

# Vitamin Determination Study in Yoghurts and Yoghurts with Fruits

VitaFast® – microbiological microtiterplate test for quality control procedures of folic acid, vitamin B12 (cyanocobalamin), vitamin B7 (biotin), vitamin B3 (niacin), vitamin B5 (pantothenic acid), vitamin B1 (thiamine), vitamin B2 (riboflavine), vitamin B6 (pyridoxine) and inositol

Author: Wolfgang Weber\*, Sylvia Stengl\*\*, Sigrid Haas-Lauterbach\*\*  
ifp\*, R-Biopharm AG\*\*

## Introduction

Food products are often enriched with vitamins. Thus the industry has expanded the number and variety of yoghurts and yoghurts with fruit preparations available on the market. Fruit preparations naturally contain ascorbic acid and B-vitamins however the industry fortifies food with vitamins. Food manufacturers, regulatory agencies and commercial laboratories should therefore have analytical methods on hand that are quick and reliable for the determination of the natural and fortified vitamin content. A study of storage ring trails concerning the natural yoghurts, the fruit preparations and the yoghurts with fruits is performed with a rapid system for water soluble B-vitamin determination. For the different watersoluble B-vitamins different enzymes and sample preparations are required.

In traditional microbiology, colonies of the target microorganisms must first be cultured and later maintained by regular inoculation. Before the actual assay procedure can begin, the cultures must be freshly prepared and the number of microorganisms must be regulated before the organisms are transferred to the medium. This requires a great deal of time and manpower. Chromatographic methods, such as HPLC, are also often used.

The VitaFast® tests are ideal for routine analysis since the reagents are in a ready-to-use microtiter plate format and very user friendly. These test kits are marketed by R-Biopharm AG, Darmstadt and produced by ifp, Institut für Produktqualität, Berlin.



The VitaFast® test kit contains a microtiter plate (96 wells) coated with microorganisms, an additional holder, each 3 bottles of assay-medium, standard, buffer, sterilized water and adhesive foils. The test procedure further requires sterile single disposable materials and a microtiter plate photometer.

## Method

The vitamin concentration in yoghurts and yoghurts with fruits was determined by using innovative microbiological assays in test kit format (VitaFast®). R-Biopharm presents a system of watersoluble B-vitamin determination which is rapid and based on AOAC, EN and DIN reference methods.

### Sample preparation of natural yoghurt, fruit preparation and yoghurt with fruits (total folic acid content):

- weigh exactly 1 g (ml) homogenized sample and 10 mg Chicken Pancreatin into a 50 ml sterile centrifuge vial
- add 30 ml phosphate buffer (0.05 mol / l; 0.1 % ascorbate; pH 7.2, freshly prepared), shake well and fill up to 40 ml with phosphate buffer
- incubate 2 h at 37 °C (98.6 °F) in the dark (shake at times); thereafter heat 30 min at 95 °C (203 °F) in a water bath; chill down quickly to below 30 °C (86 °F)
- dilute the supernatant and transfer 1 ml of the dilution in a 1.5 ml sterile reaction vial

After extraction of the vitamins, pipette 150 µl of the assay-medium and 150 µl of the diluted extract or standard into the wells of the microtiter plate which has been coated with specific microorganisms. Incubate the microtiter plate in the dark at 37 °C (98.6 °F) for 44 - 48 h.

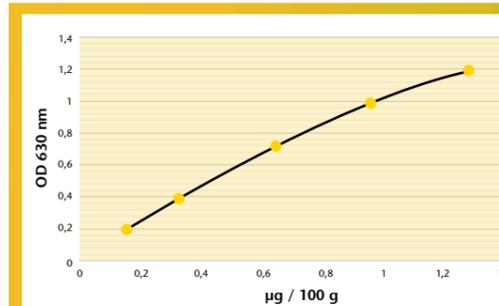
The growth of the microorganisms is dependent of the vitamin content. Following the addition of standard or sample, the bacteria will grow until the vitamin is consumed. The intensity of metabolism or growth in relation to the extracted vitamin is measured as turbidity and compared to a standard curve. The measurement is done using a microtiter plate reader at 610 - 630 nm (alternatively at 540 - 550 nm).

## Conclusion

A range of yoghurts and yoghurts with fruits were successfully tested with the microbiological VitaFast® tests as well as a range of other food types. In cooperation with ifp, the VitaFast® kits for folic acid, vitamin B12, niacin, pantothenic acid, vitamin B1, B2 and pyridoxine were validated for yoghurt samples mentioned above. The VitaFast® microtiter plate system has excellent handling and performance characteristics. Unlike other immunological assay systems, no washing step is required.

The test is suitable for determining the specific vitamin of interest with high accuracy and precision. Validation of the VitaFast® kits was carried out using recognised and reliable reference materials, as well as carrying out spike recovery tests for various food products available on the market. The coefficient of variation (CV) was below 10 %.

## Vita Fast® Folic Acid



Std n	µg/100g mean	CV (%)
Std 1	0.16	3.0%
Std 2	0.32	3.5%
Std 3	0.64	2.3%
Std 4	0.96	1.2%
Std 5	1.28	2.7%

The standard curve from the quality assurance certificate for VitaFast® Folic Acid measured at 630 nm. The coefficient of variation (CV) of the standards is less than 10 %. All test kit components are quality controlled by the ISO certified manufacturer ifp.

## Validation

### Vitamin study of preparation and storage of yoghurt products

Vitamin	Fruit Preparation			Natural Yoghurt	Natural Yoghurt + 20 % Fruit Preparation		
	natural	vitamin spiking	3 week storage 4 °C		calculated value	measured value	measured value after 5 week storage
Vit. B1 mg/100g	< 0.12	1.5	1.6	0.051	0.33	0.36	0.41
Vit. B2 mg/100g	< 0.16	1.9	1.8	0.18	0.52	0.57	0.56
Vit. B6 mg/100g	< 0.08	1.9	1.6	0.045	0.42	0.33	0.37
Vit. B12 µg/100g	< 0.30	4.7	4.6	0.30	1.2	1.3	1.2
Biotin µg/100g	< 8.0	147	141	2.3	31	29.0	27.8
Folic Acid µg/100g	18.0	490	422	5.9	103	88	81
Niacin mg/100g	< 0.64	10.3	10.4	0.12	2.2	2.0	2.1
Pantothenic Acid mg/100g	< 0.40	8.4	7.8	0.40	2.0	1.8	1.9

The vitamins have been determined with the VitaFast® test kits. The study shows excellent results, even after the storage of the yoghurts for 5 weeks at 4 - 8 °C. So an excellent reliability was observed.

The VitaFast® test kits are ideal for routine analysis since the reagents are ready-to-use and the kit is user-friendly. Therefore, food producers are now able to carry out vitamin analysis in-house.