Vitamin Determination Study in Meat and Meat 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

Meat serves people as food since from time immemorial. Meat is not only one of the most important provider for proteins, but also for vitamins and minerals. For B-vitamins meat is an important source of these vitamins stand out due to a high availability. Meat products are also often enriched with vitamins. Thus industry has expanded the number and variety of meat products available by fortification with vitamins on the market. 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 determination the vitamin content in meat and meat products 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 meat and meat 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.

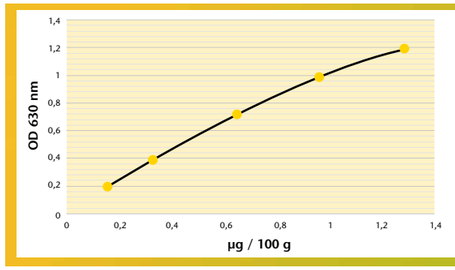
Sample preparation of meat and meat products for Folic Acid (total content of natural and added vitamin):

- 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 over night 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 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).

Vita Fast® Folic Acid



Standard curve folic acid		
Std n CV (%)	µg/100g mean	
Std 1 6 1.4 %	0.16 0.337	
Std 2 6 2.5 %	0.32 0.512	
Std 3 6 1.7 %	0.64 0.813	
Std 4 6 1.2 %	0.96 1.021	
Std 5 6 0.7 %	1.28 1.192	

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

Samples	Biotin µg/100 g			Folic Acid µg/100 g			Niacin mg/100 g			Pantothenic acid mg/100 g			Vitamin B1 mg/100 g			Vitamin B6 mg/100 g		
	Labelled	Result	CV's (%) n=4	Labelled	Result	CV's (%) n=4	Labelled	Result	CV's (%) n=4	Labelled	Result	CV's (%) n=4	Labelled	Result	CV's (%) n=4	Labelled	Result	CV's (%) n=4
Snack salami	40	52	2.1	40	64	1.9	11.7	13.7	4.8	2.3	3.5	5.9	1.1	1.0	2.4	1.0	0.92	4.9
Cream liver sausage	100	73	12	140	335	2.3	12.6	14.2	4.8	4.2	4.6	3.9	0.8	0.68	0.7	1.2	1.0	3.0
Snack pork sausage	90	79	6.5	90	133	3.7	10.8	11.4	2.9	3.6	3.7	7.8	0.9	0.74	8.1	1.2	1.2	3.8
Ham pork sausage	90	91	9.6	90	109	6.5	10.8	11.5	6.2	3.6	4.0	8.9	0.9	0.84	3.9	1.2	1.0	5.5

The vitamins have been determined with the VitaFast® test kits. The study of spiking shows excellent results and CV's.

Samples	Vitamin B1			Vitamin B6			Vitamin B12		
	Target concentration (mg/100 g)*	Results with VitaFast® (mg/100 g)	CV's (%) n=4	Target concentration (mg/100 g)*	Results with VitaFast® (mg/100 g)	CV's (%) n=4	Target concentration (µg/100 g)*	Results with VitaFast® (µg/100 g)	CV's (%) n=4
Sausages	0.37 - 0.70	0.62	3.0	0.23 - 0.45	0.39	5.3	1	0.48	7.8
Pork	0.70 - 0.89	0.65	1.4	0.33 - 0.44	0.31	6.1	1	0.45	10.0
Liver, salted	0.25 - 0.40	0.35	3.8	0.33 - 0.85	0.52	4.2	23 - 55	24	9.5
Beef, frozen	0.057	0.058	8.0	0.24	0.24	7.9	4	2	1.5
Turkey	0.09	0.12	1.9	0.30 - 0.59	0.25	7.8	0.50	3	10.0

* Lit.: Souci-Fachmann-Kraut, medpharm Scientific Publishers

Conclusion

A range of meat and meat products was 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 meat and meat 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%.

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.

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