United States Department of Agriculture

Food Safety and Inspection Service

CLG-SLT.04

Quantitation of Salt (NaCl)

This method describes the laboratory procedure for quantitation of salt in processed meat products at a level ≥ 0.08%.

Notice of Change

This method has been modified for:

- The method is reformatted.
- Removed determination and added quantitation to the title and throughout the method.
- Added reagent blank and positive control criteria.

Table of Contents

Safety Precautions

The personnel performing the analysis are to read the Safety Data Sheets for the standards and reagents used in this method. Follow all applicable federal, state, and local regulations regarding the disposal of chemicals listed in this method.

Introduction

Salt (sodium chloride, NaCl) is one of the most widely used food additives today.[1] Before the use of canning and the invention of refrigeration, salt curing was one of the primary forms of food preservation. Although we now have other means of preservation, salt is used for curing or corning foods such as country ham, corned beef and bacon.[2] Salt is also added to meat to influence water-holding capacity.

The analysis of salt is used in ensuring food is compliant with labeling requirements set by the Federal Meat Inspection Act (FMIA), the Poultry Products Inspection Act (PPIA), the Egg Products Inspection Act (EPIA), and Title 9 of the Code of Federal Regulations (CFR). The correct quantitation of the salt content of meat is important for the economic value of the food product.

Method Overview

The salt (NaCl) content is determined by the Volhard method, which determines the salt content based on the quantitation of chlorides. The sample is treated with excess silver nitrate ($AgNO_3$) to react with the chlorides in the sample through precipitation to produce silver chloride and wet-ashed through boiling with concentrated nitric acid (HNO_3) to remove organic material. Prior to titration, concentrated potassium permanganate ($KMnO_4$) is then added to oxidize any organic matter not disposed of by the HNO_3. Following boiling, cooling, and dilution, the excess $AgNO_3$ is back titrated with a potassium thiocyanate (KSCN) solution, employing an ammonium iron (III) sulfate ($FeNH_4(SO_4)_2$) solution as an indicator. The $FeNH_4(SO_4)_2$ reacts with an excess of thiocyanate, forming the ferric thiocyanate ($FeSCN^{2+}$) complex and salmon colored solution, indicating the end point. The salt content is then determined by the amount of volume of KSCN used for the titration.

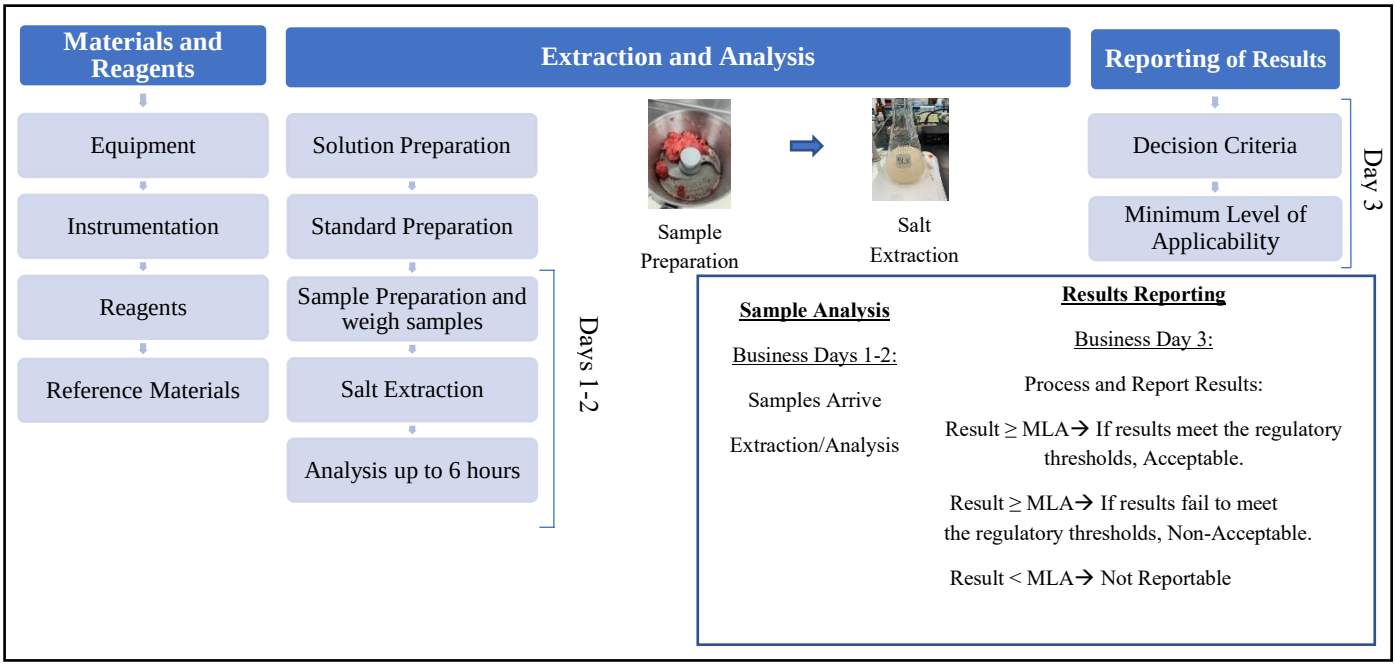

Figure 1: Overview and timeframe of salt analysis. Materials and reagents are obtained and utilized to prepare solutions and standards. Samples arrive at the laboratory, are prepared into a homogenized mixture, weighed, and analyzed on business days 1-2. Results are reported on business day 3. This chart represents the best-case scenario, but analyses may take longer due to analytical testing circumstances. Pictures courtesy of Hue Quach and Bruno Giri, USDA-FSIS.

[1]https://ask.usda.gov/s/article/What are the most widely used food additives?

[2]https://ask.usda.gov/s/article/How-is-salt-used-to-make-food-shelf-stable?

Decision Criteria

Quantitative results are reported for all samples. Sample results are compared to the regulatory thresholds found in the 9 CFR for the particular product of interest and the MLA. A sample is considered "Acceptable" if the results meet the regulations. A sample is considered "Non-Acceptable" if the results fail to meet the regulations.

KEY DEFINITIONS
MLA: Lowest level at which an FSIS method has been successfully validated for a residue in each matrix. Full definition is on the CLG website here.

Disclosure Statement

FSIS does not specifically endorse any test products listed in this method. FSIS acknowledges that equivalent equipment, reagents, or solutions may be suitable for laboratory use. The FSIS laboratory system uses method performance requirements when evaluating the equivalence of an alternative equipment, reagent, or solution for a given analyte and sample matrix pair. Significant equivalence changes would require FSIS laboratory leadership approval.

Materials and Reagents

Equipment

Table 1: Equipment Required to Perform CLG-SLT

Equipment	Supplier and Part Number	Purpose
Food Processor	Robot Coupe USA Inc.	Homogenize sample
Cutting board and knives	General lab supplier	Preparation of sample
50 mL Burette Class A, Kimax	General lab supplier	Measuring solution used
300 mL Erlenmeyer Flask Pyrex	General lab supplier	Digestion vessel
25 mL pipettes, Class A Pyrex	General lab supplier	Addition of silver nitrate to samples
Boiling Chips Carborundum #12 granules	General lab supplier	Prevention of solution spattering
1 L volumetric Flask Class A, Kimax	General lab supplier	
Drying oven, capable of maintaining 101 ± 1°C	FREAS oven Model 625, Precision Scientific	Drying of primary standard

Freezer, -10 °C	General lab supplier	Storage of sample, standards and reagents
Analytical balance, capable of reading 0.1 mg	General lab supplier	Weighing sample

Instrumentation

Table 2: Instrumentation

Instrument	Supplier and Model Number	Purpose
None		

Reagents

Table 3: Reagents

Reagent	Supplier and Part Number
Ferric alum indicator-Saturated aqueous solution of reagent grade $FeNH_4(SO_4)_2 \cdot 12 H_2O$, ACS Grade	Cat. No. 3070, RICCA Chemical Company
Nitric Acid - reagent 68-70% Solution in Water (HNO_3) ACS Grade	Cat. No. 424000026, Thermo Scientific
Potassium Permanganate ($KMnO_4$), 5% (W/V)	Cat. No.636016, RICCA
Diethyl Ether - reagent grade	Cat No. 9244, J.T. Baker
Lactose - ACS Grade,	Cat. No. 2248, J.T. Baker
Silver Nitrate ($AgNO_3$), ACS Grade, 99.9+%	Cat. No. 2169, Thermo Scientific
Potassium Thiocyanate (KSCN), ACS Grade	Cat. No. 27035.230, VWR BDH Chemicals
Potassium Chromate (K_2CrO_4), ACS Grade	Cat. No. P220, Fisher Scientific
Water, distilled or deionized	House supplied

Reference Materials

Table 4: Reference Materials

Standard	Supplier	Catalog Number
Potassium Chloride (KCl) - ACS Grade	Sigma-Aldrich	Cat. No. 3911

Purity and counterions are to be taken into account when calculating standard concentrations. In-house prepared standards are to be assigned an expiration date that is no later than the stability stated in the method.

Extraction and Analysis

Solution Preparation

Table 5: Preparation of Solutions

Solution	Procedure
Silver Nitrate ($AgNO_3$) (0.1000 N ± 0.0005) **Solution expires after 1 year.** **Standardization is verified weekly.** **Store at room temperature.**	1) Dissolve 17.04 g of silver nitrate ($AgNO_3$) in water in a 1 L volumetric flask. 2) Dilute to volume with water.
Potassium Thiocyanate (KSCN) (0.1000 N ± 0.0005) **Solution expires after 1 year.** **Standardization is verified weekly.** **Store at room temperature.**	1) Dissolve 9.72 g of reagent grade potassium thiocyanate (KSCN) in water in a 1 L volumetric flask. 2) Dilute to volume with water.
1:1 Nitric Acid (HNO_3) **Solution expires after 1 year.** **Store at room temperature.**	1) Add 100 mL of nitric acid (HNO_3) to 100 mL of water and mix carefully.
5 % Potassium permanganate ($KMNO_4$) solution **Solution expires after 1 year.** **Store at room temperature.**	1) Add 100 g of potassium permanganate ($KMNO_4$) to a 2 L graduated cylinder or volumetric flask. 2) Dilute to volume with water. 3) Mix well. 4) Store in actinic glassware or otherwise protect from light.
5 % Potassium Chromate (K_2CrO_4) solution **Solution expires after 1 year.** **Store at room temperature.**	1) Add 5 g of potassium chromate (K_2CrO_4) to 100 mL volumetric flask. 2) Dilute to volume with water and mix.

Standardization of silver nitrate ($AgNO_3$) and potassium thiocyanate (KSCN)

Table 6: Standardization of silver nitrate ($AgNO_3$) and potassium thiocyanate (KSCN)

Standardization of Silver Nitrate ($AgNO_3$) solution	1) Weigh 0.2500 ± 0.0500 g of potassium chloride (KCl) that has been dried at 101 °±1 °C for 1 hour ± 10 min into a 250 mL Erlenmeyer flask and dissolve in 40 mL of water. 2) Add 1 mL of potassium chromate (K_2CrO_4) indicator. 3) Titrate with the silver nitrate ($AgNO_3$) solution to a permanent light brown (salmon colored) endpoint.
Standardization of Potassium Thiocyanate (KSCN) solution	1) Pipette 25 mL of the standard silver nitrate ($AgNO_3$) solution into a 300 mL Erlenmeyer flask. 2) Add 80 mL of water. 3) Add 15 mL of the 1:1 nitric acid (HNO_3) solution. 4) Add 2 mL of the ferric alum indicator. 5) Titrate with potassium thiocyanate (KSCN) solution to a permanent light brown (salmon colored) end point.

Sample Preparation

Samples must be kept cold and sealed from the air before and during shipping to the laboratory. An example of a sample is shown in Figure 2. Once received at the laboratory, samples must be frozen (≤ -10 °C) prior to grinding if they cannot be prepared on the day of receipt. If frozen, temper (partially thaw) the sample while keeping it as cold as possible. As shown in Figure 3, grind the sample in blender or vertical cutter-mixer until homogeneous. Store homogenized samples frozen (≤ -10 °C) prior to analysis. Equipment must be dry before grinding. Handle sample in a way to avoid evaporation and condensation.

Figure 2: Processed meat product. Photo courtesy of Getty Images.

Figure 3: Homogenized sample. Photo courtesy of Hue Quach, USDA FSIS

Salt Extraction

Samples

QUALITY CONTROL

1) Run a reagent blank with each set of samples.
2) Run a previously analyzed sample or a sample with a known reference value as a recovery with each set of samples.
3) Weigh one additional portion for an intra-laboratory check sample, if necessary.

KEY DEFINITIONS

Recovery (positive control): A sample with a known concentration of salt.
Reference value: The value of the known concentration in the recovery.
Reagent Blank: A control sample containing only silver nitrate, nitric acid and a small amount of lactose. The lactose is added so the titration endpoint will be clearly visible.

1) Weigh 2.5-3.0 g of finely comminuted and thoroughly mixed sample into a 300 mL Erlenmeyer flask.
2) For country ham or a cured product, weigh 1.0-1.5 g into a 300 mL Erlenmeyer flask.
3) For "seasoning" samples weigh 10.0-10.5 g into a 100 mL volumetric flask and dilute to volume with water. Pipet a 1.0 mL aliquot into a 300 mL Erlenmeyer flask and record the weight as 0.10 g.

Extraction

1) Add 25.0 mL of 0.1000 N ± 0.0005 silver nitrate ($AgNO_3$) solution, swirl flask until sample and solution are in intimate contact, and then add 15 mL of concentrated nitric acid (HNO_3).

Figure 4: Sample after addition of the silver nitrate ($AgNO_3$) and nitric acid (HNO_3). Photo courtesy of Bruno Giri, USDA-FSIS

Technical Note:

The silver nitrate ($AgNO_3$) solution must be added first, followed by the addition of concentrated nitric acid (HNO_3). The order of addition is critical to ensure the complete precipitation of the chlorides. If nitric acid (HNO_3) is added first, loss of chlorides by volatilization as hydrochloric acid (HCl) could occur because hydrochloric acid (HCl) has a higher vapor pressure than nitric acid (HNO_3).

2) Add sufficient boiling chips and boil until meat digests. Add a teaspoon of lactose to the reagent blank. Solution will turn from a cloudy white color to yellow.

Figure 5: Digested sample. Photo courtesy of Bruno Giri, USDA-FSIS

3) Add enough potassium permanganate ($KMnO_4$) solution while boiling to turn the solution dark brown for several minutes. Continue boiling until color disappears. Continue adding small portions of potassium permanganate ($KMnO_4$) until solution retains dark color for several minutes before clearing. Wash sides of flask with water.

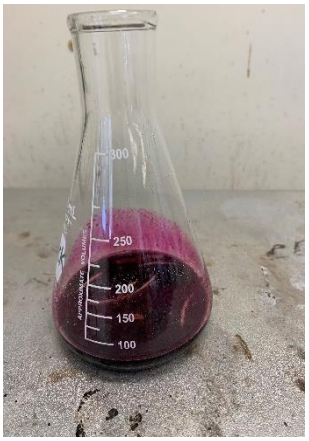

Figure 6: Digested sample with initial addition potassium permanganate ($KMnO_4$). Photo courtesy of Bruno Giri, USDA-FSIS

Figure 7: Digested sample with the addition of potassium permanganate ($KMnO_4$) and boiled for several minutes. Photo courtesy of Bruno Giri, USDA-FSIS.

Figure 8: Digested sample after addition of potassium permanganate ($KMNO_4$) and becoming clear after boiling. Photo courtesy of Bruno Giri, USDA-FSIS

4) If solution retains color and will not become colorless, add a small amount of lactose until the color disappears.
5) Add water to adjust total volume to 25 mL using the graduations on the flask. Boil for 5 min, cool to room temperature in the fume hood, rinse the neck of the flask and dilute with water to a total volume of 150 mL using the graduations on the flask.
6) Add the ferric alum indicator using a disposable transfer pipette, and swirl to coagulate the precipitated silver chloride (AgCl).
7) Titrate the excess silver nitrate ($AgNO_3$) with potassium thiocyanate (KSCN) solution to a permanent, salmon colored, end point.

Technical Note:

Excess of thiocyanate will react with the precipitated silver chloride (AgCl), therefore, the addition of nitrobenzene or diethyl ether overcomes the precipitation by coating the precipitated silver chloride (AgCl), thereby withdrawing it from the action of the thiocyanate solution.

- If diethyl ether is used, add diethyl ether using a disposable transfer pipette.

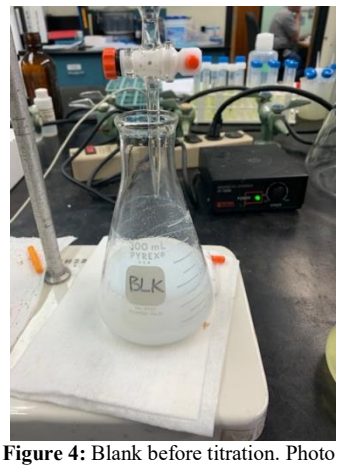

Figure 4: Blank before titration. Photo courtesy of Bruno Giri, USDA-FSIS

Figure 5: Titration endpoint. Photo courtesy of Bruno Giri, USDA-FSIS

8) If titration with potassium thiocyanate (KSCN) is less than 2 mL, repeat with a smaller sample weight.

Instrumental Analysis

No instrument required.

Sample Set

The sequence below can be modified, as needed, but must include required controls.

1) Reagent blank
2) Positive Control (Recovery)
3) Intra-laboratory check sample (if applicable)
4) Samples up to 20 samples

INTRA-LABORATORY CHECK SAMPLE

Defined on the CLG website here.

Reporting of Results

Decision Criteria

Calculations

1. Procedure

$$\text{Percent NaCl} = \frac{[25.0\ \text{mL} - (\text{mL KSCN})(R)](\text{N } AgNO_3)(5.85)}{\text{Sample Weight}}$$

$$\text{where R = ratio of } \frac{\text{mL } AgNO_3}{\text{mL KSCN}}$$

As determined in Standardization of $AgNO_3$ and KSCN

Technical Notes:

1) Use a sample weight of 2.5 grams for the reagent blank.
2) After all the silver has been back-titrated, an excess of thiocyanate will react with the precipitated silver chloride (AgCl) because the solubility product of silver thiocyanate (AgSCN) is 1/100 that of silver chloride (AgCl).

AgSCN	1.0×10^{-12}
AgCl	1.1×10^{-10}

If results are rounded to 0.1%, precipitate coating is not needed.

QUALITY CONTROL

Quality Control Procedures

For set acceptance

1) The positive control (recovery) value obtained are to have a ≤ 0.22% difference from the reference value.
2) The reagent blank (negative control) are to be less than 10% of the positive control.

Intralaboratory Check Samples (If applicable)

1) Acceptability criteria.
   a. The check samples are to have a ≤ 0.22% difference from the reference value.
      a. FSIS Field Service Laboratories are to refer to internal FSIS Quality Control Procedures when unacceptable values are obtained:
         i. Refer to LW-Q1002, Chemistry Non-Conformance Tables, for how to proceed and whether to take corrections or corrective actions.

Minimum Level of Applicability

Table 13: Minimum Level of Applicability for Screening level per species

	Matrix	%
Salt	Processed Product	0.08

References

a. Official Methods of Analysis of the Association of Official Analytical Chemists, 15th Edition: 935.47, 941.18.

b. 9 CFR 319 Definitions and Standards of Identity or Composition

Contact Information and Inquiries

Inquiries about methods can be submitted through the USDA website via the "Ask USDA" portal at https://ask.usda.gov or please contact:

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This method has been validated, reviewed, approved, and deemed suitable and fit for purpose for use in the USDA FSIS Field Service Laboratories.

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