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Growth and Metabolism of Lactic Acid Bacteria in a Model Wine System and a Red Wine with Emphasis on Carbohydrate Metabolism

A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Technology (Food Technology) in the Faculty of Technology at Massey University New Zealand

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February, 1990.

ABSTRACT

Studies were conducted to investigate the application of capillary gas-liquid chromatography in analysis of wine carbohydrates, and the growth and metabolism of wine lactic acid bacteria in a synthetic model wine system.

1. Analysis of carbohydrates in wine using capillary gas-liquid chromatography

Wine carbohydrates were analysed by capillary gas liquid chromatography of their acetate and aldononitrile acetate derivatives. A wide range of aldoses, polyols and disaccharides (30 compounds) were analysed in 55 minutes, using a single injection. All the derivatives were well-separated except for ribose and rhamnose, which almost co-eluted. The method recovered spiked carbohydrates at 86 to 110% and had adequate reliability. This technique may be applied routinely to the analysis of other alcoholic and non-alcoholic beverages.

2. Growth and metabolism of wine lactic acid bacteria

Malic acid and pH values had determinative effects on the growth of wine lactic acid bacteria. Malic acid stimulated the growth rate and cell population of L. oenos 122 and 252 at pH 4 and allowed their growth at pH 3.2. The absence of malic acid at pH 3.2 inhibited the growth of L. oenos 122 and 252. The stimulatory effect of malic acid on growth was more striking at pH 3.2. This effect was not caused by the pH increases

resulting from malic acid degradation. Malic acid had only a small stimulation on the growth rate of L. plantarum 49 and P. parvulus 93 at pH 4 and their growth was suppressed at pH 3.5, irrespective of malic acid. These results imply that pH 3.5 is a critical value for the bacteriological stability of wine after malolactic fermentation.

This study confirmed that sugars served as the main growth substrates for wine lactic acid bacteria and polyols did not act as growth substrates, with the exception of mannitol. Glucose and trehalose were the preferred substrates for all the bacteria tested. The significance of trehalose in relation to yeast autolysis in induction of malolactic fermentation was discussed. Wine lactic acid bacteria varied in the ability to utilise substrates. Malic acid, citric acid and arginine did not serve as single energy sources.

Malolactic fermentation had a profound impact on substrate utilization by L. oenos 122 and 252, yet seemed not to affect the substrate utilization of L. plantarum 49 and P. parvulus 93. The presence of malic acid resulted in an increased utilization of sugars by L. oenos 122 and 252, and decreased utilization of arabinose by L. oenos 252. Trehalose utilization by L. oenos 252 was not influenced by malolactic fermentation. The increased utilization of sugars may be the biological functions of malolactic fermentation.

pH exerted a marked effect on the metabolism of L. oenos 122 and 252. More sugars were utilized at pH 4 and above than at pH 3.31 and below. L. oenos 122 attacked only a very minor amount of glucose and a portion of malic and citric acids at pH below 3.31. L. oenos 252 also used only

a small quantity of sugars except for glucose, which was used completely, but degraded all malic and citric acid at pH below 3.42. These results strongly suggest that the degradation of malic acid, citric acid and arginine required the presence of fermentable sugars. This implies that the absence of fermentable sugars in wine may prevent malolactic fermentation. These results also justify the benefits of malolactic fermentation at low pH values (below 3.3).

The role of wine lactic acid bacteria in formation of biogenic amines was clarified. L. plantarum 49 was the only organism which reduced the levels of tyrosine and phenylalanine dramatically, indicating that this bacterium may be a potential producer of tyramine and phenylethylamine. P. parvulus 93 did not markedly decrease the levels of any amino acids. Arginine was catabolised only by L. oenos 122 and 252 with the formation of ornithine and ammonia. Arginine was not degraded at low pH values (below 3.5), suggesting that arginine may not play any role in energy supply at low pH values. L. oenos 122 and 252 did not significantly reduce the concentrations of other amino acids.

The role of malolactic fermentation may lie in energy generation. Two potential energy-yielding mechanisms of malolactic reaction were proposed: ATP production through pyruvic acid cleavage (substrate level phosphorylation, pseudo-malolactic fermentation) and chemiosmotic ATP synthesis via formation of extra lactic acid (non-substrate level phosphorylation, real malolactic fermentation). It is speculated that L. oenos 122 may employ the pyruvic acid cleavage pathway and generation of superfluous lactic acid may be adopted by L. oenos 252, L. plantarum 49 and P. parvulus 93. The biological function of the extra lactic acid

could be accounted for by the chemiosmotic theory that postulates energy (ATP) production through efflux of metabolic end-products (e.g., lactic acid). The origin of the superfluous lactic acid remains to be investigated. These findings suggest that the criteria for selection of starter cultures be redefined.

ACKNOWLEDGEMENTS

I sincerely thank my supervisors, Dr. John Brooks and Dr. Craig Davis, for their valuable advice, guidance and time.

I also appreciate the useful discussions with Dr. Gordon Pilone and Mr. Malcolm Reeves. Dr. Gordon Pilone kindly read this thesis.

Thanks are extended to the following: June Husbands for the assistance with HPLC; the Department of Chemistry/Biochemistry for the analysis of amino acids; Margaret Bewley and Garry Radford; the Department of Food Technology for providing the facilities.

The sponsorship provided by the Government of the People's Republic of China is greatly acknowledged.

I am indebted to my parents for their continuous care, support and encouragement during the course of this study.

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Chapter One
Introduction

Wine is produced by the alcoholic fermentation of grape juice carried out by yeasts such as Saccharomyces cerevisiae. Wine may also support a secondary fermentation, the malolactic fermentation (MLF), the conversion of L-malic acid to L-lactic acid and carbon dioxide by strains of lactic acid bacteria (LAB) (Kunkee, 1967). The malolactic fermentation normally occurs after completion of the alcoholic fermentation.

The malolactic fermentation is not unique to wine. It also occurs in the fermentation of other foods and beverages which contain L-malic acid such as cider (Beech, 1972). Since its discovery, MLF has become the focus of research of many oenologists and microbiologists (Vaughn, 1955; Kunkee, 1967; Pilone and Kunkee, 1972; Lafon-Lafourcade, 1983; Davis et al, 1986a,b). The occurrence and growth of LAB in wine and the practical implications of MLF have been reviewed recently by Wibowo et al (1985) and Davis et al (1985). Briefly, the beneficial effects of MLF include acidity reduction, flavour complexity and microbial stability. However, the microbial stability is now in question, since it has been recently shown that other LAB will grow after MLF (Davis et al, 1986a,b). The reduction in wine acidity, resulting from the decarboxylation of L-malic acid, is of great advantage in cold wine regions, such as New Zealand, where the wines are often too tart. The deacidification itself softens the excessive acidity of cool climate wines. Malolactic fermentation may also bring about flavour complexity of wines through the production of

flavour compounds, such as acetoin and diacetyl (Fornachon and Lloyd, 1965).

With increased recognition of the impact of MLF on wine quality, winemakers are seeking to control the MLF either by encouraging its rapid induction or inhibiting it completely. One of the problems in inducing MLF is the lack of proper MLF starter cultures with known characteristics. Spontaneous MLF is often too slow and difficult to control and may not possess suitable characteristics for producing good quality wines.

Extensive research on the occurrence and growth of LAB in wines has been undertaken throughout the world, but little has been done on the physiological and biochemical properties that will determine the ability of LAB to grow in wine and on the biochemical mechanisms by which these LAB affect wine quality (Davis et al, 1988). Furthermore, the reasons why LAB conduct MLF are still unknown, although some speculations have been given, for example, a stimulation in utilization of sugars by MLF (Kunkee and Pilone, 1972), production of ATP from malic acid by phosphoroclastic reaction (Chauvet et al, 1980) and chemiosmotic ATP synthesis through efflux of lactic acid resulting from malic acid decarboxylation (Renault et al, 1988; Cox and Henick-Kling, 1989). Such fundamental knowledge is now becoming increasingly important, because the wine industry is moving towards controlled MLF with specific strains of LAB (Beelman et al, 1979; King et al, 1984; Kunkee, 1984). Basic information on the physiological and biochemical properties of strains of lactic acid bacteria will not only help in the selection for commercial use, but will also indicate the conditions under which these

LAB will be most effectively utilized in the wine industry. Furthermore, an understanding of how they grow in wine and what factors affect this growth may eventually lead to the means of limiting or stimulating such growth.

Therefore, the aim of this project was to investigate the metabolism of LAB so that some basic physiological and biochemical information useful for selection of LAB starter cultures could be obtained. Considerable information is available in the literature on the metabolism of lactic acid bacteria in semi-synthetic media such as MRS (de Man et al, 1960) or media containing tomato juice (Rogosa et al, 1953). Little data, however, are available on their metabolism in wine, apart from the recent reports of Davis et al (1986a,b). It becomes important, however, to have complete control over the composition of the growth media to derive specific information on growth substrates and end-products. Therefore, a major part of this work involves the use of a model wine system of totally defined composition which simulates the growth conditions during conservation of wine after alcoholic fermentation.

Associated with this is the requirement of a sensitive and accurate carbohydrate assay which can be used with this model wine system and with wine. Therefore, another major aim of this project was the development of a suitable capillary gas-liquid chromatography method for this purpose.

Much research has considered the carbohydrate metabolism of lactic acid bacteria from dairy products (Lawrence et al, 1976). By contrast, however, little research has focussed specifically on the wine LAB,

particularly when they are actually growing in wine. Much of their basic biochemistry is unknown. Clearly, there is need for further information on what compounds serve as energy sources, under what conditions they are utilized and how this affects wine quality.

Special emphasis was placed on the carbohydrate metabolism of wine LAB during the course of this research, since the metabolism of carbohydrates by LAB is an area of wine biochemistry which is still poorly understood. Also, the understanding of the metabolism of particular carbohydrates could be an important criterion for selection of LAB for induction of MLF.