HISTOCHEMISTRY AND FOOD SCIENCE*

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In modern food science, microscopes are equally important tools as back in the past, in the early days of food chemistry. Numerous problems arise in everyday laboratory routine which could not be solved without microscopes.

A few decades ago, morphological examinations by microscope were almost exclusively used for identifying and checking the origin of animal and vegetable raw materials and products, with other words, for quality control and detection of food adulteration. Often, specific preparatory operations were not required at all, or else only simple histological techniques like cutting, bleaching and colouring were applied.

With the progress in food science, research fields and problems changed. Studies became more profound and necessitated the introduction of novel instruments and methods. At present, e.g., we are not satisfied any more with determining the percentage of raw proteins in wheat flour, but investigate the protein structure, its distribution within the grain, its behaviour and its modifiability by industrial processing. Novel methods in modern research include histochemical procedures making use of microscope techniques.

Histochemistry is a branch of biological sciences concerned with "in situ" examinations of the chemical composition and structure of tissues and cells. Such studies are of interest not only for botany and medicine but also for food technology, since the quality of up-to-date preserves is more and more related to histological problems of the raw material in connection with either the development of the raw material or the preserving operations (freezing, irradiation etc). In the majority of cases histochemistry yields qualitative results. However, also quantitative determinations are feasible [1].

In food science, histochemical techniques were first applied in the hygienic control of meat and meat products. In addition, such methods are used for studying the occurrence of tissues and tissue parts of various origin. Histochemical investigation on the activity of tissue enzymes yields an interesting picture of the ageing process in the case of dry sausages. Such methods can be applied e.g. to follow the mould ripening of salami by determining the penet-

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ration depth of the microorganisms, i.e. the depth up to which they exert chemical or biochemical action in the product. The penetration depth is measured by the distance from the surface where enzymes of the microorganism can still be detected.

Another food product of animal origin whose structure has recently been investigated by light and electron microscopy is cheese [2, 3, 4]. Such studies have been reported in several countries. In Hungary, PULAY [5] was the first to study cheese structure by histochemical methods. The same methods are used for studying mould ripening of cheese as for dry sausages.

Until recently, only simple histological techniques were used for studying food products of vegetable origin. The reason for the slow permeation of histochemical methods in this field is due to the fact that these methods have mainly been developed and applied in medical science where the study of lesions and anomalies in tissues is of vital importance both from therapeutical and diagnostical views. Methods developed for tissues of mammals cannot, however, be applied without modification to vegetable raw materials. Difficulties are encountered already at the first step, since vegetable tissues contain much more water and the high ice content of the frozen material makes it difficult to cut thin sections. Though, these difficulties have been mastered in several cases. Thus, urease enzyme was detected in vegetable tissues already 40 years ago. Results of histochemical studies — though infrequently — have been reported on in the literature. Objects of such studies included the occurrence of Ca, Fe and Mg, polysaccharides (starch, cellulose, hemicelluloses), sulfhydryl groups, carotenoids, enzymes (in addition to the mentioned urease, adenosin triphosphatase, cytochrome oxidase, dehydrogenases, phosphatases, phosphorylase etc.) [6, 7].

In addition to the mentioned products, many other raw materials and products can be found in the field of food science to which histochemical methods might be extended. For instance, studies on the structure of dough and of bakery products might help to explain the changes occurring during dough preparation and baking. In other branches of the food industry, e.g. in mills and in canneries, various materials and products are found whose structures and changes might be studied by histochemical methods.

At the Department of Food Chemistry, we applied histochemical methods for studying mushrooms. The first objective of these studies was to find the location of different acid and neutral polysaccharides and other constituents in the fruit body of the cultivated mushroom. In further work, we identified the enzymes present in the mushroom. We first confirmed the presence of the carbohydrate metabolism (Krebs cycle) enzymes and subsequently the presence of several enzymes belonging to the hydrolase group, including adenosin triphosphatase, acid phosphatase, glucose-6-phosphatase, carboxylesterase, aminopeptidase. Among oxidoreductases, we detected the presence of succinic

acid dehydrogenase, malate dehydrogenase, i-citrate dehydrogenase, lactate dehydrogenase, glucose-6-phosphate dehydrogenase, mannitol dehydrogenase, cytochrome oxidase, ascorbate dehydrogenase, cysteine dehydrogenase. In addition to the detection of these enzymes, we also studied the distribution of enzyme activity in the fruit body of the mushroom in which virtually no differentiation of the tissues exists. We investigated whether the distribution is uniform or differences exist between the pileus and the stipe, or between different parts within the pileus and the stipe. In conformity with our expectations, we found greatest enzyme activity to exist in the lamellae where — owing to sporulation — the intensity of the metabolic processes is highest. Also, in the upper part of the pileus and in the parts of the stipe close to the mycelium higher activity was found [8].

We compared the activity of some enzymes in various parts of the mushrooms at different stages of their development. Parallelly to the histochemical studies, the enzyme activity was also determined in the homogenized material. We found e.g. that the activity of ascorbic acid dehydrogenase in the upper and lower part of the stipe increases proportionally to the weight of the fruit body. In the lamellae, the activity increase is slighter. The lowest activity was found in the pileus. In over-mature mushrooms (after the termination of the sporulation) enzyme activity decreases.

Histochemical methods were also applied for investigating the effect of gamma rays in doses used for preserving experiments (50 to 100 krad). We found that irradiation slightly increases the activity of hydrolases and decreases the activity of oxidoreductases. A remarkable effect was observed: ascorbic acid dehydrogenase was totally inactivated by irradiation. The disturbed metabolism led to a protracted development and ageing of the uninjured fruit bodies. Sporulation was slowed down and spores were shed sluggishly [9].

Summary

Histochemical methods are suited for studying the structure of food raw materials and products. These methods allow to follow biochemical changes and changes caused by processing operations. The enzyme systems of cultivated mushroom (Agricus bisporus) and the effect of gamma rays on their activity were investigated.

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