, a more microbially stable wine can potentially result, like certain lactic cultures can produce a host of products during MLF depending on the available substrates (acids, sugars, polyols, etc). These compounds are claimed by many to potentially modify vegetal characters, and potentially impart nutty, lactic, and/or earthy aromas. A reduction in colour intensity can be caused by these lactic cultures and not due to the metabolic activity of bacteria on phenolic compounds (Lonvaud-Funel, 1995). MLF can also decrease astringency and increase the polymerisation of tannins and anthocyanins (Riesen, 1999).

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Lactic acid bacteria possess three possible enzymatic pathways for the conversion of L-malic acid to L-lactic acid and CO_2 (Roissart and Luquet, 1994). The first is the direct conversion of malic acid to lactic acid via malate decarboxylase, also known as the malolactic enzyme (MLE) (Bartowsky, 2005). The enzyme has been purified from various species, isolated from wines and grapes, including species of *Lactococcus*, *Lactobacillus* and *Leuconostoc* (Wibowo *et al*., 1985; Lonvaud and Funel, 1995; Nakarai *et al*., 2000). The other metabolic routes of conversion of malate to lactate is by conversion of malate directly to pyruvate and then to lactate or by conversion of malate to oxaloacetate to pyruvate and then to lactate (Henick and Kling, 1993; Lonvaud and Funel, 1999; Versari *et al*., 1999; Konings, 2002). *Lactococcus lactis* var. *cremoris* strains are preferred because of their superior contribution to product flavor via unique metabolic mechanisms (Sandine, 1988; Salama *et al*., 1991).

This study was aimed to find out if organisms like *Lactococcus* species would be able to bring about this fermentation and enhance the flavor. Again it must be remembered that the flavor enhancement is a value addition process and the manufacturer should find it profitable to do this additional work. Keeping all these in mind this feasibility study was conducted.

Materials and Methods

Lactococcus lactis *var* cremoris *NCIM 2402*

The organism was grown anaerobilaly at 30°C on MRS medium containing peptone (1%), meat extract (1%), yeast extract (0.5%), glucose (2%), tween 80 (0.1% v/v), dipotassium hydrogen phosphate (0.2%), sodium acetate (0.5%), tri-ammonium citrate (0.2%), magnesium sulphate (0.02%), manganese sulphate (0.005%), agar (2%). The cells were harvested by centrifuging at 6000 x g for 20 minutes and washed with 0.85% NaCl solution to remove all medium components. This was suspended in suitable amount of the same NaCl solution to get a cell density of 1 x 10^8 cells mL^{-1}, which was used as inoculum, for further studies, at 0.1 %(v/v) concentration.

Study of malolactic fermentation

A bottle of, moderately priced, white wine was purchased from a local dealer. The above mentioned culture of *Lactococcus lactis* var *cremoris* NCIM 2402 was inoculated as mentioned above. Fermentation was carried out at different incubation temperatures such as 14°C, 25°C and 37°C for 21 days. At every 7th day, 50 ml of this fermented wine was centrifuged at 6000 x g. for 15 min., to which equal volume of diethyl ether was added and thoroughly mixed. The ether layer was dried over sodium sulphate and evaporated. The residue was dissolved in 2 ml of methanol and analysed using HPLC. The instrument used in this study was 2 columns reverse phased HPLC (Waters 2690 system) using C8 column (4.6×250 mm). Elution was done with 70% acetonitrile at a flow rate of 1.0 ml min^{-1}, which was monitored by measuring UV A215 with a Waters Lambda- Max model LC Spectrophotometer. The results were confirmed by GCMS analysis of the same samples.

Result and Discussion

The results of HPLC analysis were compared with standard Lactic acid and malic acid. It was observed that, the commercial white wine which was used in this experiment had a very flat taste. The HPLC analysis showed neither malic nor lactic acid in it. This is as shown in Figure1. The wine was then inoculated with the culture of *Lactococcus lactis* var *cremoris* NCIM 2402 and incubated at 14°C for 7 days. The results are as shown in Figure. 2. It can be seen from this figure, that at low temperature lactic acid production was at a very slow rate and it was forming esters with ethanol and hence no free lactic acid could be detected by HPLC.

However when the same experiment was carried out at 25°C for 7 days, it is possible to see free lactic acid along with the esters, as lactic acid formation was much faster. The results are as shown in Figure 3. Unfortunately, malic acid or its esters were not detected, by these methods. It is probably due to the fact that malate was being immediately converted to lactate by the organism. The product showed significant flavor enhancement with less time and at an affordable cost as compared to bottle aged wine available in market.

However, there was very scanty growth at 37°C and hence there was no MLF (results not shown). It is evident from Figure 4, that the ethanolic ester of lactic acid is more prominent as compared to free lactic acid. This is due to the fact that there was very little thermally decomposed lactic acid detected by the mass spectrometer. Figure 5, shows also the presence of the ester but some thermally decomposed lactic acid too indicating the presence of free lactic acid in the medium.

This is the first time report, of Lactococcus spp being used to carry out malo-lactic fermentation of wine. The previous reports have indicated that MLF is carried out by lactic acid bacteria (LAB), from the genera *Oenococcus*, *Lactobacillus*, *Pediococcus* and *Leuconostoc* (Wibowo *et al*., 1985). Of these,

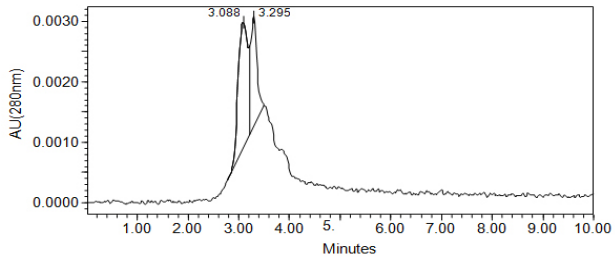

Figure 1. HPLC analysis of market wine showing absence of lactic and malic acid

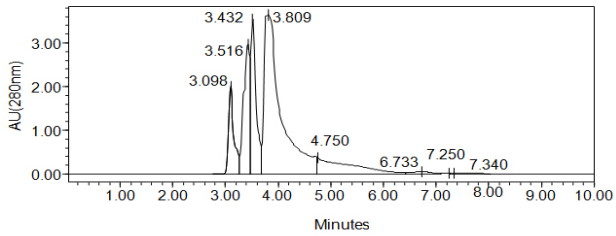

Figure 2. HPLC analysis of market wine inoculated with *Lactococcus lactis* var *cremoris* NCIM 2402 incubated at 14^{0}C for 7 days showing lactic acid ester of ethanol

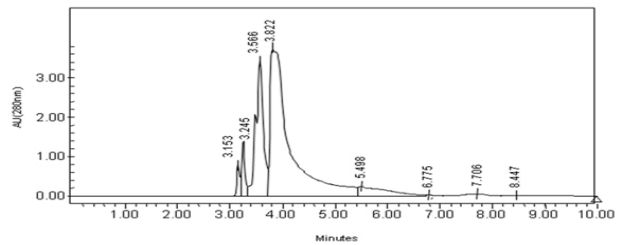

Figure 3. HPLC analysis of market wine inoculated with *Lactococcus lactis* var *cremoris* NCIM 2402 incubated at 25^{0}C for 7 days

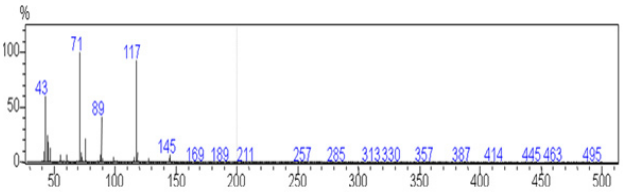

Figure 4. GCMS analysis of wine inoculated with *Lactococcus lactis* var *cremoris* NCIM 2402 incubated at 14^{0}C for 7 days

Oenococcus oeni is best adapted to the harsh wine environment, including conditions of high alcohol, low pH and the presence of sulphur dioxide (SO_2) (Wibowo *et al*., 1985; Davis *et al*., 1988; Drici-Cachon *et al*., 1996; Lonvaud- Funel, 1999).

Finally, the wine was tasted for its organoleptic qualities and it was found to be scoring 6 out of a score of 10 (results not shown). However, further studies are required to be carried out for a suitably large spectrum of white wines to check, whether the same holds true in all cases.

Conclusion

It can be concluded that MLF is a very important process to improve the consumer acceptability of grape wine, especially that of white wine which are moderately priced and not aged for a long time. It is a value-addition process and can be carried out by *Lactococcus* spp. aimed at improving the productivity of the manufacturing unit. The wine gets a combination of tangy flavors of butter, and very little of coconut. The blend of such flavors has been found to be highly acceptable amongst the consumer in general.

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References

Bartowsky, E. J. 2005. Oenococcus oeni and malolactic fermentation- moving into the molecular arena. Australian Journal of Grape Wine Research 11: 174-187.

Davis, C. R., Wibowo, D., Fleet, G .H. and Lee, T. H. 1988. Properties of wine lactic acid bacteria: Their potential enological significance. American Journal of Enology and Viticulture 39: 137-142.

Drici-Cachon, A., Guzzo, J., Cavin, F. and Divies, C. 1996. Acid tolerance in *Leuconostoc oenos*. Isolation and characterisation of an acid resistant mutant. Applied Microbiology and Biotechnology 44: 785-789.

Henick-Kling, T. 1993. Malolactic fermentation. In: Fleet, G. H. (Ed.). Wine Microbiology and Biotechnology, Harwood Academic Publishers, Chur, Switzerland 289-326.

Henick-Kling, T. 1993. Malolactic fermentation. In: Fleet, G.H. (Ed.). Wine Microbiology and Biotechnology, Harwood Academic Publishers, Chur, Switzerland 289-326.

Konings, W.N. 2002. The cell membrane and the struggle for life of lactic acid bacteria. Antonie Van Leeuwenhoek 82: 3-27.

Kunkee, R. E. 1991. Some roles of malic acid in the malolactic fermentation in wine making. FEMS Microbiology Letters 88: 55-72.

Liu, S.Q. 2002. A review: Malolactic fermentation in wine - beyond deacidification. Journal of Applied Microbiology 92: 589-601.

Lonvaud-Funel, A. 1995. Microbiology of the malolactic fermentation: Molecular aspects. FEMS Microbiology Letters 126: 209-214.

Lonvaud-Funel, A. 1999. Lactic acid bacteria in the quality improvement and depreciation of wine- Antonie van Leeuwenhoek 76: 317-331.

Maicas, S., Gil, J. V., Pardo, I. and Ferrer, S. 1999. Improvement of volatile composition of wines by controlled addition of malolactic bacteria. Food Research International 32: 491.

Nakarai, T., Morita, K., Nojiri, Y., Nei, J. and Kawamori, Y. 2000. "Liver abscess due to *Lactococcus lactis* cremoris". Pediatrics International 42: 699-701.

Riesen, R. 1999. Modification of Wine Characteristics by Malolactic Fermentation *http://www.oardc.ohio-tate.edu/grapeweb/vinevan/van0699.htm*

Robinson, J. 2006 a."The Oxford Companion to wine", Third Edition, Oxford University Press, (ed.) ISBN 0-19-860990-6., 421-422.

Robinson, J. 2006 b."The Oxford Companion to wine", Third Edition, Oxford University Press, (ed.) ISBN 0-19-860990-6., 387-388.

Roissart, H. and Luquet, F. M. 1994. Bacteries lactiques: aspects fondamentaux et technologiques. Uriage, Lorica, France. 1: 605. ISBN 2 9507477 0 1.

Salama, M., Sandine, W. E. and Giovannoni, S. 1991. Isolation of *Lactococcus lactis* subsp. *cremoris* from nature by colony hybridization with rRNA probes. Applied Environmental Microbiology 57: 1313-1318.

Ugliano, M., Genovese, A. and Moio, L. 2003. Hydrolysis of wine aroma precursors during malolactic fermentation with four commercial starter cultures of *Oenococcus oeni*. Journal of Agriculture and Food Chemistry 51: 5073-5078.

Versari, A., Parpinello, G. P. and Cattaneo, M. 1999. Leuconostoc oenos and malolactic fermentation in wine: A review. Journal of Industrial Microbiology and Biotechnology 23: 447- 455.

Wibowo, D., Eschenbruch, R., Davis, C.R., Fleet, G.H. and Lee, T.H. 1985. Occurrence and growth of lactic acid bacteria in wine: A review. American Journal of Enology and Viticulture 36: 149-153 / 302-312.