Carrageenans in the meat industry: detection using microscopic methods

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Abstract

Carrageenans are used in the meat industry primarily as stabilisers and binders and belong to the group of additives with the code E407. Three types of food carrageenans (κ-kappa, λ-lambda and ι-iota) were analysed using light and electron microscopy in this study. The individual types of carrageenans were analysed in the form of a loose powder. Samples for analysis under a light microscope were stained with Lugol-Calleja, Toluidine Blue and PAS-Calleja. PAS-Calleja was adjudged to be the most suitable staining for detection of κ-, λ- and ι-carrageenan on the basis of the intensity of colour and the size of the stained area in individual fragments of carrageenans. Images obtained by a scanning electron microscope indicate the morphological differences in structure between ι-carrageenans and κ- and λ-carrageenans. Cooked hams with 1% added κ-, λ- and ι- carrageenan were also examined under a light microscope. Differences in the localisation or spatial arrangement of individual types of carrageenan were found in this study.

Additives, cooked hams, electron microscope, SEM, staining, structure

Introduction

Carrageenans have been used as safe food additives for a number of decades and are identified in the system of E-codes in the European Union as E407 (Weiner 2014). They are naturally occurring anionic linear sulphated polysaccharides extracted from red seaweeds of the genus *Rhodophyceae* (Chen et al. 2002). The principal constituent of these seaweeds are co-polysaccharides with a linear chain created by β-D-galactose and 3,6-anhydro-α-D-galactose with a varying number of sulphate groups (Campo et al. 2009). The food industry uses 70 to 80% of total global production of carrageenans, estimated to be around 45 000 tons a year, of which around 45% is used in dairy products and 30% in meat and meat products. Only three dominant types are used in the food industry, i.e. κ-, λ- and ι- carrageenan. Commercial carrageenan is generally a mixture of carrageenans, with κ-carrageenan (gelling) predominating over λ-carrageenan (non-gelling) at a ratio of around 3:2 (Velíšek and Hajšlová 2002). Carrageenans are widely used due to their excellent physical and functional properties such as thickening, gelling and stabilising qualities. Carrageenans are used to improve the texture of cheeses, puddings and desserts, and as binding agents and stabilisers in the meat industry, for instance in hams and smoked meats (McHugh 2003). The microstructure of meat products plays an important role in hams and other specialities, influencing stability, colour, texture, sliceability and taste. Under normal conditions, the microstructure of a meat product results from the disruption and subsequent loosening of muscle proteins; a viscous three-dimensional network is created in which small particles of muscle tissue, ligament and fat are caught. We can influence the stability of this system either in the production process or by the use of additives. Carrageenans (E407) are one such additive affecting the microstructure (stability, sliceability, binding) of the cooked hams to which they are regularly added. It should, however, be noted that it is prohibited to added fibre, starch (including starch modified physically or by enzymes), plant or other animal proteins to hams of the highest quality and select hams under the

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national legislation (Degree no 326/2001, Coll.). Carrageenans, which are considered fibre, are ingredients that may not be used in hams of the highest quality (Kong and Ziegler 2013). Due consideration should be paid to this fact at the present time in view of the increasing pressure from consumers for the production of additive-free foods without "E-numbers".

Light and electron microscopy have been used to detect carrageenans as a food additive in a number of scientific works (Ayadi et al. 2009, Farouk et al. 2011; Heertje 2014). Carrageenans are imported in the form of a loose powder which necessitates verification of their identity (origin, occurrence) and perhaps type. It is difficult to obtain conclusive evidence using chemical methods, though observation of microscopic fragments stained histochemically can enable their detection (Flint 1985).

This study aimed to differentiate between individual types of carrageenan (γ, κ, ι) by selecting specific staining and using a light microscope and by studying their morphological structure with a scanning electron microscope (SEM).

The study also investigated the possibility of detecting κ-, λ- and ι- carrageenans directly in cooked hams (with 1% added carrageenan) by light microscopy and histological staining using the PAS-Calleja method.

Materials and Methods

Three types of carrageenan were analysed – κ-carrageenan, λ-carrageenan and ι-carrageenan – in pure powder form isolated from red seaweed (Eurogum A/S, Herlev, DE).

Scanning electron microscopy (SEM)

The tested carrageenans (κ, λ, ι) in the form of a powder were applied to support mounts, covered with double-sided carbon adhesive tape and plated with a 10 nm layer of gold. Images of carrageenans were obtained using a MIRA3 FEG SEM scanning electron microscope (TESCAN, CR).

Light microscopy (LM)

For the purposes of investigation under a light microscope, carrageenans were spread evenly on a slide (Menzel-Gläser, GER), coated with egg white and placed on a warmed hotplate (Vezas, CR) for 2 hours. The carrageenans were then stained with three kinds of histological staining. Staining with Toluidine Blue (TB) – stains hydrocolloids containing acid groups in various shades of blue, purple and pink (Flint 1994). The combined staining procedure PAS-Calleja was also used, which highlights structures of the polysaccharide type in colour (the PAS component of the stain) to produce purple-red colouring.

Lugol-Calleja is a combined stain that stains starch blue to brownish red. Five slides of each type of carrageenan were tested with each stain. The slides were mounted after staining and observed under a light microscope (Nikon, Eclipse E200, JPN) and images of individual carrageenans were taken at magnifications of 100x and 400x with a digital camera (Canon EOS 1100D, JPN).

Production of cooked hams

Pork leg (S1) from a Czech farm was ground using kidney-shaped grinding plates. The individual batches used in production weighed 400 g of minced meat, to which 20% brine containing a nitrite salting mix, polyphosphates and 1% κ, λ or ι-carrageenan was added. The raw mixture was tumbled for 10 minutes in a kitchen mixer (ETA-GRATUS, CR) on a cycle of 1 minute tumbling and 1 minute rest. The hams were stored for 24 hours at 5 °C before being cooked in such a way that the internal temperature of 70 °C was attained for a period of 10 minutes. The ready products were chilled and stored before analysis at 5 °C.

Histological testing of cooked hams

Samples for histological testing 1x1 cm in size were taken from 3 kinds of ham (with added κ, λ and ι-carrageenan). Three paraffin blocks were prepared from each ham, from which nine histological slices were prepared. The samples were prepared using the classic histological technique of paraffin slices cut on an RM 2255 rotary microtome (Leica, GER) to a slice thickness of 4 μm. The staining used was taken from the previous testing, i.e. Pass-Calleja staining was used. The histological staining procedures were based on traditional staining procedures modified for food matrices. The stains are given in the "Manual of Methodologies for Food Histology" (Manual 2005). The stained slices were examined in an Eclipse E220 light microscope (NIKON, JPN) at 100x and 400x magnifications.

Results and Discussion

We can see the structure of individual types of carrageenans in the images from the scanning electron microscope (Plate III, Fig. 1; Plate IV, Fig. 3 and Plate V, Fig. 5), and details of their surface in (Plate III, Fig. 2; Plate IV, Fig. 4 and Plate V, Fig. 6). We can see markedly different properties in structure and surface in ι-carrageenan as compared with κ- and λ-carrageenans. In ι-carrageenan, the surface is comprised of small formations similar to starch grains adjoining one another (Plate V, Fig. 6). It is more difficult to distinguish κ and λ-carrageenans from one another as their surface details show a certain similarity (Plate III, Fig. 2 and Plate IV, Fig. 4), though individual fragments of κ-carrageenan (Plate III, Fig. 1) differ from λ-carrageenan (Plate IV, Fig. 3). A structure similar to agarose is described for these polysaccharides. These three fractions of carrageenan differ chemically in terms of their degree of sulphation and content of 3,6-anhydrogalactose, and this differing composition is reflected in their varying functional properties: unlike λ-carrageenan, κ- and ι-carrageenans can form gels. The formation of a gel in κ-carrageenan occurs as a result of interhelical aggregation (Spagnuolo et al. 2005).

Examination under a light microscope showed that the best results in terms of the differentiation of individual types of carrageenans are shown by PAS-Calleja staining (Plate V-VIII, Fig. 6–12). Staining using the PAS-Calleja method provided a range of rose-coloured carrageenan structures, as is also confirmed by Bancroft and Cook (2000). λ-, κ- and ι-carrageenan can be differentiated under a light microscope on the basis of the differing intensity of staining, from deep pink in ι-carrageenan (Plate VIII, Fig. 11–12), through light pink with pronounced blue edges in κ-carrageenan (Plate VI, Fig. 7–8), to purple in λ-carrageenan (Plate VII, Fig. 9–10), and the size of the stained areas inside fragments and the bordering of individual fragments.

The examination of final products, i.e. cooked hams with 1% added κ-, λ- and ι-carrageenan, under a light microscope showed that the polysaccharides in carrageenans were stained from pink to light purple and are clearly different from other tissues. However, varying differences were seen in the localisation or spatial arrangement of individual types of carrageenans. An interconnected network with the network of muscle proteins was observed in κ-carrageenan, as is confirmed by the work of Ayadi et al. (2009), who tested a 1.5% addition of carrageenan. However, no compact network of muscle proteins with these carrageenans was observed in λ- and ι- carrageenans, and these carrageenans were crammed into interstitial spaces. The given results indicate that microscopic examination may explain changes reflected in the textural properties of cooked hams.

Conclusions

The results of this study indicate that microscopic techniques are appropriate for the differentiation of κ-, λ- and ι-carrageenan. Microscopic methods are also suitable for identification of additives of plant material, i.e. in this case including the additive E407. PAS-Calleja staining was adjudged the most suitable on the basis of observation of histological slices under a light microscope. Images from a scanning electron microscope provide us with more-detailed information about the structure and surface of individual fragments of carrageenans, and can be used to differentiate ι-carrageenan from κ- and λ-carrageenan.

Histological examination of cooked hams indicates that microscopic examination may explain changes reflected in the technological properties of final products such as, e.g., texture properties. It should be noted that these properties have a pronounced influence on the selection of meat products by the consumer.

Acknowledgements

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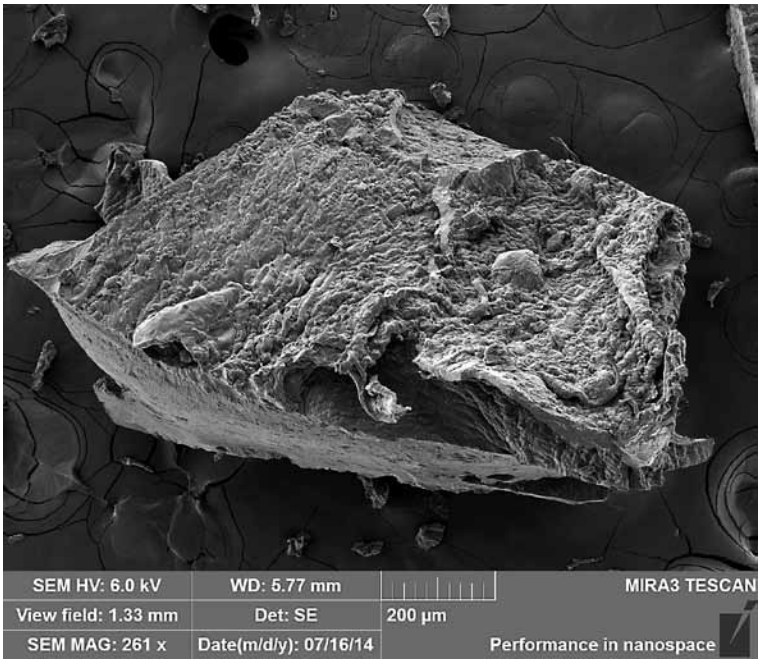

Fig. 1. A fragment of κ-carrageenan (SEM)

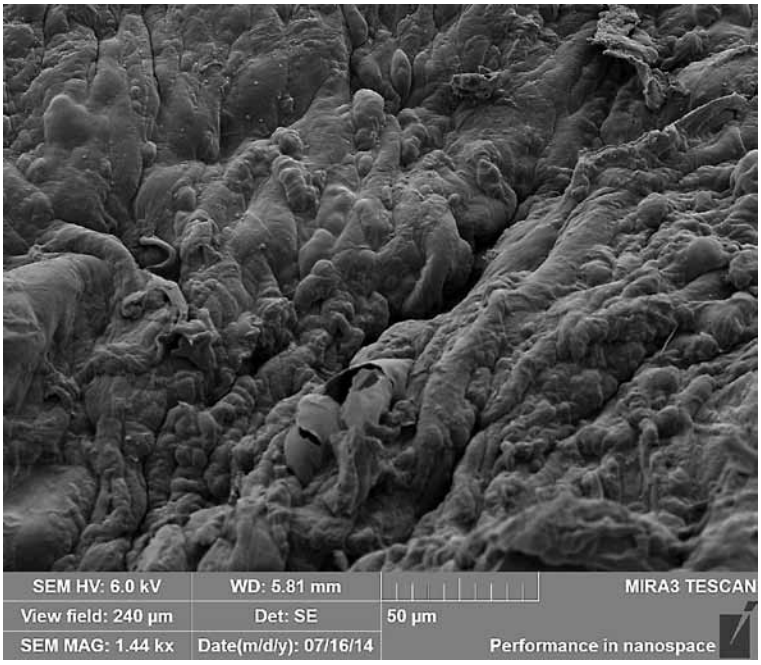

Fig. 2. A κ-carrageenan – surface detail (SEM)

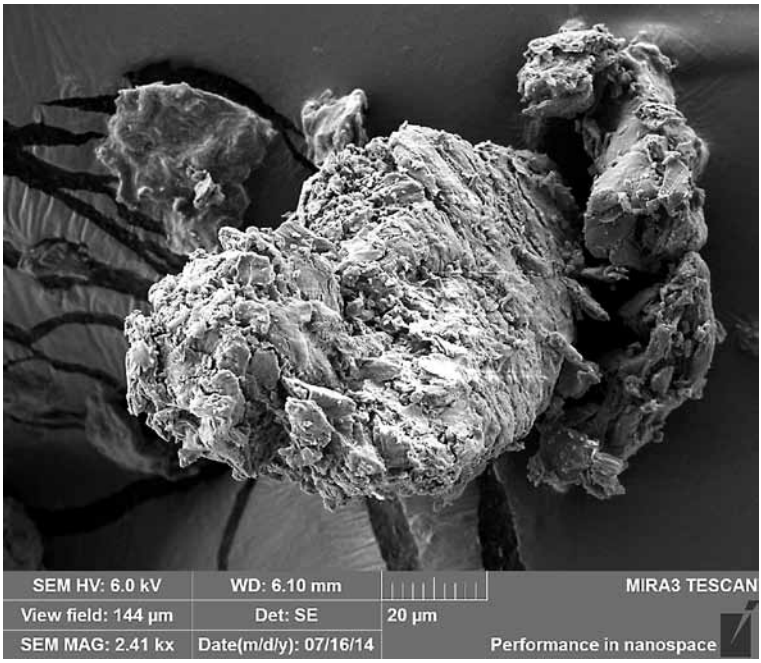

Fig. 3. A fragment of λ-carrageenan (SEM)

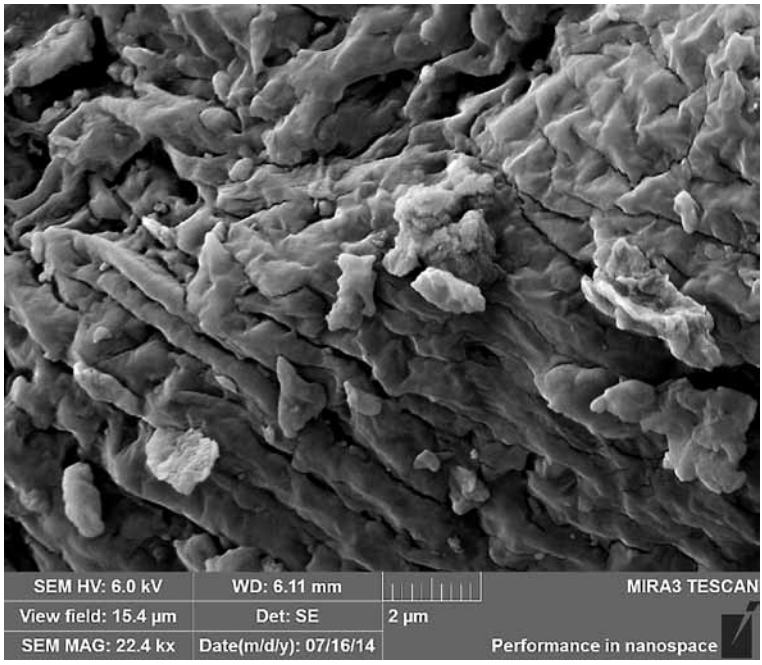

Fig. 4. A λ-carrageenan – surface detail (SEM)

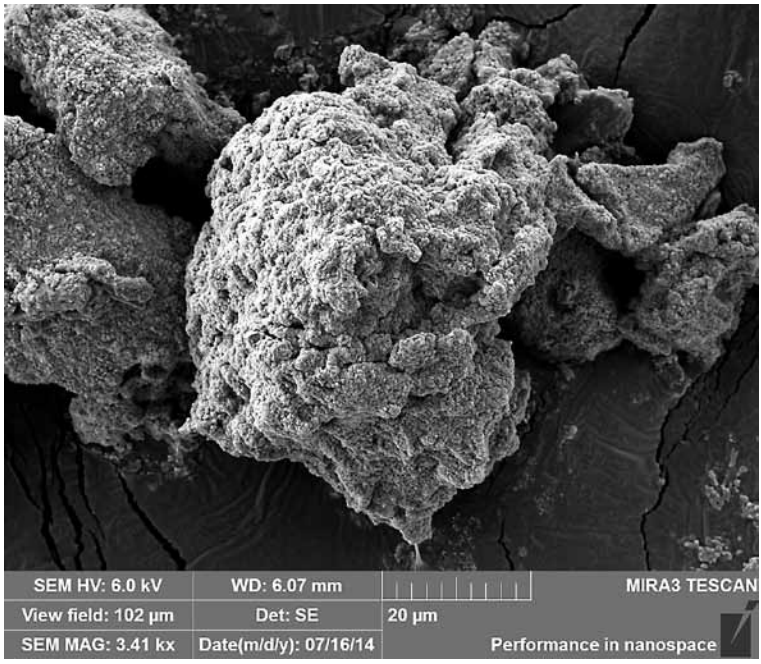

Fig. 5. A fragment of ι-carrageenan (SEM)

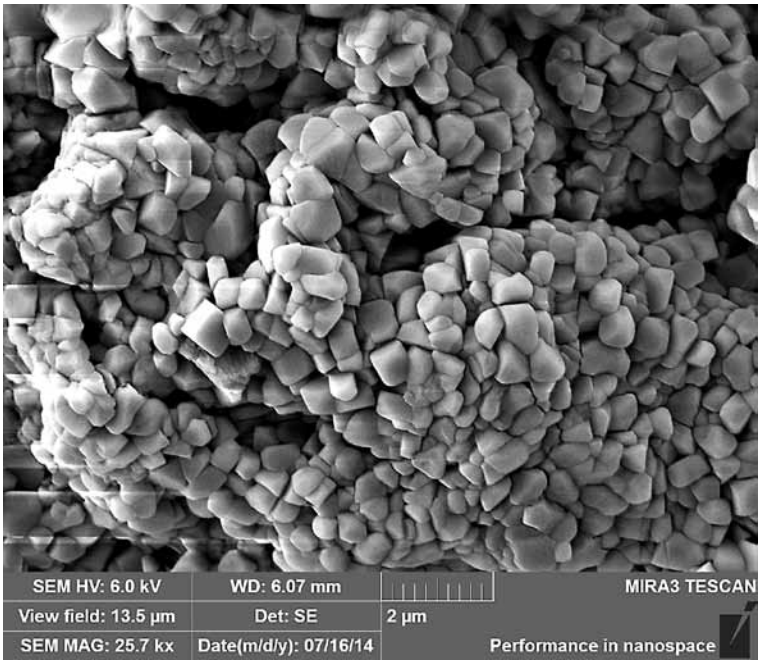

Fig. 6. A ι-carrageenan – surface detail (SEM)

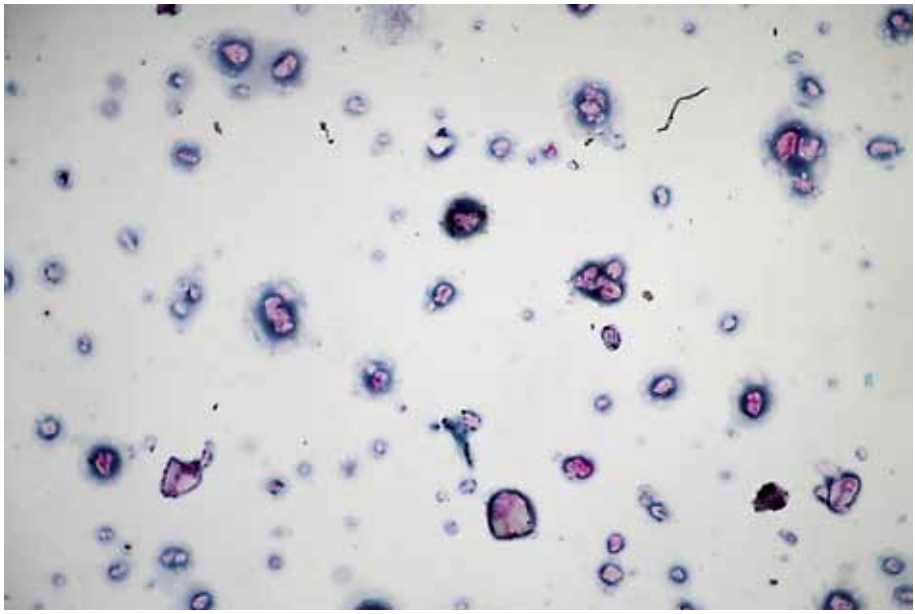

Fig. 7. A κ-carrageenan 100x (LM)

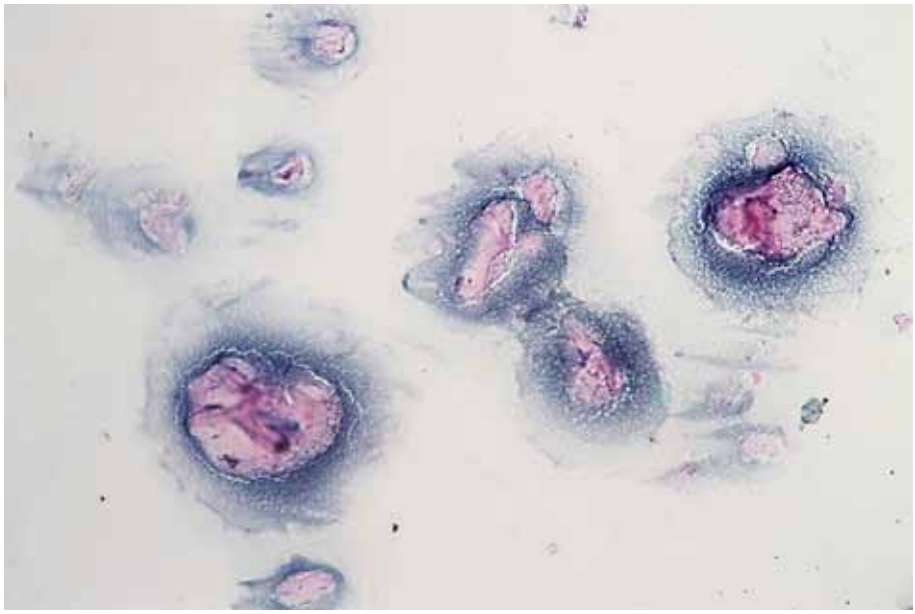

Fig. 8. A κ-carrageenan 400x (LM)

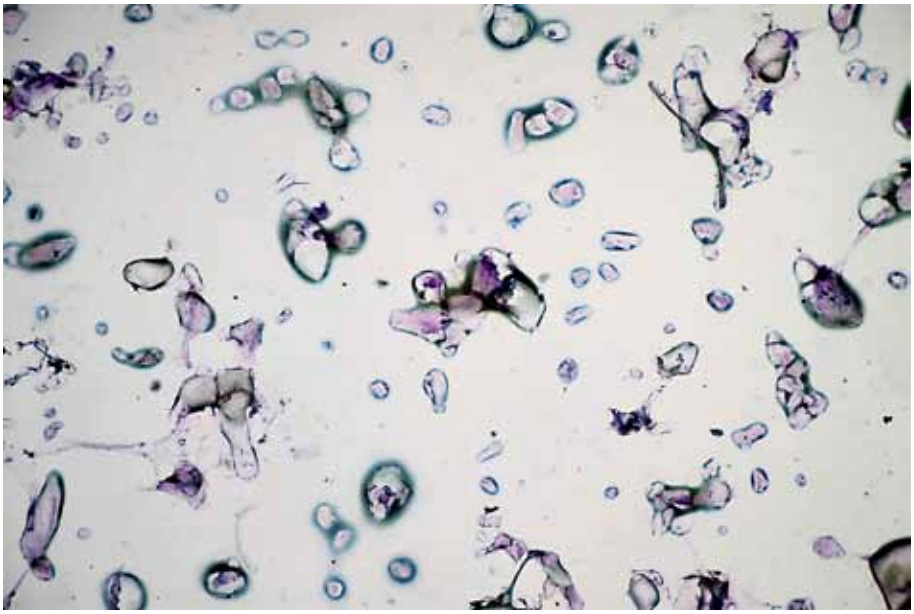

Fig. 9. A λ-carrageenan 100x (LM)

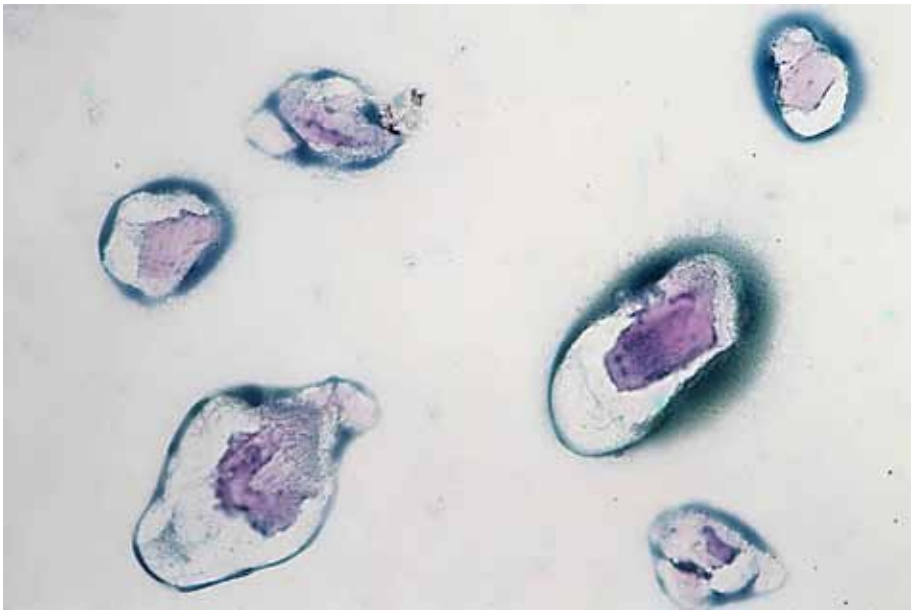

Fig. 10. A λ-carrageenan 400x (LM)

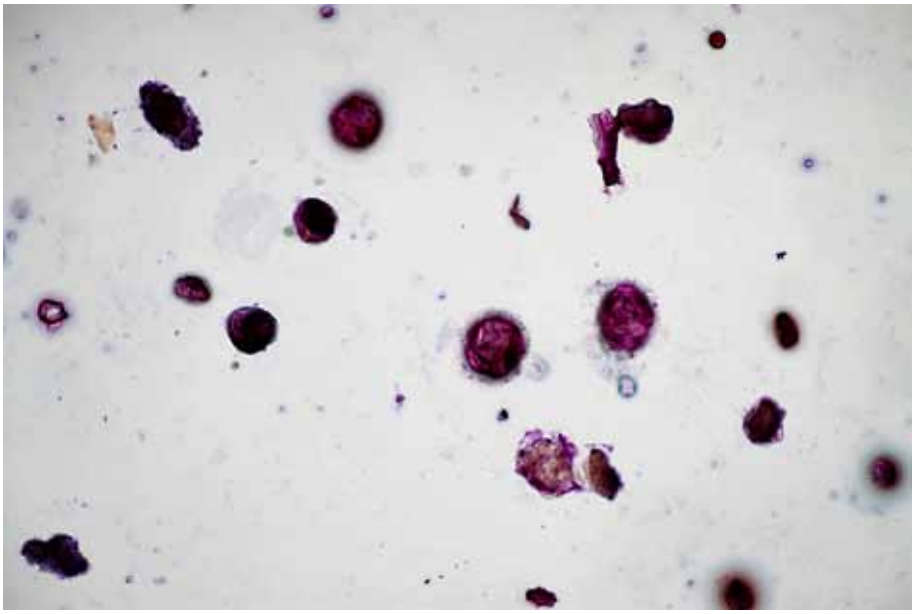

Fig. 11. A ι-carrageenan 100x (LM)

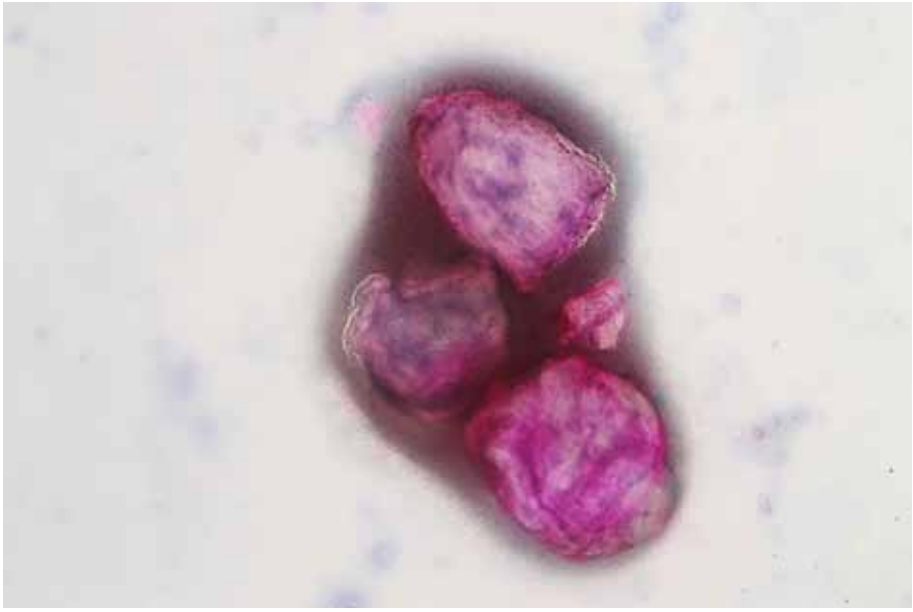

Fig. 12. A ι-carrageenan 400x (LM)