



ISSAAS - SEAPPRO

The International Society for Southeast Asian Agricultural Sciences
Southeast Asia Plant Protection

Joint International Seminar and Congress 2022
"Smart Agriculture: Challenges and Opportunities"



The International Society for
Southeast Asian Agricultural Sciences



Organized by:

IPB University
— Bogor Indonesia —

Department of Plant Protection
Faculty of Agriculture



Society for Agricultural Education
Research Development Abroad

Certificate

This certificate is presented to:

AGUS SUPRIADI

as

ORAL PRESENTER

ISSAAS - SEAPPRO

(The International Society for Southeast Asian Agricultural Sciences - Southeast Asia Plant Protection)

Joint International Seminar and Congress 2022

"Smart Agriculture: Challenges and Opportunities"

November 3-5, 2022 - Bogor, Indonesia

Head of
Department of Plant Protection

Dr. Ali Nurmahsyah

President of
ISSAAS

Dr. Suwardi

Chairman of
ISSAAS-SEAPPRO
International Seminar & Congress 2022

Prof. Dr. Dadang

The Study of Pempek Morphology Based on the SEM Image

Agus Supriadi¹, Daniel Saputra², Gatot Priyanto³, Eka Lidiasari³, Sigit Purwanto⁴, Siti Nurhasanah⁵

¹Department of Fisheries, Fish Product Technology Study Programs/Sriwijaya University

²Department of Agricultural Technology, Agricultural Engineering Study Program/Sriwijaya University

³Department of Agricultural Technology, Agricultural Product Technology Study Program/ Sriwijaya University

⁴Department of Nursing, Faculty of Medicine/Sriwijaya University

⁵Departement of Food Technology, Faculty of Agroindustrial Technology/Padjajaran University

Corresponding author at: Department of Fisheries, Fish Product Technology Study Programmes/Sriwijaya University, Indralaya 30862, South Sumatra, Indonesia. Email: agussupriadi_thi@unsri.ac.id

Abstract

The standardised 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 quality of the taste and especially the texture of the pempek. One method of measuring the taste quality of pempek is to examine the microstructure of pempek. The study of the microstructure of pempek is important for understanding the relationship between the composition, processing and final properties of pempek. This study provides quantitative data on microstructure to develop a high quality Pempek. Microstructure studies can be performed by visual analysis of SEM (scanning electron microscopy) images. The study shows that there are three forms of surface morphology of pempek: a sponge (hole), a 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 morphological formation structure of Pempek was influenced by the ratio of protein to carbohydrates and the homogeneity of the composite during the Pempek kneading process.

Keywords: Pempek, SEM, microstructure, protein-network

1. 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 [1][2][3], 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 meat), namely lenjer dough, adaan dough (using coconut milk) and 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 and fish mince 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. During the heating process, two different processes take place. The first is heating above the gelatinisation 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 [4].

The interaction between cassava starch, which acts as a gelling agent, and minced fish meat, which acts as a tissue former [5], plays an essential role in forming texture quality and food stability [6][7]. One of the ways to improve product quality and stability is to understand the structure of the food, which is expressed in the morphology of the product [8]. The structure is the spatial arrangement of different structural elements and their interactions. A proper understanding of 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) [9], 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 [10][11]. SEM has been widely used to observe the surface of various foods: Sausage [12], Cheese [13][14], Rice flour [15], Dough [16], Gluten [17] and Bread Dough [18]. This study aimed to investigate the surface morphology of pempek using SEM images quantitatively.

2. Material and methods

2.1. Material

The material used in this study was pempek lenjer purchased from 10 brands in Palembang, Indonesia. The retailers were selected based on the type of fish used in preparing pempek. The fish were snakehead fish (G), mackerel fish (T) and a mixture of snakehead and mackerel fish (TG). The ratio of fish meat to flour used in the preparation of pempek is 1:1.

2.2. Methods

The study of the surface morphology of pempek was conducted in the Central Agricultural Postharvest Testing Centre (government-accredited) laboratory of the Indonesian Ministry of Agriculture in Bogor. The cylindrical pempek was sliced and then freeze-dried before being coated very thinly with Au metal. The sample was then placed in the 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 in 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 the form of SEM images were further processed using MountainsMap@ SEM Topo 7.4.8226 software from Digital Surf to determine the morphological features. Pempek protein matrix analysis was performed with the AngioTool software from the National Cancer Institute Center for Cancer Research [19][20].

3. Results and discussion

3.1. 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 Figure 1.

The morphology represented by the image SEM in Figure 1 is the result of 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).

The height of the contours of the samples was categorised as 0 to 250 nm, 250 to 750 nm and above 750 nm. The percentage of surface contours at the height of 250 nm for the samples is shown in Figure 2. 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%.

The highest number of holes (pores) was found in the TG sample (3.1×10^{17}), followed by the G sample (2.7×10^{17}) and the T sample (1×10^{17}) (Figure 3). The formation of holes and fractals on the surface results in different roughness (Figure 4).

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 gelatinisation. As a rule, cavities form whose size is larger than that of the starch grains. The heating process forms fractals or cracks and fissures due to water flow/water intrusion, which increases as the cooking temperature rises [21].

The stability of the composite structure that forms polysaccharides (cassava starch) and protein (minced fish meat) is influenced by one of the ratios of protein to carbohydrate [22]. Thermal influences cause protein and starch to undergo an independent transformation, denaturation and aggregation in the case of protein and gelatinisation in the case of starch [23].

Protein gels are divided into two types, namely particle gel and strand 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 gels consisting of polymeric fibrils that form interlacing and connecting zones. The distribution of the homogeneous protein (strand gel) in the composite formed a smooth structure (Figure 1, G and TG), while the distribution of the inhomogeneous protein (particle gel) (T) formed an open structure because the fracture stress of the gel particles was lower 23 (kPa) than that of the strand gel (26 kPa). The breaking stress depends on the protein concentration. The higher the protein concentration, the lower the fracture stress [9]. Areas of low protein concentration in the composite that are not homogeneous become weak points, resulting in low stress.

3.2. Protein Network

The protein network plays an essential role in the properties of starch composites with proteins such as texture, structure and morphology [24], so the quantification of the protein network in Pempek is essential. The images from SEM were then processed using Angio Tool software, which was able to show the protein network. However, the Angio Tool software has the disadvantage

that it cannot show the entire structure of the protein network on the image. The parameters for quantifying the network are determined using the attributes explant area (region of interest in which the entire network is embedded), vessel area (area occupied by the protein network, μm^2), 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 threads/distance between two branches), average vessel length (μm), the total number of endpoints (open-ended protein threads), mean toxicity (a measure of the degree of gaps and irregularities), branching rates (number of branches/protein area), endpoint rates (number of endpoints/protein area), protein width (protein area/total length), the last three attributes describe the strength of the protein networks [25].

The result of the pempek network analysis confirmed the differences in surface morphology consisting of hole contours, fractal contours and mixed contours, as well as the morphological quantification data based on SEM images (Figur 5). The hole contour is formed by strand gel, a polymer molecule of fine fibres that form interlacing and connecting zones. In contrast, fractal contours formed by particle gel consist of protein deposits that form round strands [9].

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 junctions on the hole contour is smaller (200) than on the fractal contour (261), and there is more junction protein on the mixed contour (240). The low amount of protein junction 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, with the ionic strength far from the isoelectric point of the protein, so the ability to open the protein structure becomes lower [26].

Since the hole contour consists of stranded 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 consist of particle gel and mixed contours (471). Therefore the degree of lacunarity of hole 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 endpoint rate of 0.0029), shows the concentration of protein added to the higher pempek formula compared to pempek with fractal contours and mixed contours, because the higher the protein concentration, the lower the protein decay stress, so many protein networks are open [9].

4. Conclusions

The ratio of protein to carbohydrate and the homogeneity of the mixture are thought to influence the formation of the morphological structure of pempek. The surface morphology of Pempek was found to take three forms, namely holes (G), fractals (cracks) (T) and mixtures (TG). Analysis of the protein network shows that pempek with hole contours has a vessel area, a lower total number of protein compounds and a higher endpoint than pempek with fractal contours and mixed contours.

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

Author contribution statement

Supriadi and Priyanto compiled and designed the programme. Supriadi designed and experimented, Lidiasari assisted in experimenting. Purwanto provided expertise and guidance on matrix analysis. Supriadi and Saputra wrote the paper.

Acknowledgements

The authors thank the Sriwijaya University Research Development Fund (2022) for supporting this research.

References

- [1] A. Trichopoulou, S. Soukara, and E. Vasilopoulou, "Traditional foods: a science and society perspective," *Trends Food Sci. Technol.*, vol. 18, no. 8, pp. 420–427, 2007, doi: 10.1016/j.tifs.2007.03.007.
- [2] L. Guerrero *et al.*, "Consumer-driven definition of traditional food products and innovation in traditional foods. A qualitative cross-cultural study," *Appetite*, vol. 52, no. 2, pp. 345–354, 2009, doi: 10.1016/j.appet.2008.11.008.
- [3] F. Vanhonacker, B. Kühne, X. Gellynck, L. Guerrero, M. Hersleth, and W. Verbeke, "Innovations in traditional foods: Impact on perceived traditional character and consumer acceptance," *Food Res. Int.*, vol. 54, no. 2, pp. 1828–1835, 2013, doi: 10.1016/j.foodres.2013.10.027.
- [4] M. Fan, T. Hu, S. Zhao, S. Xiong, J. Xie, and Q. Huang, "Gel characteristics and microstructure of fish myofibrillar protein / cassava starch composites," *Food Chem.*, vol. 218, pp. 221–230, 2017, doi: 10.1016/j.foodchem.2016.09.068.
- [5] M. Joshi, P. Aldred, J. F. Panozzo, S. Kasapis, and B. Adhikari, "Food Hydrocolloids Rheological and microstructural characteristics of lentil

- starch e lentil protein composite pastes and gels,” *Food Hydrocoll.*, vol. 35, pp. 226–237, 2014, doi: 10.1016/j.foodhyd.2013.05.016.
- [6] M. D. Alvarez, C. Fernández, M. D. Olivares, and W. Canet, “A rheological characterisation of mashed potatoes enriched with soy protein isolate,” *Food Chem.*, vol. 133, no. 4, pp. 1274–1282, 2012, doi: 10.1016/j.foodchem.2011.05.111.
- [7] S. Tietze, M. Jekle, and T. Becker, “Possibilities to derive empirical dough characteristics from fundamental rheology,” *Trends Food Sci. Technol.*, 2016, doi: 10.1016/j.tifs.2016.08.016.
- [8] J. M. Aguilera and D. J. Park, “Texture-modified foods for the elderly: Status, technology and opportunities,” *Trends Food Sci. Technol.*, vol. 57, pp. 156–164, 2016, doi: 10.1016/j.tifs.2016.10.001.
- [9] I. Heertje, “Structure and function of food products : A review,” *Food Struct.*, vol. 1, no. 1, pp. 3–23, 2013, doi: 10.1016/j.foostr.2013.06.001.
- [10] A. Yoshida, Y. Kaburagi, and Y. Hishiyama, *Scanning Electron Microscopy*. Tsinghua University Press Limited, 2016.
- [11] M. D. A. Pereira-da-silva and F. A. Ferri, *1 - Scanning Electron Microscopy*. Elsevier Inc., 2017.
- [12] C. N. Horita, V. C. Messias, M. A. Morgano, F. M. Hayakawa, and M. A. R. Pollonio, “Textural, microstructural and sensory properties of reduced sodium frankfurter sausages containing mechanically deboned poultry meat and blends of chloride salts,” *Food Res. Int.*, vol. 66, pp. 29–35, 2014, doi: 10.1016/j.foodres.2014.09.002.
- [13] M. El-bakry and J. Sheehan, “Analysing cheese microstructure : A review of recent developments,” *J. Food Eng.*, vol. 125, pp. 84–96, 2014, doi: 10.1016/j.jfoodeng.2013.10.030.
- [14] H. M. Chong, I. K. Mohammed, B. Linter, R. Allen, and M. N. Charalambides, “Mechanical and microstructural changes of cheese cracker dough during baking,” *LWT - Food Sci. Technol.*, vol. 86, pp. 148–158, 2017, doi: 10.1016/j.lwt.2017.07.035.
- [15] M. I. Saleh, “Protein-starch matrix microstructure during rice flour pastes formation,” *J. Cereal Sci.*, vol. 74, pp. 183–186, 2017, doi: 10.1016/j.jcs.2017.02.005.
- [16] M. Jekle and T. Becker, “Dough microstructure: Novel analysis by quantification using confocal laser scanning microscopy,” *Food Res. Int.*, vol. 44, no. 4, pp. 984–991, 2011, doi: 10.1016/j.foodres.2011.02.036.
- [17] J. Li, M. P. Yadav, and J. Li, “Effect of different hydrocolloids on gluten proteins, starch and dough microstructure,” *J. Cereal Sci.*, 2019, doi: 10.1016/j.jcs.2019.03.004.
- [18] R. Upadhyay, D. Ghosal, and A. Mehra, “Characterization of bread dough: Rheological properties and microstructure,” *J. Food Eng.*, vol. 109, no. 1, pp. 104–113, 2012, doi: 10.1016/j.jfoodeng.2011.09.028.
- [19] I. Bernklau, L. Lucas, M. Jekle, and T. Becker, “Protein network analysis — A new approach for quantifying wheat dough microstructure,” *FRIN*,

- vol. 89, pp. 812–819, 2016, doi: 10.1016/j.foodres.2016.10.012.
- [20] E. Zudaire, L. Gambardella, C. Kurcz, and S. Vermeren, “A computational tool for quantitative analysis of vascular networks,” *PLoS One*, vol. 6, no. 11, 2011, doi: 10.1371/journal.pone.0027385.
- [21] X. Zhuang, M. Han, Y. Bai, Y. Liu, L. Xing, and X. Xu, “Food Hydrocolloids Insight into the mechanism of myofibrillar protein gel improved by insoluble dietary fiber,” vol. 74, pp. 219–226, 2018, doi: 10.1016/j.foodhyd.2017.08.015.
- [22] P. Taylor *et al.*, “Critical Reviews in Food Science and Nutrition Structure and Technofunctional Properties of Protein- Polysaccharide Complexes : A Review Structure and Technofunctional Properties of Protein- Polysaccharide Complexes : A Review,” no. March 2013, pp. 37–41, 2010.
- [23] D. D. Hamann, M. C. Wu, and T. C. Lanier, “Thermal Transitions of Admixed Starch/Fish During Heating,” vol. 50, pp. 20–25, 1985.
- [24] M. Fan, T. Hu, S. Zhao, S. Xiong, J. Xie, and Q. Huang, “Gel characteristics and microstructure of fish myofibrillar protein/cassava starch composites,” *Food Chem.*, vol. 218, pp. 221–230, 2017, doi: 10.1016/j.foodchem.2016.09.068.
- [25] I. Bernklau, L. Lucas, M. Jekle, and T. Becker, “Protein network analysis — A new approach for quantifying wheat dough microstructure,” *Food Res. Int.*, vol. 89, pp. 812–819, 2016, doi: 10.1016/j.foodres.2016.10.012.
- [26] L. Wu *et al.*, “Effects of cations on the ‘salt in’ of myofibrillar proteins,” *Food Hydrocoll.*, vol. 58, pp. 179–183, 2016, doi: 10.1016/j.foodhyd.2016.02.028.

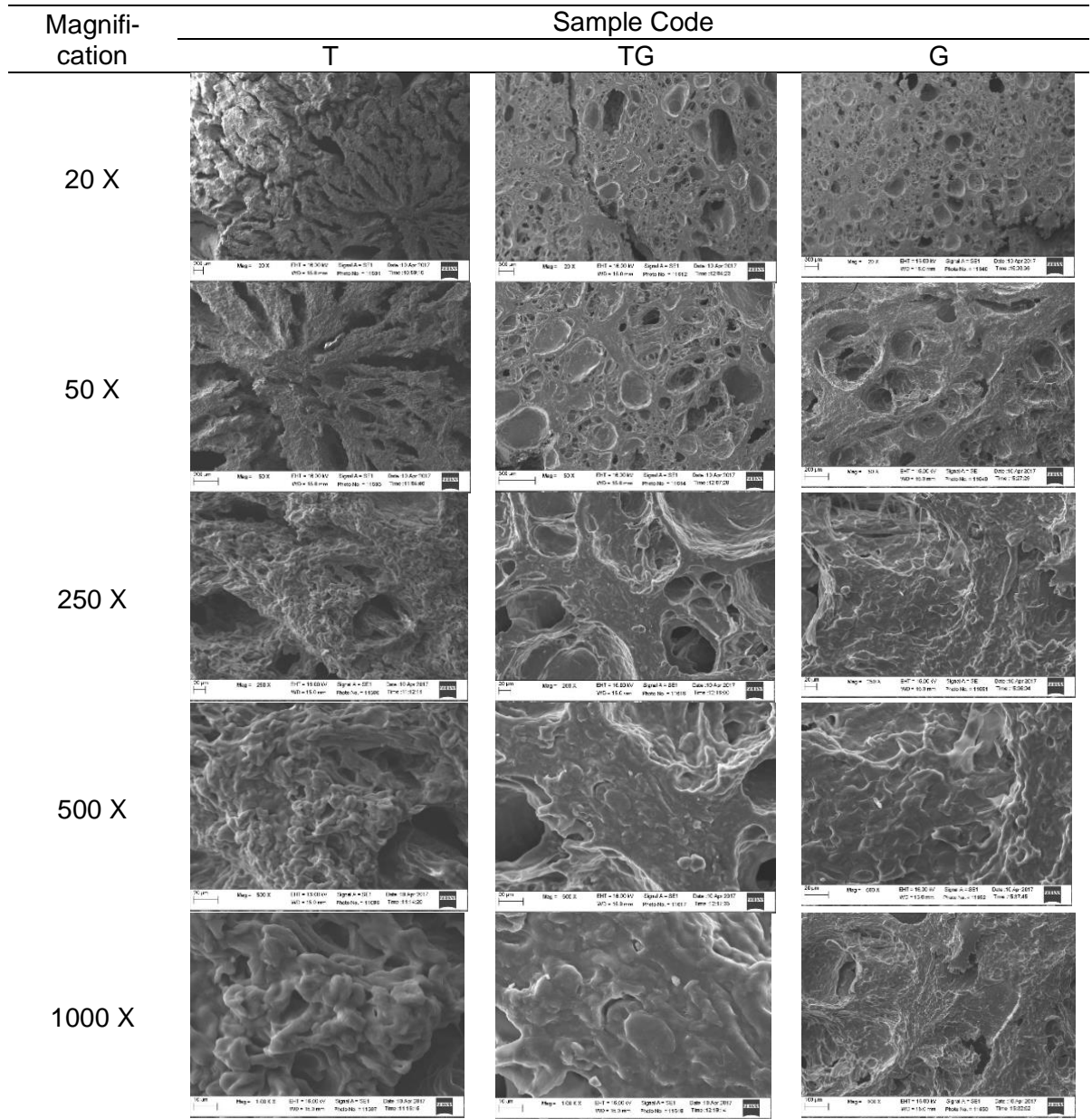
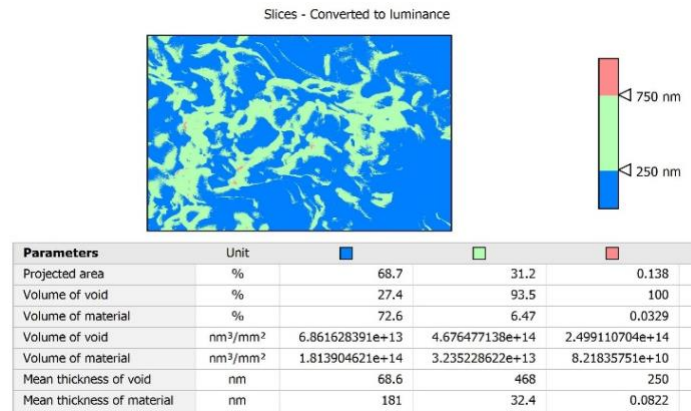
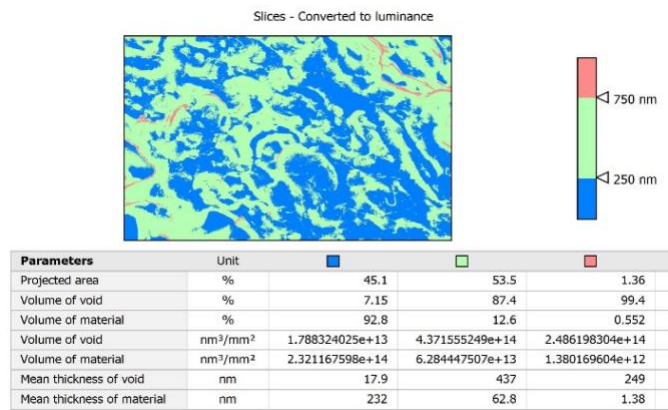


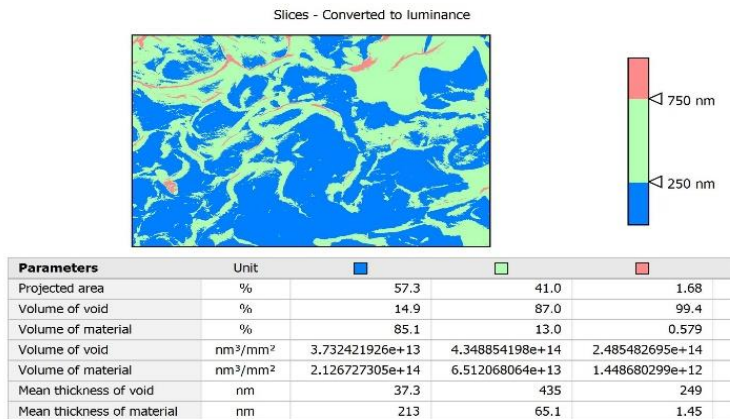
Figure 1. SEM Image of Pempek



T

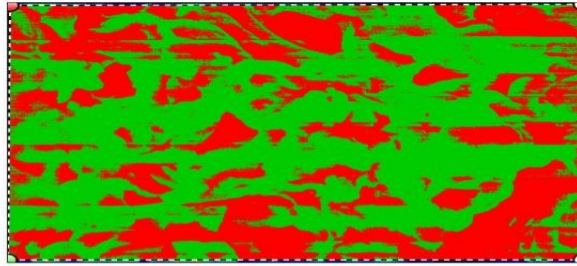


TG



G

Figure 2. Void volume of pempek surface



Parameters	Unit	Hole	Peak
Surface	mm ²	1366	1988
Volume	nm ³	1.033645561e+17	2.34803183e+17
Max. depth/height	nm	396	841
Mean depth/height	nm	75.7	118

T

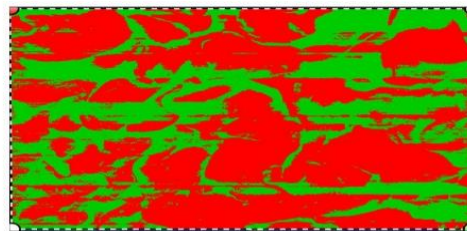
Volume of a hole or a peak - Converted to luminance



Parameters	Unit	Hole	Peak
Surface	mm ²	2203	1200
Volume	nm ³	3.170247338e+17	1.485310348e+17
Max. depth/height	nm	705	835
Mean depth/height	nm	144	124

TG

Volume of a hole or a peak - Converted to luminance



Parameters	Unit	Hole	Peak
Surface	mm ²	2103	1345
Volume	nm ³	2.772361441e+17	1.696741118e+17
Max. depth/height	nm	716	826
Mean depth/height	nm	132	126

G

Figure 3. The hole volume of pempek surface

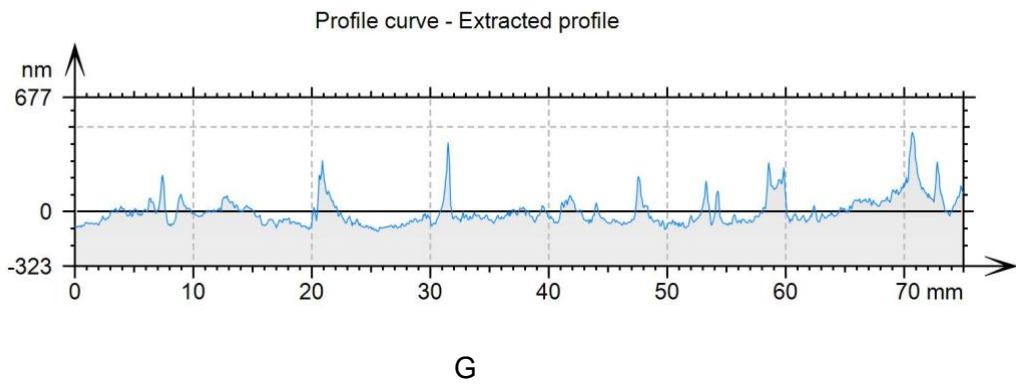
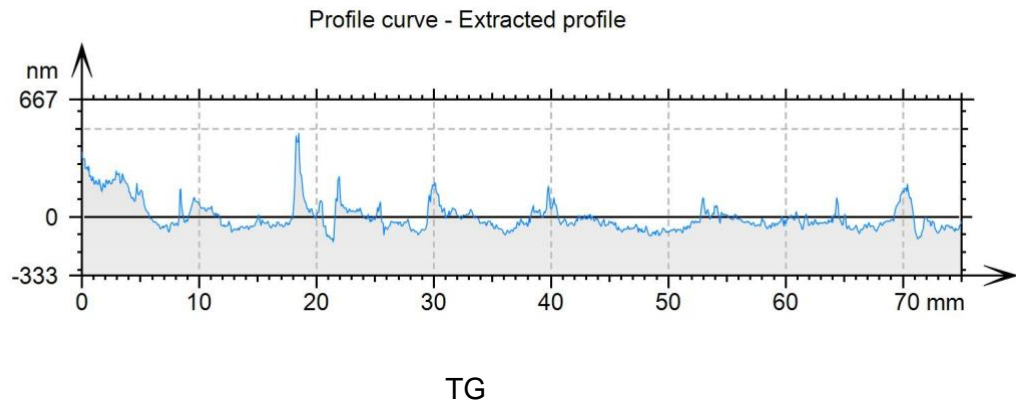
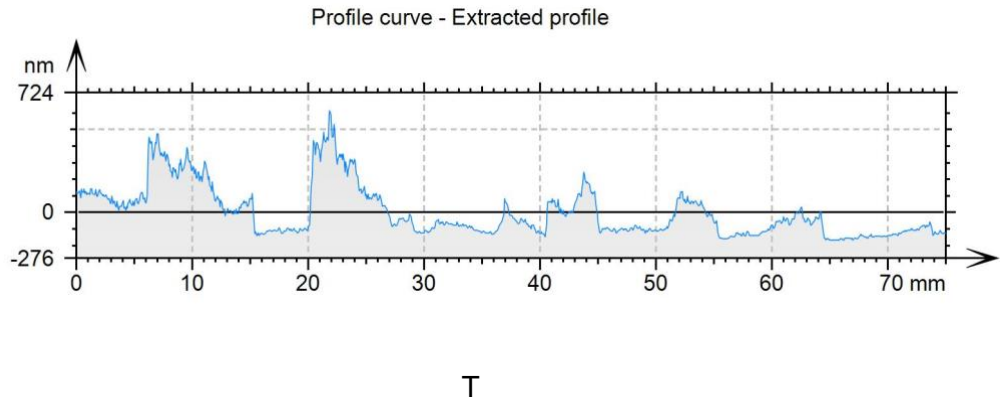
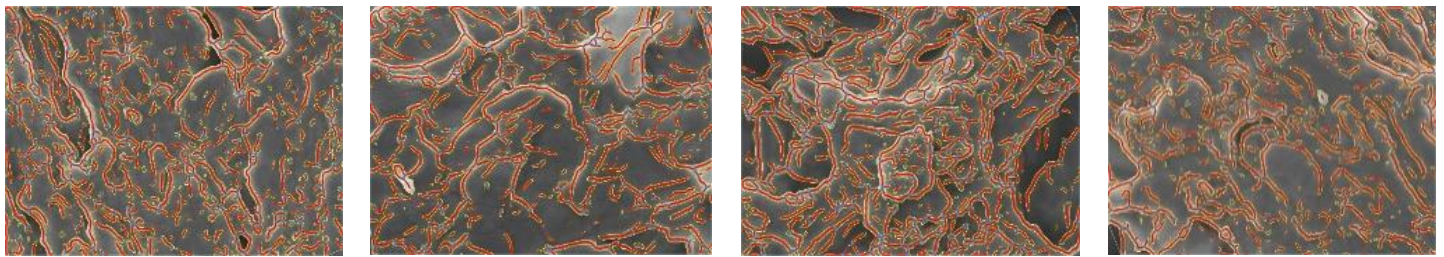


Figure 4. The roghness of the pempek surface



CD

SDM

SLM

WW

Sponge/holes group

(CD: Candy brand, SDM: Sudimampir brand, SLM: Selamat brand, WW: Wawa brand)



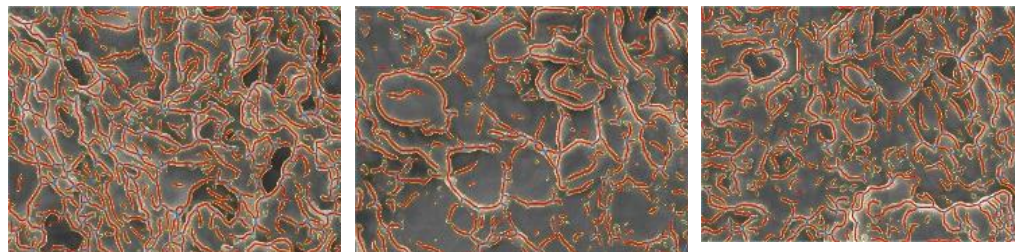
NN

PR

SWD

Fractal/cracked group

(NN: Nony brand, PR: Pak raden brand, SWD: Suwandi brand)



BR

EL

VC

Mix between sponges and fractal group

(BR: Beringin brand, EL: Ellen brand, VC: Vico brand)

Figure 5. Protein network analysis of pempek after processing by Angio Tool
(Blue= junction, Red= protein skeleton, yellow= protein outline/area)