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Microstructure and Protein Network Study of Pempeks Based on the SEM Image

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ABSTRACT

The standardized and quality-oriented technical process for the production of pempek is determined by various components. One of the quality components is determined by the protein-to-carbohydrate ratio of the ground fish meat used to make Pempek. The ratio of protein to carbohydrates determines the taste quality and especially the Pempek's texture. One method of measuring the flavour quality of Pempek is to examine its microstructure and protein network. The study of Pempek's microstructure and protein network is crucial to understanding the relationship between its composition, processing, and final properties. This study provides quantitative data on microstructure to develop a high-quality Pempek. Microstructure and protein network studies can be performed by visual analysis of SEM (scanning electron microscopy) images. The study shows three forms of pempek surface morphology: sponge (hole), fracture (fractal), and a mixture of sponge and fracture. The structure of the sponge, the fractal and the mixture of sponge and fracture form different surface roughnesses. Protein network analysis shows that pempek with hole contours has a vascular area, a lower total number of protein linkages and a higher endpoint than pempek with fractal contours and mixed contours. The protein influenced the morphological formation structure of the Pempek to carbohydrate ratio and the homogeneity of the composite during the Pempek kneading process.

Keywords : Pempek; SEM, microstructure; protein network; Palembang

INTRODUCTION

Pempek, as a traditional food, has become an essential part of cultural identity, history and lifestyle, especially for the people of South Sumatra. According to (Trichopoulou *et al.,* 2007) (Guerrero *et al.*, 2009)(Vanhonacker *et al.*, 2013), traditional foods have local characteristics (place of origin) that include aspects of materials (traditional ingredients), composition (traditional composition) and technology (traditional way of production processing).

Pempek comes in many variations due to its evolution. It is made from three types of basic doughs consisting of the two main ingredients of carbohydrate (cassava starch) and protein (minced fish), namely lender dough, again dough (using coconut milk) and fish skin dough. The variations of the three doughs can be classified by raw materials (flour, fish meat and fish skin meal), process (boiled/steamed, fried and baked) and shape (cylindrical, round and corrugated).

The composition of cassava starch, minced with water and without fat, is decisive for gel formation. In the raw state, stirring causes the starch granules to become physically entrapped in the protein matrix

(electrostatic and hydrophobic), making them a continued fraction. Two different processes take place during the heating process. The first is heating above the gelatinization temperature of the starch. The starch granules swell by absorbing water until they break apart so that amylose and amylopectin are dispersed, forming a thick solution that fills the empty spaces in the protein matrix. Secondly, denatured proteins exhibit gelling behaviour and form a matrix together with the starch through cross-linking bonds that transform the starch into a continuous fraction (Fan *et al*., 2017).

The interaction between cassava starch, which acts as a gelling agent, and minced fish, which acts as a tissue former (Joshi *et al*., 2014), plays an essential role in forming texture quality and food stability (Alvarez *et al*., 2012) (Tietze *et al.,* 2016). Understanding the morphology of a food product is vital to improving its quality and stability (Aguilera and Park, 2016) The structure is the spatial arrangement of different structural elements and their interactions. Correctly understanding the relationship between structure and function helps in designing spatial arrangements to produce foods of good quality and stability. Structures can be studied through visual observation techniques.

A reasonably good tool for visual observation to produce surface images to study morphology is the scanning electron microscope (SEM) (Heertje, 2013), which uses the interaction between irradiated electrons and the sample. The essential function of SEM is the magnification and high-resolution imaging of surface structures (Yoshida *et al*., 2016) (Pereira-da-silva and Ferri, 2017). SEM has been widely used to observe the surface of various foods: Sausage (Horita *et al*., 2014), Cheese (El-bakry and Sheehan, 2014) (Chong *et al*., 2017), Rice flour (Saleh, 2017) and Bread Dough (Upadhyay *et al*, 2012). This study aimed to quantitatively investigate the surface morphology of pempek using SEM images.

MATERIALS AND METHODS

Material

The study used pempek lenjer purchased from 10 brands in Palembang, Indonesia. The retailers were selected based on the type of fish used in preparing pempek, which included snakehead fish (G), mackerel fish (T), and a combination of both (TG). The ratio of fish meat to tapioca used in making pempek was 1:1.

Methods

The surface morphology of pempek was studied in The Integrated Laboratory for Research and Testing, Gadjah Mada University. The pempek lenjer was sliced and freeze-dried before being thinly coated with Au metal. Afterwards, the specimen was carefully positioned inside the designated sample chamber of the scanning electron microscope (SEM, JEOL serial number 6510 LA), and the tube was vacuumed to 10-6 Torr to ensure that no air was trapped in the SEM column and the sample. Pempek surface images were viewed at magnifications of 20x, 50x, 100x, 250x, 500x and 1000x. The images and composition of the sample with an SEM instrument were performed by placing and sticking the sample on the SEM sample holder with the longitudinal section facing upwards of the objective lens.

The data in SEM images were further processed using MountainsMap@ SEM Topo 7.4.8226 software from Digitalsurf to determine the morphological features. Pempek protein matrix analysis was performed with the angiotool software from the National Cancer Institute Center for Cancer Research (Bernklau *et al*., 2016) (Zudaire *et al*., 2011).

Research Procedure

Slice-shaped samples that have been dried with a freeze dryer and coated with aurum (Au) metal are placed in the sample position in a Scanning Electron Microscope (SEM) and the tube is vacuumed. Images of the pempek surface were observed with magnifications of 20x, 50x, 100x, 250x, 500x

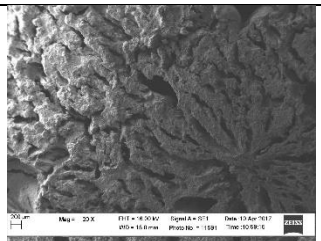
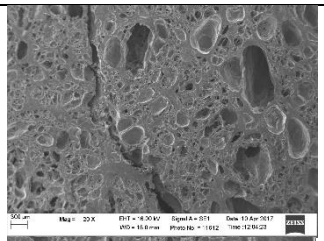
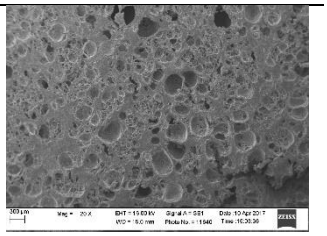
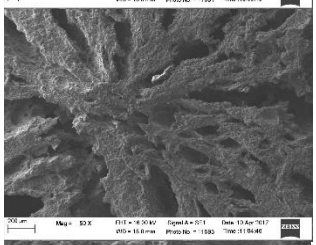
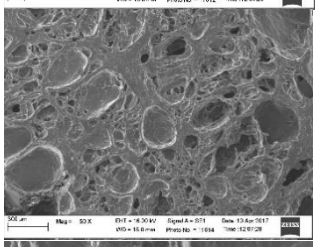
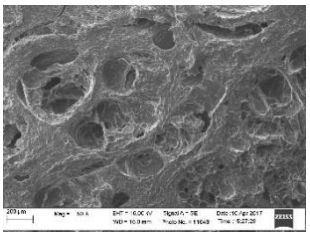
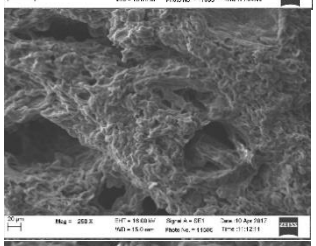
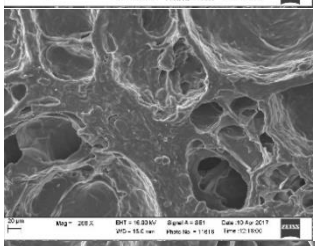
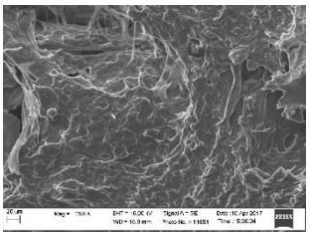
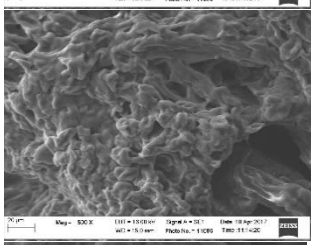
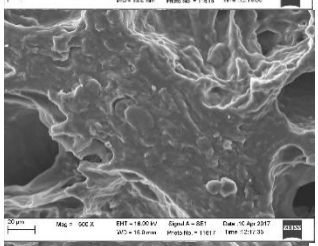
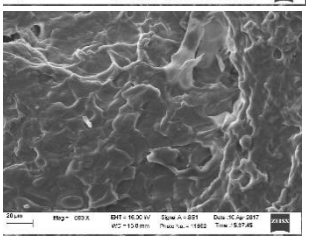
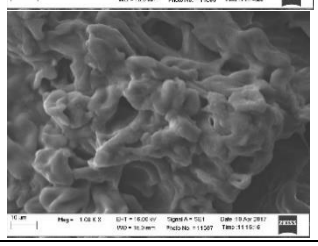
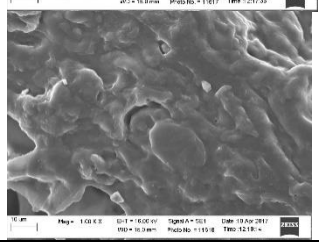
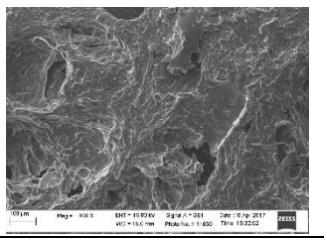
and 1000x. If you get a good image, then save it as a file. Data in the form of SEM images were further processed to determine morphological characteristics. Quantification of surface morphology from images taken using SEM was carried out using the MountainsMap@SEM Topo 7.4.8226 software from Digitalsurf. The protein matrix from pempek was analyzed using Angiotool software from the National Cancer Institute Center of Cancer Research.

RESULTS AND DISCUSSION

Microstructure

A total of ten samples of pempek were scanned using SEM. The resulting images were then grouped into the same morphological form. The results of the morphological grouping of pempek are shown in Table 1.

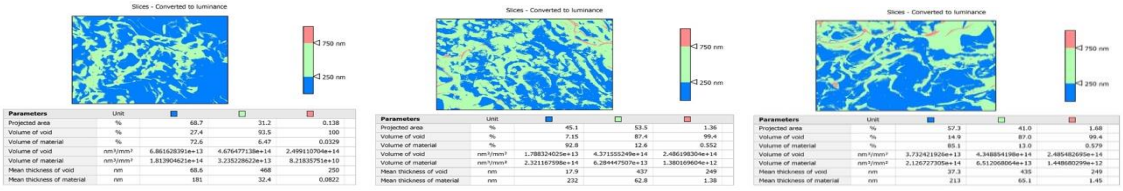
Table 1. Microstructure of pempek

Magnification	Sample Code		
	T (Mackerel)	TG (Mix)	G (Snakehead)
20 X			
50 X			
250 X			
500 X			
1000 X			

The morphology represented by the image SEM in Table 1 results from capturing Pempek specimens at different magnifications. The overall distribution of the morphology can be seen in three forms, namely, sponge/hole (G) creating a space (void), fractal/open/cracked (T) and a mixture of fractals and holes (TG).

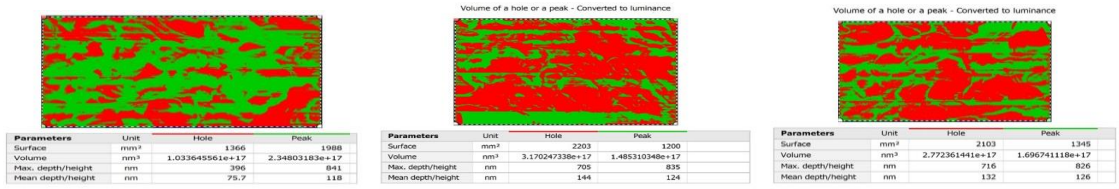
It is shown in Figure 1. It was highest for the T sample (68.7%), compared to the G sample (57.3%), and lowest for the TG sample (45.1%). The second level of contours between 250 - 750 nm shows that the T-sample has the lowest percentage (31.2%), followed by the G-sample (41%) and the highest percentage for the TG-sample (53.5%). For the contour above 750 nm, the T-sample and the TG-sample had the same percentage (1.3%), and the G-sample had the highest percentage (1.6%).

The percentage of void volume (space) on the contour below 250 nm shows that the T-sample has the highest percentage (27.4%), followed by the G-sample (14.9%) and the lowest of the TG-sample (7.15%). For the contour between 250 - 750 nm, the T-sample had the highest percentage (93.5%) compared to the G-sample (87%) and the TG-sample (87%). For contours above 750 nm, the T-sample had 100% pore formation, followed by the G- and the TG - samples with 99.4%.



T TG G

Figure 1. Void volume of pempek surface (T: Mackerel, TG: Mix, G: Snakehead)



T TG G

Figure 2. The hole volume of pempek surface (T: Mackerel, TG: Mix, G: Snakehead)

The highest number of holes (pores) was found in the TG sample (3.1 x 1017), followed by the G sample (2.7 x 1017) and the T sample (1 x 1017) (Figure 2). The surface roughness is influenced by the formation of holes and fractals (Figure 3). Voids form in the protein matrix caused by the inclusion of starch granules and air in the dough. Starch grains with limited availability of free water in the protein network lead to disruption of starch gelatinization. As a rule, cavities whose size is larger than that of the starch grains form. The heating process as the cooking temperature rises (Zhuang *et al.*, 2018).

The stability of the composite structure of polysaccharides (cassava starch) and protein (minced fish meat) is influenced by one of the ratios of protein to carbohydrate (Taylor *et al.*, 2010). Thermal influences cause protein and starch to undergo independent transformation, denaturation and aggregation in the case of protein and gelatinization in the case of starch (Hamann, Wu and Lanier, 1985).

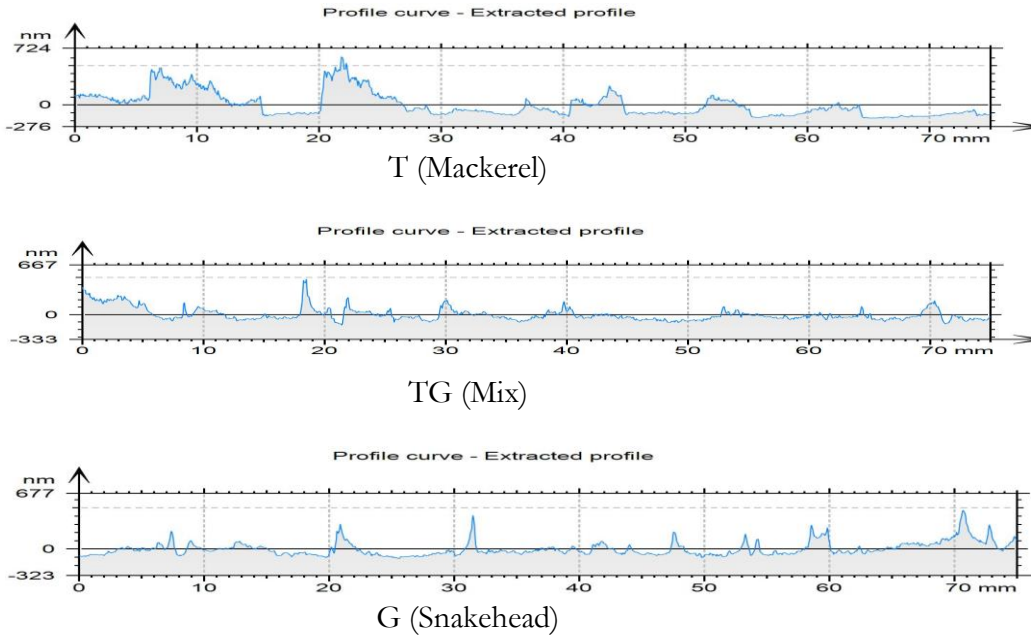


Figure 3. The roghness of the pempek surface

Protein gels are divided into two types, namely particle gel and string gel. Gel particles consist of protein deposits that form spherical strands in small or large quantities and have an irregular fractal structure. Strand gels are delicate polymeric fibre gels that form interlacing and connecting zones. The distribution of the homogeneous protein (strand gel) in the composite formed a smooth structure (Table 1, G and TG), while the distribution of the inhomogeneous protein (particle gel, T) formed an open structure because the breaking stress of the gel particles was lower 23 (kPa) than that of the strand gel (26 kPa). Protein concentration affects the gel-breaking stress; high protein concentration causes the gel-breaking stress to be low. (Heertje, 2013). Areas with low protein concentration in the composite that are not homogeneous become weak points, resulting in low stress.

Protein Network

The protein network is crucial for the properties of starch composites with proteins, such as texture, structure, and morphology (Fan *et al.*, 2017a). Therefore, quantification of the protein network in Pempek is essential. The images from the SEM were then processed using Angio Tool software, which was able to visualize the protein network. However, the Angio Tool software has the disadvantage of not displaying the entire structure of the protein network on the image. The parameters used to quantify the network are calculated using the attributes explant area (region of interest in which the entire network is embedded), vessel area (area occupied by the protein network, μm2), percentage of vessel area (vessel area/explant area * 100), the total number of nodes (total number of nodes in the protein network), node density (number of nodes/explant area), the total length of vessels, μm (sum of all protein filaments/distance between two branches), the average vessel length (μm), the total number of endpoints (open-ended protein filaments), the mean toxicity (a measure of the degree of gaps and irregularities), the branching rates (number of branches/protein area), the endpoint rates (number of endpoints/protein area), the protein width (protein area/total length), the last three attributes describe the strength of the protein networks (Bernklau *et al.*, 2016).

The result of the Pempek network analysis confirmed the differences in surface morphology, consisting of pitted contours, fractal contours and mixed contours, and the morphological quantification data based on

the SEM images (Figure 4). The hole contour is formed by strand gel, a polymer molecule composed of fine fibres that form interlacing and connecting zones. In contrast, the fractal contours formed by particle gel consist of protein deposits that form round strands (Heertje, 2013).

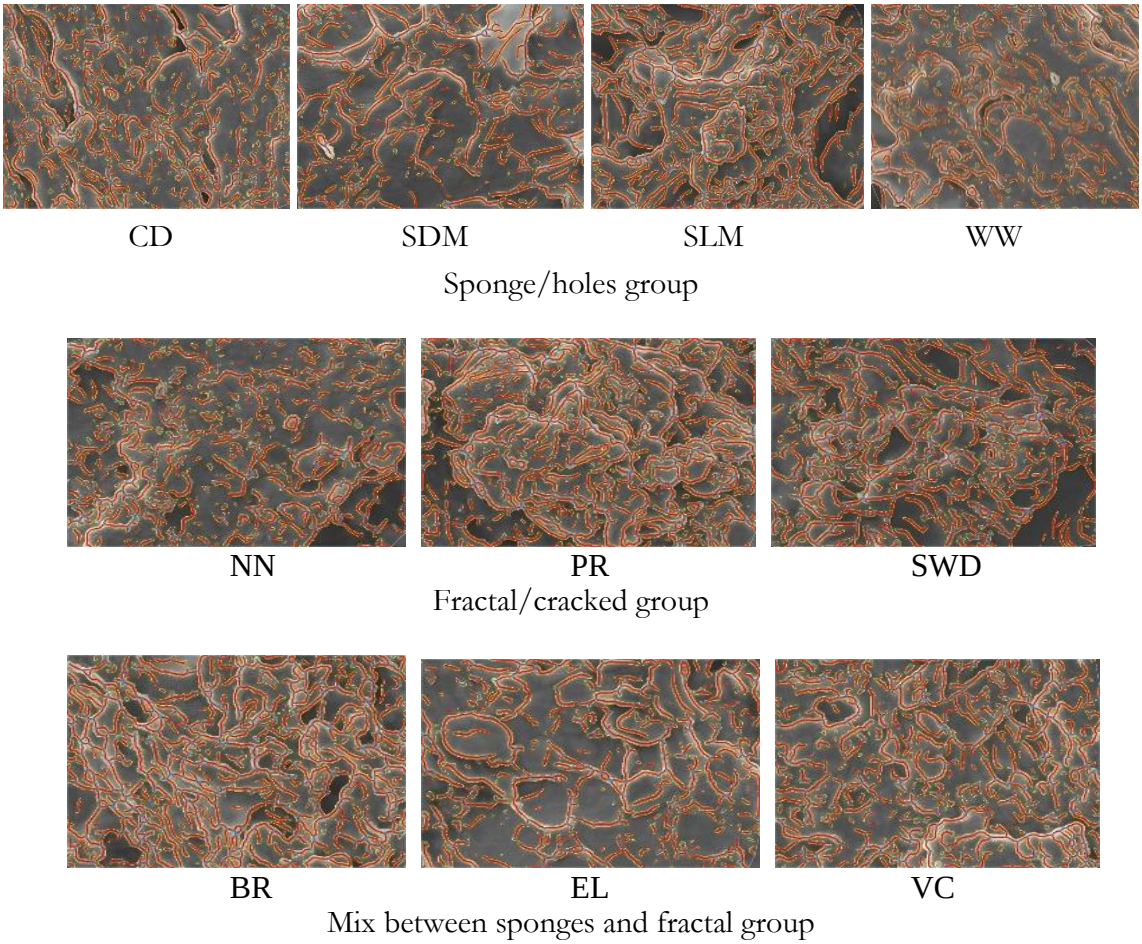


Figure 4. Protein network analysis of pempek after processing by Angio Tool (Blue = junction, Red = protein skeleton, yellow = protein outline/area)

For the average hole contour, the percentage of protein area is smaller (26.76%) than for the fractal contour (29.9%), and the mixed contour has a larger protein area (30.37%). The number of crossovers on the hole contour is smaller (200) than on the fractal contour (261), and there are more crossover proteins on the mixed contour (240). The small number of protein connections in the protein network of the hole contour is probably due to the low ionic strength of the type and the amount of salt added. The protein's ability to open its structure decreases as the ionic strength moves further away from its isoelectric point (Wu *et al.*, 2016).

Since the hole contour consists of filamentous gel with exemplary polymer fibril molecules, the total number of endpoints (the number of open protein networks) is more significant (539) than that of fractal contours (538), which are composed of particle gel and mixed contours (471). Therefore, the degree of patchiness of perforated contours is more extensive (0.18) than that of fractal contours and mixed contours (0.15). A more significant number of hole contour points means more broken or open protein matrix numbers (as evidenced by the low branching rate of 0.001 and a higher end point rate of 0.0029), shows the concentration of added protein in the

higher Pempek formula compared to Pempek with fractal contours and mixed contours, because the higher the protein concentration, the lower the stress of protein degradation, so many protein networks are open (Heertje, 2013).

CONCLUSIONS

It is assumed that the ratio of protein to carbohydrate and the homogeneity of the mixture influence the formation of the morphological structure of pempek. It was found that the surface morphology of Pempek takes three forms, namely holes (G), fractals (cracks) (T) and blends (TG). Protein network analysis shows that Pempek with hole contours has a vascular surface, a lower total number of protein connections and a higher endpoint than Pempek with fractal contours and mixed contours.

The significance of this study is that the research on the morphological quantification of Pempek is the first to be conducted. We propose that quantification of the surface morphology of Pempek can be related to functional properties to understand the structural function of Pempek better.

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