# Lab Manuals Compendium

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| **COURSE 1**<br>**MEAT PRODUCTION AND MEAT QUALITY** | **Basis of meat science and meat quality**<br><br>1. Analysis of pH<br>2. Analysis of water holding capacity in raw meat and during cooking<br>3. Myoglobin content<br>4. Instrumental analysis of raw meat colour<br>5. Analysis of cooked meat texture with Warner-Bratzler shear forcé<br><br>**Meat handling and storage procedure**<br><br>1. Total aerobic mesophilic viable counts<br>2. Analysis of thiobarbituric acid reactive substances (TBARS) |
| **COURSE 2**<br>**MEAT PROCESSING FUNDAMENTALS** | **Traditional meat preservation methods**<br><br>1. Analysis of sodium chloride content in meat products<br>2. Determination of the degree of reaction of meat dyes<br>3. Determination of nitrites content in meat products |
| **COURSE 3**<br>**ADVANCES IN MEAT PROCESSING AND NOVEL MEAT PRODUCTS** | **Emerging technologies in meat processing**<br><br>1. Extraction of bioactive compounds with Ultrasound Assisted Extraction<br>2. Extraction of bioactive compounds with Pulsed Electric Fields<br>3. Oil extraction with Supercritical Fluid Extraction |
| **COURSE 4**<br>**SAFETY, QUALITY AND REGULATORY AFFAIRS** | **Regulatory framework on meat and meat products**<br><br>1. Web search for food additives: How to search for any additive authorized in the UE<br>2. How to search for quality labels in the UE (*e*Ambrosia web database)<br><br>**Meat and meat products safety**<br><br>1. How to determine CCP using a decision tree. The determination of a CCP in the HACCP system can be facilitated by the application of a decision tree, which indicates a logical reasoning approach.<br>2. Risk Analysis Tools in the Meat Industry: tool to use in order to perform risk analysis that is needed when developing a HACCP plan for a Food Safety Management System<br>3. HACCP verification Tools in the Meat Industry: possible verification tools for the implementation of a HACCP plan in the meat industry will be described. |

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| | **Meat quality control and assurance**<br><br>1. Meat Products Quality Control: Nitrites. the method for determining the nitrite content will be described<br>2. Meat Products Quality Control: Fat content. the method for determining the nitrite content will be described<br>3. Meat Products Quality Control: Protein content. the method for determining the protein content will be described<br>4. Meat Products Quality Control: Simultaneous determination of protein, fat and moisture. the method for determining the protein, fat and moisture contents will be described<br>5. Environmental Monitoring as a prerequisite for certification schemes in the meat industry: a step-by-step development of an environmental monitoring plan, that is essential for several Food Safety Management Systems will be described. |
| **COURSE 5**<br>**ENVIRONMENTAL IMPACT OF MEAT PRODUCTION AND CONSUMPTION AND SUSTAINABILITY IN THE MEAT SECTOR** | **Meat production in relation to GHGs emissions and water consumption**<br><br>1. Calculating and analysis of the student's environmental footprint EF<br><br>**Alternatives for meat proteins**<br><br>2. Characteristics and evaluation of innovative meat products: meat snack and meat analogues |

# Lab Manual Course 1

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| **COURSE** | **MEAT PRODUCTION AND MEAT QUALITY** |
| **Training Subject** | **1.3. Basis of meat science and meat quality** |
| **Index** | **1. Analysis of pH**<br>**2. Analysis of water holding capacity in raw meat and during cooking**<br>**3. Myoglobin content**<br>**4. Instrumental analysis of raw meat colour**<br>**5. Analysis of cooked meat texture with Warner-Bratzler shear force** |

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| **Practical** | **1. <u>Analysis of pH</u>** |
| **Competences** | To be able to determine the pH value of meat in the lab<br>To understand most important applications of meat pH determination |
| **Overview** | The normal pH of meat before slaughter is between 6.9 and 7.1. After slaughter, the pH value decreases for a 12-48 h period to 5.4-6.2. During further storage this pH value is rather constant; this constant pH value is known as pHu. However, over the course of storage time pH can steadily and slightly increase due to microbial growth alkaline metabolite formation, indicating that meat is losing its shelf life.<br>Meat pH varies slightly among species, within species among individuals and within individuals among muscles. Muscles with large numbers of fats twitch fibres tend to have lower pHs.<br>The pH of meat also depends on pre-slaughter factors, such as the stress of the animal at the time of slaughter. Exhausted animals with depleted muscle glycogen reserves result in dark, firm and dry (DFD) meat with an elevated pH. Pigs and poultry with adequate muscle glycogen reserves but momentarily stressed can result in low pH meat [pale, soft and exudative (PSE) meat]. |
| **Materials** | • Graduated cylinder and beaker<br>• pH buffers (4, 7, and/or 10)<br>• Deionized water in a wash bottle<br>• Delicate task wipe<br>• Filter paper (Whatman #1 or equivalent) folded into cone shape |

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| | • Product to be tested (e.g. a 20 g portion of longissimus thoracis muscle of beef, lamb or pork or chicken breast) |
| **Equipment** | • pH metre<br>• Blender<br>• Gram scale |
| **Procedure** | 1. Calibrate pH metre using a two-point calibration (7 and 4 are recommended)<br>2. Prepare a sample by dicing and weighing approximately 10 g of meat.<br>3. Blend the 10 g diced-meat sample and 90 mL of deionized water<br>4. Transfer the mix to beaker<br>5. Place the filter paper inside the beaker so that the end of the cone is immersed in the mixture and the pH meter probe can be placed inside the immersed part of the cone (optional)<br>6. Place the pH probe into a sample inside the mix. If the filter paper is used, place the pH probe into the filter paper cone flooded with liquid<br>7. Gently and manually stir the beaker until the pH reading is stable<br>8. Record pH reading on record keeping document<br>9. Properly clean and store all equipment |
| **Applications** | • To detect presumably DFD, PSE meat, or low-pH or acidic meat. Typical/normal/acceptable values for beef, 5.4 to 6.0; pork, 5.5 to 6.2; chicken: 5.1 to 6.2.<br>• To monitor the pH changes during meat storage. The pH tends to increase when meat is spoiled due to microbial metabolisms.<br>• To differentiate muscles according to their metabolism or type of fibre. |
| **References** | 1. Dutson, T.R. (1983). The Measurement of pH in Muscle and its Importance to Meat Quality. Reciprocal Meat Conference Proceedings. Volume 36, 7 983. https://meatscience.org/docs/default-source/publications-resources/rmc/1983/the-measurement-of-ph-in-muscle-and-its-importance-to-meat-quality.pdf?sfvrsn=2<br>2. Processed Meat Quick Guide: pH and Water Activity Protocol https://www.canr.msu.edu/resources/processed-meat-quick-guide-ph-and-water-activity-protocol<br>3. Font-i-Furnols, M., Fulladosa, E., Prevolnik Povše, M. and ČandekPotokar, M., 2015. A Handbook of Reference Methods for Meat Quality Assessment. (European Cooperation in Science and Technology (COST), Brussels, Belgium) https://www.researchgate.net/publication/283730729_A_handbook_of_reference_methods_for_meat_quality_assessment |

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| **Practical** | **2. Analysis of water holding capacity in raw meat and during cooking** |
| **Competences** | To know and be able to perform water holding capacity analysis of raw meat and of meat during cooking.<br>To visualize the free water released by meat submitted to forces or heating. |
| **Overview** | Water holding capacity (WHC) is defined as the ability of the postmortem muscle (meat) to retain water even though external pressures (e.g. gravity, centrifugation) or heating are applied to it. In fresh or thawed meat, it is inversely related to the amount of drip loss or purge. During meat cooking, it is directly related to cooking yield.<br>Meat proteins are the main components responsible for chemically holding meat water. The integrity of meat structures such perimysium and epimysium also contribute to retaining water into muscle fibers. Meat protein denaturation, meat pH next to the pI of meat proteins (c.a. 5.2) or a sort of sarcomere length are associated to low WHC of meat.<br>WHC of meat and meat products determines visual acceptability, weight loss, and cook yield as well as sensory traits on consumption. WHC is directly related to meat juiciness.<br>Numerous methods have been evaluated over the years to determine WHC. Each method has advantages and limitations. What works for one operation may not work for another. Two methods for WHC analysis (one for drip in raw (uncooked) whole meat and one for cooking losses in whole meat have been approved as reference methods for WHC. |
| **Materials** | • Meat to be tested (e.g. a 100-300 g portions of *longissimus thoracis* muscle)<br>• Sealable, water-impermeable containers (or plastic bags), c.a. 200 ml capacity<br>• Sample support that allows the escape of fluid (plastic net bag or perforated support)<br>• Paper towel |
| **Equipment** | • Gram scale (0.05 g accuracy)<br>• A temperature-controlled environment<br>• Temperature-controlled water bath<br>• A puncture thermometer or thermocouples to allow temperature recording in the centre of cooked samples.<br>• Vacuum packaging equipment (recommendable) |
| **Procedure** | 1. Raw meat<br>2. Cut and weigh immediately. Samples weighing approximately 80-100 g are recommended.<br>3. Place each sample in the netting or supporting mesh and then suspend it into an inflated bag or container and seal, ensuring that the sample does not contact the bag or container.<br>4. Keep the samples for a storage period (usually 24 h) at chill temperatures (1 to 4°C) |

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| | 5. -Remove the samples from the bags or containers and weigh them again.<br>6. - The same samples can be used for further drip loss measurements, e.g. after 2, 7 days, etc., but in every case the initial weight is used as the reference point.<br>7. - At the time of measurement, samples should be taken immediately from the containers, gently blotted dry and weighed.<br>8. -Drip loss is expressed as a percentage of the initial weight.<br>9. -Triplicates are recommended.<br>10. During cooking<br>11. -Cut and weigh (initial weight) sample slices of 50 mm thick (maximum) and of a standard weight and put them in plastic bags<br>12. -Place the bags and meat in a heated water-bath at a temperature between 70 and 80 °C. Bags have to be hermetically closed or placed into the bags. Then place the containers or bags with the meat in the bath.<br>13. -Cook the samples to a defined internal temperature; 70-75°C is recommended.<br>14. -When the end- point temperature has been attained (around 30-40 min), remove the samples from the water-bath, and cool it in an ice slurry.<br>15. -Hold the samples in chill conditions until equilibrated.<br>16. -Take the samples out of the bags and blot dry and weight.<br>17. Drip loss is expressed as a percentage of the initial weight.<br>18. Triplicates are recommended. |
| **Applications** | To predict the drip loss of fresh meat during storage and to estimate the cooking yield of fresh meat. Water holding capacity is related to pH and pH changes during *rigor mortis* and with the sarcomere length. It is a technological quality trait responsible for meat quality. Meat with low values of WHC is indicative of meat with low quality. |
| **References** | 1. Font-i-Furnols, M., Fulladosa, E., Prevolnik Povše, M. and ČandekPotokar, M., 2015. A Handbook of Reference Methods for Meat Quality Assessment. (European Cooperation in Science and Technology (COST), Brussels, Belgium) https://www.researchgate.net/publication/283730729_A_handbook_of_reference_methods_for_meat_quality_assessment<br>2. Fundamentals of Water Holding Capacity of Meat. http://qpc.adm.slu.se/6_Fundamentals_of_WHC/index.htm<br>3. Honikel, K.O. (1998). Reference methods for the assessment of physical characteristics of meat. *Meat Science*, 49, 447–457. https://www.sciencedirect.com/science/article/pii/S0309174098000345 |

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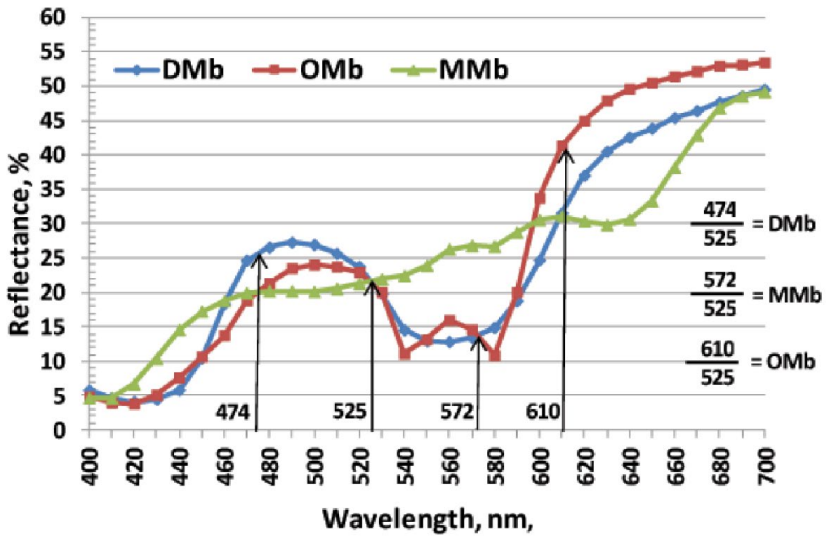
| **Practical** | **3. Myoglobin content** |
| **Competences** | To know how to determine the myoglobin content in fresh meat as a quality trait responsible for meat colour in order to compare different meats, from different types or production background.<br>To practise using a spectrophotometer. |
| **Overview** | Myoglobin (Mb), a water-soluble sarcoplasmic protein, is the main pigment of meat, and thus the main molecule responsible for meat colour. Mb content varies as the function of animal species (e.g. 2 mg/g in pork or 6 mg/g in lamb), animal age (e.g. 2 mg/g in veal or 18 mg/g in old beef) and muscle type (related to the abundance of different types of muscle fibres), among other factors.<br>In this method, Mb, in all forms (Deoxy or reduced Mb, oxygenated or OxiMb, and oxidised or MetaMb), is extracted from the muscle into a cold 0.04 M phosphate buffer, pH 6.8. The total Mb concentration is determined by absorbance at 525 nm, the isosbestic point for all 3 forms of myoglobin.<br>  <br><br>Fig. 1. Reflectance and isosbestic wavelengths used for quantitative determination of myoglobin redox forms (King et al., 2023). License CC SA BY 4.0.https://doi.org/10.22175/mmb.12473 |
| **Materials** | • Product to be tested (e.g. a 30 g portion of *longissimus thoracis* muscle)<br>• 50-ml tubes to be used in a centrifuge<br>• Cells for the spectrophotometer<br>• 40 mM potassium phosphate buffer, pH 6.8, made from $KH_2PO_4$ = 4.87 g and $K_2HPO_4$ = 2.48 g in 1000 mLdistilled/deionized water |
| **Equipment** | • Balance (0.01 g accuracy) |

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| | • Chilling room or refrigerator<br>• Blender<br>• Ultraturrax disperser or similar<br>• Spectrophotometer<br>• Centrifuge |
| **Procedure** | 1. Grind meat through a 3 mm plate or mince it into particles of less than 3-mm diameter using a blender.<br>2. Weigh in duplicate 5-g meat samples and place it into 50-mL tubes.<br>3. Add 25-mL of chilled phosphate buffer (pH 6.8, 0.04 M); the dilution factor is 25 mL + 5 g = 30 mL; 30 mL/5 = 6.<br>4. Homogenise the sample for 40 to 45 seconds at low speed, using a small diameter head Ultraturrax or similar homogenizer.<br>5. Hold the homogenised sample in ice (0 to 4°C) for 1 hour.<br>6. Centrifuge sample at 10,000-50,000 × *g* for 30 minutes at 5°C. A lower *g*-force may be used, but if the supernatant is turbid (Absorbance at 700 > 0.05), clarify the supernatant through a syringe filter (0.45 µm pore size)<br>7. Measure the absorbance at 525 nm.<br><br>**Calculations**: Mb concentration (mg/g meat) = (A525 /7.6) × 17 × 6, where 7.6 = millimolar extinction coefficient for Mb at 525 nm, and 6 = dilution factor. An average of 17 kDa can be used as Mb molecular mass. Absorbance at 700 nm is used to compensate for turbidity (if any) and is therefore subtracted from the absorbance at 525 nm. |
| **Applications** | • To compare the myoglobin content of different muscles or same muscle of animals from different production backgrounds (species, age, feeding). Mb content is a quality trait related to meat colour. |
| **References** | 1. AMSA (2012). Meat Color. Measurement. Guidelines. American Meat Science Association. https://meatscience.org/docs/default-source/publications-resources/hot topics/2012_12_meat_clr_guide.pdf?sfvrsn=d818b8b3_0<br>2. Li, Y.S.; Zhu, N.H.; Niu, P.P.; Shi, F.X.; Hughes, C.L.; Tian, G.X.; Huang, R.H. (2013). Effects of dietary chromium methionine on growth performance, carcass composition, meat colour and expression of the colour-related gene myoglobin of growing-finishing pigs. *Asian-Australasian Journal of Animal Science, 26*, 1021–1029. Fundamentals of Water Holding Capacity of Meat. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4093490/ |

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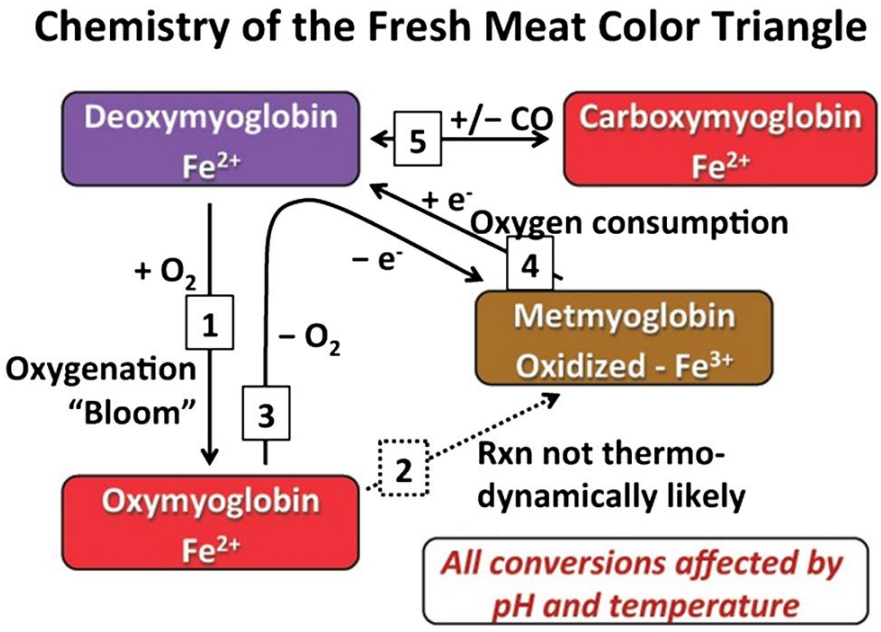
| **Practical** | **4. Instrumental analysis of raw meat colour** |
| **Competences** | • To understand colour theory using colour scales such as CIELAB, which is used to measure and compare colours objectively.<br>• -To acquire skills to use a colorimeter accurately, as well as data analysis and interpretation software.<br>• - To gain knowledge of factors that influence raw meat color stability, such as pH, oxidation, myoglobin content, and packaging conditions. |
| **Overview** | Consumers select the meat they buy based on its colour, e.g. meat discoloration is a sign of spoilage. Meat colour is the result of complex interactions between several ante- and post-mortem factors. Variables that influence the concentration and chemical state of pigments (myoglobin), as well as the microstructure of the muscles, which in turn affect light scattering in the meat.<br>Myoglobin is the principal pigment responsible for the colour in the surface of fresh meat, which depends not only on the myoglobin content but also on its chemical form: I) reduced myoglobin (Mb or deoxymyoglobin) which is the purple pigment present in deep muscle and of meat surface under vacuum, ii) oxymyoglobin (MbO2) which is bright red and considered a freshness indicator, and that occurs when Mb at the meat surface is exposed to oxygen, and iii) metmyoglobin (MetMb), brown and unattractive when oxygen partial pressure is low or meat has been stored for long time.<br>  <br>Fig. 2. Changes of myoglobin chemical forms in raw meat. (King et al., 2023). License CC SA BY 4.0.https://doi.org/10.22175/mmb.12473 |
| **Materials** | • A muscle portion (usually *longissimus thoracis et lumborum*)<br>• Tray to place the meat |

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| | • Soft tissue and 96% ethanol to clean the chroma meter measurement window<br>• Knife |
| **Equipment** | • Chromameter (e.g. Konica Minolta CR200, CR300, CR400) attached with a mask with glass protection and 11 mm aperture window<br>• Calibration tiles |
| **Procedure** | 1. Take the muscle portion<br>2. Cut a pair of slices of at least 2 cm thickness (i.e. take a cross-section perpendicular to the muscle fibres)<br>3. Select the following conditions in the chromameter<br>  • Light source of D65<br>  • Specular component included (SCI) mode<br>  • 11-mm aperture for illumination and 8-mm for measurement<br>  • Standard observer angle is 10°<br>  • Colour scale L*a*b* (CIE 1976)<br>4. Calibrate (set black standard as L*=0, and white as L*=100)<br>5. Measure the colour on the cut surface of the meat slices. Measurements should be made on different sites of the sample's exposed surface (at least triplicate)<br><br>**Notes**:<br>• Use meat over 24 h postmortem.<br>• Allow the slices to bloom before analysis (or the time it takes for the surface myoglobin to become fully oxygenated). For this, it is advised to wait a minimum of one hour from the cut to the measurement (the length of blooming needs to be specified). For blooming, the slice should be placed with the recently cut surface upwards. Surface drying must be prevented.<br>• All details regarding samples, storage, and measurement conditions including blooming time, equipment used, and procedure should be recorded/provided as experimental conditions.<br>• According to the CIELab system, the lightness L* is represented along the vertical axis and varies from 0 (dark) to 100 (white). The a* value is represented on the X axis and corresponds to the red/green opponent colours with red at positive and green at negative (scale from - 60: green to + 60: red). The b* value is represented on the Y axis and corresponds to the yellow/blue opponent colours with blue at negative and yellow at positive b* values (scale from - 60: blue to + 60: yellow).<br>• Certain muscles have considerable colour differences between lateral and medial sites on their cross-sections.<br>• Highly variable readings are likely to occur in muscles with high levels of intramuscular fat (marbling). |
| **Applications** | To assess the quality of meat colour and follow the changes of meat colour during storage (colour stability). Consumer preferences and perceptions |

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| | are influenced by raw meat colour. Sensory and instrumental evaluations of colour shown that a* values rather than L* values explained a greater portion of the range in customer scores; e.g. for Australian lamb consumers, according to analysis, customers will, on average, find the meat's colour acceptable when the a* score is higher than 9.4, and L* values are equal or higher than 34.<br><br>To assess the effect of on-farm and slaughter factors on meat colour.<br><br>Different muscles can show different colour and colour stability.<br><br>The gas concentrations of modified atmosphere packaged meat can also affect the colour and colour stability. |
| **References** | 1. Honikel, K. O. (1998). Reference methods for the assessment of physical characteristics of meat. *Meat science*, *49*(4), 447-457. https://www.sciencedirect.com/science/article/pii/S0309174098000345?via%3Dihub<br>2. Font-i-Furnols, M., Čandek-Potokar, M., Maltin, C., & Prevolnik Povše, M. (2015). A handbook of reference methods for meat quality assessment. *European Cooperation in Science and Technology (COST): Brussels, Belgium*. https://www.researchgate.net/publication/282133283_A_handbook_of_reference_methods_for_meat_quality_assessment<br>3. American Meat Science Association. (2012). *AMSA Meat Color Measurement Guidelines: AMSA*. American Meat Science Association.https://meatscience.org/publications-resources/printed-publications/amsa-meat-color-measurement-guidelines |

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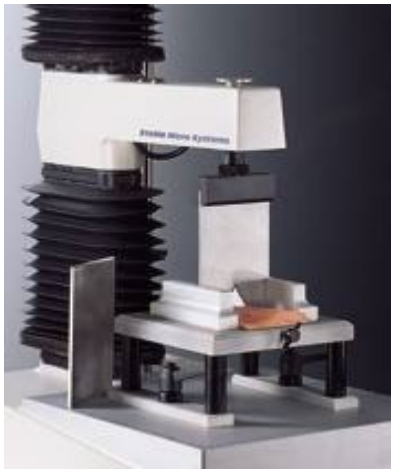
| **Practical** | **5. Analysis of cooked meat texture with Warner-Bratzler shear force** |
| **Competences** | • To know textural analysis devices, their operation, and how to interpret their results.<br>• To analyse the hardness of different cuts and varieties of meat, which is affected by muscle fibre type, fat content, and connective tissues.<br>• To analyse the effect of meat ageing and cooking time and temperature on meat science. |
| **Overview** | • Tenderness is typically regarded as one of the primary characteristics of meat quality, along with water-holding capacity. It is one of the most important aspects of meat texture. Sensory evaluations such as trained panels or instrumental measurements such as texture analysis are typically used to assess it. A Warner-Bratzler shear force (WB) device provides an objective measure of tenderness by measuring the force required to shear through a standard sample of meat.<br>• Tenderness is affected by animal-intrinsic factors such as sex, age, breed, muscle type and location. It is also affected by meat ageing and cooking conditions. |
| **Materials** | • Have a piece of approximately 150 g of muscle. The most widely used muscle is the *longissimus thoracis et lumborum*.<br>• Knife or scalpel<br>• Water bath<br>• Vacuum packaging bags<br>• Scale mm<br>• Gram scale (two decimals) |
| **Equipment** | • Texturometer type Instron (equipped with a WB shear force blade)<br>  <br>WB shear blade:<br>Blade thickness: 1.2 mm |

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| | Cutting blade V shaped (60° angle) |
| **Procedure** | **The Warner-Bratzler shear force (WB):**<br>If texture is going to be measured in raw meat, cut the meat as explained below.<br>If texture is going to be measured in cooked meat, weigh the meat portion and put the meat in a vacuum packaging bag. Once the meat has been packaged, cook it in a water bath at a temperature of 70°C for approximately 40 min (core temperature of 68 ºC). Then, put the bag with the meat in tap water for 10 min and then in a cold chamber overnight at 2 to 5°C. Afterwards, remove the bag and dry the sample surface by wiping it with paper. Weight again and calculate the weight loss during cooking.<br>Cut a minimum of five prisms of raw or cooked meat (1 x 1 x 3 cm sized) paralleled to the longitudinal orientation of the muscle fibres (use a sharp knife or a scalpel and the scale).<br>Calibrate the texturometer and determine the shear force. The blade should cut the muscle prism perpendicular to the longitudinal orientation of the muscle fibres at a cross speed of 50-100 mm/min<br>The peak force is determined by recording the maximum value from the force vs space deformation curve. The WB shear force is the average of five measurements made on each sample. The WB shear force is expressed as N. |
| **Applications** | -To determine meat tenderness is useful for carcass quality grading<br>-It is also useful to assess the effects of on-farm factors, slaughter factors, muscle location or meat ageing on tenderness.<br>-Variations in meat texture result from structural differences within raw meat/muscle tissue, including differences in contractile protein structures, connective tissue framework, as well as external factors such as cooking and sample handling. |
| **References** | 1. Honikel, K. O. (1998). Reference methods for the assessment of physical characteristics of meat. *Meat science*, *49*(4), 447-457. https://www.sciencedirect.com/science/article/pii/S0309174098000345?via%3Dihub<br>2. Font-i-Furnols, M., Čandek-Potokar, M., Maltin, C., & Prevolnik Povše, M. (2015). A handbook of reference methods for meat quality assessment. *European Cooperation in Science and Technology (COST): Brussels, Belgium*. https://www.researchgate.net/publication/282133283_A_handbook_of_reference_methods_for_meat_quality_assessment |

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| **COURSE** | **1. MEAT PRODUCTION AND MEAT QUALITY** |
| **Training Subject** | **1.5. Meat handling and storage procedure** |
| **Index** | **1. Total aerobic mesophilic viable counts**<br>**2. Analysis of thiobarbituric acid reactive substances (TBARS)** |

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| **Practical** | **1. <u>Total aerobic mesophilic viable counts</u>** |
| **Competences** | • To get skills on microbiological analysis of meat<br>• To be able to estimate the microbial content in fresh meat to assess the meat hygiene or shelf-life during refrigerated storage. |
| **Overview** | The aerobic plate count determination, also known as aerobic mesophilic count, is used to estimate the bacterial population in a food/meat sample. It provides an estimate of the level of microorganisms that can grow aerobically at mesophilic temperatures (It is not an evaluation of the entire bacterial population, nor does it indicate differences among bacterial types in a food product, but it is an index of the microbial load). A hygienic manipulation of meat during fabricating or cutting results in low aerobic mesophilic microbial counts. Values over about $10^6$–$10^7$ colony forming units per g indicate the end of shelf life by the formation of off-flavours and appearance depreciation. |
| **Materials** | • Work area, level table with ample surface in room that is clean, well-lighted (100 foot-candles at working surface) and well-ventilated, and reasonably free of dust and drafts. The microbial density of air in the working area, measured in fallout pour plates taken during plating, should not exceed 15 colonies/plate during 15 min exposure.<br>• Storage space, free of dust and insects and adequate for protection of equipment and supplies<br>• Glass flasks (500 mL to 1 L) with plastic caps (autoclavable)<br>• Petri dishes, glass or plastic (at least 15 × 90 mm).<br>• Plate count agar<br>• Bacteriological peptone<br>• NaCl |

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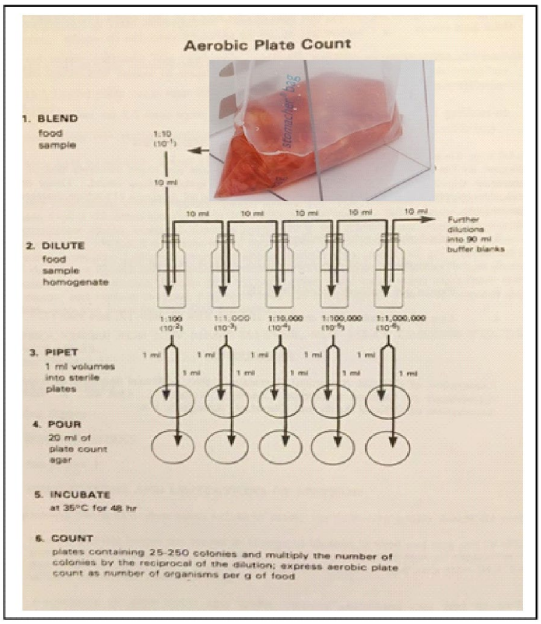
| | • Pipette with pipet aids (no mouth pipetting) or pipettors, 1, 5, and 10 ml, graduated in 0.1 ml units.<br>• Dilution tubes, 100 ml approx., borosilicate-resistant glass, with rubber stoppers or plastic caps<br>• Pipette and petri dish containers, adequate for protection<br>• Thermometers (mercury) appropriate range |
| **Equipment** | • Gram scale (0.01 g accuracy).<br>• Homogenizer for microbiology, Stomacher-400 circulator or similar.<br>• Autoclave<br>• Vortex<br>• Microwave or magnetic stirring with heating plate to prepare the media<br>• Sterile bags for the homogeniser, 400 mL capacity with filter<br>• Laminar flow hood for preparing the dilutions and plating.<br>• Circulating water bath, for tempering agar, thermostatically controlled to 45 ± 1°C<br>• Incubator, 32 ± 1°C Refrigerator, to cool and maintain samples at 0-5°C<br>• Colony counter, dark-field, Quebec, or equivalent, with suitable light source and grid plate (optional). |
| **Procedure** | 1. Prepare the required amount of media (agar) and a 0.1% peptone + 0.85% NaCl dilution in distilled water.<br>2. Sterilise the media, the peptone water solution, and all the required material for sample preparation and plating (autoclave, 121 °C, 15-30 min).<br>3. Process the samples as soon as possible. If necessary, store the samples under refrigeration.<br>  <br><br>4. Aseptically, take a 25-g meat sample and homogenise the sample in 225 mL of peptone water. |

5. Using separate sterile pipettes, prepare decimal dilutions of $10^{-2}$, $10^{-3}$, $10^{-4}$, and others as appropriate, of meat homogenate by transferring 10 ml of previous dilution to 90 ml of diluent. Avoid sampling foam.
6. Shake all dilutions 25 times in a 30 cm arc within 7 s.
7. Pipet 1 ml of each dilution into separate, duplicate, appropriately marked petri dishes.
8. Reshake dilution bottle 25 times in 30 cm arc within 7 s if it stands more than 3 min before it is pipetted into a petri dish.
9. Add 12-15 ml plate count agar (cooled to 45 ± 1°C) to each plate within 15 min of original dilution.
10. Add plate count agar (previously prepared according to the manufacturer instructions) to the latter two for each series of samples.
11. Pour agar and dilution water control plates for each series of samples. Immediately mix sample dilutions and agar medium thoroughly and uniformly by alternate rotation and back-and-forth motion of plates on flat level surface.
12. Let agar solidify.
13. Invert solidified petri dishes
14. Incubate for 48 ± 2 h at 35°C.
    (Do not stack plates when pouring agar or when agar is solidifying)
15. Report all aerobic plate counts computed from duplicate plates containing between 25 and 250 colonies. Counts outside the normal 25-250 range may give erroneous indications of the actual bacterial composition of the sample. Low counts (less than 25), and crowded plates (greater than 250) may be difficult to count or may inhibit the growth of some bacteria. Report counts less than 25 or more than 250 colonies as estimated aerobic plate counts (EAPC).

To avoid creating a fictitious impression of precision and accuracy when computing APC, report only the first two significant digits. Round off to two significant figures only at the time of conversion.

**Calculations**

$$N=(\sum C) /[(1xn_1+0.1xn_2)xd]$$

N = Number of colonies per mL or g of meat
Σ C = Sum of all colonies on all plates counted
$n_1$ = Number of plates in first dilution counted
$n_2$ = Number of plates in second dilution counted
d = Dilution from which the first counts were obtained
Example
Dilution 1:100, C= 232 and 244
Dilution 1:1000, C= 33, 28

**Equation**

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| | $$N = \frac{(232+244+33+28)}{[(1\times2)+(0.1\times2)]\times10^{-2}}$$<br><br>N = 537/0.022<br>N = 24,409<br>≈ 24,000<br><br>Express the results as $Log_{10}C$<br>Psychotropic bacteria can be measured using the same procedure except for the incubation that should be at 6.5 °C for 10 days. |
| **Applications** | The aerobic plate counts may be used to judge hygienic quality, sensory acceptability, and conformance with good manufacturing practices. It also provides information on storage conditions, and shelf-life. Detectable changes in fresh meat quality characteristics due to microbial growth generally occur when the APC increases reaches about $10^6$–$10^7$ per g. This level can be considered as the end of meat shelf life. |
| **References** | 1. Kim J.H., Yim D.G. (2016). Assessment of the Microbial Level for Livestock Products in Retail Meat Shops Implementing HACCP System. *Korean Journal of Food Science and Animal Resources*, 36(5), 594-600. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5112421/<br>2. Maturin, L., Peeler, J.T. (2001). BAM Chapter 3: Aerobic Plate Count. U.S. Food and Drug Administration. https://www.fda.gov/food/laboratory-methods-food/bam-chapter-3-aerobic-plate-count#conventional |

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| **Practical** | **2. Analysis of thiobarbituric acid reactive substances (TBARS) in meat** |
| **Competences** | • Increasing proficiency with laboratory techniques such as pipetting, sample preparation, homogenization, handling chemicals, and operating laboratory equipment such as spectrophotometers.<br>• Gaining knowledge of TBARS assay principles and lipid oxidation chemistry, to interpret results accurately, it is important to understand how thiobarbituric acid (TBA) reacts with lipid oxidation products, such as malondialdehyde (MDA).<br>• Improving analytical skills, particularly spectrophotometry is required to measure absorbance, including calibration, acquisition, and interpretation of data.<br>• Estimate TBARS values, comparing them with standards or reference values, and determining meat lipid oxidation.<br>• Be aware of factors that can influence TBARS measurements, such as sample preparation, storage conditions, and assay variability.<br>• Practicing Laboratory safety practices include proper handling of chemicals and disposal, following safety protocols, and being aware of potential hazards.<br>• Maintaining accurate records requires knowledge of experimental procedures, recording data, and preparing reports. |
| **Overview** | Lipid oxidation can be estimated with the analysis of thiobarbituric acid reactive compounds (TBARS). This procedure is widely applied to either raw and cooked meat and used to motorize lipid oxidation during storage. TBARS level is an indicator of lipid oxidation in meat, related to the rancid flavour of cooked meat.<br>One of the most important aldehydes produced during polyunsaturated fatty acid oxidation is malondialdehyde or MDA (1,3-propanedial). This molecule tends to bind to other meat components because it is highly reactive. The method consists of a colorimetric measurement of the complex formed between TBA and MDA. Following the extraction processes, a pink complex is created when one mole of MDA and two moles of TBA react. This reaction occurs at high temperatures (about 80–100 °C) and low pH values. The resulting-coloured complex has a maximum absorbance between 532 and 535 nm, and MDA is therefore quantified using a UV-Vis spectrophotometer.<br>While MDA is the most common TBA reactive substance, there are multiple aldehydes and other oxidation products that also react with TBA; thus, the reaction is not specific to MDA, although MDA is the most abundant reactive compound. To encompass all substances that react with TBA, the method is called thiobarbituric acid reactive substances (TBARs). |

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| **Materials** | • Product to be tested (e.g. 20-50 g raw or cooked meat at different times of storage, e.g.; 1, 3, 5 and 7 days for refrigerated storage of raw meat; 1 and 3 days for refrigerated storage of cooked meat; 1 day and 90 days for frozen storage or raw or cooked meat)<br>• Distilled water<br>• 2,6 Di-tert-butyl-4-methylphenol (BHT) solution at 7.2 % v/v in ethanol<br>• Trichloroacetic acid (TCA) solution at 15 % w/v<br>• 2-thiobarbituric acid (TBA) solution 20 mM in TCA at 15 % w/v<br>• 1,1,3,3-Tetraethoxypropane (TEP) as analytical standard<br>• Glass tubes with a screw cap (10 mL volume)<br>• Strainer/Sieve<br>• Beakers, 50 and 100 mL<br>• Volumetric flask, 1 L, 100 mL and 10 mL (5)<br>• Syringes (5 mL) and 0.45 µm syringe filters |
| **Equipment** | • Ultra-turrax homogenizer<br>• Balance<br>• Vortex<br>• Centrifuge<br>• Water bath<br>• Spectrophotometer and measurement cells<br>• Micropipettes and micropipette tips (0,1 ml, 1 ml and 5 mL) |
| **Procedure** | <u>For samples</u><br>1. Homogenise the meat sample with a domestic food processor<br>2. Weigh 2 g of sample (write down the exact weight)<br>3. Place the sample in a suitable beaker and add 20 ml of distilled water<br>4. Homogenise with Ultra-turrax for 1 minute at 9500 rpm<br>5. Filter with a strainer/sieve into a clean beaker<br>6. Take 1 ml of the homogenate with a micropipette and place it in a 10 ml test tube with a screw cap<br>7. Immediately add 50 µl of 7.2% BHT (reagent 2) and shake<br>8. Add 2 ml of 20 mM TBA (reagent 3); (can be added when all samples have reached this point in the process)<br>9. Vortex<br>10. Incubate in a water bath for 20 min at 80°C.<br>11. After 20 min, cool with water for at least 10 min.<br>12. Vortex<br>13. Centrifuge for 20 min at 3000 rpm and 5°C.<br>14. Filter using a syringe filter 0.45 µm pore size directly into the measurement cell<br>15. Measure the absorbance of the supernatant at 532 nm in a spectrophotometer<br><br><u>Standard curve</u><br>1. Prepare a stock solution of $3.10^{-4}$ M TEP: Pipette 81 µl of TEP into a small beaker. Note the weight. Dilute with ultrapure water and transfer to a 1 L volumetric flask. Wash the beaker several times by |

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| | adding it to the flask. Make up to 1 L. Keep this solution under refrigeration (4ºC). Do not store for more than one month.<br>2. Prepare $3.10^{-5}$ M TEP working solution: Pipette 10 mL of stock solution into a 100 mL volumetric flask and make up to the mark with ultrapure water.<br>3. Prepare 5 TEP standards points by pipetting 0; 0.33; 0.66; 1.33 and 2 ml of the working solution into 10 ml volumetric flasks and make up to the mark with ultrapure water.<br>4. Place 1 ml of each standard in 10 ml screw-capped test tubes. Add 2 ml of 20 mM TBA in 15% TCA. Vortex. Incubate in a water bath for 20 min at 80°C. After 20 minutes, cool with water for at least 10 min.<br>5. Measure the absorbance of each standard at 531 nm in a spectrophotometer.<br><br>**Calculations**<br>-Represent the graph of the measured absorbance for the TEP standard points as a function of the number of moles of TEP of those standards (in Excel Microsoft). Based on this graph, calculate the number of moles of TEP for each sample.<br>-Accordingly, the result is expressed as ppm (mg/kg) of malondialdehyde (MDA):<br><br>No. of moles of TEP = No. of moles of MDA<br><br>Molecular weight of MDA = 72.0636 g/mol<br><br>Grams of MDA = No. of moles of MDA * 72.0636 g/mol<br><br>ppm MDA= (grams MDA/ml) *(20 ml/P) *$10^3$ mg MDA/g MDA*$10^3$ g meat/kg meat<br><br>Where:<br><br>grams MDA/ml: concentration of MDA in the measurement cells calculated from the standard curve previously obtained<br><br>P = weight of the sample expressed in g |
| **Applications** | • Evaluating meat freshness, shelf-life, and quality during storage and distribution.<br>• To monitor the lipid oxidation in meat during storage using the increment in TBARS. An increased level of TBARS can indicate rancidity and off-flavours. For example, the accepted limit for rancidity in beef has been established as 2–2.5 mg MDA/kg or raw meat.<br><br>Because it is straightforward and has a strong correlation with the sensory degradation of meat, the TBARs test is therefore the most often used method for estimating the oxidative status of meat and meat products. |
| **References** | 1. Nam, K. C., & Ahn, D. U. (2003). Combination of aerobic and vacuum packaging to control lipid oxidation and off-odor volatiles of irradiated raw turkey breast. *Meat Science*, *63*(3), 389-395. |

https://www.sciencedirect.com/science/article/pii/S0309174002000980
2. Domínguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W., & Lorenzo, J. M. (2019). A comprehensive review on lipid oxidation in meat and meat products. *Antioxidants*, *8*(10), 429. https://www.mdpi.com/2076-3921/8/10/429
3. Fernández, J., Pérez-Álvarez, J. A., & Fernández-López, J. A. (1997). Thiobarbituric acid test for monitoring lipid oxidation in meat. *Food chemistry*, *59*(3), 345-353. https://www.sciencedirect.com/science/article/pii/S0308814696001148

# Lab Manual Course 2

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| **COURSE** | **MEAT PROCESSING FUNDAMENTALS** |
| **Training Subject** | **2.2 Traditional meat preservation methods** |
| **Index** | **1. Analysis of sodium chloride content in meat products**<br>**2. Determination of the degree of reaction of meat dyes**<br>**3. Determination of nitrites content in meat products** |

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| **Practical** | **1. Analysis of sodium chloride content in meat products** |
| **Competences** | The ability to assess the sodium chloride (NaCl) content in meat products |
| **Overview** | Meat products are prepared either from salted or cured meat because salt shapes not only the sensory attributes of meat products (by making them more tasty) but also affects muscle proteins which results in increased water-holding capacity of muscle tissue. This in turn enables obtaining products with good textural properties and juiciness. Salt also plays a role in preserving meat products. However, excessive salt consumption might lead to serious health problems. Therefore, it is important to monitor its concentration in meat products.<br>Below Mohr's method for the determination of sodium chloride is described. The content of table salt in the assessed food product is the content of chlorides, designated as chloride ions, which is then converted and expressed as sodium chloride - NaCl.<br>The Mohr method is based on the argentometric titration of chlorides in a neutral medium with silver nitrate in the presence of potassium chromate as an indicator:<br>$NaCl + AgNO_3 \rightarrow NaNO_3 + AgCl\downarrow$<br>After precipitation of silver chloride, excess silver nitrate reacts with silver chromate, giving a brown-red colour:<br>$2AgNO_3 + K_2CrO_4 \rightarrow Ag_2CrO_4 + 2KNO_3$ |
| **Materials** | • Meat products<br>• Distilled or deionized water<br>• saturated potassium chromate ($K_2CrO_4$) solution<br>• 0.1 N silver nitrate ($AgNO_3$) solution |
| **Equipment** | • Meat grinder with a mesh 2 mm<br>• laboratory scale |

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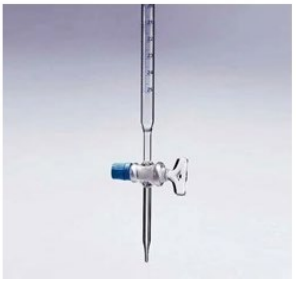
| | • 200 mL conical flasks<br>• electric hob to heat water<br>• water bath (100°C)<br>• graduated cylinder (100 mL)<br>• a stirring stick<br>• burette 25 mL<br>• glass funnels<br>• filters<br>           |
| **Procedure** | The principle of the method consists of titration of an aqueous extract prepared from the product under investigation with silver nitrate (V) against potassium chromate as an indicator.<br><br>*Reagents preparation*<br>• 0.1 N silver nitrate ($AgNO_3$) solution – in volumetric flask (1 L) dissolve 16.989 g of $AgNO_3$ and add distilled water to reach the mark on the flask<br>• saturated potassium chromate ($K_2CrO_4$) solution - in volumetric flask (1 L) dissolve 629.0 g of $K_2CrO_4$ and add distilled water to reach the mark on the flask.<br><br>*Analysis*<br>To determine the sodium chloride content in meat products follow the described below steps:<br>1. Grind the meat product twice in a meat grinder with 2 mm mesh and mix thoroughly |

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| | 2. Take 2 g (with an accuracy of 0.001 g) of ground product sample and transfer it to a 200 mL conical flask. Add 100 mL of hot distilled water and, while stirring, heat in a boiling water bath for 15 minutes.<br>3. Filter the sample and cool it down.<br>4. Add 5 drops of indicator (5% potassium chromate solution).<br>5. Titrate with a standardised 0.1 N silver (V) nitrate solution ($AgNO_3$) to obtain an orange colour.<br>6. Calculate the sodium chloride content in percentage by weight (% m/m) from the formula:<br>$$NaCl = \frac{0.00585 \cdot a}{b} \cdot 100 \ (\%)$$<br>where:<br>a – volume of 0.1 M silver nitrate (V) solution ($AgNO_3$) used for the titration of the sample (cm3);<br>b - sample weight (g);<br>0.00585 - conversion factor for sodium chloride (1 cm3 0,1 M solution of silver nitrate (V) corresponds to 0.00585 g of sodium chloride). |
| **Applications** | • Assessing the correctness of the salting/curing process<br>• Providing information about the product quality<br>• Preparing information which must be enclosed on a product label |
| **References** | 1. Pikul J. (ed). 1993. Ocena Technologiczna surowców i produktów przemysłu drobiarskiego. Wydawnictwo Akademii Rolniczej w Poznaniu, Poznań, p. 102. |

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| **Practical** | **2. Determination of the degree of reaction of dyes** |
| **Competences** | The ability to assess the efficiency of the curing process |
| **Overview** | The term "curing" means the treatment of meat with a curing mixture and/or brine (nitrite or nitrate). Nitrite is a substance whose transformation products react with myoglobin to form the dye of cured meat, i.e. nitrosylmyoglobin, which is transformed into nitrosylmyochromogen during heat treatment.<br>Meat curing is intended to:<br>● shaping the typical colour of cured meat,<br>● inhibiting the development of some undesirable and health-threatening microorganisms (e.g. *Clostridium botulinum*),<br>● shaping the taste and smell typical for cured meat,<br>● slowing down oxidation processes.<br>The effectiveness of the curing process depends on, among others, on the following factors:<br>● raw material: its type and quality,<br>● functional additives used,<br>● curing method and its parameters.<br>The intensely red colour of raw cured meat is created by a complex of nitric oxide with myoglobin, called nitrosylmyoglobin (MbNO; myoglobin nitoxide). When heated, nitrosylmyoglobin denatures and turns into the pink dye nitrosylmyochromogen. It is assumed that for the colour of cured meat to be stable, the degree of reaction of hem dyes to nitrosyl dyes should be not less than 50%.<br>The effectiveness of the curing process might be monitored by the determination of the degree of meat dye reaction.<br>The principle of the method is to optically determine the content of nitrosyl dyes contained in an acetone-water extract from meat or cured meat products. |
| **Materials** | ● Meat products<br>● acetone-water mixture<br>● acetone-water mixture with the addition of concentrated HCl |

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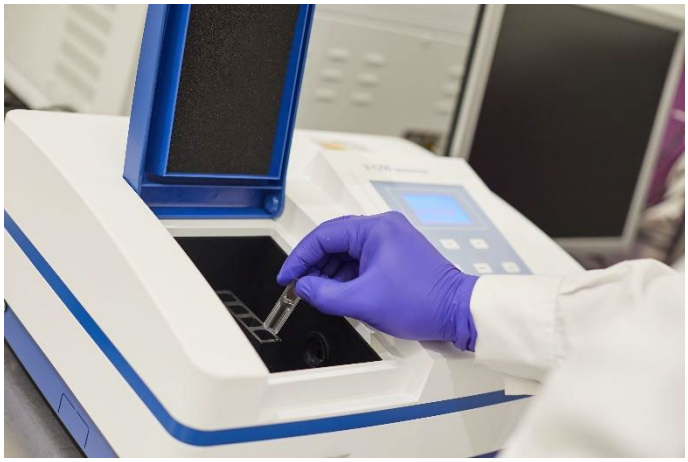
| **Equipment** | • Meat grinder with a mesh 2 mm<br>• laboratory scale<br>• 100 mL conical flasks<br>• graduated cylinder (100 mL)<br>• spectrophotometer<br>  |
| **Procedure** | • Grind the meat product twice in a meat grinder with 2 mm mesh and mix thoroughly<br>• Weight two a 5 g cured meat sample (with an accuracy of 0.001 g) to 100 mL conical flask<br>• To one of them add 21.5 mL of acetone-water (1000 mL : 75 mL) mixture and incubate in the darkness for 1 hour. Measure the extinction of the solution at a wavelength of 540 nm.<br>• To the second 5 g portion add 21.5 mL of acetone-water mixture with the addition of concentrated HCl (1000 mL : 50 mL : 25 mL for acetone, water and HCl respectively). After 1 hour incubation in the darkness measures the extinction at a wavelength of 640 nm.<br>• The duration of extraction should be the same for both samples.<br><br>The extent of discolouration (% of dye reaction) is calculated according to the formula:<br><br>$$\% \text{ reaction} = \frac{E_{540} \cdot 100}{E_{640} \cdot 2.5}$$<br><br>Where:<br>$E_{540}$ - extinction at a wavelength of λ = 540 nm expresses the content of nitrosyl dyes<br>$E_{640}$ - extinction at a wavelength of λ = 640 nm expresses the content of general dyes<br><br>The extent of discolouration indicates the amount of hem dyes that have converted into nitrosyl dyes. The degree of dye reaction should not be lower than 50%. The low degree of reaction of dyes is the reason for the |

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| | insufficient colour of cured products - too light and not very stable. The causes of the low degree of dye reaction may be:<br>- reaction time too short at a given temperature,<br>- curing temperature too low,<br>- too high oxygen content (entering the meat during grinding and stuffing),<br>- lack or insufficient amount of curing aids<br>- too little nitrite (improperly prepared curing mixture). |
| **Applications** | ● Assessing the efficiency of the curing process<br>● Assessing the quality of the curing mixture |
| **References** | 1. Dzierżyńska-Cybulko B., Kijowski J., 1982 – Zarys przetwórstwa surowców zwierzęcych (Outline of Processing Animal Raw Products). In Polish. Published by the Agricultural University of Poznań |

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| **Practical** | **3. Determination of nitrites content in meat products** |
| **Competences** | The ability to determine nitrites content in meat products |
| **Overview** | Nitrites and nitrates, which include: potassium nitrite (E 249), sodium nitrite (E 250), sodium nitrate (E 251) and potassium nitrate (E 252), are substances used for preservation and ensure the microbiological safety of food, in particular meat, fish and cheese, and to enhance their characteristic organoleptic properties. At the same time, it is recognized that the presence of nitrites and nitrates in food may lead to the formation of nitrosamines - carcinogenic organic compounds. Therefore, EU regulates their usage in food products [1,2] to minimize the risk of the formation of nitrosamines due to the presence of nitrites and nitrates in food, and, on the other hand, to maintain their protective effect against the multiplication of bacteria, in particular the *Clostridium botulinum* bacteria responsible for botulism poisoning.<br>The European Union regulation defines the amounts of nitrates and nitrites that may be used in meat products and indicates the maximum amounts of residues of these substances from all sources in relation to products ready to be placed on the market. If the maximum nitrate residue limits are exceeded, the food business operator should investigate the reason for the exceedance. |
| **Materials** | • Meat products<br>• Distilled or deionised water<br>• saturated borax solution ($Na_2B_4O_7 \cdot 10H_2O$)<br>• potassium ferrocyanide ($K_4Fe(CN)_6 \cdot 3H_2O$)<br>• zinc acetate $(CH_3COOH)_2Zn \cdot 2H_2O$)<br>• acetic acid (icy)<br>• sulphanilic acid<br>• N-(1-naphthyl)-ethylene diamine dihydrochloride<br>• sodium chloride<br>• sodium nitrite |
| **Equipment** | • Meat grinder with a mesh 2 mm<br>• laboratory scale<br>• volumetric flasks (100 mL, 200 mL and 1000 mL)<br>• colonial flasks (200 mL)<br>• water bath<br>• graduated cylinder (100 mL, 20 mL)<br>• stirring sticks<br>• glass funnels<br>• spectrophotometer<br>• laboratory shaker<br>• homogeniser with 150 mL tubes<br>• pipettes 1 mL, 5 mL, 20 mL<br>• glass tubes (length 15 cm, diameter 20 – 25 cm)<br>• Whatman 1 filter paper<br>• paper filters |

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| **Procedure** | *Reagents preparation*<br>● potassium ferrocyanide ($K_4Fe(CN)_6 \cdot 3H_2O$) – weight 106 g of $K_4Fe(CN)_6 \cdot 3H_2O$ and dissolve it in a small amount of distilled water in 1 L volumetric flask. Then, fill the water to reach the mark.<br>● zinc acetate ($(CH_3COOH)_2Zn \cdot 2H_2O$) – in a 1 L volumetric flask in a small amount of distilled water dissolve 220 g of $(CH_3COOH)_2Zn \cdot 2H_2O$, add 30 mL of icy acetic acid. Mix and fill the water to reach the mark.<br>● saturated borax solution - in a 1 L volumetric flask dissolve in a small amount of distilled water 50 g of $Na_2B_4O_7 \cdot 10H_2O$, and fill the water to reach the mark.<br>● Griess A - dissolve, by heating in a water bath, 6 g of sulphanilic acid in 200 ml of icy acetic acid and 400 ml of distilled water. Add 200 ml of 10% sodium chloride solutions. Top up with distilled water to 1 L.<br>● Griess B - dissolve, by heating in a water bath, 3 g of N-(1-naphthyl)-ethylene diamine dihydrochloride in 100 ml of distilled water. Add 200 ml of glacial acetic acid. Top up with distilled water to 1 L.<br>● Before analysis mix Griess A and B (1:1). Shelf-life of the mixture is up to 1 week.<br><br>*The standard curve preparation*<br>● Solution C - in a 1 L volumetric flask dissolve in a small amount of distilled water 1.0 g of sodium nitrite, and fill the water to reach the mark<br>● Solution D – transfer 5 mL of solution C to a 1 L volumetric flask and fill the water to reach the mark<br>● prepare dilutions from solution D by transferring 5, 10, 20, 25, 30, and 35 mL of solution D, respectively, into 100 ml volumetric flasks and fill up to the mark with distilled water. The prepared solutions contain the appropriate 0.25, 0.50, 1.0, 1.25, 1.50, 1.75 of sodium nitrite in 1 mL. For seven test tubes, take 10 ml of distilled water and 10 mL of previously prepared solutions from 100 ml volumetric flasks. Then add 10 mL of Griess' reagent to each test tube, mix and after 20 minutes measure the absorbance of the solutions at a wavelength of 520 nm against a blank sample containing 10 mL of distilled water instead of the solutions. Plot a standard curve showing the dependence of absorbance on the concentration of sodium nitrite expressed in mg per ml of solution.<br><br>*Sample preparation*<br>1. Grind the meat product twice in a meat grinder with 2 mm mesh and mix thoroughly<br>2. Weigh 5 g (or 2.5 g when you suspect the concentration of nitrites might be high) of a sample of the ground meat product with an accuracy of 0.01 g, and transfer it to a 200 mL volumetric flask with approximately 100 mL of hot water, using a funnel and a stirring stick. |

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| | 3. Add 5 mL of saturated borax solution and heat the mixture for 15 minutes in a water bath, shaking to break up all the meat aggregates.<br>4. Cool down to room temperature and add 10 mL of deproteinizing reagents - potassium ferrocyanide and zinc acetate, mixing after each addition.<br>5. Set aside for 30 minutes at room temperature, then fill up to the mark with water.<br>6. Mix everything and filter into conical glass flasks. The prepared filtrate is used to determine nitrates (III).<br><br>*Assessment*<br>1. Take 10 mL of the prepared filtrate, add 10 mL of Griess reagent and mix in a colonial flask<br>2. Prepare a blank containing 10 mL of water and 10 mL of Griess' reagent.<br>3. After 20 minutes, measure the extinction of the solution on a spectrophotometer at a wavelength of 520 nm against a blank sample.<br>4. The amount of sodium nitrate expressed in mg/mL can be read from the standard curve |
| **Applications** | • Assessing the nitrites residues in the finished product<br>• Monitoring of the curing process<br>• Monitoring complying with a law regulations<br>• Assessing the quality of the curing mixture |
| **References** | 1. Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004 Text with EEA relevance<br>2. Commission Regulation (EU) 2023/2108 of 6 October 2023 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council and the Annex to Commission Regulation (EU) No 231/2012 as regards food additives nitrites (E 249-250) and nitrates (E 251-252). https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=OJ:L_202302108<br>3. Pikul J. (ed). 1993. Ocena Technologiczna surowców i produktów przemysłu drobiarskiego. Wydawnictwo Akademii Rolniczej w Poznaniu, Poznań, p. 98-101. |

# Lab Manual Course 3

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| **COURSE** | **ADVANCES IN MEAT PROCESSING AND NOVEL MEAT PRODUCTS** |
| **Training Subject** | **Emerging technologies in meat processing** |
| **Index** | **1. Extraction of bioactive compounds with Ultrasound Assisted Extraction**<br>**2. Extraction of bioactive compounds with Pulsed Electric Fields**<br>**3. Oil extraction with Supercritical Fluid Extraction** |

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| **Practical** | **1. Extraction of bioactive compounds with Ultrasound Assisted Extraction** |
| **Competences** | Application of Ultrasound Assisted Extraction to obtain bioactive compounds with antioxidant activity. |
| **Overview** | Ultrasound-Assisted Extraction (UAE) is recognized as an effective tool used in the industrial extraction of bioactive compounds and nutrients, significantly minimizing the time required, and increasing both the productivity and the quality of the product. This technique is an economic and useful technology, since it is efficient, simple and does not require expensive instruments. Moreover, it enhances the yield of extraction, simultaneously improving the functional properties, requires less extraction time and reduced solvent consumption. It can be performed at low temperatures, which can decrease the damages caused by temperature, and reduce the loss of bioactive substances. |
| **Materials** | • Beaker (250 mL)<br>• Spoon |
| **Equipment** | • Chronometer<br>• Analytical balance<br>• Magnetic stirrer<br>• Ultrasonic equipment with probe |
| **Procedure** | **Analysis protocol**<br>1. In a 250 mL beaker, weigh the amount of sample you want to treat (it depends on the solid:liquid ratio you want).<br>2. Calibrate and establish the ultrasonicator work conditions (To know more about ultrasonicator work conditions see annex 1). |

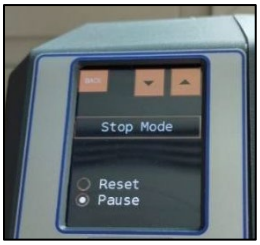
a) To access to menu press setting


b) Select calibration mode


c) Temperature upper-limit 50°C


d) Temperature Delta 10°C


e) Work in pause mode (reset to start a new extraction and calibrate again)



f) Temperature control On


g) Mode Amplitude (100 %)

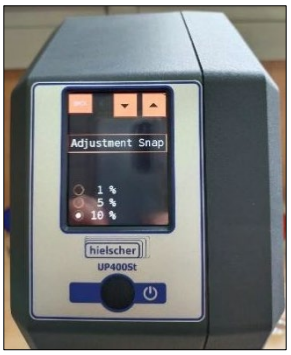

3. Add the amount of solvent you need (distilled water, ethanol, …) to the sample.
4. Stir for 45 seconds a 450 rpm with the help of a magnetic stirrer.
5. Place the sample in the ice bath and introduce both the sonotrode and temperature probes inside it. Important: sonotrode must be immersion depth 45 mm, until first mark.
6. Apply the corresponding established time for treatment (min). Press button for start treatment.
7. Timing treatment time. **IMPORTANT: All extractions are in contact with solvent for 15 minutes.**
8. Clean both sonotrode and temperature probe with distilled water and alcohol.

**Appendix. Ultrasonicator UP400 St work conditions**

1. **Important considerations**: With the current sonotrode (S24D22d), the maximum energy voltage is 250 W in rapeseed oil and 180 W in water.

2. The ultrasonicator UP400St can work in two modes Power or Amplitude:


3. In the power mode, the voltage increases gradually, while in the amplitude mode, the energy selected is applied at the moment to start treatment.
4. Amplitude mode: the units are in %. 100%= 200 W; 50%= 100W, etc.
5. Power mode: modifying adjustment snap be stablished the rate at which power increases.




6. During ultrasonic treatment, the sample temperature increases fastens. Therefore, be recommended to always work with control temperature and ice bath. Furthermore, to reduce the temperature sample increase, raise the total sample volume.

**Applications**

- Extraction of bioactive compounds from plants, seeds and agri-food co-products.

**References**

1. Alirezalu, K., Pateiro, M., Yaghoubi, M., Alirezalu, A., Peighambardoust, S. H., & Lorenzo, J. M. (2020). Phytochemical constituents, advanced extraction technologies and techno-functional properties of selected Mediterranean plants for use in meat products. A comprehensive review. Trends in Food Science & Technology, 100, 292-306. https://www.sciencedirect.com/science/article/pii/S0924224420304398?casa_token=GnXcx8-iDloAAAAA:84GKxRcPeh01Z2FRIGEz8VGYOZEy7PvmLSASLfSnM_VesiSUOAgSZ3SCs0OLYHJ2YwvxjVIZ_S4
2. Al Khawli, F., Pateiro, M., Domínguez, R., Lorenzo, J. M., Gullón, P., Kousoulaki, K., Ferrer, E., Berrada, H., & Barba, F. J. (2019). Innovative green technologies of intensification for valorization of seafood and their by-products. Marine Drugs, 17(12), 689. https://www.mdpi.com/1660-3397/17/12/689

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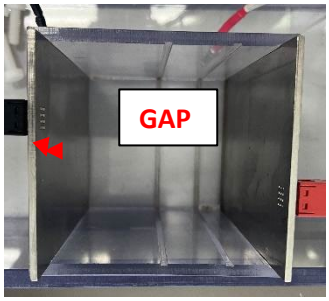
| **Practical** | **2. Extraction of bioactive compounds with Pulsed Electric Fields** |
| **Competences** | Application of Pulsed Electric Fields to obtain bioactive compounds with antioxidant activity. |
| **Overview** | Pulsed Electric Fields (PEF) extraction was developed as a useful method to enhance the effectiveness of extraction, diffusion processes drying and pressing [40]. The main mechanism that describes extraction assisted by PEF is an electric field that destroys cell membrane structure [41]. The effectiveness of this technique is specifically attributed to some parameters, such as process temperature, field strength, pulse intensity, especial energy input and sample characteristics, which allow to minimize the degradation of heat sensitive compounds. |
| **Materials** | • Distilled water<br>• Beaker<br>• Magnet stirrer<br>• Centrifuge tubes |
| **Equipment** | • Generator (EPULSUS-LPM1-10)<br>• Safety box and treatment chamber inside<br>• Centrifuge<br>• Magnetic stirrer |
| **Procedure** | **Initial considerations before working with the equipment**<br>• The PM1-10 generator must be connected to 230 Vac / 50 Hz input voltage, single phase, from the appropriate cable locate at the bottom right of the metallic enclose.<br>• Prior to the connection of the output plastic cable to the safety box ensure that the generator is off and disconnected from the mains input power. The output plastic cable comprises the coaxial HV cable, with power return ground, the safety loop.<br>• On the front panel of the PM1-10 generator, on the enclosure door, is located:<br>  ○ Right bottom, the ON/OFF knob to turn the power *on* and *off* on the generator, if the mains cable is connected to power<br>  ○ Center bottom, the Emergency button to stop the operation of the generator in the case of an emergency<br>  ○ Left bottom, the RESET blue button to re-start the generator after a turning off<br>  ○ Left middle, the red light indicates input power is ON<br>  ○ Left, the green light indicates HV ON<br>  ○ Right middle, the white light indicates Pulses ON<br>  ○ Right, the orange light indicates an alarm<br>  ○ Top, the touch screen for programming output voltage, frequency and pulse width, and for monitoring capacitors voltage, output |

voltage and current pulse, output power and internal temperature and diagnostics.

General view of the front panel

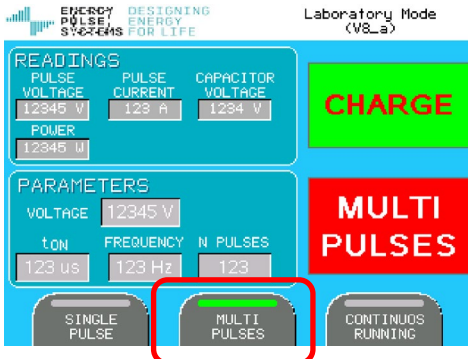
**Analysis protocol**

1. Prepare a solution with the sample and distilled water, with a 1:1 solid:liquid ratio (it may vary depending on the sample to be treated) and homogenize.
2. Measure the conductivity of the solution. High conductivities (in our case >5 mS/cm) can give rise to intensities that are too high (above the equipment limit, in our case >200 A) that do not allow treatment.
3. Select the GAP (distance between electrodes) and the amount of sample to be treated. The smaller the distance between the GAP electrode and the greater the amount of sample, the greater the probability of exceeding the intensity limit of the equipment and not being able to perform the treatment.




GAP

4. Select Multi Pulses on the main screen. When this mode is selected its button will be highlighted.



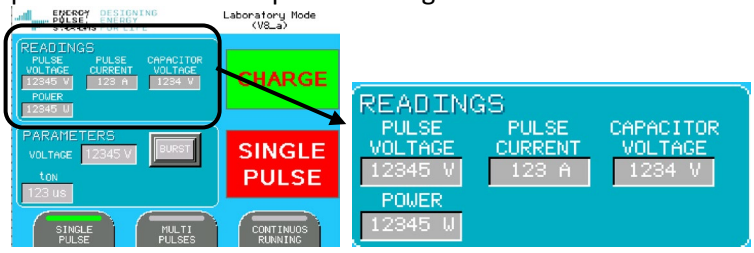

Program Selection with program highlighted

5. In this mode the user can define the pulse voltage, pulse width and number of pulses:
   *Parameters limit:*
   - Voltage – [1000V to 10000V]
   - Pulse width – [2µs to 200µs]
   - Frequency – [1 Hz to 200 Hz]
   - Number of pulses – [2 to 220]

6. After the parameters are set the charge button will become available.
7. Press Charge to start the charging of the capacitors. When the voltage on the capacitors reaches the set value the Multi Pulse button becomes available.
8. Press the Multi Pulse button to apply the pulses to the load.
   - During the pulses the user can stop by pressing STOP and start the process again by pressing Multi Pulses.

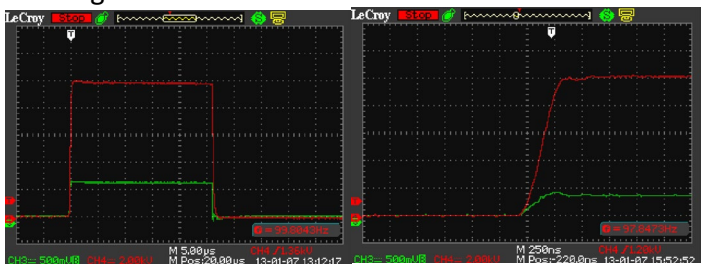
Multi pulses mode – Stop

9. On the main menu the field Readings is where the user can see the actual readings of the pulse, peak current and voltage, the output power and the first capacitor voltage.



Readings

10. Typical voltage and current waveforms



Left image is a typical 10 kV, 60 A, pulse waveform on resistive load, 26 µs width pulse and 100 Hz repetition rate, and on the right image the pulse rise time for similar working conditions.

11. Stir, protected from light (120 min, 150 rpm).
12. Centrifuge at 4200 rpm / 20 minutes and finally filter.

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| **Applications** | • Extraction of bioactive compounds from plants, seeds and agri-food co-products. |
| **References** | 1. Gómez, B., Munekata, P. E. S., Gavahian, M., Barba, F. J., Martí-Quijal, F. J., Bolumar, T., Campagnol, P. C. B., Tomasevic, I., & Lorenzo, J. M. (2019). Application of pulsed electric fields in meat and fish processing industries: An overview. Food Research International, 123, 95-105. https://www.sciencedirect.com/science/article/pii/S0963996919302819?casa_token=mfGLjKsMK_YAAAAA:4BgdX6SJn_wqjmoizDZttxULO09zUpXKtSlMic9nxMuwmVc92dGlUVskKZXWe4BbVnVVgwOeC6U<br>2. Barba, F. J., Parniakov, O., Pereira, S. A., Wiktor, A., Grimi, N., Boussetta, N., Saraiva, J. A., Raso, J., Martin-Belloso, O., Witrowa-Rajchert, D., Lebovka, N., & Vorobiev, E. (2015). Current applications and new opportunities for the use of pulsed electric fields in food science and industry. Food Research International, 77, 773-798. https://www.sciencedirect.com/science/article/pii/S0963996915301897?casa_token=hul7Q1ckxOEAAAAA:n1i9M9PlSchyByeqabzz7fJMDDLgbDwhurpS30iLFzAAopcYusNEk6qz5jfoA54ry76S_lrRttE |

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| **Practical** | **3. Oil extraction and concentration of other nutrients with supercritical fluid equipment** |
| **Competences** | Potential alternative to conventional extraction to obtain oils rich in compounds with high added value. |
| **Overview** | Supercritical Fluid Extraction (SFE) is an alternative extraction method that has attracted growing attention in food industries over the last decades. The most frequently used co-solvent is ethanol, because it meets the green technology requirements. Among the main characteristics and advantages of this technology are: highly unchanged extract obtained without solvent residues and without quality degradation due to high temperature or oxidation; use of non-toxic solvents such as carbon dioxide; variation of solubility through the characteristics of the solvent; and high selectivity for the extraction of non-polar compounds. |
| **Materials** | • $CO_2$ cylinder |
| **Equipment** | • Supercritical fluids equipment |
| **Procedure** | **Initial commissioning**<br>• The following connections are provided on the common connection plate on the right side of the system, which must be connected on site:<br>a) Compressed air connection for the pipe with outer diameter of 10 mm (or hose with inside diameter of 10 mm), Serto screw connection. |

b) $CO_2$ feed for HP 3/8" pipe, HP screw connection (only use $CO_2$ cylinders with dip tube!)
c) Exhaust gas connection (discharge lines) for the pipe with outer diameter of 12 mm (or hose with inside diameter of 13 mm), Serto screw connection.
d) Exhaust gas connection (rupture discs) for the pipe with outer diameter of 22 mm (or hose with inside diameter of 19 mm), Serto screw connection.

- In addition, the cooling water lines at the lower rear must also be connected as follows:

e) Cooling water inlet for the pipe with outer diameter of 10 mm (or hose with inside diameter of 10 mm), Serto screw connection.
f) Cooling water outlet for the pipe with outer diameter of 10 mm (or hose with inside diameter of 10 mm), Serto screw connection.

- The following cables must be connected for the power supply:

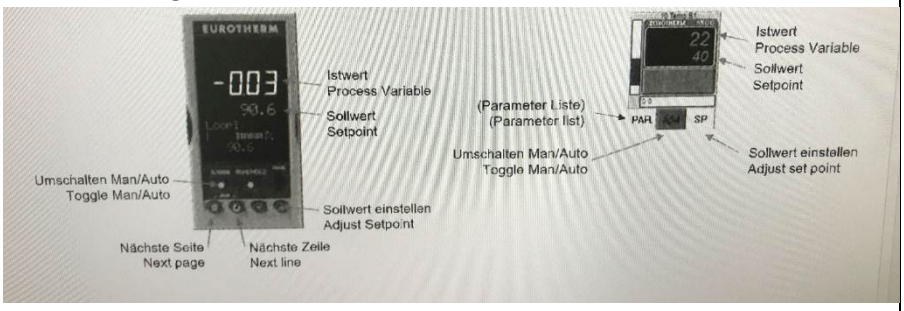
g) 5-wire connection cable for 3 x 400 V / 50 Hz - 12 A.

**Analysis protocol**

- The following instructions describe all manipulations, step by step, which must be taken for safely starting, up and shutting down the system:

1. Make sure that the level detector in separator B2 is clean and seal separator B2.
2. Make sure that the **apparatus is supplied with compressed air** and set the corresponding pressure regulator as follows:
   - Pressure regulator for SITEC valves -> Regulator PCI (right side): **6 bar**
   - Pressure regulator for proportional control valve C1 -> Regulator PC2 (right side): **2.5 bar**
3. **Switch on cooling water supply** for pilot system (building side).
4. Switch on the system using the **main switch (0/1).**
5. Power enabled by **pressing the green push-button <<RUPTURE DISCS OK>>**.
   - When the pressure switch detects a brief pressure rise in the rupture disc line, the power relay drops out and the inlet valve C5 is closed automatically. This process can only be reversed by pressing the <<RUPTURE DISCS OK>> button.
6. Prepare valves for filling the low-pressure part and opening the extractor B1:
   - Close: Turn clockwise
   - Open: Turn counter-clockwise
     - ✓ Closed valves: V1, V20, V3, V5, C1 carefully, V19 carefully, V16, V15
     - ✓ Open valves: V2, V30, V4, V6, V79

7. **Switch on the PC** and then start the operator software with <<SV3>>:
8. **Open CO2 cylinder**.
   - **Slowly open valve V1**. If no level of liquid CO2 is detected in K1, the pneumatic valve C5 opens automatically to fill fresh CO2 in the system.
9. **Slowly open valve V15**.
   - The pressure in <<SEPARATOR B2>> and in the <<CO2 tank K1>> now corresponds to the cylinder pressure and should be approx. 50 - 60 bar (compare with indicators on corresponding pressure gauges). If the apparatus is completely empty, the filling process might take a few minutes.
10. **Make** sure that the **cooling thermostat TC4 has started automatically** and the **setpoint** for the bath temperature is set to -7°C. (Details about the operation of this thermostat can be found in chap. 11.) The pump head is now pre-cooled.
11. Make sure that all **temperature and pressure controllers are in automatic mode** (<<MAN>> should not be illuminated). (Details can be found in the separate operating instructions in chap. 13.)
    - The red button below the regulation symbol indicates the controller is in <<manual>> mode. If the button is grey, the controller is working in <<automatic>> mode.




12. The separation mode can be selected by the toggle switch next to the separator symbol, <<SEPARATOR B2>>. The following cases are possible:
    - Toggle switch down (-> <<LEVEL>>) -> Separation in liquid CO2 (subcritical separation; The level control controls the heat input into Separator B2).
    - Toggle switch up (-> <<TEMPERATURE>>) -> Separation in the gas phase (supercritical separation; temperature is regulated by the temperature controller in Separator B2).
      - Proceed by setting the toggle switch in the "up" position (-> << TEMPERATURE>>).
13. Set the **temperature controller** for <<SEPARATOR B2>> to 32°C using the << ^ /v >> keys (details can be found in the separate operating instructions in chap. 13).
14. Set the setpoint for the output of the **controllers <<TEMPERATURE EXTRACTOR B1>> to 40°C and <<TEMPERATURE W1>> to 43°C** using

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| | the << ^ /v >> keys (details regarding controller operation can be found in the separate operating instructions in chap. 13). This way, the heating valves are opened, and the large steel masses of the extractor and the heat exchanger are preheated to the extraction temperature (as soon as thermostat TC1 is switched on).<br>15. Set the **pressure controller <<PRESSURE C1>> to 200bar** using the arrow keys. (Details regarding controller operation can be found in the separate operating instructions in chap. 13.)<br>● The pressure controller <<PRESSURE CONTROL C1>> changes the setpoint by approx. 50 bar/min.<br>16. **Switch on** the <<**PULSATION DAMPENER**>> heater by pressing the corresponding push-button in the pulsation damper icon (green lamp is on).<br>● Check the setpoint for the heater of the pulsation damper. Setpoint of 250°C should be set (lower display on the temperature controller <<TEMPERATURE W1>>).<br>17. **Switch on** the thermostat **TC1** and set the setpoint for **the bath temperature to 40°C.** (Details regarding the operation of the thermostats can be found in the separate instructions in chap. 12.)<br>18. **Switch on** the thermostat **TC2**, which delivers the heat energy for <<SEPARATOR B2>> and set the setpoint for the **bath temperature to 43°C** on the thermostat. (Details regarding the operation of the thermostats can be found in the separate instructions in chap. 12.)<br>19. Make sure that valves **V6 and V4** are also still open. Then open the cover of <<EXTRACTOR B1>>.<br>20. Pull the insertable container out of <<EXTRACTOR B1>> with lifting tool, open the sinter metal fastener, fill in raw material, and refasten the sinter metal fastener **very lightly! In the case of unknown or swelling raw materials, only fill the insertable container halfway.**<br>21. Carefully push the insertable container back into <<EXTRACTOR B1>> with the lifting tool and press it downward to the mechanical stop. Finally, **refasten** the lid of <<**EXTRACTOR B1**>>.<br>22. Prepare <<EXTRACTOR B1>> for the pressure build-up by **closing valves V4 and V6**.<br>23. **Slowly open** valve **V3**. The pressure in <<EXTRACTOR B1>> is now approximately that in <<TANK K1>> (50-60 bar; compare the corresponding pressure gauges). **Slowly open valve V5**.<br>24. Wait until the thermostat **TC1 has reached the previously set setpoint**.<br>25. Make sure that the **flow controller <<$CO_2$ MASS FLOW>> is set to automatic mode (<<MAN>>** should not be lit up) and set the **$CO_2$ mass flow to 10 kg/h** for the extraction test using the arrow keys (details regarding controller operation can be found in the separate operating instructions in chap. 13). |

26. Set a **stroke of approx. 23 mm** on the $CO_2$ pump (for details, please refer to chap. 10 of these operating instructions).
    - Start the CO2 pump P1 using the <<CO2 PUMP P1>> key switch (corresponding green operating lamp is on).
27. **Start the cooling unit TC5** with the corresponding **<<REFRIG.>> push-button**. The corresponding green push-button will light up.
28. **Switch on <<HEATING TANK K1>>** with the corresponding push-button (the corresponding operating lamp must be on).
    - Set a setpoint of approx. 17°C on the temperature controller <<TEMPERATURE TANK K1>> using the arrow keys. The value should lie approx. 6°C below the ambient temperature.
    - The pressure in the low-pressure part of the system should be held steady at approx. 50 bar by heating <<TANK K1>>.
29. **Wait** until the inlet temperature for the $CO_2$ pump P1 <<**Temp P1 IN**>> **is less than approx. 8°C** (on the 2nd page of the temperature controller <<TEMPERATURE TANK K1>>, <<P1 IN>>) and the **temperature <<TANK K1>> is less than approx. 20°C**.
    - If these temperatures are fallen below and the green level lamp <<LEVEL MIN.>> is also lit up, close valve V2.
30. Set the setpoint of **thermostat TC1 to 43°C**.
31. Now the pressure in <<EXTRACTOR B1>> slowly starts to rise.
32. As soon as the desired extraction pressure has been reached, the $CO_2$ pressure control valve begins to expand into <<SEPARATOR B2>>.
    - Since the delivery pressure now no longer changes, an optimal pump stroke for the CO2 Pump P1 can also be set. The pump stroke is optimal when the control output on the flow controller <<CO2 MASS FLOW>> is approx. 40 -90%.
33. In the case of a relatively high $CO_2$ mass flow at a relatively low gas density, the **<<PRESSURE CONTROL>>** might be overburdened (overshooting of the pressure...). In this case, **by sensitively opening the fine dosing valve C1**, **the control valve can be <<returned>> to a sensible control range (opening degree approx.40- 90 %)**
34. Check the Temperature of the <<SEPARATOR B2>> after a few minutes and **adjust the bath temperature of the thermostat TC2** corresponding.
35. **Close valve V79** to start the extraction process with <<EXTRACTOR B1>>.
36. Start data recording by pressing the <<STARTLog>> key in the operator software. This way, a separate measurement file is generated for this measurement. The data is stored while the operator software is running independent of the of the <<STARTLog>> key.
37. During the extraction process, extract can be taken out of <<SEPARATOR B2>> manually by slowly opening the dosing valve V19 and close it afterwards without force.

38. During the extraction process from plant material, water might collect in the system. The collected water can be removed by periodically briefly opening the bottom drain valve V20 of <<TANK K1>>.
39. After ending the test, proceed as follows:

a) Stop the data recording file using the <<STOP Log>> key in the operator software.
b) For extractions **above 60°C**, reduce the setpoint for **TC1 in steps of 40°C to 20°C**. <<EXTRACTOR B1>> and the heat exchanger W1 are cooled now.
c) On the flow controller <<**MASS FLOW $CO_2$**>>, set the flow to **approx. 2 kg/h** using the arrow keys and on the $CO_2$ pump, set a **stroke of approx. 10 mm**.
d) To save $CO_2$, the setpoint of the <<**PRESSURE CONTROL C1**>> can be set **in steps of 50 bar to approx. 60 bar** and $CO_2$ can be condensed back into the <<TANK K1>>. The pressure controller <<PRESSURE CONTROL C1>> changes the setpoint by approx. 50 bar/min.
   - During the entire expansion process, the pressure in <<SEPARATOR B2>> must never exceed 70 bar! If this value is still reached, stop the expansion process and open V16 until the pressure is below this again.
e) **Wait** until the <<PRESSURE C1>> has reached approx. **70 bar**.
f) **Wait** until the temperature in <<EXTRACTOR B1>> has fallen **below 50°C**.
g) **Slowly open valve V2**, to stop the flow through <<EXTRACTOR B1>>.
h) **Switch off heating thermostats** TC1 and TC2.
i) **Switch off heater <<PULS. DAMPENER>>** by pressing the corresponding green push-button (green lamp should be off).
j) **Switch off heater <<TANK K1>>** by pressing the corresponding green push-button (green lamp should be off).
k) **Switch off the cooling unit** with the **<<REFRIG>>** button (green lamp should be off).
l) **Switch off <<$CO_2$ PUMP P1>>** with the corresponding key switch.
m) **Slowly open** bypass valve **V79**.
n) **Close** valves **V3 and V5**.
o) Completely expand <<EXTRACTOR B1>> by **slowly opening valve V6. At the end of the expansion process, also open valve V4**.
p) Open the cover of <<EXTRACTOR B1>>.
q) Pull the insertable container out of the extractor with the lifting gear and open the upper sinter metal fastener. Then remove the residual product and clean the insertable container.
r) Replace the cover of <<EXTRACTOR B1>> to protect the thread from contamination and damage.

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| | s) Close valve V15 and **immediately start slowly relieving** <<SEPARATOR B2>> by opening valve V16. At the same time, remove extract from the separator by slowly opening the dosing valve V19.<br>t) Once <<SEPARATOR B2>> is completely expanded, open the cover and remove the extract residues manually.<br>u) Replace the cover of the separator to protect the thread from contamination and damage.<br>40. **Close valve V1**.<br>41. **Close the Software and switch off the computer**.<br>42. **Close** the valve on the **$CO_2$ cylinder and shut off the cooling water and compressed air on the building side**.<br>43. **Switch off** the **main switch of** the apparatus.<br>44. At the beginning of a new testing day, start with step 1 again. |
| **Applications** | • Extraction of fat<br>• Improve the quality of protein isolates |
| **References** | 1. Al Khawli, F., Pateiro, M., Domínguez, R., Lorenzo, J. M., Gullón, P., Kousoulaki, K., Ferrer, E., Berrada, H., & Barba, F. J. (2019). Innovative green technologies of intensification for valorization of seafood and their by-products. Marine Drugs, 17(12), 689. https://www.mdpi.com/1660-3397/17/12/689<br>2. Roselló-Soto, E., Barba, F. J., Lorenzo, J. M., Dominguez, R., Pateiro, M., Mañes, J., & Moltó, J. C. (2019). Evaluating the impact of supercritical-CO2 pressure on the recovery and quality of oil from "horchata" by-products: Fatty acid profile, α-tocopherol, phenolic compounds, and lipid oxidation parameters. Food Research International, 120, 888-894. https://www.sciencedirect.com/science/article/pii/S0963996918309396?casa_token=pcStHwOBXE0AAAAA:8xhNFp-VCP3Un3PUmwxrRlb6mmRN2yVN5eS-5iyUnu7GXLympxGP2H25VMuBzelur7vl0epXArM |

# Lab Manual Course 4

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| **COURSE** | **SAFETY, QUALITY AND REGULATORY AFFAIRS** |
| **Training Subject** | **4.1. Regulatory framework on meat and meat products** |
| **Index** | **1. Web search for food additives**: How to search for any additive authorized in the UE<br>**2. How to search for quality labels in the UE** (*e*Ambrosia web database) |

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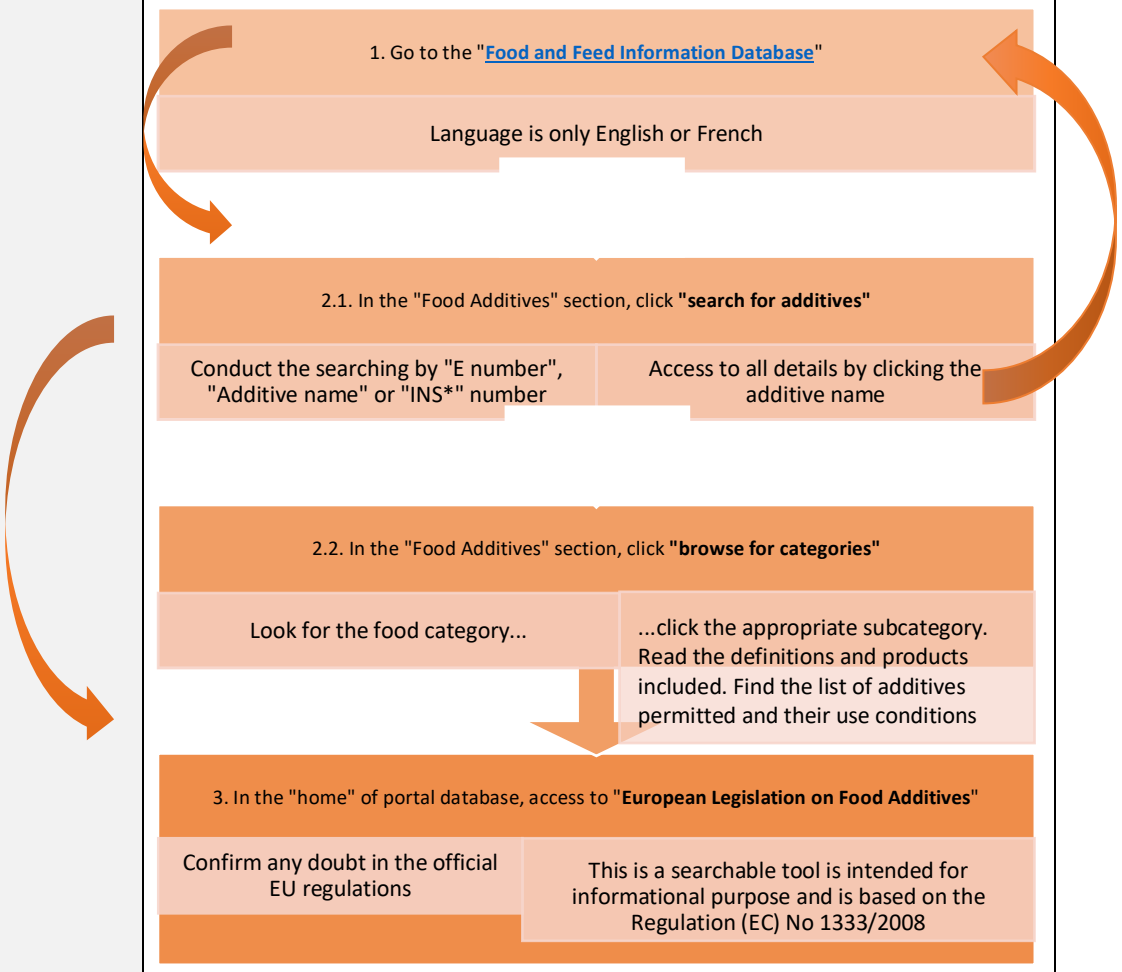
| **Practical** | **1. Exploring quickly (web search) food additives permitted in meat and meat products in the European Union (EU)** |
| **Competences** | To effectively conduct web searches to find food additives authorised for meat and meat products within the EU.<br>To develop the ability to conduct effective online searching to identify authorized food additives in meat and meat products within the EU. This involves navigating the official EU database and understanding how to interpret regulatory information. |
| **Overview** | In the EU, any substance used as a food additive must be approved and listed in the EU's positive list, along with its permitted conditions of use. This lab practical will walk you through a simple process for conducting online searches to find authorized food additives for the meat sector within the EU. |
| **Materials** | No need. |
| **Equipment** | Computer and internet connection |

**Procedure**




*INS, International Numbering System for Food Additives

**Applications**

It is possible to easily handle this web database with the food additives approved for use in food in the EU. It is an informative tool (not legal) to inform about them and their conditions of use in accordance with Regulation (EC) 1333/2008.

Examples:

a) Decide if the E 260 can be legally used in pre packed 100% fresh minced meat.

Procedure: *Step 1 Step 2.1 write down "260" on the box "E No." Click on "acetic acid" Go to "conditions of use" and read in category "8.2 Meat preparations" and check that is permitted*

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| | *quantum satis only in "prepacked preparations of fresh minced meat and meat preparations to which other ingredients than additives or salt have been added".*<br><br>b) Find the additives legally authorised to be used in a canned meat ball.<br>Procedure: *Step 1 Step 2.2 click on the category entitled "Heat treated processed meat (8.3.2)". Now, read which products this subcategory includes. After, check the list of authorised additives in this subcategory. They are displayed with their E-number, additive name, the maximum limit and restrictions or exception. Moreover, clicking on a particular additive name to find more details.* |
| **References** | 1. Regulation (EC) 1333/2008 (food additives list in Annex II): http://data.europa.eu/eli/reg/2008/1333/oj.<br>2. Database on food additives (EU): https://ec.europa.eu/food/food-feed-portal/screen/home<br>3. Food additives in the EU, an overview: https://food.ec.europa.eu/safety/food-improvement-agents/additives_en |

# Lab Manual Course 4

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| **Practical** | **2. How to search for quality labels in the UE (*e*Ambrosia web database)** |
| **Competences** | To effectively conduct web searches aimed at identifying the most pertinent quality labels (legal registration data) for meat and meat products within the EU.<br>To develop the proficiency in utilizing the "eAmbrosia" web search tool, which aggregates legal information for food and agricultural products, wine, spirits and aromatised wine. |
| **Overview** | Various product names within the meat sector have sought recognition through either Geographical Indication (GI) or Traditional Specialty Guaranteed (TSG) statuses. These designations are documented across different EU registers. For the meat industry, the primary online resources for locating such designations are the "eAmbrosia" and the "GIview" registers, the latter specifically cataloging all GI protected at the EU level. |
| **Materials** | No need. |
| **Equipment** | Computer and internet connection |

**Procedure**

**eAmbrosia**

Go to the EC website

Click box...

"**Agricultural products and foodstuffs register**"

**Geographical indications -**

Use one or several of the provided fields to display the required information

In the field "**Combined nomenclature code**", type “meat”, choose codes 02 16 (related to the meat sector); click "i" icon to obtain details

**Export**

Beside the displayed records, at the right side, find the **"xls" button** to download the retrieved information

**TSGs**

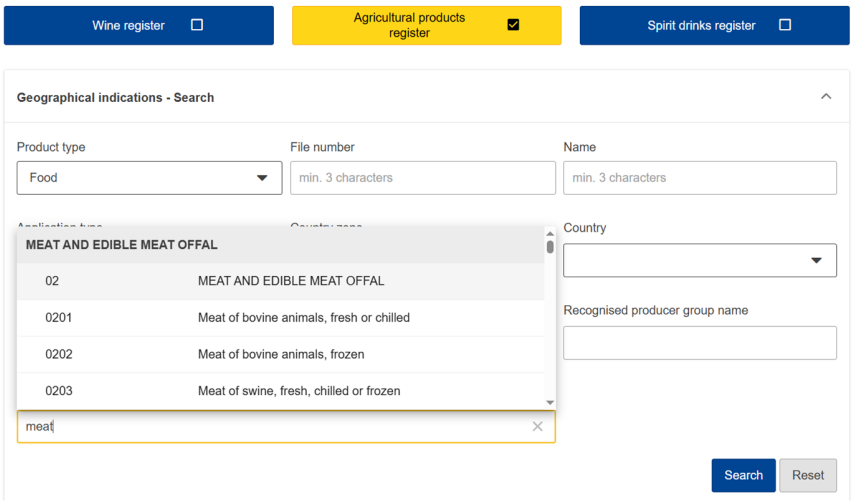
Go to the EC website, see at the bottom of webpage

Proceed as the eAmbrosia indications

**Glview**

Go to the website

Directly search for the required information: use “product category”, type of quality label, etc




**Applications**

Examples:

a) Find the meat products with Protected Designation of Origin (PDO) in Italy and Greece registered between 2000 and today.

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| | Procedure: *Go to "eAmbrosia" → search "meat" (codes 02 16) → select "European Union" in "Country zone" → in "Country", choose "Greece" and "Italy" → in the "Type" field, mark "PDO" → Search button → "xls" button → open the "xls" file and filter information for "Date of registration" ranging 2000 up to date.*<br><br>b) Identify if the name "Jabugo" is protected under any geographical indication in the EU and, correspondly, its main characteristics.<br><br>Procedure: *Go to "eAmbrosia" → mark "Food" (Product type) → write down "Jabugo" in "Name" → click "I" icon to obtain all details: download the file containing the product specifications.*<br><br>c) Identify all "fresh meat" registered as Traditional Specialities Guaranteed (TSGs) across the EU.<br><br>Procedure: *Go to "eAmbrosia" → click on mark "Traditional Specialities Guaranteed" → mark "1.1 Fresh Meat" (Product category) → select "European Union" in "Country zone" → "xls" button to download the information.* |
| **References** | 1. Regulation (EU) 2024/1143:<br>on geographical indications for wine, spirit drinks and agricultural products, as well as traditional specialities guaranteed and optional quality terms for agricultural products, amending Regulations (EU) No 1308/2013, (EU) 2019/787 and (EU) 2019/1753 and repealing Regulation (EU) No 1151/2012<br>myyu?44ifyf3jzwtuf3jz4jq4wjl47579466984to<br><br>2. Commission Delegated Regulation (EU) No 664/2014 on the logos to be used for PDOs, PGIs and TSGs:<br>myyu?44ifyf3jzwtuf3jz4jq4wjldijq475694;;94to<br><br>3. Geographical indications registers (EU):<br>https://agriculture.ec.europa.eu/farming/geographical-indications-and-quality-schemes/geographical-indications-registers_en |

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| **COURSE** | **SAFETY, QUALITY AND REGULATORY AFFAIRS** |
| **Training Subject** | **4.2. Meat and meat products safety** |
| **Index** | **1. How to determine CCP using a decision tree**. The determination of a CCP in the HACCP system can be facilitated by the application of a decision tree, which indicates a logical reasoning approach.<br>**2. Risk Analysis Tools in the Meat Industry:** tool to use in order to perform risk analysis that is needed when developing a HACCP plan for a Food Safety Management System<br>**3. HACCP verification Tools in the Meat Industry:** possible verification tools for the implementation of a HACCP plan in the meat industry will be described. |

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| **Practical** | **1. How to determine CCP using a decision tree** |
| **Competences** | To develop the ability to determine critical control points (CCPs) in the context of the HACCP system using a decision tree. |
| **Overview** | The determination of a CCP in the HACCP system can be facilitated by the application of a decision tree, which indicates a logical reasoning approach. According to Codex Alimentarius, training in the application of the decision tree is recommended. |
| **Materials** | Downloadable documents.<br>· General principles of food hygiene<br>· A Generic HACCP Model for Fresh Ground Pork Sausage Patties (Food Safety Inspection Service, US Department of Agriculture) |
| **Equipment** | Computer and internet connection |

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| **Procedure** | · Find the decision tree in p. 35 of General principles of food hygiene<br>· Find and check a flow diagram for the preparation of fresh ground pork sausage patties in A Generic HACCP Model for Fresh Ground Pork Sausage Patties<br>· Focusing on *Salmonella* as a potential biological hazard, apply the decision tree until a CCP is determined to control the aforementioned hazard |
| **Applications** | The application of the decision tree must allow for determination of a CCP. *Salmonella* is known to be present in raw materials and to outgrowth if proper measures are not implemented<br>· The flow diagram has the following steps:<br>1. Receiving and storage of raw materials<br>2. Weighing meat and non-meat ingredients<br>3. Chopping and grinding<br>4. Forming<br>5. Finished product labeling, storage and distribution<br>· Answers to questions in the decision tree will be as follow:<br>1. Receiving and storage of raw materials: Q1, No; Q2, No. Not a CCP<br>2. Weighing meat and non-meat ingredients: Q1, No; Q2, No. Not a CCP<br>3. Chopping and grinding: Q1, No; Q2, Yes; Q3, No; Q4, Yes. **CCP** |
| **References** | 1. General principles of food hygiene<br>2. A Generic HACCP Model for Fresh Ground Pork Sausage Patties |

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| **Practical** | **2. Risk Analysis Tools in the Meat Industry** |
| **Competences** | The competencies required to conduct risk analysis, especially within the meat industry, include a wide range of skills, knowledge areas and attributes.<br><br>Some key skills are:<br><br>Technical knowledge in food microbiology, food safety principles and risk analysis methodologies.<br><br>Experience in the ability to identify, assess and prioritize risks associated with microbial hazards in meat products.<br><br>Problem solving ability is also very important, as well as critical thinking to address challenges and uncertainties inherent in risk analysis processes.<br><br>Ability to collaborate with multidisciplinary teams including microbiologists, food scientists, epidemiologists, statisticians and industry professionals is very important.<br><br>Finally, adherence to professional codes of conduct and regulatory requirements governing food safety, public health and research ethics is a very important qualification for the person conducting risk analysis. |
| **Overview** | The meat industry faces many challenges related to food safety and hygiene due to the possible presence of biological, chemical and physical hazards both in raw materials and throughout the production process, storage and distribution of the final product. Applying a risk analysis framework to all these stages of production can help to identify, assess and manage these risks effectively, thereby ensuring the production of safe and high-quality meat products for consumers. |
| **Materials** | Risk analysis tools |
| **Equipment** | PC |

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| **Procedure** | Performing a risk analysis in the meat industry involves a systematic approach to identifying, assessing and managing the risks associated with the production, processing and distribution of meat and meat products. Such a systematic approach can be done by the following steps:<br><br>**Define scope and objectives:**<br>- Clearly define the scope of the risk analysis, including the specific areas of the meat production process to be assessed (e.g. slaughter, processing, packaging, storage, distribution etc).<br>- Define clear objectives for the risk analysis, such as improving food safety, strengthening hygiene practices or reducing the risk of contamination.<br><br>**Identify Hazards:**<br>- Identify all potential hazards in the meat production process, including biological (e.g. pathogens such as Salmonella, E. coli), chemical (e.g. cleaners, pesticides) physical hazards (e.g. foreign objects) and allergen inventory that take part in the production or are in the raw materials.<br>- Examine all stages of meat production, from rearing and transport to slaughter, processing, packaging and distribution.<br><br>**Evaluate the consequences:**<br>- Assess the potential consequences of each identified risk to public health, consumer safety and business operations.<br>- Consider factors such as the severity of the illness or injury, financial losses, reputational damage and regulatory implications.<br><br>**Evaluate likelihood:**<br>- Assess the likelihood or likelihood of each hazard occurring at various points in the meat production process.<br>- Consider factors such as the prevalence of hazards in raw materials, the effectiveness of control measures, hygiene practices and regulatory compliance.<br><br>**Risk Based calculation:**<br>- Calculate the level of risk associated with each hazard by multiplying the estimated consequences by the probability of occurrence. This results in a risk score or score that reflects the overall level of risk for each identified risk in the meat production process.<br><br>**Prioritize risks:**<br>- Prioritize risks based on their risk scores or assessments, focusing on those with the highest potential impact on food safety and public health.<br>- Consider factors such as severity of consequences, likelihood of occurrence, and feasibility of risk mitigation measures. |

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| | **Identify Risk Controls**:<br>- Identify and evaluate potential risk control measures or mitigation strategies to manage each prioritized risk. This may include implementing good manufacturing practices (GMPs), sanitation procedures, HACCP plans, temperature controls, pest control measures and employee training programs.<br><br>**Implementation of risk management actions:**<br>- Implementation of selected risk controls and mitigation measures to reduce or eliminate priority risks in the meat production process. |

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| **Applications** | • Meat processing operations<br>• Storage & Refrigeration<br>• Cleaning and sanitation<br>• Hygiene practices<br>• Pest control<br>• Allergen management<br>• Recall and crisis management |
| **References** | 1. Food safety and quality: Risk assessments (fao.org)<br>2. carrefour cover.eps (fao.org)<br>3. https://www.fao.org/food-safety/food-control-systems/risk-and-evidence-base/risk-based-approaches-and-tools/en/ |

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| **Practical** | **3. HACCP Verification Tools** |
| **Competences** | Basic knowledge of food safety management systems with emphasis on the verification principle |
| **Overview** | • Validation is "obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome."<br><br>• Monitoring is "the act of conducting a planned sequence of observations or measurements of control parameters to assess whether a control measure is under control."<br><br>• Verification is "the application of methods, procedures, tests and other evaluations, in addition to monitoring, to determine whether a control measure is or has been operating as intended." |
| **Materials** | HACCP plan |
| **Equipment** | N/A |
| **Procedure** | 1. Internal Audit<br>2. Calibration of measuring equipment<br>3. Microbiological and chemical analysis for assessing the acceptable limit of the identified hazards |

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| **Applications** | • reviewing monitoring records to confirm that CCPs are kept under control;<br>• reviewing corrective action records, including specific deviations, product disposition and analysis conducted to determine the root cause of the deviation;<br>• calibrating or checking the accuracy of instruments used for monitoring and verification;<br>• observing control measures to determine if they are being conducted according to the HACCP plan;<br>• sampling and testing, for example, for microorganisms (pathogens or their indicators), chemical hazards such as mycotoxins, or physical hazards such as metal fragments, to verify product safety;<br>• sampling and testing the environment for microbial contaminants and their indicators, such as Listeria spp.;<br>• reviewing the HACCP system, including the hazard analysis and the HACCP plan (for example, by means of internal and/or third-party audits). |
| **References** | 1. FAO. 2023. Validation and verification – Step 11, Principle 6. FAO Good Hygiene Practices (GHP) and Hazard Analysis and Critical Control Point (HACCP) Toolbox for Food Safety. Rome. https://doi.org/10.4060/cc6272en |

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| **COURSE** | **SAFETY, QUALITY AND REGULATORY AFFAIRS** |
| **Training Subject** | **4.3. Meat quality control and assurance** |
| **Index** | **1. Meat Products Quality Control: Nitrites.** the method for determining the nitrite content will be described<br>**2. Meat Products Quality Control: Fat content.** the method for determining the nitrite content will be described<br>**3. Meat Products Quality Control: Protein content.** the method for determining the protein content will be described<br>**4. Meat Products Quality Control: Simultaneous determination of protein, fat and moisture.** the method for determining the protein, fat and moisture contents will be described<br>**5. Environmental Monitoring as a prerequisite for certification schemes in the meat industry:** a step by step development of an environmental monitoring plan, that is essential for several Food Safety Management Systems will be described. |

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| **Practical** | **1. <u>Meat Products Quality Control: Nitrites</u>** |
| **Competences** | Analytical skills |
| **Overview** | The present method describes a spectrophotometric method for the determination of nitrite based on the reaction of nitrite with sulfanilamide to form a diazonium salt, then coupling the diazotized sulfanilamide with N- (1 Napthyl) ethylenediamine dihydrochloride to form an intensely purple colored azo dye which is measured spectrophotometrically. |

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| **Materials** | **Reagents:**<br><br>a) NED reagent - Dissolve 0.2 gm N- (1 Napthyl) ethylenediamine dihydrochloride in 150mL, 15% (v/v) acetic acid. Filter if necessary and store in a glass stoppered brown glass bottle.<br><br>b) Sulphanilamide reagent- Dissolve 0.5 gm sulphanilamide in 150 mL 15% acetic acid(v/v). Filter, if necessary and store in a glass stoppered brown bottle.<br><br>c) Nitrite standard solution-<br><br>(i) Stock solution - 1000 ppm $NaNO_2$ - Dissolve l.000 mg pure $NaNO_2$ in water and make up to 1 lit.<br><br>(ii) Intermediate solution - 100 ppm - Dilute 100 mL of stock solution to 1 lit<br><br>with water.<br><br>(iii) Working solution- l ppm - Dilute 10 mL of intermediate sol to 1 lit with water.<br><br>(d) Filter paper - Test for nitrite contamination by analyzing 3-4 sheets at random. Filter approx 40 mL water through each sheet. Add 4 mL of sulphanilamide reagent, mix, let stand for 5 minutes, add 4 mL of NED reagent, mix and wait for 15 minutes. If any sheets are positive do not use them. |
| **Equipment** | Photometer |

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| **Procedure** | 1. Weigh 5 gm prepared sample in a 50 mL beaker. Add about 40 mL of water heated to 80°C. Mix thoroughly with a glass rod taking care to break all lumps and transfer to a 500 mL volumetric flask. Thoroughly wash the beaker and glass rod with successive portions of hot water adding all washings to flask.<br>2. Add enough hot water to bring volume to about 300 mL. Transfer the flask to steam bath and let it stand 2 hours shaking occasionally.<br>3. Cool to room temperature, dilute to volume with water and remix. Filter. If turbidity remains after filtration, centrifuging will usually clear the solution.<br>4. Add 2.5 mL of sulphanilamide sol to aliquot containing 5-50 µg $NaNO_2$ in 50 mL vol. flask and mix.<br>5. After 5 minutes add 2.5 mL NED reagent, mix dilute to vol, mix and let colour develop 15 minutes.<br>6. Transfer portion of solution to photometer cell and determine absorbance at 540 nm against blank of 45 mL water and 2.5 mL of sulphanilamide reagent and 2.5 mL of NED reagent.<br>7. Determine Nitrite present by comparison with standard curve prepared as follows:<br><br>a) Add 10, 20, 30, 40 mL of nitrite working solution to 50 mL vol flasks. Add 2.5 mL of sulphanilamide reagent and after 5 minutes add 2.5 mL of NED reagent and proceed as above.<br><br>b) Standard curve is straight line up to 1 ppm $NaNO_2$ in final solution. |
| **Applications** | Meat products, sausage. |
| **References** | 1. AOAC Official method 17th edition 2000, 973.31 Nitrites in cured meats - Colorimetric method, Adopted as Codex Reference method (Type II). |

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| **Practical** | **2. Meat Products Quality Control: Fat content** |
| **Competences** | Analytical skills |
| **Overview** | Crude fat content is determined by extracting the fat from the sample using a solvent, then determining the weight of the fat recovered. The sample is contained in a porous thimble that allows the solvent to completely cover the sample. The thimble is contained in an extraction apparatus that enables the solvent to be recycled repeatedly. This extends the contact time between the solvent and the sample and allows it time to dissolve all the fat contained in the sample. For the solvent to thoroughly penetrate the sample it is necessary for the sample to be as finely comminuted as possible. |
| **Materials** | - Petroleum ether boiling point 60-80ºC<br>- Cotton wool free of fat<br>- Acid washed sand |
| **Equipment** | • Analytical balance (at least 1 mg sensitivity).<br>• Electrical drying oven to be operated at 102ºC± 1ºC.<br>• Soxhlet extraction unit comprising: – Round bottom flask, 150 mL<br>• Soxhlet extractor with 60 mL siphoning capacity and condenser.<br>• Cellulose extraction thimbles (28 x 80 mm)<br>• Fume cupboard<br>• Heat source, either electric heating mantle, or steam bath 100 mL beaker<br>• Desiccator with silica gel desiccant<br>• Glass rod |
| **Procedure** | 1. Rinse all glassware with petroleum spirit, drain, dry in an oven at 102ºC for 30 min. and cool in a desiccator.<br>2. Place a piece of cotton wool in the bottom of a 100 mL beaker. Put a plug of cotton wool in the bottom of an extraction thimble and stand the thimble in the beaker.<br>3. Accurately weigh 5 g of sample into the thimble. Add 1 - 1.5 g of sand and mix the sand and sample with a glass rod. Wipe the glass rod with a piece of cotton wool and place cotton wool in the top of the thimble. (Addition of sand is not required for analysis of meat meal). Dry the sample in an oven at 102ºC for 5 hours. The drying step may be omitted in the analysis of meat meal.<br>4. Allow the sample to cool in a desiccator.<br>5. Take the piece of cotton wool from the bottom of the beaker and place it in the top of the thimble. |

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| | 6. Insert the thimble in a Soxhlet liquid/solid extractor (Figure 1).<br>7. Accurately weigh a clean, dry 150 mL round bottom flash and put about 90 mL of petroleum spirit into the flask.<br>8. Assemble the extraction unit over either an electric heating mantle or a water bath.<br>9. Heat the solvent in the flask until it boils. Adjust the heat source so that solvent drips from the condenser into the sample chamber at the rate of about 6 drops per second.<br>10. Continue the extraction for 6 hours<br>For sausage meat and other emulsified products, the extraction should be performed in stages: Extract for about 4 hours, then remove the heat source and drain the solvent from the extractor in the flask. Remove the thimble from the extractor and transfer the sample to a 100 mL beaker. Break up the sample with a glass rod. Return the sample to the thimble and replace the thimble in the extractor. Rinse the beaker with petroleum spirit and pour rinsing's into the extract. Continue extraction for a further two hours.<br>11. Remove the extraction unit from the heat source and detach the extractor and condenser. Replace the flask on the heat source and evaporate off the solvent. (The solvent may be distilled and recovered).<br>12. Place the flask in an oven at 102ºC and dry the contents until a constant weight is reached (1-2 hours).<br>13. Cool the flask in a desiccator and weigh the flask and contents. Weight of empty flask (g) = $W_1$ Weight of flask and extracted fat (g) = $W_2$ Weight of sample = S % Crude fat = (W2 – W1) x 100 S |
| **Applications** | Meat and meat products like Salami, ham |
| **References** | 1. AOAC Official Methods for Fat (960.39) |

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| **Practical** | **3. <u>Meat Products Quality Control: Protein content</u>** |
| **Competences** | Analytical skills |
| **Overview** | **Kjeldahl method** determines the nitrogen content of meat and meat products. The sample is digested in a mixture of concentrated sulphuric acid and potassium sulphate, using a digestion block system. Salt and a catalyst are added to speed up the conversion of organic nitrogen to ammonium sulphate. An excess of sodium hydroxide is added to the cooled digest to liberate ammonia. The liberated ammonia is trapped in a weak boric acid solution and titrated with a stronger standardized hydrochloric acid, using colorimetric end-point detection. The nitrogen content is calculated from the amount of the determined ammonia. |
| **Materials** | (a) Catalyst tablets. —Containing 3.5 g $K_2SO_4$ and 0.175 g HgO (Kjeltabs "MT" available from Tecator, Inc., 2875C Towerview Rd, Herndon, VA 22071, USA, or equivalent).<br><br>(b) Boric acid solution. —4%. Dis solve 4 g $H_3BO_3$ in $H_2O$ containing 0.7 mL 0.1% alcoholic solution of methyl red and 1.0 mL 0.1% alcoholic solution of bromocresol green and dilute to 100 mL with $H_2O$.<br><br>(c) Sodium hydroxide–sodium thiosulfate solution. —Dissolve 2000 g NaOH and 125 g $Na_2S_2O_3$ in $H_2O$ and dilute to 5 L (ca 50 mL is used per anal ysis).<br><br>(d) Hydrochloric acid standard solution. —0.2M.<br><br>(e) Hydrogen Peroxide.—30–35%. (f) Sulfuric acid. —Concentrated. |
| **Equipment** | (a) Digestion block and as sociated glass ware. —Tecator DS-6 or DS-20 (Tecator), or equivalent.<br><br>(b) Distillation unit and as sociated glass ware. —Kjeltec 1003 (Tecator), or equivalent. |

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| **Procedure** | **Digestion**<br><br>- Accurately weigh ca 2 g well-ground and mixed test sample on 7 cm N-free filter paper (e.g., Whatman 541), fold, and trans fer to 250 mL di gestion tube.<br><br>- Place tubes in fume hood and add 2 or 3 boiling chips, 2 catalyst tablets, 15 mL $H_2SO_4$ , and slowly 3 mL 30–35% $H_2O_2$ . Let reaction subside and place tubes in block digestor preheated at 410°C. (Digestor must be placed in perchloric acid fume hood or be equipped with exhaust system.<br><br>- Rapid addition of 30–35% $H_2O_2$ may cause the reaction to become violent.)<br><br>- Digest at 410°C until mixture is clear, ca 45 min.<br><br>- Remove tubes and let cool ca 10 min. Do not let precipitate form; if precipitate forms, reheat.<br><br>- Once the tubes are sufficiently cool to handle, remove the exhaust manifold and carefully add 50-75 ml of water to each tube.<br><br>**Distillation**<br><br>Transfer the digestion tube to the distillation unit. The addition of 50-75 ml alkali NaOH 40 % w/v and water as well as the steam distillation and titration will be made automatically.<br><br>Place a conical flask containing 25 ml of the boric acid solution under the outlet of the condenser in such a way that the delivery tube is below the surface of the boric acid solution. Operate the distilling unit in accordance with the user manual.<br><br>**Titration**<br><br>Titrate the contents of the conical flask with the hydrochloric acid standard volumetric solution using a burette and read the amount of titrant used. The end-point is reached at the first trace of pink color in the contents. Estimate the burette reading to the nearest 0,01 ml. An illuminated magnetic stirrer plate or a photometric detector may aid visualization of the end-point.<br><br>**Blank test**<br><br>Carry out a blank test following the procedure described above, taking about 0,20 g of sucrose instead of the test portion. Keep a record of blank values. If blank values change, identify the cause.<br><br>Calculations |

Calculate the % Nitrogen according to the formula below

$$\%N = \frac{(T-B) \times N \times 14.007}{W} \times 100$$

Where:

T =Titration volume for sample (ml)

B =Titration volume for blank (ml)

N=Normality of acid to 4 places of decimal

W = sample weight (mg)

Calculate the % protein according to the formula below:

**% Crude Protein = % Kjeldahl Nitrogen X F**

F= factor to convert nitrogen to protein F-factor is 6.25 for meat and meat products

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| **Applications** | Meat and meat products like Salami, ham |
| **References** | 1. AOAC Official Method 981.10 Crude Protein in Meat |

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| **Practical** | **4. <u>Meat Products Quality Control: Simultaneous determination of protein, fat and moisture</u>** |
| **Competences** | Analytical skills |
| **Overview** | The method uses the FOSS FoodScan™ (FOSS North America, 8091 Wallace Rd, Eden Prairie, MN 55344, USA) with artificial neural network (ANN) calibration and associated database. The method is based on near-infrared (NIR) reflectance spectroscopy, a secondary, correlative technique to predict the concentration of various constituents in biological or organic samples. The ground sample is placed in a cup and positioned inside the FoodScan sample chamber. The sample cup is rotated during the analysis process to subscan 16 zones of the test sample, which are then merged together for the final result. The ANN calibration model is derived from a database of sample spectra and associated chemical analysis values. The ANN calibration quantifies the relationship between the spectral characteristics and the constituent values to interpret the test spectra and return the results for protein, fat, and moisture. This method refers to Simultaneous determination of fat, moisture, and protein in meat and meat products (fresh meat, beef, pork, and poultry, emulsions, and finished products) in the constituent ranges of 1–43% fat, 27–74% moisture, and 14–25% protein |
| **Materials** | Not necessary |

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| **Equipment** | **a)** FOSS FoodScan system.—NIR transmission, with a moving grating monochromator scanning the region from 850 to 1050 nm.<br><br>**b)** FoodScan ANN calibration for meat and meat products, version 3.00, with the associated database.—The FoodScan for meat comes complete with the operating software, ANN calibration, and required accessories.<br><br>**c)** Polysulfone or glass-bottom sample cups.—140 mm (diameter) 17.5 mm (height). Because of optical variations, the use of polystyrene dishes, such as Petri dishes, is not recommended.<br><br>**d)** Personal computer (PC).<br><br>—With the following minimum specifications: XP (SP2) operating system, Intel Celeron or Pentium 4 processor, 2.8 GHz, 512 Mb RAM, 40 MB hard-drive space, CD and floppy drives, and USB ports. Items (a)–(d) are from FOSS Analytical (Slangerupgade 69, DK-3400 Hillerød, Denmark) from FOSS North America (Tel: 1-952-974-9892, Fax: 1-952-974-9823, www.foss.dk). |
| **Procedure** | **Preparation of Analytical Sample**<br>Grind or homogenize representative sample, using standardized protocol as AOAC 983.18.<br>Pack approximately 180 g sample into the FoodScan sample cup. Avoid air pockets in the sample, and pack the sample level with the top of the sample cup and in a consistent manner.<br>The optimal sample temperature is 10–20 C; however, if measurements outside this range are needed, ensure that the temperatures of the samples do not vary by more than ±5 C and/or condensation on the collimator lens.<br>**Determination**<br>1) Turn power on for the unit, allow unit to warm up, and perform self-test diagnostics.<br>2) Select the appropriate operator ID and product profiles. The product profile must specify the use of the FoodScan ANN calibration for meat and meat products, version 3.00.<br>3) Place prepared sample into the sample cup.<br>4) Place the sample cup in the holder in the instrument. Ensure that the sample cup engages the index pin in the holder. Close and lock the door.<br>5) Start the analysis by pressing the “Start” button.<br>6) Enter sample ID and/or sample description.<br>7) When analysis is complete, remove sample from the instrument. (8) Process and/or record results.<br>**Calculations**<br>The FoodScan software calculates the results for fat, moisture, and protein, which are displayed as percentages (g/100 g) to 2 decimal places. |

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| **Applications** | Meat and meat products. |
| **References** | 1. AOAC Official Method 2007.04<br><br>Meat Analyser for meat product analysis (fossanalytics.com) |

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| **Practical** | **5. Environmental Monitoring as a prerequisite for certification schemes in the meat industry** |
| **Competences** | Basic knowledge of food safety management systems with emphasis on the prerequisite for environmental monitoring plan |
| **Overview** | The environmental monitoring plan covers all areas where meat processing and handling occur, including production facilities, storage areas, and transportation. |
| **Materials** | HACCP plan |
| **Equipment** | N/A |
| **Procedure** | 1. Responsibilities<br>2. Key Environmental Factors to Monitor<br>3. Sampling Plan<br>4. Testing Methods<br>5. Data Analysis and Reporting<br>6. Verification and Validation<br>7. Training and Communication<br>8. Continuous Improvement |

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| **Applications** | 1. **Responsibilities:**<br>● Designate a trained team responsible for implementing and managing the environmental monitoring program.<br>● Define roles for personnel involved in sampling, testing, and recording data.<br><br>2. **Key Environmental Factors to Monitor:**<br><br>a) **Microbiological Contamination:**<br><br>● Regular swabbing of surfaces, equipment, and utensils for pathogens like Salmonella, Listeria, and E. coli.<br>● Testing air quality for microbial presence in critical areas.<br><br>b) **Allergen Cross-Contamination:**<br><br>● Monitoring shared equipment and processing lines for allergen residues.<br>● Verifying effectiveness of cleaning procedures in allergen control zones.<br><br>c) **Chemical Hazards:**<br><br>● Testing water sources for contaminants such as heavy metals and pesticides.<br>● Monitoring chemical storage areas and ensuring proper labeling and handling protocols.<br><br>d) **Physical Contaminants:**<br><br>● Conducting regular checks for foreign objects like metal fragments, glass, or plastic in processing areas.<br>● Inspecting packaging materials for integrity and cleanliness.<br><br>3. **Sampling Plan:**<br>● Establish a sampling schedule based on risk assessments, production volume, and critical control points (CCPs).<br>● Use validated sampling techniques and equipment to ensure representative samples.<br>● Document sampling locations, frequencies, and methods for each environmental factor.<br><br>4. **Testing Methods:**<br>● Utilize appropriate testing methods for different contaminants, such as swabbing, ATP testing for cleanliness, PCR for pathogens, and ELISA for allergens. |

- Ensure that testing procedures are validated, calibrated, and conducted by qualified personnel.

5. **Data Analysis and Reporting:**
   - Analyze monitoring results to identify trends, deviations, or potential hazards.
   - Establish action levels and corrective actions for out-of-specification results.
   - Maintain comprehensive records of monitoring activities, including test results, corrective actions, and verification procedures.

6. **Verification and Validation:**
   - Regularly review and validate the effectiveness of the environmental monitoring program through internal audits, third-party inspections, and proficiency testing.
   - Verify that corrective actions are implemented promptly and are effective in preventing recurrence.

7. **Training and Communication:**
   - Provide training to personnel involved in environmental monitoring on procedures, equipment usage, and hygiene practices.
   - Communicate monitoring results, trends, and best practices to relevant stakeholders, including management, production teams, and quality assurance personnel.

8. **Continuous Improvement:**
   - Conduct periodic reviews and updates to the environmental monitoring plan based on new scientific information, regulatory changes, and lessons learned from incidents or near-misses.
   - Encourage feedback and suggestions for improvement from employees involved in monitoring activities.

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| **References** | 1. https://www.fdareader.com/blog/environmental-monitoring-program<br>2. https://www.eurofinsus.com/food-testing/resources/a-guide-to-environmental-monitoring/ |

# Lab Manual Course 5

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| **COURSE** | **ENVIRONMENTAL IMPACT OF MEAT PRODUCTION AND CONSUMPTION AND SUSTAINABILITY IN THE MEAT SECTOR** |
| **Training Subject** | **5.2. Meat production in relation to GHGs emissions and water consumption**<br>**5.4. Alternatives for meat proteins** |
| **Index** | **1. Calculating and analysis of the student's environmental footprint EF**<br>**2. Characteristics and evaluation of innovative meat products: meat snack and meat analogues** |

| | |
|---|---|
| **Practical** | **<u>1. Calculating and analysis of the student's environmental footprint</u>** |
| **Competences** | Knows and understands what the ecological footprint created by humans is. It determines its impact on the planet.<br>Lists the factors influencing its size.<br>Is able to calculate the ecological footprint using available calculators. |
| **Overview** | Ecological footprint - an analysis of human demand for natural resources. In this case, human consumption of natural resources is compared with our planet's ability to regenerate them. An environmental footprint is the estimated number of hectares of land and sea surface required to sustain current production and consumption. Such an ecological footprint is the need for space that satisfies our lifestyle. It allows us to understand how much we take from environmental resources and how long these resources will last us to maintain today's standard of living. It is measured in global hectares (Gha) per person. |
| **Materials** | - |
| **Equipment** | Computer or smartphone, Internet |
| **Procedure** | 1. Familiarise yourself with the methods and formulas needed to calculate your environmental footprint - 5.2.1.<br>2. Go to https://footprint.wwf.org.uk/<br>3. Complete the required items from the EF calculator one by one.<br>4. Record the result in table 1.<br>5. Summarise your environmental footprint – what contributed most to its value. |

6. Compare your result with the results of other students.

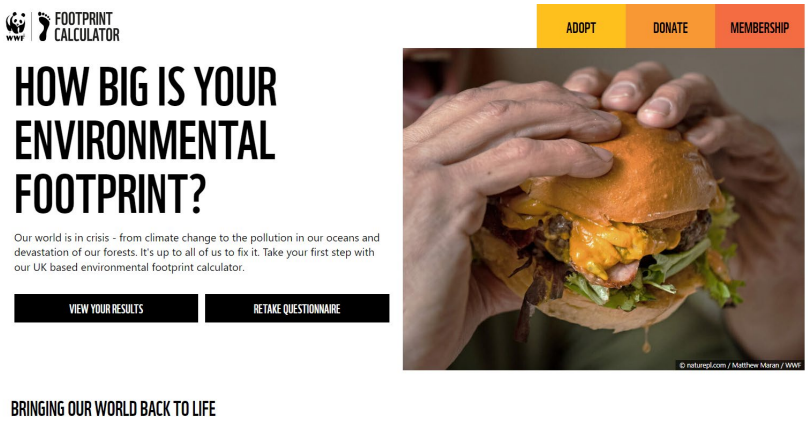
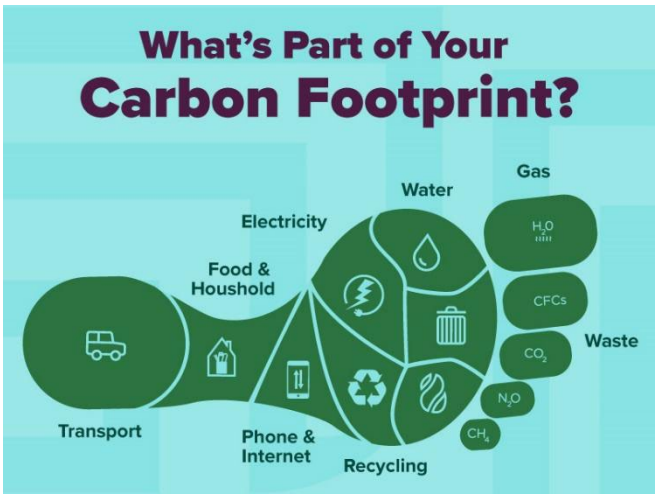



Table 1. Environmental footprint of different person

| Student | EF ha/person |
|---|---|
| Poland/2015 | 5.24 |
| Europe/2017 | 5.50 |
| USA/2018 | 9.50 |
| Summer school/ International/2023 | 11.50 |
| Innomeatedu | |

**Applications**

- to determine the impact of human activity on the environment
- building awareness of sustainable development




**References**

1. Mottet A., Steinfeld H. (2018). Cars or livestock: which contribute more to climate change? https://news.trust.org/item/20180918083629-d2wf0
2. Manzano P., Rowntree J., Thompson L., del Prado A., Ederer P., Windisch W., Lee MRF, (2023). Challenges for the balanced attribution of livestock's environmental impacts: the art of conveying simple messages around complex realities, Animal Frontiers, Volume 13, Issue 2, , Pages 35–44, https://doi.org/10.1093/af/vfac096

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| **Practical** | **<u>2. Characteristics and evaluation of innovative meat products: meat snack and meat analogues</u>** |
| **Competences** | Student is able to characterize, evaluate and compare meat products and meat analogues. |
| **Overview** | The aim of the class is to familiarize students with trends present on the meat products market. Students characterize the quality of innovative products (meat and meat analogues ) in terms of chemical composition and raw materials used in the production, the correctness of labelling, and attractiveness of chosen snacks. The colour and sensory quality of products are determined. The critical analysis of chemical composition of nutritional value is conducted. |
| **Materials** | 6 packaged meat snacks, meat analogues and products with added insect protein   |
| **Equipment** | • Konica-Minolta Chroma meter |

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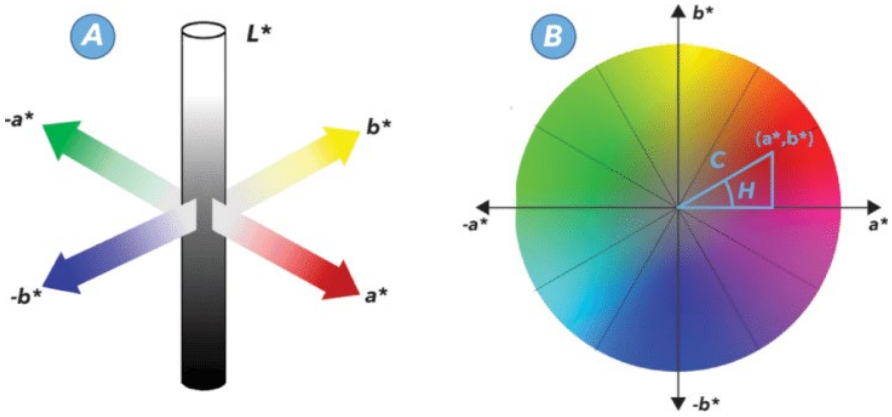
| **Procedure** | **1**. At the beginning, write the names of all products in the Table 1 and based on packaging and products' appearance evaluate the attractiveness of packaging and products using scale from 1 – not attractive at all, 5 – extremely. Please indicated also the consumer group for which each product are targeted.<br><br>**2.** Choose 3 products for further analyses such as: (Table 2).<br><br>a) The correctness of labelling based on the most recent law requirements by verification if all necessary information was provided by a producer. Indicate the obligatory information, which were placed on packaging's and additional (voluntary one) – use Doc. 1169/2011<br>b) Evaluate if the packaging is hermetic (using your sight and by touching), write down the information of the packaging material used and the way of packaging (vacuum or modified atmosphere)<br>c) Evaluate the functionality of a packaging such as a size, the ease of opening and removing the product (point 1 – not easy to open or extremely difficult to remove the product out of the packaging; size too big or too small; to points 5 – easy to open, easy to remove the product, possibility to close the packaging, size comfortable to use).<br><br>**3**. Using the same 3 products, critically analyse their composition and the results write down in Tables 3-6.<br><br>**4.** Conduct an organoleptic evaluation of all snacks. Apply the ranking method to the evaluation. 6 samples should be prepared for each panellist (the person making the evaluation). The panellist writes down his/her rating into Table 7 from the most desirable (1$^{st}$ place) to the least desirable (6$^{th}$ place). Rank products according to their desirability. |

**5.** Determine the colour of 3 products using Konica-Minolta Chroma meter in three different positions for each samples cross-section (L* lightness from 100 white to 0 black, a* positive values indicate red and negative green; and b* - positive values indicate yellow, and negative blue) – Fig.A (Table 8). Calculate the chroma (C) and hue (H) – Fig. B

$$C^* = \sqrt{(a^{*2} + b^{*2})} C^* = \sqrt{(a^{*2} + b^{*2})}$$

$$h^\circ = \text{arctangent}\left(\frac{b^*}{a^*}\right) h^\circ = \text{arctangent}\left(\frac{b^*}{a^*}\right)$$




**Calculate the colour difference (ΔE) between 2 chosen products:**

$$\Delta E = [(L^*_0 - L^*)^2 + (a^*_0 - a^*)^2 + (b^*_0 - b^*)^2]^{0.5}$$

Where: ΔE- colour difference; $L^*_0$, $a^*_0$, $b^*_0$ - colour attributes of first product; L*,a*,b*- colour attributes of second product

The difference (ΔE) interpretation – if the obtained value is with a range

| **ΔE value range** | **Interpretation** |
|---|---|
| 0 – 0.5 | trace |
| 0.5-1.5- | insignificant |
| 1.5-3.0 | noticeable |
| 3.0 - 6.0 | visible |
| 6.0 – 12.0 | clearly visible |
| 12.0 and higher | evident |

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|---|---|
| | Compare the colour of the tested products. Describe how the studied colour coordinates differ between different products.<br><br>**6**. Conclusions - analyse each point made during the exercises and wright down your remarks. |

Table 1. The list of meat snacks and meat analogues from the Polish market with the evaluation of their attractiveness

| **Product's name** | **Attractiveness of packaging** | **Attractiveness of products** | **Target group** |
|---|---|---|---|
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**1 – not attractive at all, 5 – extremely attractive**

Table 2. Evaluation of packaging – labelling, packaging material

| **Product's name** | **Information** |
|---|---|
| **Packaging –** hermetic or not, material type, packaging method | |
| **Labelling** (indicate the type of information provided, e.g. products name, composition etc.) | Obligatory information: |
| | Additional (voluntary) information: |

| | |
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| **The evaluation of the correctness of labelling** (indicate the information correctly given and all mistakes in labelling) | Information given in a right way: |
| | Mistakes: |
| **Evaluation of packaging functionality** (from 1 to 5) | |

Complete table 2 for each of the 3 selected products

**Table 3.** The meat and fat materials used in products

| Product's name | Raw material (meat and fat) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Pork (%) | Chicken (%) | Turkey (%) | Beef (%) | Veal (%) | Other (%) (specify what and proportion if given) | Fat (%) |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |

**Table 4. The list of non-meat ingredients and additive**

| | Ingredients other than meat and fat and food additives |
|---|---|

| Product ‘name | Water | Salt | Seasalt | Sugar | Glucose | E250 sodium nitrite | Plant oil/specify the kind | Vegetables, plant extracts and other plant derivatives | Lactose (from milk) | Yeast extract | Semolina | Poultry skins | Collagen protein | Other/specify what |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | |
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**Table 5. The list of functional additives**

| | Stabilizers | Antioxidants | Acidity regulators | |
|---|---|---|---|---|

| Product's name | E450 Diphosphates | E451 Triphosphates | E452 Polyphosphates | E425 Konjac | E407 Carrageenan | Other (specify what) | E300 Ascorbic acid | E301 Sodium ascorbate | E316 Sodium erythorbate | Other (specify what) | E508 Potassium chloride | E325 Sodium lactate | E330 Citric acid | E331 Sodium citrates | E326 Potassium lactate | Other (specify what) | Other (specify what) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | | | |
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**Table 6. Nutritional value analysis**

| Product's name | Energy value (kcal) | % RWS of a portion | Fat (g) | Saturated fatty acids (g) | Carbohydrates (g) | Sugars (g) | Protein (g) | Salt (g) |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
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**Table 7. Evaluation of the products quality using ranks**

| | Product's name | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | |
| Panellist I | | | | | | |
| Panellist II | | | | | | |
| Panellist III | | | | | | |
| Mean value | | | | | | |
| Rank | | | | | | |

Table 8. Values of measured L*, a* and b* and calculated C, h and ΔE

| **Product's name** | **repetition** | **$L^*$** | **$a^*$** | **$b^*$** | **C** | **h** |
|---|---|---|---|---|---|---|
| | 1 | | | | | |
| | 2 | | | | | |
| | 3 | | | | | |
| | mean value | | | | | |
| | 1 | | | | | |
| | 2 | | | | | |
| | 3 | | | | | |
| | mean value | | | | | |
| | 1 | | | | | |
| | 2 | | | | | |
| | 3 | | | | | |
| | mean value | | | | | |

**ΔE between two products**

| Products compared | **ΔE value** | Interpretation |
|---|---|---|
| | | |

**Summary of colour determinations:**

| | |
|---|---|
| **Applications** | ● for the evaluation of meat products and meat analogues |
| **References** | 1. M. Siegrist, C. Hartmann, Why alternative proteins will not disrupt the meat industry, Meat Science, Volume 203, 2023, 109223, https://doi.org/10.1016/j.meatsci.2023.109223.<br>2. Benny, A., Pandi, K. Upadhyay, R. Techniques, challenges and future prospects for cell-based meat. Food Sci Biotechnol 31, 1225–1242 (2022). https://doi.org/10.1007/s10068-022-01136-6<br>3. https://eur-lex.europa.eu/legal-content/PL/ALL/?uri=CELEX%3A32011R1169, Doc. 1169/2011 |