The contribution of microscopy to food science: State of the art

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SUMMARY

Especially in recent years, public opinion's interest for food quality has considerably grown, and the safety of food has become a main priority for consumers as well as for industry holders. Accordingly, during the last ten years the number of scientific articles on food has increased of about 160%. To cope with the emerging issues on food safety, innovative analytical methods are being developed to better characterize foodstuff quality, and to achieve higher sensitivity for detecting food natural components or contaminants. Microscopy techniques proved to be powerful tools in food science, often in association with more traditional methods of physical and chemical analysis; in fact, the use of microscopy techniques in scientific articles showed an increase of about 200% since 2008. The present paper exemplifies the multiple possibilities microscopy may offer the researchers in this field.

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Introduction

Food science encompasses a wide variety of topics, from research on raw material production and features, to technology for food processing, transformation and preservation, to quality and safety control, to nutrition studies. The growing interest for food science is demonstrated by the progressively increased number of scientific articles on this subject (source: Web of Science - all databases): in fact, in 2017 nearly 96,000 papers were published, which correspond to about 160% compared to 2008 (Figure 1a).

Food research has usually been performed by chemical and physical methods; however, browsing the scientific literature of the last ten years, it is evident that the application of microscopy techniques underwent a slight but progressive increase: the “microscopy” articles on food where 2.9% of the whole literature on subject in 2008, while they raised to 3.5% in 2017; this corresponds to an increase in the article published of about 200% (Figure 1 b,c). The positive trend of microscopy is in line with the growing application to food science of various imaging techniques such as nuclear magnetic resonance, X-ray tomography, positron emission tomography, flow cytometry, whose utilisation has nowadays more than doubled since 2008.

In recent years, technological progress led to significant improvement in the analytical potential of the different microscopy (and, more generally, of the imaging) techniques, allowing to obtain compositional and functional data in parallel with the morphological information. This has likely promoted the application of microscopy, often as part of a multidisciplinary approach, in a scientific area such as food science where chemical and physical methods were traditionally more often applied.

A wide spectrum of microscopic techniques has been employed to study any kind of food, from meat and fish, to vegetables and dairy products. The examples listed below do not intend to exhaustively summarize the contribution of microscopy to food science; rather, they will illustrate the multiple possibilities that the microscopical techniques may offer the researchers in this field.

Food safety

Microscopy plays a central role in studies on food safety. Scanning electron microscopy allowed to evaluate the effect of thermal and non-thermal treatments on spore inactivation, in order to prevent microbial spoilage of food (Rozali *et al.*, 2017). Fluorescence microscopy and immunohistochemical analyses were applied to investigate colonisation of meat by *Escherichia coli* (Barizuddin *et al.*, 2015; Chagnot *et al.*, 2017) or *Listeria* (Baysal, 2014), and by transmission electron microscopy the thermal-stability of *Listeria* phages at temperatures mimicking the preparation of ready-to-eat meats was monitored (Ahmadi *et al.*, 2017). Inhibition of *Listeria* growth was evaluated by scanning electron microscopy in cheese treated with chitosan-based packaging (Sandoval *et al.*, 2016). Immunofluorescence microscopy allowed to visualize viable bacteria on different meats (Nishino *et al.*, 2013; Comery *et al.*, 2013).

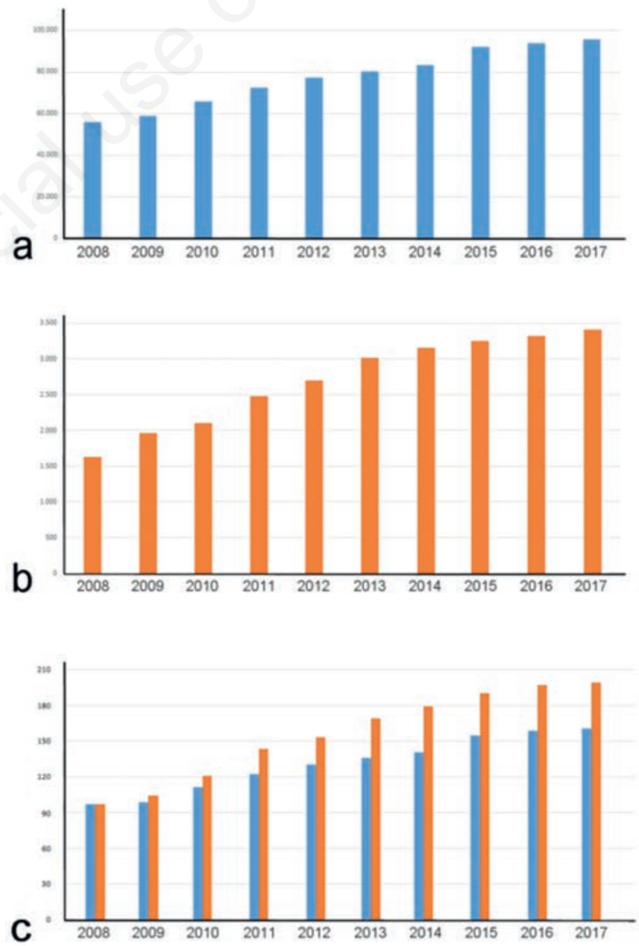

Figure 1. Number of articles on food science (a) and number of articles on food science where microscopy techniques were employed (b) published during the period 2008-2017, as from the database of Web of Science. (c) Percentage increase since 2008 of the articles in a and b.

The fish parasite, *Anisakis simplex* is a public health risk to people consuming raw or undercooked marine fish and cephalopods, because of their possible infestation by viable larvae: *Anisakis* antigens in fish muscle were detected by both fluorescence microscopy and transmission electron microscopy (Solas *et al*., 2008), and the effect of heat on the viability of *Anisakis simplex* larvae isolated from hake was studied by these techniques in order to reduce their antigenicity for consumers (Vidacek *et al*., 2010). Fluorescent *in situ* hybridization allowed the rapid detection of *Salmonella* in surface biofilms formed on tomatos and other fresh produces (Bisha and Brehm-Stecher, 2010), and made it possible to assess the efficacy of a novel biopolymer for removing *Salmonella* from poultry wastewater (Ghosh *et al*., 2009). Fluorescence microscopy proved to be also suitable for detecting wheat allergens in meat products (Lukášková *et al*., 2011).

Shellfish allergy is a relatively common disorder among consumers: to diagnose the presence of allergens and prove their reduction by food processing, scanning electron microscopy was found to be effective and able to identify a novel allergen in crab (Hu *et al*., 2017). This electron microscopy technique also allowed to detect and characterize *Pseudomonas fragi*, a bacterium which is dominant in aerobically stored chilled meats: it was thus possible to investigate the effects of modified atmosphere packaging on bacterial growth (Wang *et al*., 2017a) as well as the result of the new food preservation strategy based on moderate electric field technology (Chen *et al*., 2017).

Transmission electron microscopy was used to test the efficacy of CaO as antimicrobial agent for manufacturing frozen meat products (Ro *et al*., 2015), while fluorescence and scanning electron microscopy were employed for studying the potential of a novel food biopreservative (Chopra *et al*., 2015). Immunofluorescence was used to evaluate chicken egg-yolk-derived antibodies against *Campylobacter jejuni*, whose primary source for human infection is chicken meat (Al-Adwani *et al*., 2013). Scanning and transmission electron microscopy allowed to investigate the effect of different post-harvest treatments of grape berries on *Botrytis cinerea*, the causal microorganism for grey mould (Adrian and Jeandet, 2012; Celikkol and Turkben, 2012; Junqueira-Goncalves *et al*., 2013).

The relationship between bacteria and fat globules during cheese working was monitored by fluorescence and transmission electron microscopy (D'Incecco *et al*., 2015).

Atomic force microscopy was suitable to evaluate *Salmonella* contamination in materials used in poultry processing (Chia *et al*., 2009).

Effects of processing on foodstuffs

The effects of processing on different foodstuffs was also successfully evaluated by microscopy techniques. Histochemistry and fluorescence microscopy were used to detect oxidized proteins in bovine muscle (Astruc *et al*., 2007), while scanning electron microscopy allowed to study the effect of freeze-drying on the microstructure, texture, and tenderness of bovine muscles (Pieniazek and Messina, 2016). Marinated and cooked meat were characterized by bright-field and fluorescence microscopy (Liu *et al*., 2011), while histological and ultrastructural studies were performed to define the cooking-related changes in the lipid droplets of the duck "foie gras" (Théron *et al*., 2011).

High pressure processing is a food processing technology by which high pressure is applied to solid or liquid foods in the attempt to increase their safety: to monitor the effect of this procedure on the meat features of bovine (Kaur *et al*., 2016) and buffalo (Kiran *et al*., 2016), transmission electron microscopy was used; furthermore, scanning and transmission electron microscopy proved that high pressure processing may be a novel method for modifying the textural properties of pork sausages with reduced-salt, reduced-fat and no-fat replacement additions (Yang *et al*., 2015).

Proteases often cause softening of the fish meat during refrigerated storage or slow cooking: fluorescence recovery after photobleaching (FRAP) and confocal laser scanning microscopy (CLSM) were crucial to measure the diffusion into halibut muscles of fluorescently-labelled protease inhibitors which may help to prevent this undesired effect (Carvajal-Rondanelli *et al*., 2010).

Characterization of food features

Microscopy contributed to studies aimed at describing food features and/or improving food production. Histological and morphometric analyses were central to characterise the muscles of pigs fed with different feeds (Zancanaro *et al*., 2011) or to evaluate the occurrence of myopathy in broilers which is an emerging concern in the poultry industry (Sesterhenn *et al*., 2017).

Scanning and transmission electron microscopy allowed to examine the ultrastructural changes of rice seeds after drying at different temperatures, which is a critical step influencing the final properties of this cereal (Wang *et al*., 2017b).

Light microscopy helped to characterise the intraspecific hybrids of *Vitis vinifera* (Apolinar-Valiente *et al.*, 2017).

In the field of dairy industry, light ad transmission electron microscopy were used to describe the microstructure of hard and extra-hard cheeses (D'Incecco *et al*., 2016), and CLSM and cryo-scanning electron microscopy allowed to understand the process of coagulation of casein micelles for cheese production (Zhang *et al*., 2017), as well as the changes in mechanical properties at the different stages of baking of cheese crackers (Chong *et al*., 2017). Cryo-scanning electron microscopy was also useful to investigate the microstructure of yogurts with different concentrations of guar gum (Hussain et al., 2017). CLSM allowed to evaluate the ability of diary matrices to enhance microorganism survival during digestion (Hernandez-Galan *et al*., 2017).

Concluding remarks

Especially in recent years, public opinion's interest for food quality has considerably grown, and food safety has become a main priority for consumers as well as for industry holders. To cope with the emerging issues on food safety, innovative analytical methods are being developed to better characterize foodstuff quality and to achieve higher sensitivity for detecting food natural components or contaminants.

In parallel with more traditional methods of physical and chemical analysis, microscopy techniques proved to be powerful tools in food science. These techniques are especially appropriate for evaluating food because they produce results in the form of images which provide direct evidence of the sample structural characteristics; in their digital form, images can then be quantitatively processed for morphometric and compositional analyses, following histochemical or spectroscopic procedures. Microscopy techniques are therefore useful to assess the characteristics of a given food, and to elucidate the relationships between its microstructure and the properties, behaviour and sensory attributes (such as softness); microscopy techniques are also suitable for describing the changes occurring to the row material after heating, cooling or addition of products (such as thickeners) which may lead to textural changes. Consequently, microscopy is effective for understanding the basic structure of both the food raw materials and final products, but may be also helpful to elucidate the effects on food of the manufacturing machineries as well as of the materials used for packaging. Hence, microscopy techniques become essential to support the work of food technologists (Edwards, 2007).

Unfortunately, as much as other conventional methods for the quality assessment of foods, most of the microscopy techniques may often be time-consuming and destructive. Thus, especially for longitudinal studies a number of novel more rapid and non-invasive imaging techniques have emerged as effective analytical tools in food science: among them, in particular nuclear resonance imaging, optical imaging, spectral and hyperspectral imaging, and computed tomography showed great potential for food science (Xiabo *et al*., 2016).

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