Application note

Food protection methods assessed by Surface Enhanced Raman Spectroscopy

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Thermo Scientific DXR3 Raman Microscope.

The effectiveness of two types of packaging for preserving ground pork meat from lipid oxidation was investigated using Surface Enhanced Raman Spectroscopy.

Introduction

Lipid oxidation is a major issue for the meat industry as it can not only affect sensory quality in terms of flavor and odor but can also lead to the formation of toxic compounds such as malondialdehyde (MDA) and cholesterol oxidation products, as well as the accumulation of carbonyls, alcohols and volatile acids. The degree of oxidation depends on various factors such as the type of meat, the fat content, the storage temperature, the availability of oxygen and the type of packaging[1, 2]. To prevent oxidation, synthetic antioxidants are commonly added to meat products, but consumers are now demanding more natural products[3]. Several studies have been carried out to evaluate the antioxidant activity of many essential oils or extracts from plants or spices such as rosemary, cinnamon or oregano, and the new trend is to develop innovative packaging by adding these natural antioxidants directly to the packaging materials[4–6]. These materials, often films, are known as “active packaging” because they enable the controlled release of active agents either on the surface or in the internal atmosphere of the product, or by trapping the radicals responsible for harmful effects on food quality. However, the antioxidant performance of these films must be accurately determined to enable the selection of the right packaging for each foodstuff.

Among all the techniques available, Raman spectroscopy is a promising candidate for detecting most molecular species with high specificity and sensitivity, particularly in the presence of Surface-Enhanced Raman Scattering (SERS). Indeed, SERS is a physical phenomenon that occurs in the presence of a rough nano-structured metallic surface that causes the intensification of Raman signals from molecules adsorbed to the surface thanks to the combination of a chemical effect and an electromagnetic effect[7]. The metals used are generally gold or silver, which can be prepared by electrochemical roughening, metallic coating, or deposition of nanoparticles (often in a colloidal form). Commercial solutions are available, but many researchers prefer to create their own SERS substrates. The scope of this work is to investigate the potential of a home-made SERS substrate to compare the lipid oxidation over time in ground pork preserved either in a low-density polyethylene (LDPE) conventional packaging or in an active packaging containing oregano extract.

Material and method

Fresh ground pork meat was purchased in bulk from a local supermarket and 22 g samples of meat were placed in 5-cm diameter polystyrene Petri dishes. Each sample was covered with an active film and packed in an LDPE bag under a normal atmosphere. Reference samples were also prepared using conventional LDPE films. All samples were stored in a refrigerator at 5 °C and their lipid extracts were analyzed after 0, 7, 9, 11, 14 and 16 days using a home-made SERS substrate based on silver nanoparticles deposited on a glass Petri dish. A special home-made cooling system was used to perform the analyses at low temperature and SERS spectra were measured at -2 °C on a Thermo Scientific™ DXR3 Raman Microscope (Waltham, MA, USA) with a 532 nm laser source, a 900 lines/mm grating covering the 50-3500 cm^{-1} spectral range and an x10 objective (NA 0.40). The laser power was set at 5 mW, and 20 exposures of 30 s each were selected to acquire all data. Thermo Scientific™ OMNIC™ v. 9.2 software was used for data collection and analysis. All the measurements were performed in triplicate. As the decrease in the peak at 1655 cm^{-1} (stretching of C=C bonds from unsaturation) can be attributed to lipid peroxidation, and the 1439 cm^{-1} (scissoring deformation of CH_2) is not affected by it, the area ratio between 1655 cm^{-1} and 1439 cm^{-1} bands was calculated to follow the lipid oxidation.

Results and discussion

Figure 1 shows the Raman and the SERS spectra of fat extracted with n-hexane/diethyl ether (1:1) from meat after 7 days of storage in both conventional LDPE and active packaging. The SERS spectrum shows bands of much higher intensity than the conventional Raman spectrum, where the conventional and active packaging spectra were almost identical. During the study, it was found that the difference between fresh and oxidized samples was more pronounced in the SERS spectra measured at low temperatures (data not shown).

The enhancement factor (EF) for a given shift can be calculated as follows[8]:

$$EF_{\lambda} = \frac{I_{SERS}/N_{SERS}}{I_{Raman}/N_{Raman}}$$

Where I_{SERS} is the signal intensity at the selected shift with the silver substrate, I_{Raman} is the signal intensity at the same shift without the silver substrate, N_{SERS} is the number of molecules responsible for the enhancement and N_{Raman} is the number of molecules in the detection volume. Table 1 shows the calculated enhancement factors for the two bands of interest in this study: 1439 cm^{-1} and 1655 cm^{-1}.

EF_{1439}	$1.64 \cdot 10^7$
EF_{1655}	$8.58 \cdot 10^6$

Table 1. EF calculated for the 1439 cm^{-1} and 1655 cm^{-1} bands.

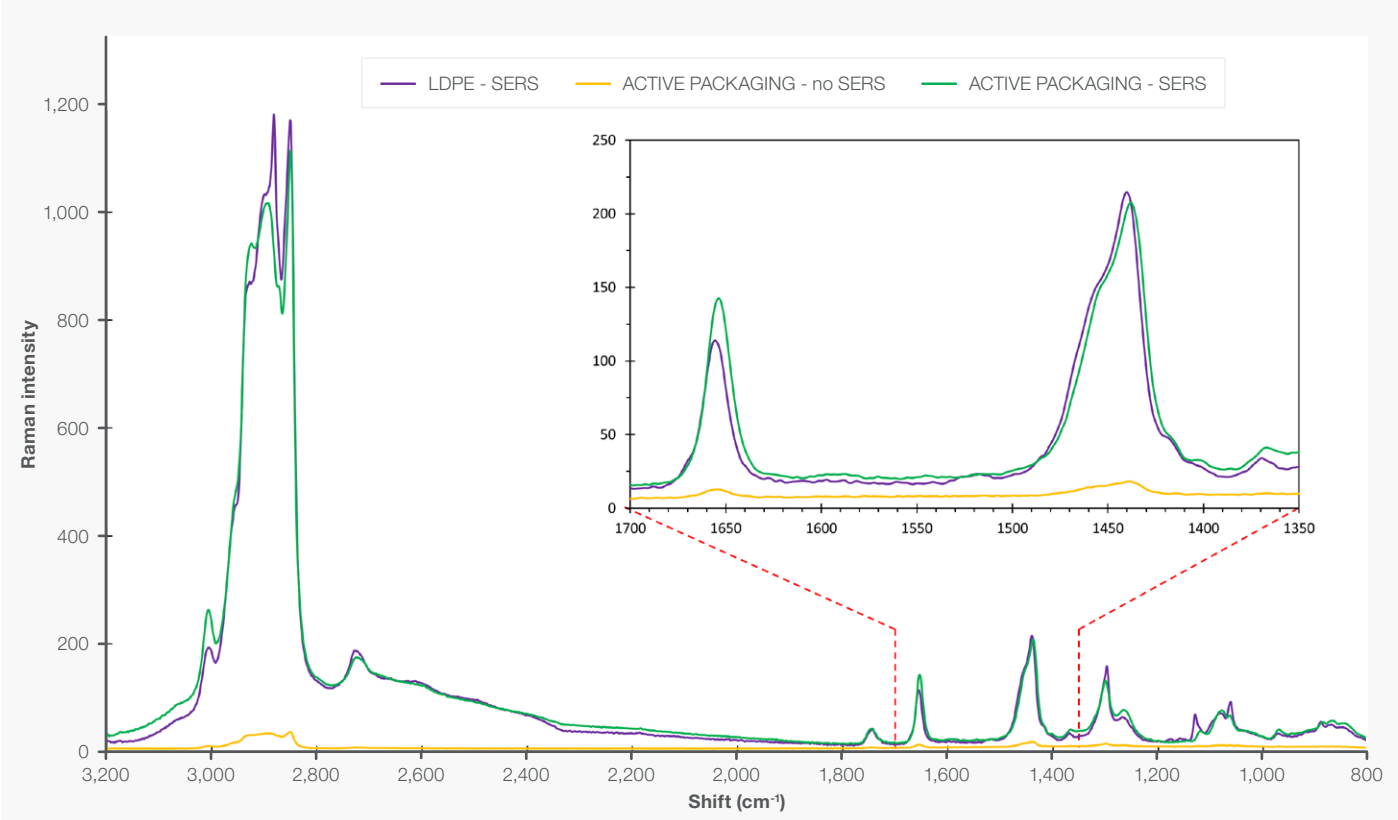

Figure 1. Conventional Raman (yellow) and SERS spectra of fat extracted from meat on the 7th day. The higher peak at 1655 cm^{-1} of active packaging (green) indicates effective protection in comparison with LDPE (purple).

These are on the order of a factor of 10^7, demonstrating the very high efficiency of the home-made SERS substrate. The use of such a substrate means higher sensitivity and therefore faster acquisition.

Packaging efficiency was assessed by measuring the relative change of unsaturation (RCU%) as follows:

$$RCU\% = \frac{AR_0 - AR_n}{AR_0} \cdot 100$$

where AR_0 and AR_n are the area ratio between the 1655 cm^{-1} and the 1439 cm^{-1} SERS bands respectively at day 0 and after n days of storage. The higher the RCU% value, the higher the level of lipid oxidation.

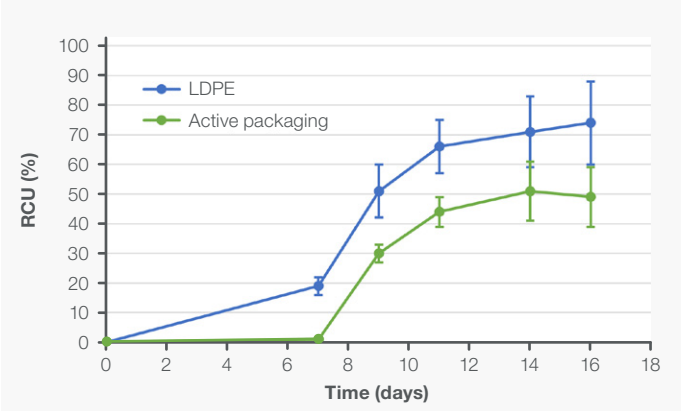

Figure 2. Relative change of unsaturation (RCU%) over time for conventional LDPE packaged meat and active packaged meat.

Figure 2 shows the graphical results of the RCU% obtained for the meat stored in both conventional and active packaging. The values calculated in this study indicate an increase in lipid oxidation over time, which appears to accelerate from day 7 of storage. The RCU% values are consistently lower for the active packaging than for the conventional LDPE packaging, confirming that this type of packaging is more effective in preventing the unsaturation of fats in ground pork due to their oxidation over time.

Conclusion

The effectiveness of a home-made SERS substrate coupled with a home-made cooling system was demonstrated for monitoring lipid oxidation in packaged ground pork meat over time. The advantages of this technique are its speed and the minimal sample handling requirements. An improvement factor of the order of 10^7 over conventional Raman was observed. This study also confirmed the greater efficacy of active packaging containing oregano extracts against lipid oxidation in ground pork, compared to conventional LDPE packaging.

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