# Detection of amaranth as an additive in meat products using histochemical methods

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**Abstract**

This work deals with the microscopic characterisation of amaranth seed and amaranth flour in model meat products using histochemical methods. Amaranth is used as an additive in meat products. Grit made of crude amaranth seeds had a positive effect on water-holding capacity of stuffing and allowed a reduction to cooking losses in cans. This improved water-holding capacity resulted in an improvement to the tenderness, juiciness and taste of canned meat. With the assistance of the histological method, several model samples consisting of a mixture of beef and pork musculature with pure amaranth flour in various concentrations were created. We used three types of staining in order to pick out the flour in the individual chains of samples for microscopic detection: basic staining with haematoxylin and eosin, special staining by means of the PAS – Calleja method, and staining with toluidine blue. Special staining by means of the PAS – Calleja method has proved the most evidentiary method for detecting even the tiniest parts of amaranth flour in the musculature. Grains of starch were subsequently examined to find the best staining method for picking out and the conclusiveness of the starchy grains. The best method seems to be toluidine blue staining.

*Amaranth seeds, amaranth flour, staining, microscopic detection, starch grains*

## Introduction

The genus amaranth (*Amaranthus L.*) belongs to the amaranth family (*Amaranthaceae*) and is comprised of more than 60 species (Kalač and Modrý 2000). These are annual dicotyledonous plants with a root that penetrates deep into the ground. The stalk is branched and reaches a height of from 1 to 3 metres. Some species have terminal inflorescence, while in others the inflorescence grows in the axilla of the leaves. The fruit is a lens-shaped samara of 1 to 2 mm in diameter. A single plant can produce as many as 500 000 seeds. The seed is comprised of 48 – 69% starch (Bressani 2003), which is, according to Belton and Taylor (2002), stored in the perisperm. The starch grains of the amaranth are polygonal, lenticular, circular or elliptical in shape (Cai et al. 2004). Marcome (2001) states that amaranth starch granules are 1 to 2 micrometres in diameter, and in comparison with rice starch this is the smallest commercially produced starch. Other saccharides present are saccharose, raffinose, stachyose and maltose, which occur as small particles (Bressani 2003). The principal component of amaranth grain are, therefore, saccharides. The grain also contains significant quantities of calcium, iron, potassium, phosphorus, vitamins and fibre (Marionetti et al. 2009). Amaranth was known to the Incas (from where it got the name "Gold of the Incas"), the Mayas and the Aztecs, for whom it was a holy crop plant (Sauer 1950). The Mayas made flour from the grains of this plant and also cooked its leaves as vegetables. Today the leaves are used as salad, cooked like spinach, and added to soup as vegetables (Bressani 2003). Amaranth seeds ground into flour can be used to make cakes, biscuits and various kinds of pudding. Amaranth in the form of flour is also used in bread and rolls. Amaranth also finds a use in meat production. Ostoja et al. (2002) describe the use of amaranth seeds in canned meat, where they had a significant effect in improving the taste, tenderness and succulence of the meat. The expansion in the range of

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uses of amaranth in foodstuffs is accompanied by the increasing importance of developing methods of detection for determining the presence of amaranth in various food matrices. These methods include histochemical methods employing both common and targeted staining that can determine the majority of animal and plant components contributing to the structure of meat products (Pospiech 2010).

### Materials and methods

The material examined were model samples obtained by mixing minced meat with amaranth flour in concentrations of 0.01%, 0.1%, 1.0% and 10.0% in a VORWERK Thermomix TM 31 mixer for a period of 5 minutes with the addition of salt and distilled water until the desired consistency of the mixture was achieved. The samples prepared in this way were fixed in a 10% solution of neutral formol, following which they were prepared by the paraffin section technique. Four blocks were prepared for each sample. Three sections were then sliced from each block on a single slide. Ninety sections were prepared for histochemical examination, of which 72 were sections of muscle mixed with amaranth flour (18 sections for each concentration) and 18 sections with flour alone. The samples were then stained with haematoxylin and eosin, PAS – Calleja, or toluidine blue – in each case 2 slides of each sample in each of the three types of dye. The samples of amaranth flour were stained in the same way. Following staining and repeated dehydration, the samples were mounted with the use of Solacryl. Examination was performed in a Nikon ECLIPSE E200 optical microscope, adjusted to project the observed object on a computer monitor, and with a Canon PowerShot 69 digital camera.

## Results and discussion

The most apparent details were depicted in a model sample with the use of the PAS – Calleja staining technique, see (Plate III, Figures 1 and 2). This staining highlighted the sample in greatest detail in spite of the fact that it targets polysaccharides. Using this staining, we were able to detail amaranth flour, which was stained dark pink as it is primarily starch perisperm. In the image in Fig. 1 stained with the PAS – Calleja technique, the fat tissue, which is usually unstained, i.e. white, is also evident. With the use of this staining technique, we can also notice collagen tissue, which appears in blue. Muscle, which appears as a grey–green colour, forms the background in the samples stained using the PAS – Calleja method. The only things we were unable to detect with this type of staining were the nuclei of the muscle cells and connetive tissue. These structures were very clearly imaged by haematoxylin and eosin staining and toluidine blue staining, which did not, however, show other structures in such detail as staining using the PAS – Calleja method. The results obtained show the PAS – Calleja method to be the most suitable. The differing results are most probably the result of the fact that haematoxylin and eosin and toluidine blue staining are common forms of staining, during which, as stated by Pospiech et al. (2010), the entire sample is stained the same colour, merely in varying shades. For this reason, the inexperienced observer may be unable to pick out all the details and be unable to detect all the particles of amaranth flour. With PAS – Calleja staining, in contrast, the staining targets polysaccharides (Pospiech 2010), including starch. The greatest quantities of starch are stored in the perisperm (Belton and Taylor 2002), for which reason PAS – Calleja staining would seem to be the most suitable. The most suitable staining for the microscopic detection of grains of amaranth flour was toluidine blue staining, which was the only staining to clearly show starch granules, see (Plate IV, Figure 3).

## Conclusions

Both the leaves and the seeds of the amaranth plant are used for food. Light microscopy can be used to detect amaranth flour and amaranth seeds admixture in food products. Pure amaranth flour, when mixed with muscle tissue, acts as an extremely good binding agent. To obtain microscopic evidence of amaranth flour or amaranth seed in food products, it is important to perform the correct histological processing of these products, and then to select the most suitable type of staining to detect the amaranth. On the basis of the staining of the

individual samples examined, we can state that the best staining to demonstrate the presence of amaranth flour in muscle tissue was staining by the PAS – Calleja technique. With the use of this staining, the individual structures in the samples were stained in varying shades of colour, making it possible to differentiate the flour from the other components. The most suitable staining for the purposes of microscopic detection of amaranth starch grains was shown to be toluidine blue staining, which was the only one to clearly demonstrate the presence of starch granules.

**Acknowledgement**

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**References**

Belton PS, Taylor JRN 2002: Pseudocereals and Less Common Cereals: grain properties and utilization potential. Berlin: Springer, 269 ISBN 3-540-42939-5

Bressani R 2003: Amaranth, Encyklopedia of Food Sciences and Nutrition, Oxford, 166-173 ISBN 978-0-12-227055-0

Cai YZ, Corke H, Wu HX 2004: Amaranth, Encyclopedia Grain of Science. Hong Kong: The University of Hong Kong, 113 ISBN 978-0-12-76-5490-4

Kalač P, Moudrý J 2000: Chemické složení a nutriční hodnota semen amarantu. Czech J Food Sci **5**: 201–206

Marcone FM 2001: Starch properties of Amaranthus pumilus (seabeach amaranth): a treatened plant species with potential benefits for the breeding / amelioration of present Amaranthus cultivars. Food Chem **1**: 61 – 66

Mariotti M, Lucisano M, Pagani MA, Perry KW 2009: The role of corn starch amaranth flour, pea isolate, and psyllium flour on the rheological properties and the ultrastructure of gluten-free doughs. Food Research International. Ng. **42**: 963-975

Ostoja H, Cierach M, Konopko H, Majewska K 2002: Effect of addition of grit made of crude and expanded amaranth seeds on the quality of canned meat. Nahrung **46**: 270-275

Pospiech M 2010: Mikroskopické metody v analýze potravin. Maso **4**: 29-31

Sauer JD 1950: The grain amaranths: A survey of their history a classification. Annals of The Missouri Botanical Garden **37**: 561

HE staining toluidine blue staining PAS - Calleja staining

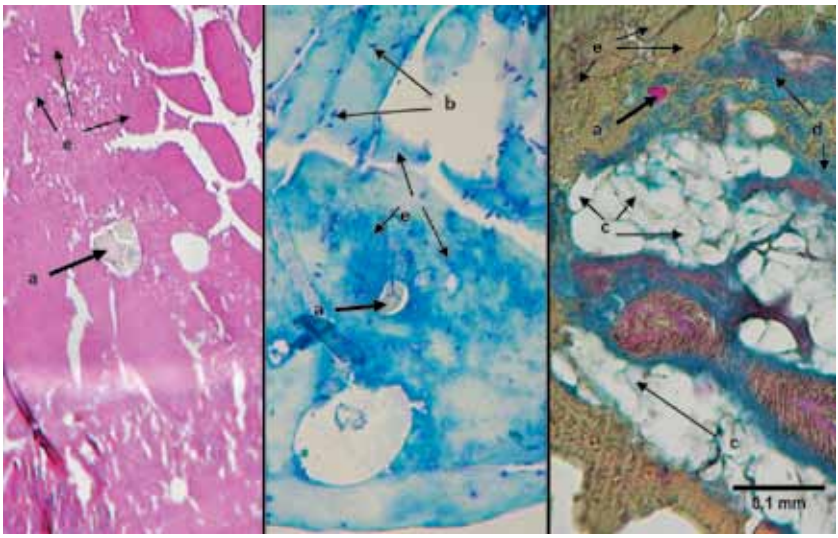


Fig. 1. Model sample – 100 × magnification
Key: a – amaranth perisperm particles, b – nucleus of muscle fibers, c – fat tissue, d – collagenous tissue, e – muscle tissue

HE staining toluidine blue staining PAS - Calleja staining

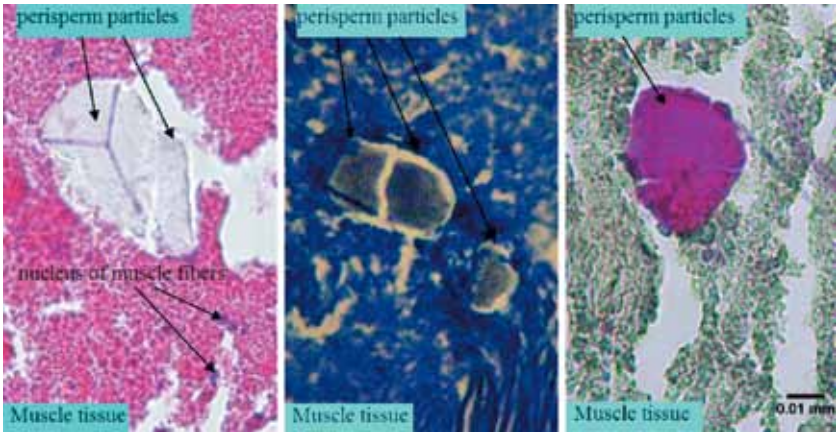


Fig. 2. Model sample – 400 × magnification

Plate IV

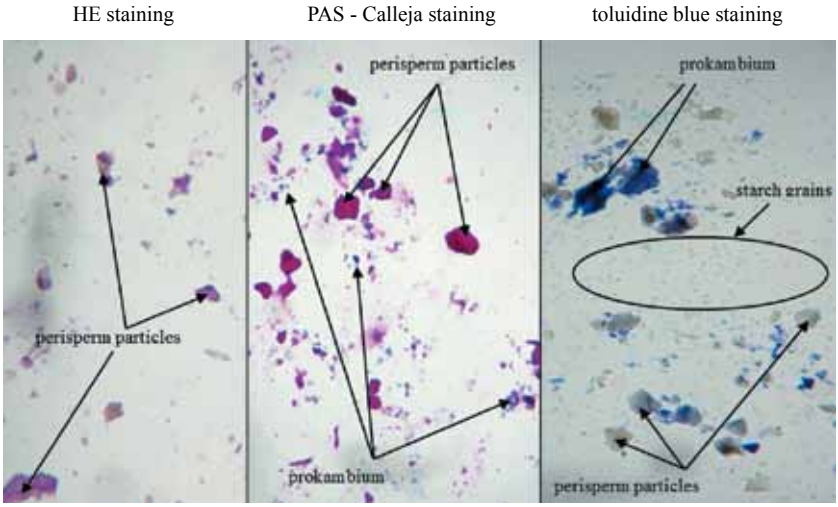


Fig. 3. Pure amaranth flour – 100 × magnification