Supelco®
Analytical Products

Application Note

Total Phosphorus in Meat and Meat Products

(According to German Food and Feed Code §64 LFGB 06.00-9)

Note

Pursuant to the valid copyright regulations this application note contains only a rough description of the content of the official method followed by a detailed description of the specific measurement procedure with the Spectroquant® Prove Spectrophotometers. A detailed description of the method specific handling steps can be found in the official method of the German Food and Feed Code §64 LFGB 06.00-9.[1]

Introduction

Phosphorus is an essential nutrient required for energy metabolism, signalling pathways, phosphorylation processes, and mineralization of bones and teeth.[2] Its main dietary sources are foods of animal origin, including dairy, meat, poultry, and fish, with cereals and legumes providing additional contributions.[3] Inorganic phosphates from food additives (E 338–341, E 343, E 450–452) have emerged as a major contributor to total intake, particularly through processed and convenience foods. In the meat industry, such additives are widely employed to enhance water-binding, emulsification, and color stability, and are estimated to account for up to half of daily phosphorus intake in Western diets.[4] Unlike protein-bound phosphorus, which is absorbed at about 60%, phosphate salts from additives are almost completely bioavailable.[5]

Excessive phosphorus intake has been associated with cardiovascular morbidity and mortality, especially in patients with chronic kidney disease, underscoring its importance as a public health concern. To limit risks, the Scientific Committee for Food established an acceptable daily intake (ADI) of 40 mg phosphorus/kg body weight per day.[6] Additive-derived phosphates are of particular concern due to their rapid dissociation and high absorption efficiency, whereas plant-derived phosphorus, bound to phytates, shows a bioavailability of only 20–30%.[6] This difference in absorption emphasizes the need to monitor total phosphorus intake, particularly among vulnerable populations.

Accurate determination of phosphorus in meat and sausage products is essential for regulatory compliance, dietary exposure assessment, and quality control. For this purpose, the German Food and Feed Code §64 LFGB 06.00-91[1] provides a validated reference procedure.

Experimental

Method

The total phosphorus content in meat and meat products is determined after dry ashing followed by acid hydrolysis. The prepared sample reacts with ammonium vanadate and ammonium heptamolybdate to form orange yellow molybdovanado-phosphoric acid that is measured photometrically at 430 nm.

This method is based on the official method of the German Food and Feed Code §64 LFGB 06.00-91[1] and describes the determination of the total phosphorus in in meat and meat products.

Measuring Range

Method 2533	Phosphorus Meat §64 LFBG 06.00–9
	0.000–2.500 g/100 g P_2O_5

Applicable Sample

Meat and meat products

Reagents, Instruments and Materials

Reagents

- Nitric acid 65% for analysis EMSURE® (**1.00452**)
- Ammonium monovanadate GR for analysis (**1.01226**)
- Ammonium heptamolybdate tetrahydrate GR for analysis (**1.01182**)
- Phosphate standard solution traceable to SRM from NIST, KH_2PO_4 in H_2O 1000 mg/L PO_4 Certipur® (**1.19898**)

MilliporeSigma is the U.S. and Canada Life Science business of Merck KGaA, Darmstadt, Germany.

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Instrument(s) & Devices

For the measurement one of the following Spectroquant® photometers is necessary

- Spectroquant® VIS Spectrophotometer Prove 100 plus (**1.73026**)
- Spectroquant® UV/VIS Spectrophotometer Prove 300 plus (**1.73027**)
- Spectroquant® UV/VIS Spectrophotometer Prove 600 plus (**1.73028**)

This application note pertains to the above listed photometers and all discontinued instruments from the Spectroquant® Prove series.

Software for Data transfer

- Optional Spectroquant® Prove Connect to LIMS software package (**Y.11086**) to transfer your data into an existing LIMS system.

Instrument Accessories

- Rectangular cells 10 mm (**1.14946**)

Other Reagents and Accessories

- Quartz or porcelain dishes
- Watch glass
- Muffle furnace
- Crucible tongs
- Exicator
- Water bath
- Folded filter, phosphate free
- Graduated cylinders, 10 mL, 20 mL
- Volumetric flasks, 100 mL, 1000 mL
- Standard laboratory glassware (e.g. glass beakers) and pipettes
- Analytical balance

Analytical Procedure

Reagent Preparation

- **Ammonium monovanadate solution** – The solution must be prepared according to German Food and Feed Code §64 LFGB 06.00-9.[1]
- **Ammonium heptamolybdate solution** – The solution must be prepared according to German Food and Feed Code §64 LFGB 06.00-9.[1]
- **Reagent solution** – The solution must be prepared according to German Food and Feed Code §64 LFGB 06.00-9.[1]

Sample Preparation

According to German Food and Feed Code §64 LFGB 06.00-9.[1]

Preparation of Measurement Solutions

Dry ashing

- Weigh sample to a dish and follow the procedure according to German Food and Feed Code §64 LFGB 06.00-4.[7]
- Note the sample weight.

Sample Solution preparation

- Hydrolyze the obtained ash according to the procedure of German Food and Feed Code §64 LFGB 06.00-9, chapter 7.3.[1]

Phosphorus determination

- **Reagent blank** - Mix 2 mL of distilled water with 8 mL Reagent solution and incubate for 15 min at room temperature. The color of the measurement solution remains stable for 30 min.
- **Sample** - Mix 2 mL of the prepared Sample solution with 8 mL Reagent solution and incubate for 15 min at room temperature. The color of the measurement solution remains stable for 30 min.

Measurement

Note: It is advisable to measure the reagent blank and the sample using the same cell as the one used for the zero adjustment or else a cell with identical optical characteristics and an identical absorption (matched pair).

- Open the methods list (<Methods>) and select Method No. 2533 "Phosphorus Meat §64 LFGB 06.00-9”.
- The instrument automatically prompts a "Zero adjustment”.
- For the zero adjustment fill a clean and dry 10 mm rectangular cell with distilled water.
- After prompting, insert the filled rectangular cell into the cell compartment. The zero adjustment is performed automatically.
- Confirm the performance of the zero-adjustment procedure by clicking on <OK>
- A window with an input field to enter the sample weight pops up.
- Enter the weight of the sample in grams (g), accurate to 0.001 grams (g), confirm with <OK> and click on <START> to switch to the measurement procedure.

  Note: It is possible to enter a sample weight in range of 0.010 to 10.000 g.
- Fill the prepared reagent blank into a clean and dry 10 mm rectangular cell. Insert the cell into the cell compartment. The measurement is performed automatically. A (✓) symbol appears behind the cue "Insert Reagent Blank”.
- Confirm the measurement by clicking on <OK>.
- Finally fill the prepared sample solution into a clean and dry 10 mm rectangular cell. Insert the cell into the cell compartment. The measurement is performed automatically. A (✓) appears behind the cue "Insert Sample”.

- Confirm the measurement by clicking on <OK>
- Read off the result in g/100 g P_2O_5 and the absorption for the reagent blank (A_{RB}) and the sample (A_{Sample}) from the display.
- Tap the <START> button to start the measurement procedure for the next sample.

Analytical quality assurance

- The method can be checked using Phosphate standard solution traceable to SRM from NIST KH_2PO_4 in H_2O 1000 mg/L PO_4 Certipur® (**1.19898**), which corresponds to 747.3 mg/L P_2O_5. This solution is diluted to 10 mg/100 mL P_2O_5 (= 100 mg/L P_2O_5) with water for analysis or distilled water. To do this, place 2.676 mL Cat. No. 1.19898 Phosphate standard solution 1000 mg/l PO_4 into a 20 mL volumetric flask and fill up to the mark with distilled water.
- Mix 2 mL of the prepared Standard solution with 8 mL Reagent solution and incubate for 15 min at room temperature. The color of the measurement solution remains stable for 30 min.
- Measure this solution versus a reagent blank as described in the section "Measurement". Hereby enter a weight of 1.00 g.

Note: Due to the different sample preparation procedure and phosphorus determination procedure of the 10 mg/100 mL P_2O_5 standard solution compared to a sample analysis it is necessary to recalculate the displayed result manually as follows:

$$\text{Measured concentration standard} \left[\frac{\text{mg}}{\text{100 mL}}\right] =$$

$$\text{Displayed result [g/100 g]} \cdot \frac{F1}{F2} =$$

$$\text{Displayed result [g/100 gm]} \cdot \frac{1000}{100}$$

$$\textbf{Measured concentration standard} \left[\frac{\textbf{mg}}{\textbf{1000 mL}}\right] = \textbf{Displayed result [g/100 g] · 10}$$

Whereas

F1 = 1000 = recalculation g/100 g to mg/100 mL
F2 = 100 = Factor sample preparation for real sample

Adjustment

- In case of significant deviations in the method control procedure the preprogrammed factor of 27.99 or the current factor used in the calculation of the displayed results can be adjusted by the user.
- The corrected factor must be recalculated as follows:

$$\text{Factor corrected} = \text{Current factor} \cdot \frac{\text{target value standard}}{\text{measured and recalculated value standard}}$$

- To edit the preprogrammed factor, select method 2533 from <Methods>.
- Close the window for the “Zero adjustment” by clicking on <X>.
- Close the input field for the sample weight by clicking on <X>.
- Click <Settings> and select the list “FACTORS”.
- Tip on the input field “Factor”, enter the corrected factor and confirm by clicking on <OK>.
- Close the window for the “Zero adjustment” by clicking on <X>.
- For the next measurement restart the method by selecting the method anew from <Methods>.

Note: To find the used factor, select Method 2533 from <Methods>.
- Close the window for the “Zero adjustment” by clicking on <X>.
- Close the input field for the sample weight by clicking on <X>.
- Click <Settings> and select the list “FACTORS”.

References

1. German Food and Feed Code §64 LFGB 06.00-9:2008 Bestimmung des Gesamtphosphorgehaltes in Fleisch und Fleischerzeugnissen.
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3. EFSA Panel on Food Additives and Flavourings (FAF), Younes M, Aquilina G, Castle L, Engel KH, Fowler P, Frutos Fernandez MJ, Fürst P, Gürtler R, Husøy T, M`ennes W. Re-evaluation of phosphoric acid–phosphates–di-, tri-and polyphosphates (E 338–341, E 343, E 450–452) as food additives and the safety of proposed extension of use. *EFSA Journal*. **2019**;17(6):e05674. DOI:**10.2903/j.efsa.2019.5674**
4. Tonelli M, Sacks F, Pfeffer M, Gao Z, Curhan G. Cholesterol and Recurrent Events (CARE) Trial Investigators. Relation between serum phosphate level and cardiovascular event rate in people with coronary disease. *Circulation*. **2005**;112(17):2627–33. DOI:**10.1161/CIRCULATIONAHA.105.553198**
5. Milešević J, Vranić D, Gurinović M, Koricanac V, Borović B, Zeković M, Šarac I, Milićević DR, Glibetić M. The intake of phosphorus and nitrites through meat products: A health risk assessment of children aged 1 to 9 years old in Serbia. *Nutrients*. **2022**;14(2):242. DOI:**10.3390/nu14020242**
6. Kemi VE, Kärkkäinen MU, Lamberg-Allardt CJ. High phosphorus intakes acutely and negatively affect Ca and bone metabolism in a dose-dependent manner in healthy young females. *British journal of nutrition*. **2006**;96(3):545-52. DOI:**10.1079/BJN20061826**
7. German Food and Feed Code §64 LFGB 06.00-4:2017 Bestimmung der Asche in Fleisch, Fleischerzeugnissen und Wurstwaren.

Featured Products

Description	Cat. No.
Instruments and Accessories	
Spectroquant® VIS Spectrophotometer Prove 100 plus	**1.73026**
Rectangular cells 10 mm	**1.14946**
Reagents	
Nitric acid 65% for analysis EMSURE®	**1.00452**
Ammonium monovanadate GR for analysis	**1.01226**
Ammonium heptamolybdate tetrahydrate GR for analysis	**1.01182**
Phosphate standard solution traceable to SRM from NIST KH_2PO_4 in H_2O 1000 mg/L PO_4 Certipur®	**1.19898**

Related Products

Description	Cat. No.
Instruments	
Spectroquant® UV/VIS Spectrophotometer Prove 300 plus	**1.73027**
Spectroquant® UV/VIS Spectrophotometer Prove 600 plus	**1.73028**

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