VITAMIN ANALYSIS OF BEVERAGES –

HOW MANY VITAMINS DOES FRUIT JUICE REALLY CONTAIN?

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Food products are now being enriched and fortified with vitamins in many forms because many people do not follow the National Nutrition Association recommendations to eat five portions of fruits and vegetables each day. As a result, the industry has expanded its range of vitamin-fortified fruit juices. The vitamin content of natural fruit juices mainly consists of vitamin C and B vitamins. One portion of vitamin-enriched fruit juice (100 ml) should contain at least 15 % percent of the daily recommended dose of the vitamins specified on the label. The question is: How high is the actual vitamin content in the final product? Today, food manufacturers, monitoring authorities and commercial laboratories require analytical test methods that allow quick and reliable determination of both the natural and the added vitamin content of foods and beverages.

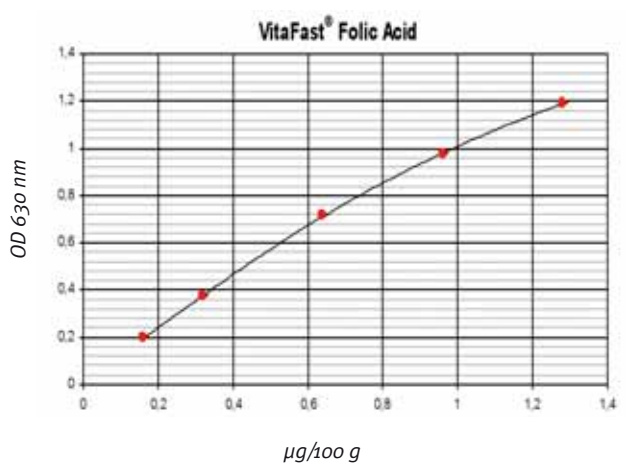
The established microbiological methods for analysis of water-soluble B vitamins, which have been on the market for several decades, are both highly sensitive and highly specific. Complementary to microbiological assays, HPLC, LC/MS/MS and other chromatographic methods are also available. HPLC with UV detection is the method of choice for analysis of fat-soluble vitamins.

Certain microorganisms replicate only in the presence of specific vitamins. These microorganisms must first be cultured in an optimal culture medium before testing. When inoculated on a culture medium that does not contain the specific vitamin, the organisms do not grow. When a standard or sample containing the missing vitamin is added, the organisms begin to grow. Growth of the organisms is reflected by opacity of the culture medium. The degree of opacity can be measured using a photometer.

In the traditional microbiological method for vitamin determination, bacterial colonies 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 viable cells must be regulated before the organisms are transferred to the medium. Maintaining these cultures requires a great deal of time and labor. Studies of accuracy and precision have shown that these methods often do not produce satisfactory results.

The ifp-Institute of Product Quality- in Berlin has developed a series of microbiological vitamin analysis products with a ready-to-use microtiter plate format. This product line is marketed by R-Biopharm AG (Darmstadt) under the trade name VitaFast®.

FIG. 2: STANDARD CURVE FOR VITAFAST® FOLIC ACID



STANDARD CURVE FOLIC ACID	
Standard n CV (%)	µg/100 g mean
Standard 1 6 3,0 %	0.16 0.197
Standard 2 6 3,5 %	0.32 0.374
Standard 3 6 2,3 %	0.64 0.714
Standard 4 6 1,2 %	0.96 0.975
Standard 5 6 2,7 %	1.28 1.188

To the following points please read the publication in FRUIT PROCESSING 1-2009, pages 14 ff.

Sample extraction · Assay medium · *Sample dilution* · *Standard curve* · Microtiter plate · Measurement and analysis

Three microbiological vitamin assays—VitaFast® Folic Acid, VitaFast® Pantothenic Acid and VitaFast® Vitamin B1—will be included in the new brewing technical analysis methods publications of MEBAK, the Central European Brewing Analysis Commission, scheduled for publication early next year. The volumes of the MEBAK "Method Collection" have been sold worldwide and are used as textbooks by many training and continuing education centers. Further VitaFast® test kits will follow.

The wells of the microtiter plate are coated with specific microorganisms that metabolize the target vitamin. The technically demanding work of preparing and maintaining microorganism cultures and suspensions is not necessary. The number of microorganisms in the wells is set and optimized in accordance with the respective target vitamin. In addition to assay-medium and sterile water, the test kit contains a characterized standard, which must be serially diluted in simple dilution steps. For test preparation, it is only necessary to add the assay-medium, standards and sample extracts to the wells. There are no washing steps necessary. Once this is done, the microtiter plates are incubated and vitamin measurements and analyses are subsequently performed using a microtiter plate photometer.

VitaFast® test kits have been tested and validated with reference materials in numerous comparison studies. The products were also tested in official interlaboratory ring trials. Some of the data on fruit juices is presented in Table 1. Further data on fruit juice and other matrices is available from R-Biopharm on request.

Tables 2 – 6 contain further study data on VitaFast® tests for vitamin analysis in juices. As with the reference materials, there is excellent correlation between the concentrations determined using the VitaFast® test kits and the nominal vitamin contents of the samples.

As shown in Tables 7 and 8, studies comparing VitaFast® with traditional microbiological assays and HPLC also show excellent correlation of results.

The VitaFast® microtiter plate system has excellent handling and performance characteristics. Unlike other immunological assay systems, no washing steps are required. The test demonstrates high accuracy and precision. The coefficient of variation (CV) is less than 10 %. In analyses of real matrices, the recovery rates ranged from 95 to 105 %. The high level of accuracy can be attributed to the test format. All test steps and test reagents (stan-

TAB. 1: RESULTS FOR VITAMIN CONTENT ANALYSIS OF REFERENCE MATERIALS USING VITAFAST®

Test material		Niacin mg/100 g	Vitamin B1 mg/100 g	Vitamin B2 mg/100 g	Vitamin B6 mg/100 g
FAPAS® 2133 Liquid vitamins	Target		6.60 (5.5 - 7.7)		7.71 (6.5 - 9.0)
	VitaFast®		7.29		7.60
FAPAS® 2139 Liquid concentrate Target	Target		8.12 (6.8 - 9,5)	8.86 (5.3-12.4)	9.02 (7.6 - 10.5)
	VitaFast®		8.60	8.43	9.50
LVU ring trial Blood orange	Target	8.9 (5.06 - 11.40)			1.22 (0.97 - 1.85)
	VitaFast®	8.74			1.22

TAB. 2: FOLIC ACID: INTRA-ASSAY VARIANCE IN LIQUID SAMPLES AND FRUIT

	labeled folic acid content (µg/100 g or ml)	VitaFast®-measured folic acid content, average (µg/100 g or ml)	Coefficient of variation(%)
Pineapple-tangerine juice	30	28 29	1.7 1.7
Multivitamin juice	30	40 41	1.2 1.2
Grape-apple-lemon juice	30	37	3.1
Multivitamin nectar	30	30 33 34	2.8 2.8 2.8
Strawberries	16*	27	2.4
Kiwi fruit, fresh	20*	22	7.9
Mangoes	31*	40	1.9
Oranges	26*	26	1.8

* Content according to www.naehrwertrechner.de

TAB. 3: BIOTIN CONTENT: INTRA-ASSAY VARIANCE IN LIQUID SAMPLES

	labeled biotin content (µg/100 g or ml)	VitaFast®-measured biotin content, average (µg/100 g or ml)	Coefficient of variation(%)
Multivitamin juice	75	76	1.3
12-fruit juice	23	28	2.1

TAB. 4: NIACIN CONTENT: INTRA-ASSAY VARIANCE IN LIQUID SAMPLES AND FRUIT

	labeled niacin content (mg/100 g or ml)	VitaFast®-measured niacin content, average (mg/100 g or ml)	Coefficient of Variation (%)
Energy drink	7	7.94	3.7
Beer	1.2	1.98	3.3
Fruit juice beverage	2	2.45	3.2
Oranges	0.3*	0.29	1.9

* Content according to www.naehrwertrechner.de

TAB. 5: VITAMIN B1: INTRA-ASSAY VARIANCE IN LIQUID SAMPLES

	labeled vitamin B1 content (mg/100 ml)	VitaFast®-measured vitamin B1 content, average (mg/100 ml)	Coefficient of Variation (%)
Vanilla drink	0.15	0.17	3.0
Multivitamin juice	0.60	0.84	0.5
Orange juice	1.06	1.06	0.7
Fruit juice beverage	0.21	0.29	2.5
Energy drink	0.15	0.16	4.5

* Content according to www.naehrwertrechner.de

dard, assay-medium, microorganisms) are optimally adjusted and in harmony with each other. Samples with an added or natural vitamin content can be analyzed. The sample preparation varies accordingly. With fortified samples, a single hot extraction step is generally sufficient for vitamin extraction. Extraction of natural vitamins should be performed by hydrolysis (enzyme digestion).

The microtiter plate format permits a high degree of automation, and the investment costs for automation are relatively low. Compared to traditional microbiological vitamin assays, the time required for the VitaFast® assay is roughly 60-70 % less, and materials consumption is around 30 times lower. The assay was developed for analysis of all water-soluble vitamins and their isomers. Selected amino acids (lysine, methionine and cysteine) are also available.

R-Biopharm additionally supplies EASI-EXTRACT® immunoaffinity columns (IAC) for preparation of samples prior to HPLC analysis. EASI-EXTRACT® Folic Acid and EASI-EXTRACT® Vitamin B12 immunoaffinity columns are available. The use of conventional HPLC methods for analysis of vitamin B12 and folic acid in complex food products has proved difficult because of the very low concentrations of vitamins often present in these products. Pigments and other interfering substances can mask folic acid and vitamin B12 on HPLC chromatograms. EASI-EXTRACT® immunoaffinity columns solve this problem: Highly specific monoclonal antibodies in the columns isolate and concentrate the target vitamins in the sample while pigments and interfering substances are washed out of the samples. EASI-EXTRACT® Folic Acid and EASI-EXTRACT® Vitamin B12 columns improve the sensitivity of HPLC and significantly improve the purity of samples used for HPLC analysis. The efficacy of the columns for the respective vitamins has been validated in a number of different food products. The immunoaffinity columns make it possible to analyze samples containing 10 to 100,000 µg folic acid or vitamin B12 per

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TAB. 6: PANTOTHENIC ACID: INTRA-ASSAY VARIANCE IN LIQUID SAMPLES

	labeled pantothenic acid content (mg/100 ml)	VitaFast®-measured pantothenic acid content, average (mg/100 ml)	Coefficient of Variation (%)
Multivitamin juice	3	3.48	0.8
Fruit juice beverage	0.9	1.07	2.5
Energy drink	2	1.83	1.0

TAB. 7: COMPARISON OF DIFFERENT METHODS FOR DETERMINATION OF VITAMIN B12 CONTENT

	labeled vitamin B12 content (µg/100 g or ml)	VitaFast®	Other methods
		Measured vitamin B12 content, average (µg/100 g or ml)	
Mixed fruit	1.2	1.61 (n=6)	1.56 HPLC (internal laboratory)
Milk drink with fruit	0.20	0.20 (n=4)	0.17 VitaFast® (external laboratory)
Banana-milk pudding	0.65	0.82 (n=6)	0.90 (external laboratory 1) 0.76 (external laboratory 2) 0.89 (external laboratory 3) Traditional microbiological assay AOAC 960.46

TAB. 8: COMPARISON OF DIFFERENT METHODS FOR DETERMINATION OF VITAMIN B1 CONTENT

	labeled vitamin B1 content (µg/100 g or ml)	VitaFast®	Other methods
		Measured vitamin B12 content, average (µg/100 g or ml)	
Orange juice	0.7	0.74 (n=6)	0.72 HPLC (internal laboratory)
Multivitamin juice	0.21	0.23 (n=12)	0.23 HPLC (internal laboratory)

TAB. 9: RESULTS OF VITAMIN B12 ANALYSIS USING EASI-EXTRACT® VITAMIN B12 IMMUNOAFFINITY COLUMNS

Sample	labeled vitamin B12 content (µg/100 g)	IAC Vitamin B12-measured vitamin B12 content, average (µg/100 g)	Coefficient of Variation (%)
Energy drink 1	0.1	0.107 (n=3)	3.9
Energy drink 2	0.1	0.095 (n=3)	4.2

TAB. 10: VITAMIN B12 SPIKING TEST IN LIQUID SAMPLES

Sample	IAC Vitamin B12-measured vitamin B12 content, average (µg/100 g)	Recovery (%) (Samples were spiked with 10µg/100g vitamin B12 and recovery measured)
Orange and Peach Fruit Shoot	8.5 (n=2)	85
Blackcurrant and Apple Fruit Shoot	8.0 (n=2)	80
Strawberry Fruit Shoot	8.0 (n=2)	80

TAB. 11: DETERMINATION OF FOLIC ACID CONTENT USING EASI-EXTRACT® FOLIC ACID IMMUNOAFFINITY COLUMNS

Sample	Nominal folic acid content (µg/100 g)	IAC Folic Acid-measured content, average (µg/100 g)	Variation coefficient(%)
Banana breakfast drink	100	118 (n=6)	8.3

100 g sample. Apart from fruit juices and drinks, EASI-EXTRACT® columns have been successfully used for analysis of vitamin tablets, vitamin premixes, cereals, flour, baby foods (powder and milk), dietary milk powder, and soy milk. They have also been evaluated in tests using reference materials of powdered baby food supplied by the National Institute of Standards and Technology (NIST) and FAPAS® cereal samples. All of the results were within the target range.

Table 9 shows the results for different beverages tested using EASI-EXTRACT® Vitamin B12 immunoaffinity columns. Table 10 shows the recovery results for spiked liquid samples.

Table 11 shows the average measured folic acid content and variation coefficient determined in a banana-containing breakfast drink. Data for other sample matrices are available from R-Biopharm AG on request.

The range of available vitamin analysis products has been expanded by the enzyme immunoassays introduced several years ago, namely, RIDASCREEN®FAST Vitamin B12, RIDASCREEN® FOLIC ACID and RIDASCREEN® Biotin.

R-Biopharm's wide range of analytical methods guarantees its customers individual solutions for a range of different analytical problems.

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