

Determination of Watersoluble B – Vitamins with VitaFast® Tests in Fruits and Fruit Products

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

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Introduction

Over the years the food industry has expanded the number and variety of fruit products that are available on the market. Some fruit juices and fruit products naturally contain ascorbic acid and B-vitamins however the industry is also enriching and fortifying food with vitamins in many forms. Food manufacturers, regulatory agencies and commercial laboratories should therefore have analytical methods on hand that are quick and reliable for determination of the natural and fortified vitamin content. A range of fruits, fruit samples and other food types were successfully tested with VitaFast® tests. In order to determine the total vitamin content (natural and fortified vitamins) an enzymatic treatment is necessary to release the vitamin from the matrix. 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 ifp – Institute of Product Quality, Berlin – has developed a series of microbiological vitamin analysis products with a ready-to-use microtiter plate format. The trade name is VitaFast® and the test kits are marketed by R-Biopharm AG (Darmstadt).

Method

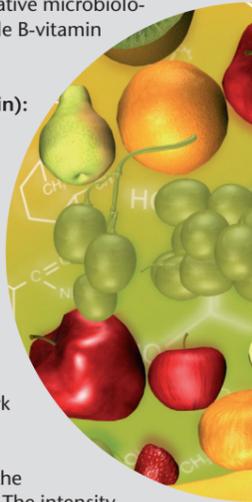
The vitamin concentration in fruits and fruit products was determined by using innovative microbiological assays in test kit format (VitaFast®). R-Biopharm presents a system of watersoluble B-vitamin determination which is rapid and based on AOAC, EN and DIN reference methods.

Fruit sample preparation for Folic Acid (total content of natural and added vitamin):

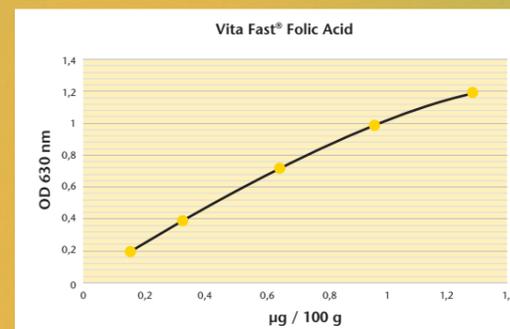
- weigh exactly 1 g (ml) homogenized sample and 10 mg Chicken Pancreatin into a 50 ml sterile centrifuge vial
- add 30 ml phosphate buffer (0.05 mol / l; 0.1 % ascorbate; pH 7.2, freshly prepared), shake well and fill up to 40 ml with phosphate buffer
- incubate 2 h at 37 °C (98.6 °F) in the dark (shake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

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

The growth of the microorganism is dependent on the supply of vitamin. 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).



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



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 and Quality Control

Test material		Niacin mg / 100 g	Vitamin B1 mg / 100 g	Vitamin B2 mg/100g	Vitamin B6 mg / 100 g
FAPAS® 2133 Liquid Vitamin	Target concentration		6.60 (5.5 - 7.7)		7.71 (6.5 - 9.0)
	VitaFast®		7.29		7.60
FAPAS® 2139 Liquid Vitamin	Target concentration		8.12 (6.8 - 9.5)	8.86 (5.3 - 12.4)	9.02 (7.6 - 10.5)
	VitaFast®		8.60	8.43	8.43
LVU ring trial Pataya blood orange (juice)	Target concentration	8.9 (5.06 - 11.40)			1.22 (0.97 - 1.85)
	VitaFast®	8.74			1.22

Vitamin B1 content intra-assay variance of fruit juices

Sample description	Concentration indicated on label (mg / 100 ml)	Amount recovered with VitaFast® (mg / 100 ml)	Coefficient of variation (%)
Vanilla drink	0.15	0.17	3.0
Multi vitamin juice	0.60	0.84	0.5
Orange juice	1.06	1.06	0.7
Fruit juice	0.21	0.29	2.5
Energy drink	0.15	0.16	4.5

Comparison of results from various analytical methods for determination of Vitamin B1

Sample description	Concentration indicated on the label (mg / 100 ml)	VitaFast®	other methods
Orange juice	0.7	0.74 (n=6)	0.72 internal HPLC
Multi vitamin juice	0.21	0.23 (n=12)	0.23 external HPLC

Folic Acid content intra-assay variance of fruit juices and fruits

Sample description	Concentration indicated on label (µg / 100 g (ml))	Amount recovered with VitaFast® (µg / 100 g (ml))	Coefficient of variation (%)
Pineapple tangerine drink	30	28	1.7
		29	1.7
Multi vitamin juice	30	40	1.2
		41	1.2
Grape-apple-lemon juice	30	37	3.1
Multivitamin nectar	30	33	2.8
		34	2.8
		35	2.8
Strawberry	16*	27	2.4
Kiwi	20*	22	7.9
Mango	31*	40	1.9
Orange	26*	26	1.8

* Nutrition table

Comparison of results from various analytical methods for determination of vitamin B12

Sample description	Concentration indicated on the label (µg / 100g (ml))	VitaFast® (µg / 100g (ml))	other methods (µg / 100g (ml))
Fruit mix	1.2	1.61 (n=6)	1.56 intern HPLC
Milk with Fruits	0.20	0.20 (n=4)	0.17 external VitaFast®
Pudding with bananas	0.65	0.82 (n=6)	0.90 (external Lab 1)
			0.76 (external Lab 2)
			0.89 (external Lab 3)
			classic microbiological assay AOAC 960.46

Conclusion

A range of fruit juices and fruit samples were successfully tested with the microbiological VitaFast® tests as well as a range of other food types. In conjunction with ifp, the VitaFast® kits for folic acid, vitamin B12, biotin, niacin, pantothenic acid, vitamin B1, B2, pyridoxine and inositol were validated for fruit 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 %.

Compared to traditional microbiological vitamin assays, the time required for analysis using the VitaFast® test kits is approximately 60 - 70 % less, and the determination costs are reduced by approx 70 %. In conclusion these microbiological tests can be successfully used to determine the fortified, natural and total vitamin content in food.