2.11 Determination of Vitamin B_1 and B_2 in Wort and Beer

The term vitamin (from the Latin *vita* for life and *amin* meaning to contain nitrogen) was coined by FUNK in the year 1912, on his belief that all substances that sustain growth and life contain an amine group.

Vitamins in the Brewing Process

Barley and malt are rich in vitamins and are localized in the living tissues of the embryo and of the aleurone layer. They are involved in building enzymes as part of a prosthetic group. Of the vitamins in the B-complex, vitamin B_1 is present in barley in amounts ranging from 1.2–7.4 mg/kg barley, dry matter. During germination, the concentration of vitamin B_2 increases to 1.5 times the quantity found in barley. This corresponds to a concentration of 1–3.7 mg/kg malt, dry matter.

Aside from numerous other active organic substances, vitamins are also necessary for yeast during reproduction and fermentation. Paramount among these vitamins are thiamin (vitamin B_1), riboflavin (vitamin B_2), niacin (vitamin B_3), pantothenic acid (vitamin B_5), pyridoxine (vitamin B_6), biotin (vitamin H) and inositol. Organic growth promoters serve as functional metabolic building blocks, which allow cellular enzymes to be effective. Niacin is a component of coenzyme I, which transfers hydrogen in the phosphoglyceraldehyde dehydrogenase system during cell metabolism. Niacin is present in the form of nicotinamide, while pantothenic acid is a constituent of coenzyme A, which is vital for carbohydrate metabolism.

Pyridoxal 5'-phosphate is the active coenzyme of pyridoxine and of great significance for amino acid metabolism. In addition to biotin, which plays an important role in yeast growth and serves as a coenzyme in all ATP-dependent carboxylation reactions, thiamin and riboflavin promote yeast metabolism. Thiamin as the coenzyme for carboxylase is central to carbohydrate metabolism. Riboflavin, in the form of flavin mononucleotide, participates in oxidation-reduction reactions in the prosthetic group of dehydrogenases. Beer only contains small amounts of thiamin. However, brewing yeast is rich in thiamin, as it is very rapidly taken up by yeast from the wort. In contrast, greater quantities of riboflavin are present in beer, since this enzyme, as described above, is affected by the malting process and is primarily derived from malt. The yeast absorbs only relatively small quantities of riboflavin during fermentation.

Riboflavin supports a number of processes, which increase the rate of aging in beer, because the Strecker degradation reaction is promoted by the photo-oxidation of riboflavin. This results in an increase in volatile substances, among them a large number of long-chain, and in part unsaturated, carbonyl compounds primarily attributable to off-flavors associated with aging in beer. Analogous to Strecker degradation, the oxidation of higher alcohols through the influence of light in the presence of riboflavin occurs more rapidly. Oxidation reactions in the side chains of iso-humulones are also accelerated in the presence of light and riboflavin. Photoactivation of riboflavin leads to the formation of carbonyl compounds and mercaptans. The compound responsible for the light-struck flavor (3-methyl 2-butene 1-thiol) is created along with numerous other mercaptans. However, riboflavin slows the auto-oxidation of long-chain fatty acids into shorter-chain aldehydes.

Principle

The reagent kit for the determination of vitamins B_1 and B_2 in blood from Chromsystems GmbH in Munich, Germany was used for the analysis. This company specializes in the manufacture of reagent kits for clinical applications. The kits are easy to use and do not require much additional equipment; a simple isocratic HPLC system is sufficient. In cooperation with the company, this kit was modified to accommodate a different sample matrix (mash, wort and beer).

This analysis method for vitamins allowed the concentration of vitamins B_1 and B_2 to be determined quickly and easily and could serve as a tool for utilizing vitamins as analytical indicators in the brewing process.

HPLC methods have been successfully used for the analysis of vitamin content in non-alcoholic beverages. However, for the most part, these methods cannot be employed with a wort or beer matrix due to the vitamin concentrations present. Tests for very narrow concentration ranges exist primarily for clinical applications due to the need for rapid and precise analysis in this area.

Reagents

Extraction buffer
Precipitation reagent
Derivatization reagent
Stabilization reagent
Reaction vials, protected from light
Eluent
HPLC columns

Sample Preparation for Vitamin B_1

- add 200 µl centrifuged mash or wort + 100 µl extraction buffer into a light-protected reaction vial
- mix for 2 s (vortex)
- add 300 µl precipitation reagent
- mix 30 s (vortex)
- centrifuge for 5 min at 9000 g
- add 200 µl derivatization reagent into a separate, light-protected reaction vial
- add 100 µl of the supernatant and mix thoroughly
- add 100 µl neutralization reagent
- add 100 µl stabilization reagent and mix
- after 20 min has elapsed, inject 50 µl into the HPLC system

Device Settings

Injection volume:	50 µl
Run time:	8 min
Flow rate:	1.0 ml/min
Column temperature:	25 °C
Fluorescence detector:	EX 367 nm, EM 435 nm

Sample Preparation for Vitamin B_2

- combine 200 µl of centrifuged mash, wort or beer + 200 µl extraction buffer + 400 µl precipitation reagent in a reaction vial protected from light
- mix for 30 s (vortex)

- incubate for 10 min at 2–8 °C
- add 400 µl stabilization buffer
- mix for 30 s (vortex)
- centrifuge at 9000 g for 10 min
- transfer supernatant immediately to a vial protected from light
- inject 50 µl into the HPLC system

Device Settings

Injection volume:	50 µl
Run time:	12 min
Flow rate:	1.5 ml/min
Column temperature:	25 °C
Fluorescence detector:	EX 465 nm, EM 525 nm

To achieve the correct settings for the vitamins, a five point calibration was performed using pilsner wort (12 % w/w) or beer. Thiamin or riboflavin pure standards were obtained from Sigma-Aldrich, Germany. The coefficient of variation for the analyses is 3.1 % for vitamin B_1 and 1.8 % for vitamin B_2 (n = 7).

Figures 1 and 2 represent chromatograms for vitamin B_1 and vitamin B_2 measured in wort, respectively.

Figure 1: Chromatogram for vitamin B_1

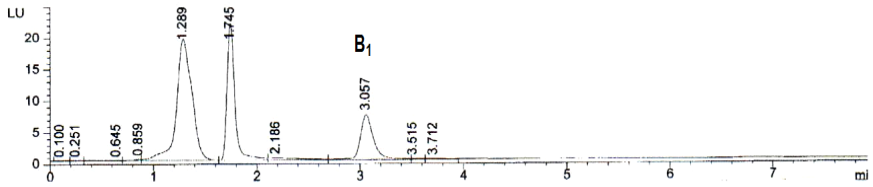
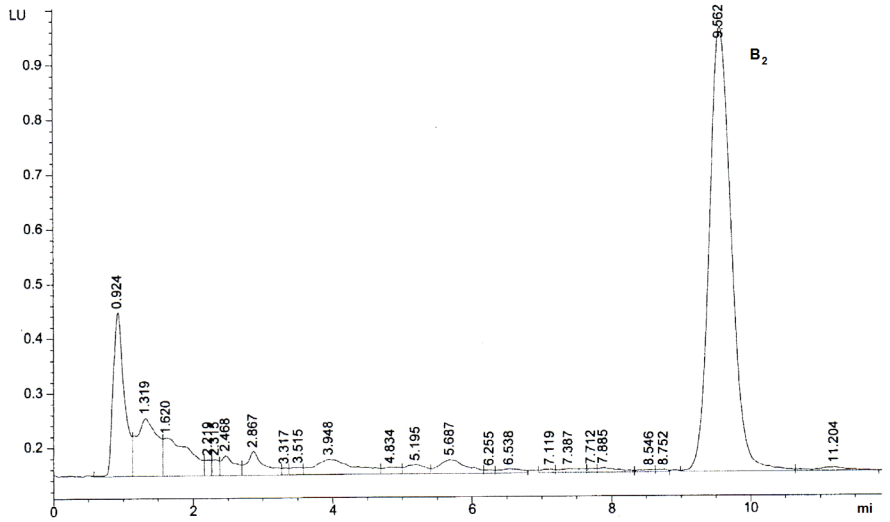


Figure 2: Chromatogram for vitamin B_2



References

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