Kasetsart J. (Nat. Sci.) 35 : 311 - 317 (2001)

Microstructural Changes in Instant Noodles During Production *via* Triple Staining and Confocal Laser Scanning Microscopy and Scanning Electron Microscopy

Lalana Chewangkul[1], Onanong Naivikul [1], Wunwiboon Garnjanagoonchorn [1], Christopher G. Oates [2], Klanarong Siroth [3] and Thongchai Suwonsichon [4]

ABSTRACT

Confocal Laser Scanning Microscopy (CLSM) was used to study changes in microstructure throughout the process of instant noodle production. Multiple staining of acridine orange, fluorolink Cy-3 and sulforhodamine was applied in order to differentiate protein, starch and lipid, respectively, in the same image. Scanning Electron Microscopy (SEM) was also applied, complementarily, to contribute stereoscopic images. The microscopic data obtained from triple-labeled samples examined by CLSM were found to correspond to the stereoscopic SEM micrographs. Morphological changes and microstructural arrangement of starch granules and protein matrix, and the presence of lipids were found to respond to different regimes of process: addition of water, inputs of mechanical force and heat and introduction of frying. Thus, triple staining, via CLSM, could be regarded as a potential tool for monitoring microstructural differences resulting from varied processing conditions.

Key words: microstructure, instant noodle, Confocal Laser Scanning Microscopy, Scanning Electron Microscopy

INTRODUCTION

Microscopy is, particularly, useful in food research development. It provides a clue to understanding the relationship between the product's microstructure and functionality of a food material (Varriano-Marston, 1977).

Confocal Laser Scanning Microscopy (CLSM) has introduced to food research new possibilities for microstructure studies of food systems (Adler *et al*., 1994; Vodovotz *et al*., 1996; Lynn *et al*., 1997). The main advantage of CLSM in food applications, in addition to its optical sectioning capabilities, is that it requires minimal sample preparation: the specimen does not require prior embedding, sectioning, or fixing (Vodovotz *et al*., 1996).

In order to achieve sensitivity and specificity from CLSM application, an appropriate fluorochrome with a proper wavelength of excitation and emission and fluorescence stability, and component stability are necessary. Some fluorochromes used in cereal based products are FITC, fluorolink Cy3, nile blue A, nile red, congo red, sulforhodamine, pyronin Y, pararosaniline and acridine orange (Adler *et al*., 1994; Vodovotz *et al*.,

1 Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, Bangkok 10900, Thailand.
2 FAO Consultant, Asian Region, Bangkok, Thailand.
3 Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Bangkok 10900, Thailand.
4 Department of Product Development, Faculty of Agro-Industry, Kasetsart University, Bangkok 10900, Thailand.

1996; Lynn *et al*., 1997).

Scanning Electron Microscopy (SEM) of surface morphology provides stereoscopic images with high magnification. Noodles and pasta samples have been studied by SEM using a liquid nitrogen slush for rapid freezing (Marion *et al*., 1987; Moss, 1985). Moss *et al*. (1987) studied the influence of ingredients and processing variables on the microstructure of several kinds of noodles, including instant noodles by employing SEM, complemented by conventional light microscopy. Microstructure differences at the dough stage of instant noodles were observed to be similar to Cantonese noodles. Microstructure changes affected by additional processes of steaming, frying and final cooking were also reported (Moss *et al*., 1987).

CLSM and SEM were employed to examine the microstructure changes of samples from the production line of instant noodles in this study.

MATERIALS AND METHODS

Confocal laser scanning microscopy

Each of the six samples were randomly taken, immediately following the stage of mixing, compression, reduction, steaming, frying and cooking. The samples were stabilized by the pre-heated chilling to 4°C, not over a period of 3 hours and stored in an air-tight container prior to staining. Wheat flour and mixed dough samples were placed on the cover slips directly. Dough samples taken from compression and reduction were viewed at the volume of approximately 1×3×3 mm^3. Noodle samples taken from steaming, frying and cooking were viewed at 5 mm long. Six specimens were randomly selected for examination.

Triple staining for observing protein, starch and fat simultaneously was modified from the methods of Vodovotz *et al*. (1996); Alder *et al*. (1994); Blonk and van Aalst (1993), respectively and performed on each individual sample. Flourolink Cy3 was directly applied (Amersham Life Science, Inc. IL) for protein marking. Acridine orange (0.01% aq., Sigma Chemical, Poole, Dorset BH17, UK) and sulforhodamine (0.1% aq., Sigma Chemical, Poole, Dorset BH17, UK) were applied directly for starch and lipid, respectively. For low moisture samples (flour and fried noodles), fluorochrome solutions were placed onto the sample and stood for a pre-tested period of 10 minutes before examining. Samples that contained intermediate (mixed or rolled or sheeted dough) to high (cooked noodle) moisture content were viewed immediately after placing fluorochromes.

CLSM examination was performed using Carl Zeiss 410 LSM system attached to an Axiovert 135M-inverted optical microscope fitted with a 100x/1.3 oil Fluar immersion objective lens. The software used to control the microscope was LSM 410 (also supplied by Carl Zeiss). The wavelengths used to generate fluorescence were 488, 514, and 543 nm. The lasers were set at 3.2% of their maximum power. Emission filters 515-565LP, 510, 525, 575-640, 590, 665 and 590-610 were selected. Filter block 1 contained a VHS-510DCLP filter. Cover slips 22×50mm, no.1 were used to support the samples. Each specimen was randomly viewed at 3 areas at ×630 magnification. Optical sections were taken every 1.0 mm through the samples. Forty images were recorded for each series and then 3-D reconstructed by employing the software, Confocal Assistant V 3.1 and LSM Dummy, respectively. The representative 3-D images from each processing step were selected for illustration.

Scanning electron microscopy

Samples for SEM examination were collected, concurrently, with CLSM samples. The specimens were prepared by the method described by Pomeranz and Meyer (1984) and Moss (1985). Samples were immediately frozen in liquid nitrogen and freeze-dried at 0.8 Torr and –50°C, for 18 hours (Edwards, Modulyo 4K Freeze Dryer, England). The freeze-dried specimens were first fractured to expose interior structure, then affixed to aluminum SEM stubs using either double-sided tape with

silver-colloid paint or dental gum with carbon cement for longitudinal sections and cross sections, respectively, at the base of each specimen. Then the prepared specimens were coated with gold using a sputter coater (Balzers SCD 004, Balzers Akteingesellscaft, Leichenstein). The coating was achieved by applying a vacuum of 0.005 mbar and a current of 15 mA for 215 s, resulting in approximately a 20 nm thick coating. The coated specimens were examined with a scanning electron microscope (JSM-5600 LV, Jeol, Japan) operated at an accelerating voltage of 10-15 kV at pertinent magnification (x85-x3000). Photomicrographs were taken on Agfaplan APX 100 Professional 120 film (Agfa, Germany). The representative micrographs from each processing step were selected for illustration.

Formulation of instant noodles and processing

Instant noodle samples were prepared from a dough comprising of wheat flour (78%); water (20%), sodium chloride, phosphates, carbonates and other texture modifiers (2%).

A commercial Australian wheat noodle flour with 87.0% total solid, 10.0% protein, 25.5% wet gluten and 0.5% ash was used in this experiment.

The processing of instant noodles was performed by a continuous production line. Wheat flour was mixed with the dissolved salts and texture modifiers in water for 20 min. The compression and reduction stage were operated by 7 pairs of rollers to achieve a dough thickness of 1.0 mm, the sheeted dough was then proceeded to a sitting roller to obtain a 0.8 mm width of noodle strands. Subsequently, noodles were steamed by being subjected to a saturated steam at 4 bar for either 0, 1 or 3 min in a steaming tunnel, prior to cutting and shaping the noodles into a block of 85.0 g in a mold to convey to a fryer, adjusted at a temperature of 135°C with the speed adjusted for a 50 s frying. The steamed fried noodles were left to ensure cooling before being doubly packed in polyethylene and in vaporized metal polypropylene bags and then stored in cold storage at 4°C.

Cooking of instant noodles (80g) was performed by placing them into 450 ml of boiling water and cooking for 2 min, with occasional stirring. The cooked noodles were then transferred to 600 ml of room temperature water and drained immediately. Excess water was blotted with tissue paper.

RESULTS AND DISCUSSION

Multiple staining was used in order to differentiate protein, starch and oil in the same image. Fluorolink Cy3, acridine orange and sulforhodamine were selected as fluorochromes due to their specificity to protein, starch and oil and different emission wavelengths. Though sulforhodamine possesses a wide range of emission wavelength, one of which produced the same color as protein, plus fluorolink Cy3, it was found to be applicable. Since the morphologies of oil droplets and protein matrix are quite different, differentiation was easily achieved.

CLSM of the wheat flour sample (Figure 1.1) showed the protein bodies, stained yellow, as a compact discrete chunk in irregular shape associated with some of the starch granules. Because of the degree of protein cover, starch granules themselves were not easily identifiable. Some were stained unevenly, unstained sections were black, and slightly stained sections appeared dark green. SEM as clusters of endosperm material of different sizes and shapes (Figure 1.2) characterized the morphology of wheat flour particles. Almost all starch granules were embedded in, or closely packed with protein bodies.

After a reasonably short mixing time with a relatively low amount of added water, CLSM revealed that protein bodies were removed from the starch granules (Figure 2.1).

In addition, a radical change occurred from the mixing, in that, the protein was fused together. The dispersed starch granules were more apparent as displaying a stronger green color when compared

to the previous starch granules in flour particles. The increase in green staining indicates that more aqueous solution had penetrated parts of some granules. The surface morphology of protein bodies, as observed by SEM (Figure 2.2), was smoother and more uniting as compared with that of wheat flour. Starch granules were more noticeable as compared with wheat flour.

After mixing, dough was compressed to form a sheet by repeated passage through pairs of rollers for compression and reduction. The changes in protein morphology occurring during compres-sion was made evident by CLSM and SEM (Figure 3.1 and 3.2). Most of the protein mass was observed as spreading out and the starch granules had been released from the protein mass, resulting in their more intensive green staining (CLSM). After the reduction stage was completed, a greater degree of protein orientation was obvious in both CLSM (Figure 4.1 and 4.2) and SEM as the gluten had developed. Morphology of the starch granules remained the same, as it was, in the original wheat flour.

After entering a steaming chamber charged with saturated steam, noticeable differences of protein strands and starch granules in the noodles were detected. Figure 5.1 revealed a slight aggregation of protein. In steamed noodles, not all the starch granules were fully gelatinized. Besides the intact starch granules, a number of granules displayed a more swollen, irregular shape. Most of the deformed granules exhibited less intensity of fluorescence. Starch gel was also evident as a non-fluorescent dark green mass. SEM micrograph (Figure 5.2) taken at the surface of steamed noodles also revealed various degrees of starch swelling. The shape of starch granules was still discernable and not all the granules were ruptured or fused together. Bubbles and pores were detected. As frying was introduced, the displaying of oil droplets had initiated in CLSM images, (Figure 6.1) ranging from yellow to orange colored and exhibiting protein aggregation. Both CLSM and SEM (Figure 6.2) detected discernable starch granules and protein strands.

After the cooking of instant noodles, clumps of dark, green swollen starch and darker starch gel, accompanied with yellow swollen protein strands were observed in Figure 7.1. A gel structure at the surface of the cooked instant noodles was shown in Figure 7.2.

CONCLUSIONS

Triple labeling of starch, protein and oil examined with CLSM was achieved by an application of specific fluorochromes (acridine orange, fluorolink Cy3 and sulforhodamine) and yielded microscopic observations, which were in agreement with SEM.

CLSM images of wheat flour showed protein bodies in yellow surroundings in almost all of the starch granules that were different in sizes and unevenly stained. Unstained granules were black. Mixing with water produced a radical change, as evident, in the spreading out of the protein matrix.

Staining of starch granules increased, presumably, due to more dye solution penetration. As evident in CLSM and SEM images, orientation of the protein matrix due to compression and subsequent reduction occurred, and the protein strands were more clearly visible. After steaming, the images showed a slightly, aggregated protein and a swollen, irregularly shaped green mass of starch.

SEM confirmed the morphological changing starch granules that were packed into a dense mass. Orange oil droplets were visible in CLSM only after frying. The increase in different degrees of starch swelling and protein aggregation was detected after steaming and frying. As a result of cooking, protein strands and almost all of the starch granules were observed to be swollen. Starch gel was also detected.

It could be concluded that morphology change, the distribution and the inter-relationship of starch granules and protein matrix (protein strands) and the presence of lipids could be

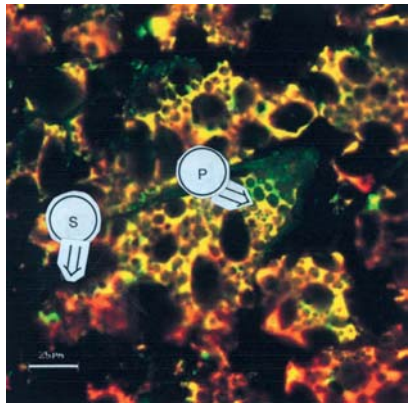

Figure 1.1 CLSM images of wheat flour.

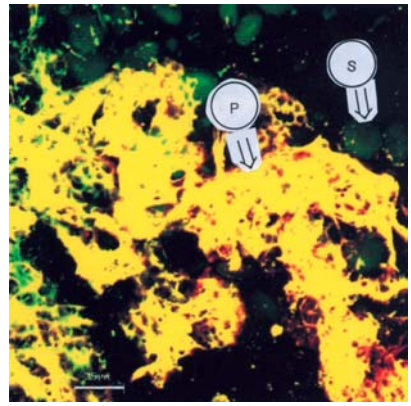

Figure 2.1 CLSM images after mixing.

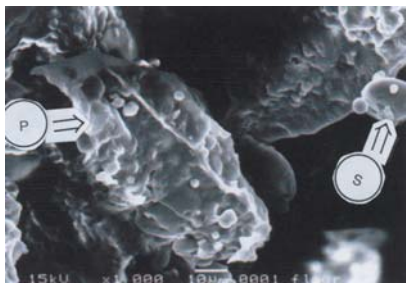

Figure 1.2 SEM micrograph of wheat flour.

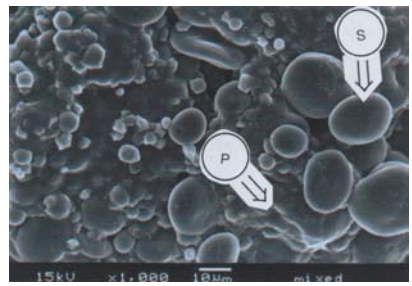

Figure 2.2 SEM micrograph after mixing.

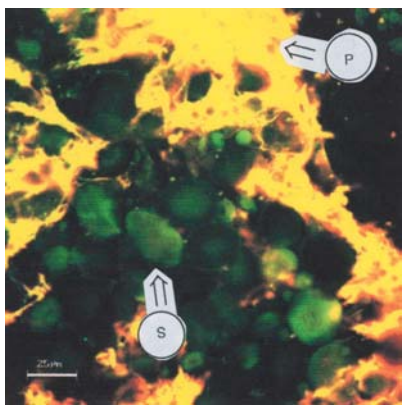

Figure 3.1 CLSM images after compression.

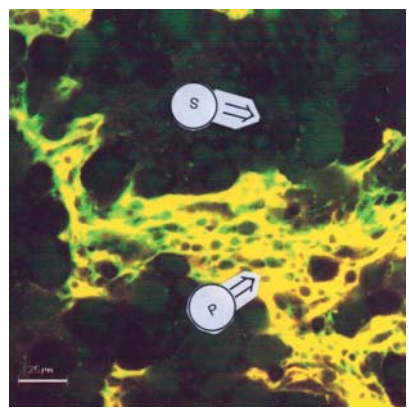

Figure 4.1 CLSM images after reduction.

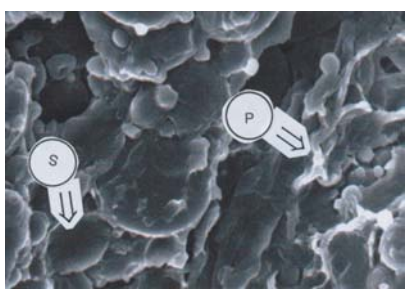

Figure 3.2 SEM micrograph after compression.

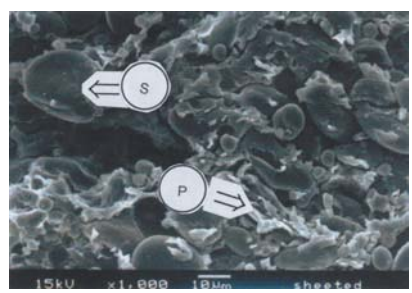

Figure 4.2 SEM micrograph after reduction.

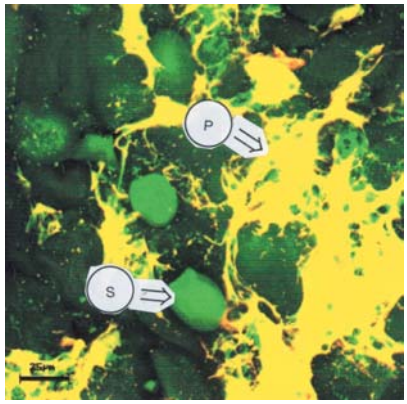

Figure 5.1 CLSM images after steaming.

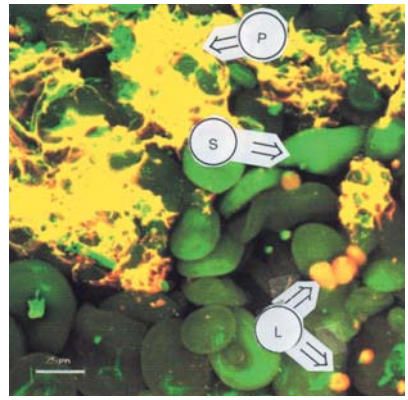

Figure 6.1 CLSM images of instant (fried) noodle.

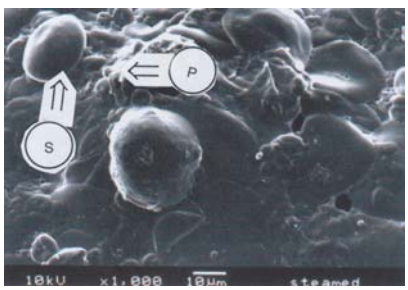

Figure 5.2 SEM micrograph of noodle surface after steaming.

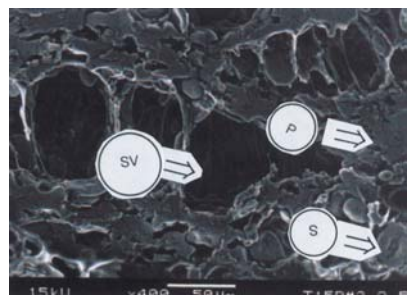

Figure 6.2 SEM micrograph of cross-sectioned instant (fried) noodle.

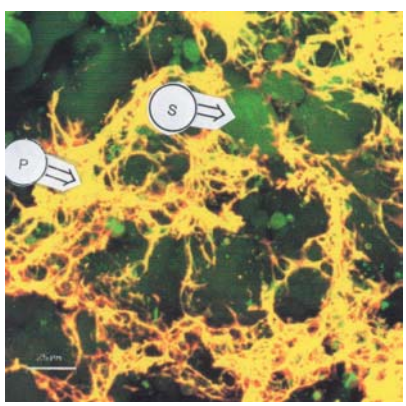

Figure 7.1 CLSM images of cooked instant noodle.

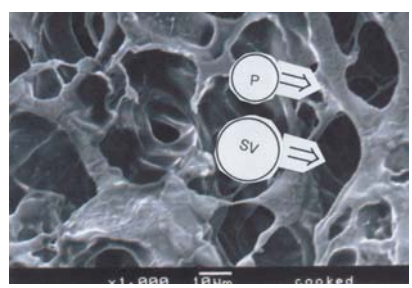

Figure 7.2 SEM micrograph of cooked instant noodle surface.

monitored, successfully, upon an addition of water with an input of mechanical force, upon mixing. The mechanical force, as the dough proceeds through the compression and reduction stage, is subjected to heat with moisture from steaming, heat input and vaporizing of the moisture, as a result of frying and boiling excess water in the final step of cooking. Thus, triple staining via CLSM could be regarded as a potential tool for understanding the effect of processing and the influence of raw materials. It could be expected that this tool would achieve and understanding of the relationship between microstructure information and sensory properties preferred by the consumer, therefore, process optimization would be met.

ACKNOWLEDGEMENTS

Financial support was provided by Nestle R&D Center (Pte) Ltd., Singapore. The Industry and Technology Relations Office, National University of Singapore and Graduate School, Kasetsart University, Thailand, did the cooperation.

Much appreciation is extended to Mr. Foo Check Woo, the Nestle R&D Center, Dr. Henry Yu and Dr. J.K. Candlish, the National University of Singapore for allowing the use of CLSM at the Clinical Research Center and SEM at the Electron Microscopy Unit, Faculty of Medicine.

LITERATURE CITED

Adler, J., P.M. Baldwin, and C.D. Melia. 1994. Starch damage Part 2: Types of damage in ball-milled potato starch, upon hydration observed by confocal microscopy. Starch 46 : 252.

Blonk, J.C.G. and H. Aalst. 1993. Confocal laser scanning microscopy in food research. Food Res. Intl. 26 : 297.

Lynn, A. and M.P. Cocharane, 1997. An evaluation of confocal microscopy for the study of starch granule enzymatic digestion. Starch 46 : 106.

Marion, D., C. Le Roux, S. Akoka, C. Tellier, and D. Gallant. 1987. Lipid-protein interactions in wheat gluten: A phosphorus nuclear magnetic resonance spectroscopy and freeze-fracture electron scanning microscopy study. J. Cereal Sci. 5 : 101.

Moss, R. 1985. The application of light and scanning electron microscopy during flour milling and wheat processing. Food Microstruct. 4 : 135.

Moss, R., P.J. Gore, and I.C. Murray. 1987. The influence and processing variables on the quality and microstructure of Hokkien, Cantonese and instant noodles. Food Microstruct. 6 : 63.

Pomeranz, Y. and D. Meyer. 1984. Light and scanning electron microscopy of wheat and rye bread crumb. Interpretation of specimens prepared by various methods. Food Microstruct. 3(2) : 159.

Varriano-Marston, E. 1997. A comparison of dough preparation procedures for SEM. Food Technol. 31 : 2.

Vodovotz, Y., E. Vittadini, J. Coupland, D.J. McClements, and P. Chinachoti. 1996. Bridging the gap: Use of Confocal Microscopy in food research. Food Tech. 50(6) : 74.

Received : 15/05/01
Accepted : 30/06/01