# Native and modified starches in meat products – detection of raw materials using microscopy methods

Martina Eliášová[1], Matej Pospiech[1], Bohuslava Tremlová[1], Kristýna Kubíčková[2], Josef Jandásek[1,3]

[1]Department of Vegetable Foodstuff
Faculty of Veterinary Hygiene and Ecology
University of Veterinary and Pharmaceutical Sciences Brno
[2]TESCAN, a.s.
Brno, Czech Republic
[3]Raps GmbH & Co. KG, Kulmbach, Germany

**Abstract**

Starches are an important natural constituent of many food commodities that significantly influence or determine their texture and functional properties. We find various types of starches in meat products, in this country primarily potato, maize and wheat starch and various forms of modifications of them. In this study, we examined the analysis of native and modified starches isolated from potatoes and maize using light and scanning electron microscopy. Individual starches are characterised by their shape and size. Light microscopy is a useful tool for species identification of native starches based on their size and shape, though light microscopy is inappropriate for the detection of certain modified starches, while examination in a scanning electron microscope (SEM) provides much clearer information about their morphology, even in the case of highly soluble modified starches. SEM is capable of differentiating native starches from modified starches and may, therefore, serve as a tool for the control of raw materials for producers who produce "additive-free foodstuffs" (foods without E numbers).

*Modification, SEM, LM, gelatinisation, additives*

## Introduction

Starch is considered one of the most versatile food additives in the food industry. Starches are part of many food commodities and significantly influence or determine their texture and functional properties (Biliaderis 1991).

Starches are effective additives in the meat industry that increase the binding capacity of meat and meat products and improve the binding of fat in meat mixes, thereby having an influence on the texture, consistency and stability of the end product. Starches are used in meat products as binding agents for water to increase yields, reduce losses during cooking, improve structure and sliceability, increase succulence and extend durability. Heat-treated meat products must be heated to a minimum core temperature of 70 °C for a period of 10 minutes (Decree 326/2001) to ensure that they are safe for human consumption. Potato starches, which gelatinise at temperatures from 50 °C to 68 °C, are recommended for the function of starches at this temperature. In contrast, maize starch gelatinises at a temperature of 62 – 72 °C, for which reason gelatinisation may not occur adequately, particularly at the centre of meat products (Belitz et al. 2009; Phillips 2009; Directive 326/2001).

Various kinds of starch (potato, wheat, maize, tapioca) can be found in meat products in native or modified form. Native and modified starches not merely have differing effects on the technological, functional and rheological properties of the end meat product, but also differ in terms of the ways in which they are used, and of final product labelling. Native starches are produced merely by cracking natural starches (by, e.g., high temperatures or enzymes). They are not considered additives (they do not have E numbers assigned to them, and their use is not regulated by directives on additives), but are considered "foodstuffs", similarly as, for instance, salt or sugar. Different conditions apply for modified starches prepared from starches by more complicated means (by the action of acids, for

**Address for correspondence:**
Ing. Mgr. Martina Eliášová
Department of Vegetable Foodstuff
Faculty of Veterinary Hygiene and Ecology
University of Veterinary and Pharmaceutical Sciences Brno
Palackého tř. 1/3, 612 42 Brno, Czech Republic
Phone: +420 541 562 704
E-mail: eliasova.martinka@seznam.cz
www.maso-international.cz

example) than mere cracking. These starches are considered food additives, and their presence in food must be stated on the packaging under their whole name or in the form of their E number (E 1404–1451). The use of modified starches in foodstuffs in the Czech legislation is regulated by Directive 4/2008, which stipulates types and conditions of use of additives and extraction solvents in the production of foodstuffs, as amended by Decrees 130/2010 and 122/2011, and by European legislation, which has been consolidated into a single European Parliament and Council Regulation (EC) 1333/2008 on food additives (Uthumporna et al. 2010).

Native starches are characterised by the fact that they retain their physical and chemical properties and their original shape. Starch grains are insoluble in cold water and form a suspension. When this suspension is heated, the starch grains absorb water, though their integrity is not violated – this is still a reversible process, though only up to a certain temperature characteristic for the particular kind of starch. This temperature is known as the initial gelatinisation temperature, at which the starch grains begin to swell. The grains of potato starch begin to gelatinise at 50 °C (initial gelatinisation temperature) and the process ends at 68 °C. In the case of maize starch, the initial gelatinisation temperature is a little higher at 62 °C, with a final temperature of 72 °C. This process leads to native starches forming a starch paste of high viscosity, giving the products thickened a gummy and cohesive structure. After standing and cooling for some time, starch paste undergoes "retrogradation", during which the paste returns to its original crystalline state and colloidally bound water is released. The retrogradation of native starches is, therefore, a particular problem in chilled foods, including meat products. These properties limit the use of native starches, for which reason they do not find such wide-ranging technological use as modified starches (Svihusa et al. 2005).

For this reason, chemically modified starches are applied extremely frequently in meat products to improve their functional properties – in particular their stability at high temperatures and low pH values, during freezing and defrosting, clarity even at lower temperatures, and help preserve their texture. Most chemical modifications depend on the reaction of the –OH group in amylose and amylopectin. Amylose contains three –OH groups to one anhydroglucose unit, which is less than in the case of amylopectin (Eliasson 2004).

The modified starches used in the meat industry include oxidised, cross-linked and substituted starches. Even at high concentrations, oxidised starches are characterised by extremely low viscosity during cooking and high viscosity during cooling. The gels produced have a reduced tendency towards retrogradation. Oxidised starches are suitable for coating meat and fish to ensure greater adhesion of the coating (Střelcová et al. 2008).

Cross-linked starches are characterised by increased starch paste viscosity, gelatinisation temperature and mechanical resistance (in an acidic environment), and cross-linking increases the stability of the starch grain. They are used, for example, to regulate the texture of food dishes and to replace fats in foodstuffs, and prevent stratification of the product when used as a thickener (Phillips 2009).

Another group found in meat products are substituted and stabilised starches. Substitution alters certain properties of starches, such as their hydration properties (solubility), dispersion stability (retrogradation), rheological properties, dispersion and chemical properties (e.g. ionogenic starches carrying an electric charge). Monosubstituted starches combined with low cross-linked starches are used for smoked meat products because they are required to gelatinise beneath a curing temperature not exceeding 70 °C (Kadlec et al. 2009; Šárka 2011).

Starches are stored in plants in the form of grains that are characterised by a specific size and shape that changes depending on the kind of modification used. Various microscopic methods serve for the detection of the structures of individual types of starch. Knowledge of the structure of starches and their functional properties has allowed chemists to apply new methods in their processing or to modify them in such a way that they fulfil special requirements for use in foodstuffs (Mishra and Rai 2006).

Starches can be detected by light microscopy, which is considered the basic microscopic technique and is based on the observation of objects in visible light (a current of photons). The image observed is magnified (as many as 1 000 times) and inverted. The resolution of LM may be around 0.2 µm depending on the type of microscope. Rather than photons, an SEM uses a current of electrons that scans a small area on the surface of the sample to obtain an image that provides information about its surface and other properties of the object examined. The result is an image with an extended depth of field (reminiscent of a 3D image) that is magnified as much as 1 000 000 times with a resolution as good as 0.5 nm.

This study aims to analyse potato and maize starches in native and modified form using light (LM) and scanning electron (SEM) microscopy, and to choose a method on the basis of the findings obtained that would enable the differentiation of types of starches and types of modification for the purpose of primary control of input raw materials.

## Materials and Methods

Six types of starch obtained from potatoes and maize were analysed during this study. These were native potato starch, physically modified (by temperature and pressure) potato starch, chemically modified potato starch – E 1414 (acetylated distarch phosphate), native maize starch (KA), chemically modified maize starch – E 1422 (acetylated distarch adipate), and chemically modified maize starch – E 1442 (hydroxypropyl distarch phosphate). All these starches were analysed under a light microscope (Nikon Eclipse E200) and a scanning electronic microscope (MIRA 3, FE-SEM, TESCAN).

Starches prepared for analysis in the light microscope were embedded in paraffin and further processed by the paraffin sections method and stained with Pass-Calleja and Lugol-Calleja stains (Protocols 1997). Samples of starches for detection in the scanning electron microscope were attached to targets with double-sided carbon tape and coated with a layer of gold (10 nm). A set of digital photographs, in which changes to the structure of starches were observed in dependence on the modification and type of starch used, were taken to document the results. The microscopic methods used (light microscopy and scanning electron microscopy) were compared on the basis of these results.

## Results

Image documentation from the light microscope documents the fact that only native potato starch and native maize starch were identified using the Pass-Calleja and Lugol-Calleja stains. For the sake of improved interpretation and clarity of the results, we have used only selected images from the light microscope stained with the Pass-Calleja stain in this work (Figs 1B, 2B, 3B, 4B, Plate I and II). Sufficient contrast was not obtained for the identification of the modified starches (both physically and chemically modified) analysed using this method, as is documented by Figs 2B and 4B (Plate I and II). The explanation for this may be that the starches are leached out of the histological preparations during staining due to their high solubility in cold water. We are not, therefore, capable of identifying the origin or method of modification of the analysed starches with the use of LM, regardless of the stain used.

The situation was different with scanning electron microscopy. Starch grains are solid objects, for which reason analysis in a scanning electron microscope provides clearer information about their morphological structure thanks to the extended depth of field. Using SEM, we are capable of identifying native (Plate I and II, Figs 1A and 3A) and modified (Plate I and II, Figs 2A and 4A) starches. The image documentation from the scanning electron microscope demonstrated that the surface of native starch grains is smooth and practically non-porous (Plate I and II, Figs 1A and 3A), in contrast to modified starches (Plate I and II, Figs 2A and 4A). In all the modified starches, clear changes were seen in the size, shape and surface of the starch grains caused by their modifications. In potato starch physically modified by the action of temperature and pressure, the starch grains were linked into a more compact structure, which is dissected, though with a smooth surface (Plate I, Figure 2A). Water is removed from the starch paste during this modification, meaning that the hydrogen bonds cannot be renewed. The result is modified starch capable of solubility and greater swelling capacity in cold water (Belitz et al. 2009).

Changes to the starch grains were again seen in the case of chemical modifications. Chemically modified potato starch (E 1412) was modified by the action of acetyl distarch phosphate, which led to the linking of individual starch grains into irregular granules many times larger with a dissected surface on which relatively large cavities occurred. Similarly altered granules were detected in maize starches (Plate II, Figure 4A) that were also chemically modified, though by the action of other agents (E 1422 – acetyl distarch adipate, E 1442 – hydroxypropyl distarch phosphate).

## Discussion

The given results indicate that light microscopy is suitable only for detecting native starches. The choice of Pass-Calleja staining instead of iodine stains such as Lugol's solution or Lugol-Calleja is based on the differences in the chemical composition of the starches used. The waxy maize that is greatly preferred and used today is made up of as much as 99 % amylopectin and 0 – 1 % amylose. The iodine index of waxy maize therefore approaches 0 %. Another starch problematic for iodine staining is amylose maize with an iodine index of 0.1 %, which is made up of 20 – 48 % amylopectins and 52 – 80 % amylose (Phillips 2009). The iodine index indicates the staining ability of the starch with iodine solutions. The staining principle consists of the binding of the iodine complex ($I^{3-}$) to the secondary left-handed helix of amylose with subsequent staining. In amylopectin, however, $I^{3-}$ binds only to short sections of the helix in its amorphous parts. A better comparison of the staining ability of individual starches than that provided by the iodine index was conducted by means of the lightness measurement method (L*) – CIELAB, which agrees with the iodine index of individual starches (Manion et al. 2011). The Pass-Calleja staining used takes advantage of the reaction of Schiff's reagent with the aldehyde bonds in polysaccharides following prior hydrolysis with acids (Rocha et al. 2012; Protocols 1997). Pass-Calleja therefore stains all starches regardless of their amylose content. Another criterion for the diagnostics of native starches are the morphological properties of the starch, which can be described as follows for potato starch: grain size 5–70 μm, ovoid, elliptical or conchoidal shape with eccentrically placed core and clearly off-centre layering; and as follows for native maize starch: grain size 8–20 μm, a many-sided, tabular or star shape with a jagged core and indistinct layering (Stasiaka et al. 2001; Mishra and Rai 2006; Eliasson 2008). We were not able to identify the structure of modified starches stained with Pass-Calleja stain and imaged under a light microscope, either in the case of physical modification (temperature, pressure) or in the case of chemical modification.

The chemically modified starches (with an E number) that were the subject of this study were created by stabilisation in association with cross-linking. Cross-linking may be considered one of the most important chemical modifications in the starch industry. It involves the substitution of hydrogen bonds between starch chains with stronger and more stable covalent bonds. This relates, first and foremost, to distarch phosphates and adipates, with the compound produced containing a longer bond than the original one. In this case, the swelling of the starch granule is repressed by prior disintegration or by a chemical action, mechanical friction or cooking. To put it simply, the starch granule is "spot welded" at random points in the molecular space for the purpose of cross-linking. Subsequent stabilisation is expressed in a reduction to the gelatinisation temperature of the starches (particularly high-amylose starches), greater stability in respect of retrogradation during the storage of products at low temperatures, and greater stability in an acidic environment. The above indicates that native starches undergo great changes that are reflected in their structure (Biliaderis 1991; Eliasson 2008).

Changes to the size and shape of starch granules caused by modification were observed in the scanning electron microscope. Modification leads to the creation of new bonds between starch granules and the formation of a new structure of a shape that is hard to define (Plate I and II, Figs 2A, 4A), characterised by a wrinkled surface. Large pores that frequently pass through the entire structure of the modified starch can also be seen here. It must be noted that chemical modification does not always cause significant changes to the structure of the starch, as is described by, for example, the work of Stasiaka (2011), who also states that changes to the grain morphology have a significant influence on their physical properties and behaviour during technological processing. The more cross-linked the starch is, the more resistant it is to changes during product processing and the greater contribution it can make to increase the stability and to improve the texture and mechanical resistance of the end product (Kodet et al. 2001; Lia and Yehb 2003). Modified starches can, for this reason, be distinguished from native starches on the basis of these changes. It is more problematic to differentiate physically modified starches (modified by the action of temperature and pressure), which like native starches are not considered additives (they do not have E numbers), from starches altered by more complicated action then mere cracking, which are considered additives (with E numbers 1404–1451). Our results show, however, that the action of temperature and pressure (Plate I, Figure 2A) leads to changes in morphology that are different from the changes seen in chemically modified starches (Plate II, Figure 4A), i.e. the modified starches belonging to the group of additives (with E numbers) can be identified by SEM.

## Conclusions

The results of our study show that scanning electronic microscopy is more suitable for detecting native and modified maize and potato starches. Only native starches were identified by light microscopy. It was not possible to obtain any information about size, shape or other visible changes in modified starches detected by LM. Scanning electronic microscopy, in contrast, provided clear information about the size and shape of both native starch grains and modified starches, in which changes in morphology caused by the modifications used were discovered. SEM can, then, be used to differentiate individual types of starch and the type of modification used, which is important from the viewpoint of identifying input raw materials and for the labelling of foodstuffs where the manufacturer is obliged to state defined types of modified starches given by the legislation in the form of E numbers.

### References

Belitz H, Grosch W, Schieberle P 2009: Food Chem. Springer, Berlin, 1070p

Biliaderis CG 1991: The structure and interactions of starch with food constituents. Canad J Physiolog Pharmacol **69**: 60-78

Eliasson ACH 2004: Starch in food: structure, function and applications. Boca Raton, CRC Press, 605p

European Parliament and Council Regulation (EC) 1333/2008 on food additives.

Funami T, Kataoka Y, Omoto T 2005: Foodhydrocolloids control the gelatinization and retrogradation behavior of starch. Functions of guar gums with different molecular weights on the gelatinization behavior of corn starch. Food Hydrocolloids **19**: 15-24

Kadlec P, Melzoch K, Voldřich M 2009: Co byste měli vědět o potravinách: Technologie potravin. Key Publishing, Ostrava, 536p

Kodet J, Šotolová I, Štěrba S 1993: Plnící, zahušťovací, gelotvorné a stabilizační látky pro potraviny. Středisko potravinářských informací, Praha, 132p

Lia J, Yehb A 2003: Effects of starch properties on rheological characteristics of strach/meat complexes. J Food Engin **57**: 287-294

Manion B, Ye M, Holbein BE, Seetharaman K 2011: Quantification of total iodine in intact granular starches of different botanical origin exposed to iodine vapor at various water activities. Carbohydrate Research **15**: 2482-2490
Mishra S, Rai T 2006: Morphology and functional properties of corn, potato and tapioca starches. Food Hydrocolloids **20**: 456-465
Phillips G 2009: Handbook of Hydrocolloids. Woodhead Pub, Cambridge, 450p
Protocols 1997: Analysenvorschrift. Färbung nach Bauer-Calleja. Veterinäruntersuchungsanstalt
Rocha T, Felizardo SG, Jane J, Franco CML 2012: Effect of annealing on these micrystal line structure of normal and waxy corn starches. Food Hydrocolloids **29**: 93-99
Stasiaka M, Rusineka R, Molenda M, Fornalb J, Blaszczakb W 2001: Effect of potato starch modification on mechanical parameters and granulesmorphology. J Food Engin **102**: 154-162
Střelcová O, Jandásek J, Bittner J 2008: Přídatné látky v masných výrobcích. Maso **19**: 51-54
Svihusa B, Uhlenb AK, Harstad OM 2005: Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: A review. Anim Feed Sci and Tech **122**: 303-320
Šárka, E 2011: Aplikace nativního škrobu a výrobků ze škrobu v mlékárenském, masném a konzervárenském průmyslu. VŠCHT, Praha, 95 p
Uthumporna U, Zaidulb ISM, Karima AA 2010: Hydrolysis of granular starch at sub-gelatinization temperature using a mixture of amylolytic enzymes. Food and Bioproducts Processing **88**: 44-57
Vyhláška 326/2001 Sb., pro maso, masné výrobky, ryby, ostatní vodní živočichy a výrobky z nich, vejce a výrobky z nich, ve znění vyhlášky č. 264/2003 Sb., č. 169/2009 Sb.
Vyhláška 4/2008 Sb., kterou se stanoví druhy a podmínky použití přídatných látek a extrakčních rozpouštědel při výrobě potravin ve znění vyhlášky č. 130/2010 Sb., č. 122/2011 Sb.

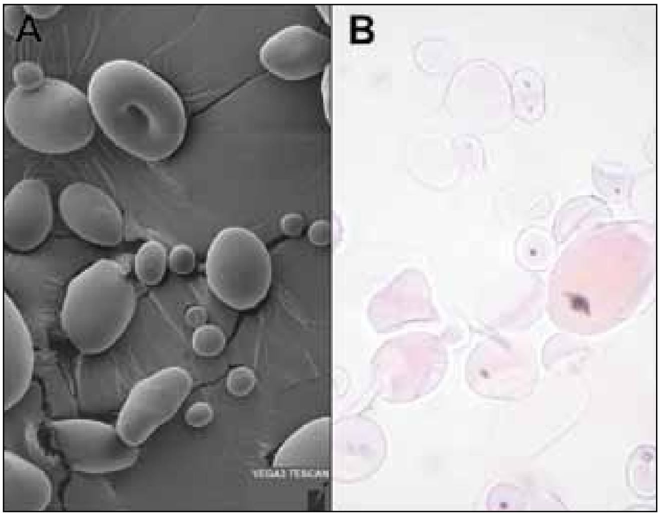


Fig. 1. Native potato starch – A (SEM),
B (LM – Pass-Calleja staining, 400x magnification)

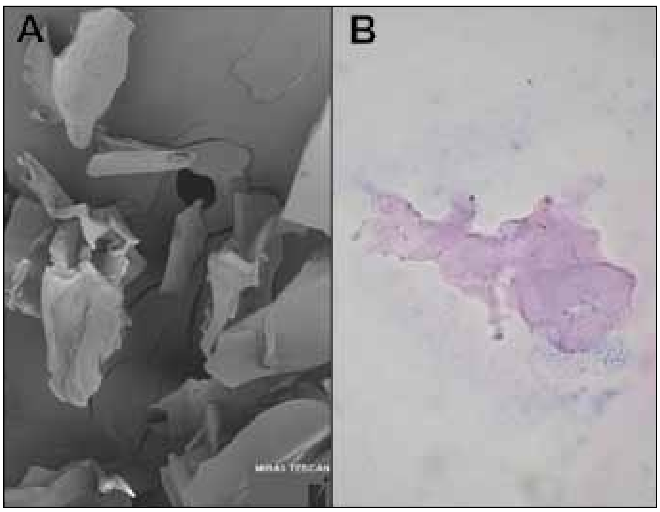


Fig. 2. Physically modified potato starch – A (SEM)
B (LM – Pass-Calleja staining, 400x magnification)

Plate II

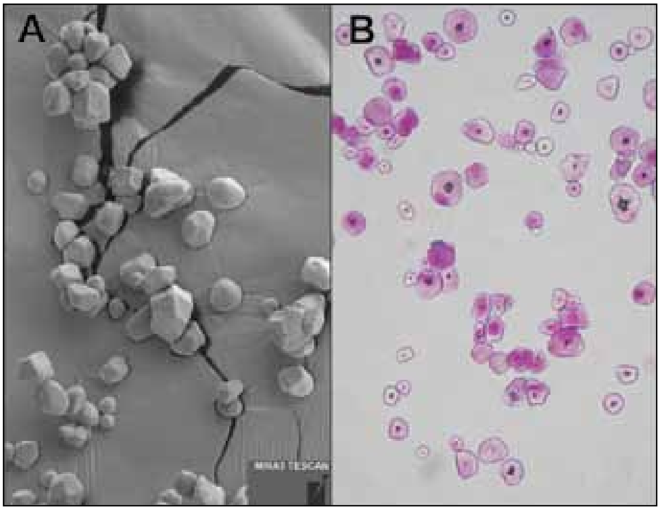


Fig. 3. Native maize starch – A (SEM),
B (LM – Pass-Calleja staining, 400x magnification)

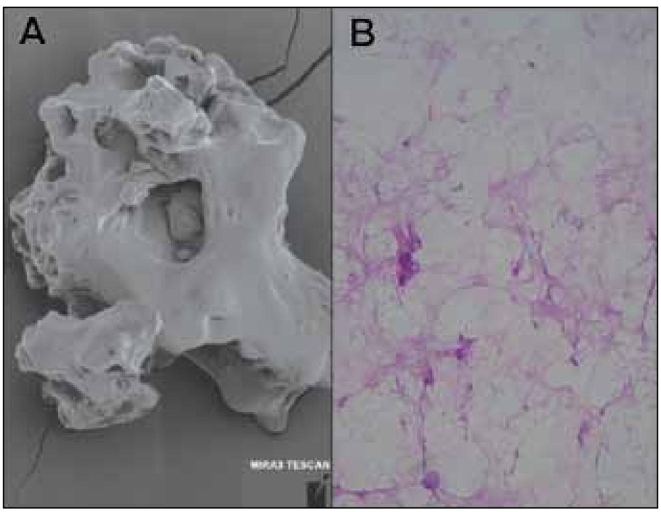


Fig. 4. Chemically mod. maize starch E 1422 – A (SEM),
B (LM – Pass-Calleja staining, 400x magnification)