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The effect of water-soluble vitamins on spoilage organisms in beer

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Abstract

The vitamin content of beer and brewing related products might not only be of interest from a human health perspective, but determining their presence and relative quantities might also help to understand the role they play in product stability. Water-soluble vitamins are important in the brewing industry, as they are essential nutrients for yeasts and potential spoilage organisms. The presence of particular vitamins in beer has been linked to deterioration (light strike) and it is possible that many vitamins may be linked to increased bacterial spoilage potential in beer. There is strong evidence to support that lactic acid bacteria require a variety of vitamins in order to grow effectively and can aid in the increased spoilage of beer and beer related products. This study investigates the effect of a variety of water-soluble vitamins on the growth of commonly found spoilage organisms, *Lactobacillus brevis* and *Pediococcus damnosus*.

Key Words: thiamine, riboflavin, beer, lactic acid bacteria

Introduction

Vitamins are essential organic compounds that are required in small amounts to maintain and support growth [10]. These individual vitamin compounds have little in common with the exception that they are typically required in enzymatic complexes involved in a variety of metabolic processes. Several authors have reported the presence of a variety of vitamins in beers [3, 11, 12]. The presence of water-soluble vitamins in beer may be due to the raw materials or the production from yeast and starter cultures. This means that the water-soluble vitamin content can vary between different styles of beer. It is currently unknown what the presence of these vitamins may have on the growth of potential spoilage organisms such as *Lactobacillus brevis* and *Pediococcus damnosus* as these vitamins have been previously described to be essential growth factors [8, 9]. However like many food products, beer has a series of intrinsic factors such as hop contents and elevated ethanol content. For instance, these intrinsic antibacterial hurdles are able to inhibit the presence of potential pathogens in beer, making it a safe product [6]. However, spoilage organisms such as lactic acid bacteria are able to grow in beers and produce a variety of off-flavours and aromas that are undesirable in final beer products. This may be attributed to the presence of high levels of vitamins in beers. This research will investigate the effect of thiamine and riboflavin contents on the growth of *L. brevis* and *P. damnosus* whilst under hop and ethanol stress.

Experimental

Survey of beers

A variety of commercially available beers were surveyed for their riboflavin and thiamine contents. These beers were grouped into four major styles (ale, lager, stout, and wheat) based on the Australian International Beer Awards Guidelines [2].

Organisms and culture conditions

The organisms *Lactobacillus brevis* and *Pediococcus damnosus* were utilized throughout this research and where previously isolated from commercially available beers (Menz, PhD thesis in progress, University of Ballarat).

In order to evaluate growth of these bacterial cultures in a medium resembling beer, we used a significantly diluted commercially available beer to lower the endogenous vitamin content. Thus, both cultures were grown in a diluted 10% v/v beer solution containing 10 g L^{-1} glucose. The ethanol was removed by distillation and confirmed by gas chromatography [5]. Hop concentrations were evaluated by IBD method IOB 9.16 and was found to contain <1 IBU. Cultures were centrifuged at 18000 rpm (SS-34 rotor) for 5 minutes in a Du Point RC5C centrifuge (FSE, Melbourne) and washed twice in beer medium without glucose prior to inoculation. All experimental media was filtered through a 0.22 μm cartridge filter (Sartorius, Germany) using a peristaltic pump (Watson-Marlow 501U). Hop stress was induced by adding an iso-α-acid extract (75 mg L^{-1} final concentration which equates to 15 IBUs); while ethanol stress was induced by adding ethanol (5% v/v final concentration). Various amounts of either thiamine or riboflavin (50 and 500 μg L^{-1}) were added to the appropriate flasks. The thiamine and riboflavin contents were adjusted to reflect either a "low" or "high" vitamin concentration, based on a survey of 60 beers. All experiments were carried out at 24°C in the dark.

Analysis of cell growth

Cell growth was measured via optical density at 550 nm using a GBC UV/Vis 911A (GBC Scientific Instruments, Aus). A standard growth curve was performed via serial dilution on each culture to determine respective cellular counts.

Analysis of thiamine and riboflavin

All samples were aseptically taken and filtered through 0.45 μm syringe filters to remove the cultures and frozen until required for analysis. All treatments were performed in triplicate and experiments were performed in duplicate.

Thiamine analysis was performed via pre-column derivatisation fluorescence RP-HPLC, using a fluorescence detector at 360/425 nm (excitation/emission); while riboflavin analysis was performed by RP-HPLC using a fluorescence detector at 270/516 nm (excitation/emission). All samples were filtered through 0.45 μm syringe filters prior to injection.

Results

A survey of 60 commercially available beers were analysed in this study and vitamin contents are shown in Table 1. These results show that the riboflavin concentration is higher in all beers compared to thiamine, and that the ale style beers contain the highest levels of both thiamine and riboflavin. This may be due to the brewing method or because these beers are traditionally bottled conditioned. The longer contact with the yeast may cause an increase in vitamin levels, especially as the cells are dying and these vitamins may be released. The fact

that the vitamin levels in wheat beers are higher than that of the stouts may be directly related to the addition of the wheat to the mash. From Table 1 two levels of vitamins were chosen, 50 μg L^{-1} and 500 μg L^{-1}, as these represent "low" and "high" levels of each vitamin, to determine potential effects of the addition of these vitamins.

Table 1. Average thiamine and riboflavin content of a variety of commercially available beers

Style	Thiamine (μg L^{-1})	Riboflavin (μg L^{-1})
Ale (12)[a]	199.2 (± 2.5)[b]	237.6 (± 3.7)
Lager (27)	42.1 (± 2.2)	150.6 (± 4.0)
Stout (8)	63.5 (± 2.3)	236.3 (± 4.0)
Wheat Beer (3)	86.6 (± 3.8)	216.5 (± 3.2)

[a] denotes number of beers tested
[b] denotes standard deviation in μg L^{-1}

In order to determine the best levels of ethanol and hops to use in these cultures a series of experiments were performed that investigated different stress levels. From these experiments 15 IBU and 5% v/v ethanol stresses were utilised (data not shown). These were chosen as they proved to be a significant stress to both cultures, while still allowing small amounts of growth.

Figure 1 shows the effect of thiamine on hop and ethanol stressed *L. brevis* culture.

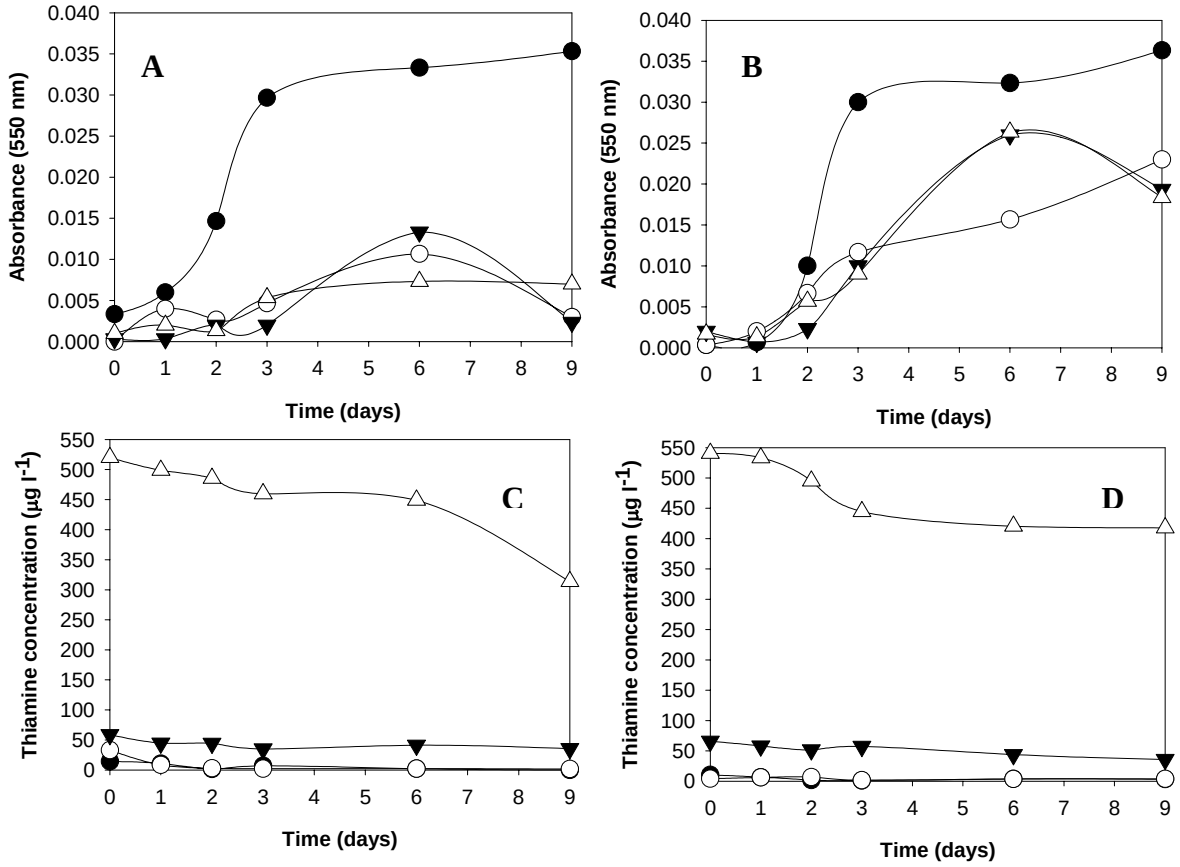

Figure 1. Effect of thiamine on ethanol and hop stressed *L. brevis*. A – Hop stressed; B – Ethanol stressed; C – Thiamine concentrations of hop stressed cultures; D – Thiamine concentrations of ethanol stressed cultures; ● - Non stressed control; ○ – Stressed control; ▼ - Stressed control + 50 μg L^{-1} of thiamine; △ – Stressed control + 500 μg L^{-1} of thiamine

The addition of 50 µl L^{-1} of thiamine to a hop stressed *L. brevis* culture resulted in a slight increase in growth in comparison to the stressed control (Fig. 1A), however, this was not so with the 500 µg L^{-1} addition. The addition of thiamine to an ethanol stressed culture of *L. brevis* appeared to improve the growth rate (Fig. 1B), suggesting that the addition of this vitamin may help alleviate the ethanol applied stress. The concentration of thiamine decreases over time as growth increases (Figs. 1C and D) which suggests there is a requirement of thiamine by the culture. A similar response is observed with the addition of riboflavin to the same stressed conditions and is shown in Figure 2.

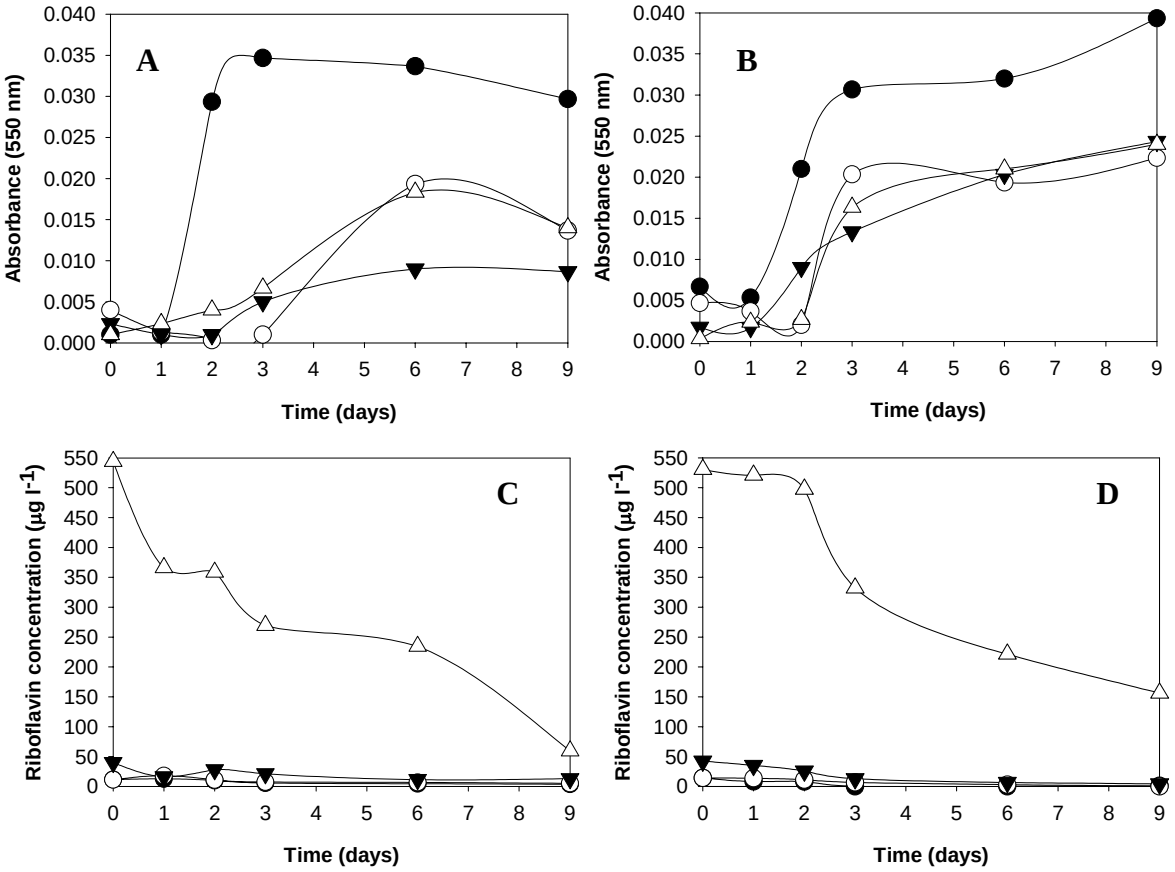

Figure 2. Effect of riboflavin on ethanol and hop stressed *L. brevis*. A – Hop stressed; B – Ethanol stressed; C – Riboflavin concentrations of hop stressed cultures; D – Riboflavin concentrations of ethanol stressed cultures; ● - Non stressed control; ○ – Stressed control; ▼ - Stressed control + 50 µg L^{-1} of Riboflavin; △ – Stressed control + 500 µg L^{-1} of Riboflavin

The elevated presence of riboflavin to a hop stressed culture of *L. brevis* appears to have little effect on growth of the culture (Fig. 2A), and no discernable effect could be found when riboflavin was added to an ethanol stressed culture (Fig. 2B). In both hop and ethanol stresses the addition of riboflavin reduces the lag time slightly but does not significantly increase the overall growth rate in comparison to the stressed control. Unlike the thiamine experiments the concentration of free riboflavin decreases more rapidly. This suggests a greater requirement for this vitamin in comparison to the thiamine. However this increased requirement did not lead to an increase in overall growth. These results are similar to those observed with the *Pediococcus* culture as shown in Figures 3 and 4.

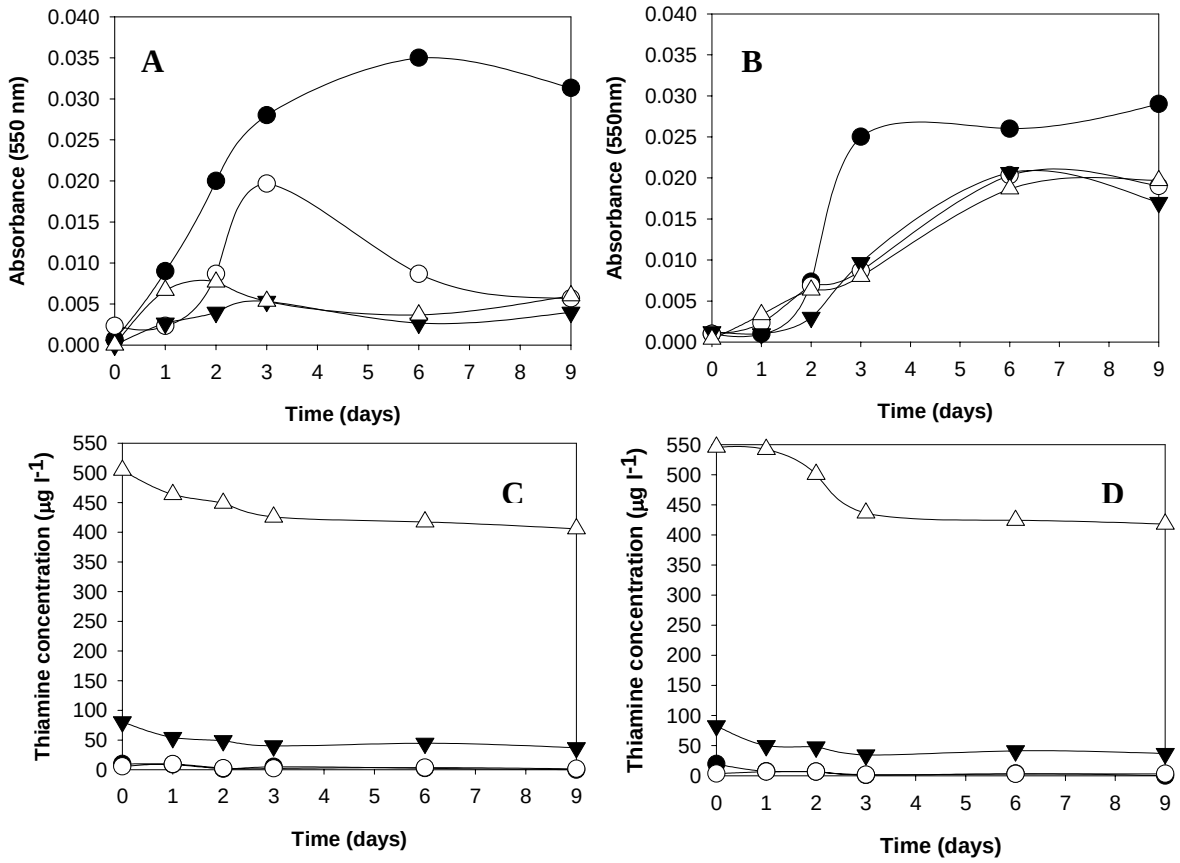

Figure 3. Effect of thiamine on ethanol and hop stressed *P. damnosus*. A – Hop stressed; B – Ethanol stressed; C – Thiamine concentrations of hop stressed cultures; D – Thiamine concentrations of ethanol stressed cultures; ● - Non stressed control; ○ – Stressed control; ▼ - Stressed control + 50 µg L^{-1} of thiamine; △ – Stressed control + 500 µg L^{-1} of thiamine

The addition of thiamine or riboflavin to a *P. damnosus* culture appears to have no significant effect on the growth of hop and ethanol stressed cultures (Figs 3 and 4). There was little to no increase in the growth rates of the stimulated cultures in comparison to the stressed control. As seen with the *L. brevis*, the requirement for riboflavin was greater than that of the thiamine based experiment (Figs 3C, D and 4 C, D). However, as shown with *L. brevis* this higher requirement did not lead to a significant increase in overall growth.

Discussion

The survey of beers revealed that ale style beers contained the highest level of thiamine and riboflavin. These results are similar to those reported previously [1, 3, 4, 11, 12] and may be directly related to the fact that these beers were all bottle conditioned in comparison to the lager beers which were all filtered. Despite these findings an increase in thiamine or riboflavin does not increase the growth of hop and ethanol stressed *L. brevis* and *P. damnosus* cultures. It is important to note that the thiamine and riboflavin concentrations in the media did decrease over time suggesting that they are required for survival but have no bearing on overcoming applied stresses. These results are quite different to previous authors [7, 9] , who reported a stimulatory effect/essential requirement for the growth of brewery isolated lactobacilli. However these previous studies were performed in synthetic media and not in real beer samples and the cultures were not tested for their stress responses. Despite the

apparent requirement for these vitamins, they do not aid in the increase of growth whilst under ethanol or hop stresses. These findings suggests that despite different styles having different vitamin contents, an increase concentration of thiamine or riboflavin will not increase the risk of bacterial spoilage and therefore one style will not be more susceptible than another.

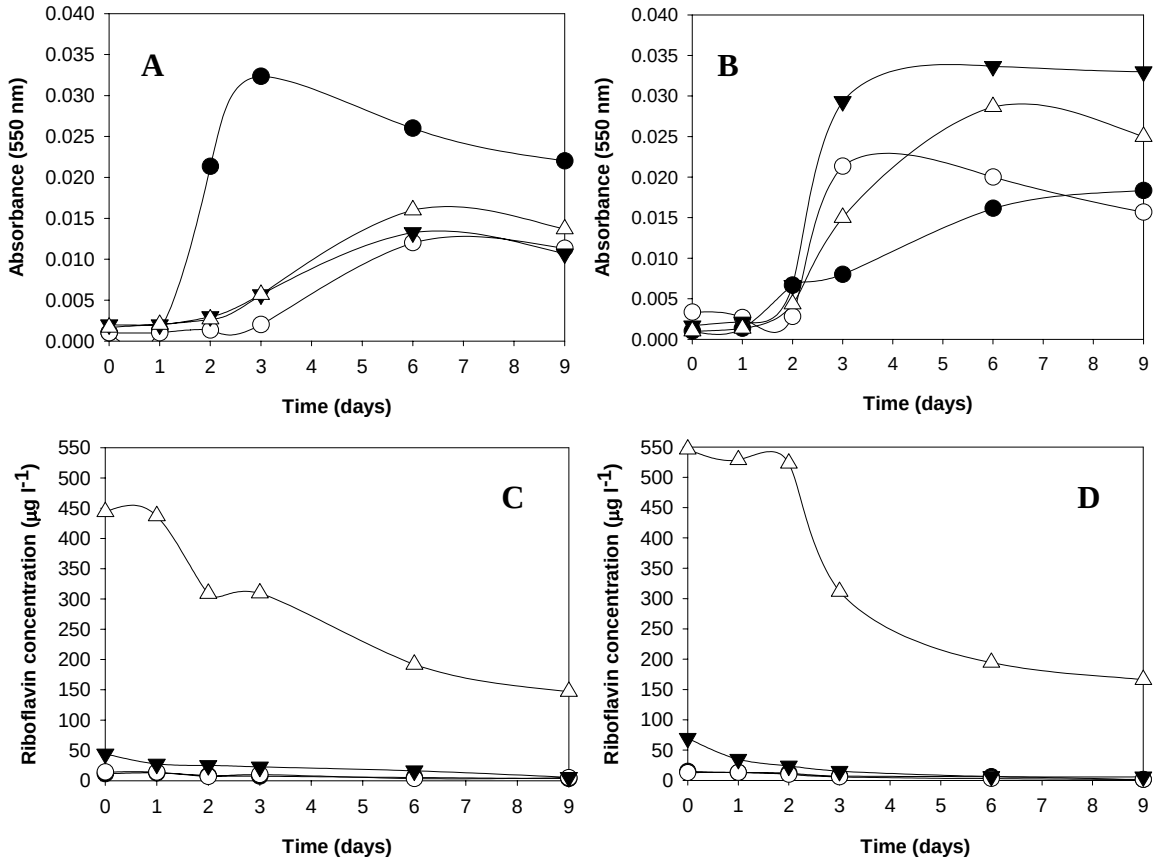

Figure 4. . Effect of riboflavin on ethanol and hops stressed *P. damnosus*. A – Hops stressed; B – Ethanol stressed; C – Riboflavin concentrations of hop stressed cultures; D – Riboflavin concentrations of ethanol stressed cultures; ● - Non stressed control; ○ – Stressed control; ▼ - Stressed control + 50 µg L^{-1} of Riboflavin; △ – Stressed control + 500 µg L^{-1} of Riboflavin

Summary

This research has only investigated whether these vitamins effect the growth of a culture whilst under one of the applied stresses in beer. The addition of a particular vitamin may have an effect where combinations of multiple stresses are applied. This research also has not investigated whether the addition of particular vitamins can increase the growth of stress-adapted cultures. This research would aid in understanding an ongoing spoilage problem in a brewery.

References

1. Andrés-Lacueva, C., F. Mattivi, and D. Tonon, *Determination of riboflavin, flavin mononucleotide and flavin-adenine dinucleotide in wine and other beverages by high-performance liquid chromatography with fluorescence detection.* Journal of Chromatography A, 1998. **823**: p. 355-363.
2. Australian International Beer Awards. *Beer Style Guidelines*. 2009; Available from: www.beerawards.com.
3. Bamforth, C., *Nutritional aspects of beer - A review.* Nutrition Research, 2002. **22**: p. 227-237.
4. Duyvis, M., R. Hilhorst, C. Laane, D. Evans, and D. Schmedding, *Role of riboflavin in beer flavor instability: Determination of levels of riboflavin and it's origin in beer by fluorometric apoprotein titration.* Journal of Agricultural and Food Chemistry, 2002. **50**: p. 1548-1552.
5. Hucker, B. and F. Vriesekoop, *Acetaldehyde stimulates ethanol-stressed Saccharomyces cerevisiae, grown on various carbon sources.* Folia Microbiolica, 2008. **53**(6): p. 505-508.
6. Menz, G., P. Aldred, and F. Vriesekoop, *Pathogens in Beer*, in *Beer in Health and Disease Prevention*, V. Preedy, Editor. 2009, Academic Press: USA. p. 403-413.
7. Moore, W.B. and C. Rainbow, *Nutritional requirements and biochemical activities of brewery Lactobacilli.* Journal of General Microbiology, 1955. **13**(1): p. 190-197.
8. Russell, C., R. Bhandari, and T. Walker, *Studies of lactic acid bacteria associated with brewery products. IV. Interrelationships in requirements for purines, folic acid and p-aminobenzoic acid.* Journal of Agricultural and Food Chemistry, 1955. **6**: p. 633-636.
9. Russell, C., R.R. Bhandari, and T.K. Walker, *Vitamin requirements of thirty-four lactic acid bacteria associated with brewery products.* Journal of General Microbiology, 1954. **10**(3): p. 371-376.
10. Sharp, D.W.A., ed. *Dictionary of Chemistry*. 2nd ed. 1990, Penguin Books Ltd: Great Britian. 442.
11. Vinas, P., N. Balsalobre, C. Lopez-Erroz, and M. Hernandez-Cordoba, *Liquid chromatographic analysis of riboflavin vitamers in foods using fluorescence detection.* Journal of Agricultural and Food Chemistry, 2004. **52**(7): p. 1789-1794.
12. Vinas, P., C. Lopez-Erroz, N. Balsalobre, and M. Hernandez-Cordoba, *Determination of thiamine and its esters in beers and raw materials used for their manufacture by liquid chromatography with post column derivatization.* Journal of Agricultural and Food Chemistry, 2003. **51**: p. 3222-3227.