

Evaluating Tyndallisation's potential for composite food products and quality considerations

Agus Supriadi ^{1,*}, Daniel Saputra ², Gatot Priyanto ³, Eka Lidiasari ³, Siti Nurhasanah ⁴ and Sigit Purwanto ⁵

¹ Department of Fisheries, Fish Product Technology Study Program, Sriwijaya University, Indonesia.

² Department of Agricultural Technology, Agricultural Engineering Study Program, Sriwijaya University, Indonesia.

³ Department of Agricultural Technology, Agricultural Product Technology Study Program, Sriwijaya University, Indonesia.

⁴ Department of Food Technology, Faculty of Agroindustrial Technology, Padjajaran University, Indonesia.

⁵ Department of Nursing, Faculty of Medice, Sriwijaya University, Indonesia.

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Abstract

Tyndallization presents potential as a cost-effective alternative sterilisation method for small-scale producers. This research aimed to assess the effectiveness of tyndallization at various temperatures in sterilising pempek, a composite food product, and its impact on product quality. The study employed the SDS-PAGE technique to investigate protein polymerisation mechanisms and FTIR to evaluate protein structure, colour, pH, and texture changes. The results indicated protein polymerisation occurred, evidenced by a decrease in Molecule High Chain (MHC) and an increase in actin, corresponding to the rising temperature and tyndallization cycles with a sinusoidal pattern. FTIR spectra demonstrated that the β -helix structure dominated secondary protein structures, consistently observed across different levels and temperature variations. This study contributes new insights into applying thermal processes in preparing composite food products. Regarding quality, tyndallization caused a shift towards a more yellow colour in the pempek and a softer texture, although there were no significant pH changes. The alterations in protein patterns resulted from the varying cycles and temperatures experienced during the tyndallization process. The findings suggest that tyndallization can be considered an effective alternative for sterilising composite products like pempek; however, its implementation still requires the appropriate combination of time, temperature, and tyndallization cycles while considering the potential impact on quality changes during the sterilisation process.

Keyword: Pempek; Tyndallization; SDS-Page; FTIR

1. Introduction

Many manufacturers strive to extend the shelf life of pempek to maximize storage stability and facilitate distribution. Some efforts include coating pempek with flour for a maximum distribution period of 24 hours and storing it at freezing temperatures combined with vacuum packaging for up to 30 days (1). However, these efforts still need to be more effective to address the pempek shelf-life issue, necessitating the exploration of commercial sterilization approaches.

One commonly used food sterilization method in the industry is thermal sterilization. Thermal sterilization involves heating food to a temperature of 121°C for a specific duration to eliminate microorganisms, typically heat-resistant bacterial spores. Although widely applied, thermal sterilization requires substantial investment in equipment, operation, and large production capacities, making it uneconomical, particularly for small-scale pempek manufacturers.

* Corresponding author: Agus Supriadi

One thermal sterilization alternative that small-scale manufacturers can implement is tyndallization, a fractional thermal method (2). Typically, tyndallization involves performing three heating cycles and incorporating incubation periods between each cycle. Incubation enables the growth of microbes in a vegetative cell form, and subsequent heating eliminates them. Removing microbes in their vegetative form is more accessible than in their spore form. Heating vegetative cells do not necessitate temperatures as high as 121°C under high pressure. This process is similar to the heating method employed in pempek production using boiling water, which makes it technically straightforward for manufacturers to adopt. Furthermore, temperatures below 121°C during processing can contribute to preserving pempek quality (3). Therefore, tyndallization has the potential as an affordable alternative sterilization method for small-scale producers.

Despite its advantages, tyndallization also has some drawbacks, such as the potential for microbial recontamination during the incubation period and the possibility of chemical and physical syneresis in pempek as a product based on a mixture of tapioca and fish. Consequently, relevant efforts to maximize the effectiveness of tyndallization involve packaging. Pempek is made from a blend of tapioca (cassava starch) and minced fish, with a maximum formulation ratio of 80% fish content. Tapioca acts as a gel-forming agent, while fish is a matrix-forming agent. Traditionally, the pempek process begins with adding salt to the fish, followed by stirring, transforming the fish into a sol. The sol is then combined with tapioca and water to create a dough. In the final step, the shaped dough is boiled in water, changing into a composite due to the complex interaction between the tapioca and the sol. This composite leads to a single-phase structure expressed in the product known as pempek.

Consequently, pempek contains nutrients suitable for microbial growth. Additionally, the intensive heating application's destruction affects pempek quality parameters, such as colour and texture. Therefore, sterilization using tyndallization is likely a better option for preserving pempek quality.

The research offers a fresh perspective on using thermal processes in pempek production. Researchers have widely employed fractional sterilization in various applications, such as traditional Korean rice-based beverages (4), Colombian honey (5), Gulabjamun (6), milk (7), and kamaboko products (8). The research results demonstrate that when combined with antibacterial agents, tyndallization is highly effective, particularly for targeting the sterilization of *C. botulinum* and when combined with CO₂ for targeting the sterilization of *B. cereus*. Furthermore, researchers successfully applied tyndallization to kamaboko products but found it unsuccessful for honey and milk products. Tyndallization has been successfully applied to carbohydrate and protein-based products. Therefore, this study aims to determine the effectiveness of tyndallization at different temperatures for the sterilisation of pempek as a composite product and its impact on product quality. This study can provide a new perspective on utilising thermal processes in pempek production.

2. Material and Methods

2.1. Material

Obtain Pempek with a slice dimension (diameter of 5 cm, thickness of 3 cm) from one of the pempek producers in Palembang, Indonesia, and store it at 4°C before use

2.2. Procedure tyndallization

After packaging, the samples were individually in airtight containers, which are divided into three tyndallization temperature treatments: 100°C, 105°C, and 110°C. Perform the tyndallization process three times and then analyze the results.

2.3. Total Plate Count

A 25 g samples were mixed aseptically with 25 ml of aquades and 225 ml of a 0.1% BPW solution. The mixture is then sterilised and homogenised with a stomacher for 1 to 2 minutes, called a suspension. A 1 to 10-fold dilution is then performed. Total plate count (TPC) indicates the number of microorganisms present in a product by counting bacterial colonies grown on agar media (9). For each dilution, 1 ml of the suspension is doubled in a Petri dish and supplemented with 15 to 20 ml of chilled PCA. The Petri dish were incubated for 24 hours at 32°C, turning the dish upside down. Calculation of colony count (TPC) is performed for each dilution, except for Petri dishes containing scattered colonies. Colonies are counted in Petri dishes with a colony count between 25 and 250.

2.4. Color and Whiteness

The color of the pempek was measured using colorimeter CS10 space (L^* , a^* , b^*). The whiteness index was calculated by following equation (10).

$$\text{Whiteness index} = 100 - [(100 - L^*)^2 + a^* + b^*]^{1/2}$$

2.5. Texture

The texture profile analysis (TPA) is conducted using a texture analyzer (TA-XT Express Plus, Stable Micro Systems) equipped with a P/36R probe. Cylindrical samples with a diameter of 15 mm and a height of 20 mm are compressed by 50% at a pre-test speed of 1 mm/s and a post-test speed of 5 mm/s. Duplicate measurements are performed, and the graph is plotted using the Exponent Lite Express software.

2.6. pH

pH measurements are carried out using a pH meter (HANNA instrument HI98107), calibrated beforehand with distilled water to reach neutrality (at a displayed pH of 7). Then, the samples mixed with distilled water up to 50 ml in a beaker are measured three times. The pH values are recorded by the pH meter.

2.7. Fraksi protein terlarut (SDSPage)

Molecular weight analysis is performed with an SDS-PAGE instrument (e-PAGEL, Atto Co., Ltd., Tokyo, Japan). A 1 mg sample is dissolved in 0.1M Na_3PO_4 (pH 7.2) at 1 mg/mL concentration. Then 20 μL of the sample were taken and mixed with sample buffer (10% β -mercaptoethanol, 20% glycerol, 1.5% Tris-HCl buffer (pH 6.8), 4% SDS and 0.1% bromophenol blue) at a ratio of 5:1 (v/v). The samples were then subjected to electrophoresis at 60 V and a current of 25 mA for 3 hours. The sample gel obtained after electrophoresis is soaked for 3 hours in a solution of 30% ethanol and 10% acetic acid in a 1:5 ratio of gel to the solution. Then the solution is replaced with 30% ethanol and shaken for 30 minutes. The ethanol solution is replaced by distilled water during shaking, which is continued for 10 minutes. The sample gels were immersed in a 0.1% AgNO_3 solution with a ratio of 1:5 (b/v) for 30 minutes. The marker used is the multicoloured broad-spectrum protein ladder. The sample gel is rinsed with distilled water for 20 seconds and placed in a 2.5% Na_2CO_3 solution and 0.02% formaldehyde in a 1:5 ratio between the gel and solution, and the gel is rinsed with distilled water. The molecular protein weight of the sample is estimated from the molecular weight marker.

2.8. Struktur sekunder protein (FTIR)

The changes in the secondary structure of proteins are measured using Fourier transform infrared spectroscopy (FTIR) Tensor 37 (Bruker Optik GmbH, Karlsruhe, Germany) with a DTGS detector in the mid-infrared range (4000-400 cm^{-1}) at a resolution of 4 cm^{-1} with 4.2 co-added scans. The FTIR spectrum in data point table format is saved as an ASC file. The data are read with the software Spectragryph1.2 and processed according to the Nature protocol. Subsequently, the FTIR data in the Amide I region are read and interpreted. Smoothing is performed using the Savitzky-Golay equation, and deconvolution is performed using OriginPro® version 2020b software.

3. Results and Discussion

3.1. Total bacterial count

The results of the microbiological count are illustrated in Figure 1. The total plate count (TPC) for the control pempek is 3.3 log CFU/g. A difference of 1 log between the TPC for the control and the initial TPC following the first heating cycle indicates that spores grew during the incubation period after the first heating cycle. This finding suggests that pempek contains beneficial nutrients for microbial growth, even though the TPC values vary at the onset of different heating temperatures. This condition implies that some spores develop into vegetative cells while others remain dormant (11).

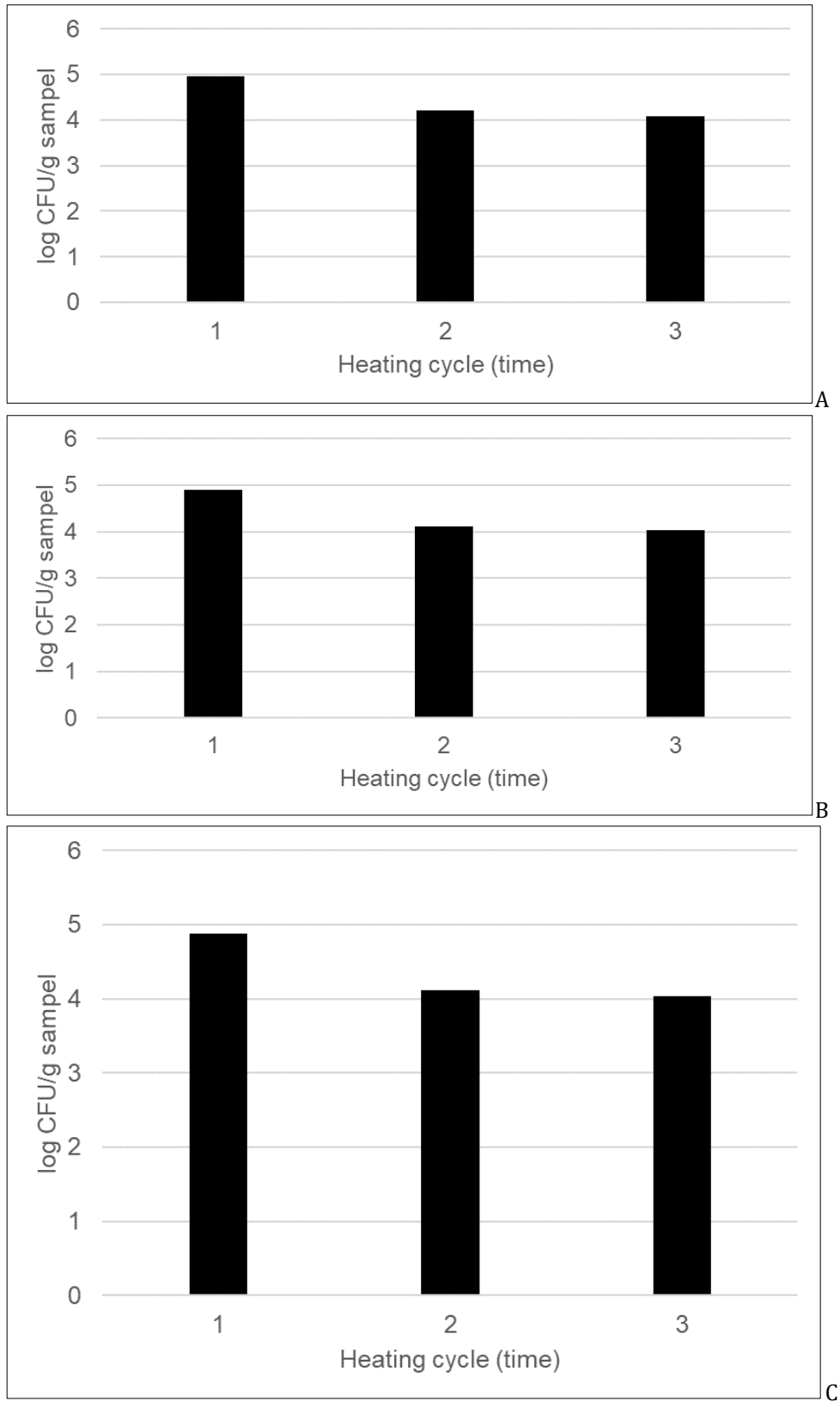


Figure 1 Total Plate Count (TPC) of pempek during each cycle of tyndallization using 100 OC (a), 105 OC (b), and 110 OC (c) as the processing temperatures

The results reveal that at a heating temperature of 100°C, microbial growth peaks during the first heating cycle and decreases with progressive heating cycle. This observation suggests that initial heating effectively controls microbial growth. Similarly, at a heating temperature of 105°C, the pattern resembles that observed at 100°C, albeit with a lower

peak value. This finding indicates that an increase in the process temperature leads to more effective control of microbial growth. The same trend is observed at a temperature of 110°C, with the highest log TPC value reached during the first heating cycle and decreased afterwards. These results demonstrate that higher heating temperatures better control microbial growth (11).

The available data indicate that increasing the process temperature generally reduces microbial growth. The same trend is observed at each process temperature, wherein the log TPC value peaks at the first heating cycle and decreases afterwards. The initial heating cycle has a more significant impact on reducing microbial growth than subsequent heating cycles.

As anticipated, each heating cycle at different temperatures reduces the TPC count. Although heat-resistant bacterial spores appear present in large numbers, as evidenced by the vegetative bacteria count of 4 logs, which is higher than the control by 1 log cycle, this heat treatment temperature is not considered capable of inactivating the spores present in the pempek.

Another observation from the data is that the TPC count decreases with each heating cycle, both at cycles 1, 2, and 3 across all heating temperature levels. However, the TPC number exhibits a similar trend and does not change significantly in the second and third heating cycles at 100°C, 105°C, and 110°C. Heating conditions at temperatures above 100°C with three heating cycles should be able to reduce the TPC number to a lower value than the control. This study contrasts with the findings of (12), wherein tyndallization effectively reduced TPC and spore counts in Kamaboko products. Nonetheless, this study aligns with the reports by (5) that tyndallization is ineffective in honey and dairy products. The contradictory results with (8) could be attributed to differences in raw material composition, as kamaboko is a single-ingredient product, while pempek is a mixed-ingredient product, which certainly has different heat penetration properties. In conclusion, this study could not achieve sterilization conditions with tyndallization at up to 110°C. A combination of additional cycles and temperature variations may be required to achieve commercially sterile conditions.

3.2. Pempek color and whiteness

The colour of pempek changes after heat treatment, becoming yellow due to the decrease in L^* , a^* , and whiteness index values (Table 1). Conversely, the b^* value of pempek increases. The highest yellowing indicator, b^* , is observed in the third cycle at 110°C, followed by 105°C, and then 100°C within the same heating cycle. Heat-resistant protease enzymes likely become inactive at these temperatures, resulting in the Maillard reaction (13). The L^* and whiteness index values display a similar decreasing trend as the processing temperature increases. Higher temperatures lead to denaturation, which contributes to the yellowing of pempek. However, there is no significant difference between the L^* and whiteness index values at the same processing temperature. It indicates that the primary factor influencing pempek's colour is the heating temperature, not the heat cycle. These findings are in line with the research results of (8).

Table 1 Color and whiteness index of pempek during tyndallization at various temperatures

Process temperature (oC)	Heating cycle (time)	L^*	a^*	b^*	Whiteness index
Control	0	60,15	-4,03	8,32	59,09
100	1	62,80	-6,14	8,42	61,37
	2	60,89	-5,88	9,00	59,44
	3	60,65	-5,61	9,88	59,04
105	1	61,24	-5,21	9,86	59,67
	2	58,35	-4,62	11,20	56,63
	3	58,15	-4,23	12,17	56,21
110	1	58,60	-4,85	10,54	57,00
	2	57,61	-3,97	12,14	55,73
	3	57,05	-3,20	13,48	54,87

In Table 2, the pempek tindallized is distinguished by its colour from the control pempek. The rate of colour change in L^* accelerates as the heating temperature increases. Similarly, the a^* value indicates an increasing redness, and the b^* value indicates an increasing yellowness. The total colour difference between the heated pempek at 100°C and the control pempek is -0.55, 105 °C is 1.02, and at 110 °C is 1.62. It shows that the heated pempek is darker and more yellow than the control pempek. The difference in darker and more yellow colour increases with the rise in heating temperature. The colour change of pempek during heating indicates the Maillard reaction or non-enzymatic browning due to the influence of heat. The increase in heating temperature causes a reaction between the reducing sugars from tapioca, used as a base ingredient for pempek, and the free amino groups from the fish protein.

Table 2 Colour difference between heated pempek and control

Color notation	Proces temperature (°C)	Heating cycle (time)			Slope
		1	2	3	
ΔL^*	100	2,65	0,74	0,5	-1,075
	105	1,09	-1,8	-2	-1,545
	110	-1,5	-2,54	-3,1	-0,8
Δa^*	100	-2,11	-1,85	-1,58	0,265
	105	-1,18	-0,59	-0,2	0,49
	110	-0,82	0,06	0,83	0,825
Δb^*	100	0,1	0,68	1,56	0,73
	105	1,54	2,88	3,85	1,155
	110	2,22	3,82	5,16	1,47
ΔE	100	3,39	2,1	2,28	-0,55
	105	2,29	3,45	4,34	1,025
	110	2,83	4,58	6,07	1,62

3.3. Textural properties

The texture of Tyndallized pempek, as shown in Table 3, indicates that hardness undergoes a positive change, with increasing stages of tyndallization resulting in softer pempek, and the rate of change is more significant as the temperature rises.

That is due to the complexity of the physical interactions between proteins and polysaccharides induced by temperature. During heating, conformational and structural changes occur in the biopolymer proteins and carbohydrates within pempek, altering the stability of the protein-polysaccharide interaction complex and decreasing pempek hardness.

Table 3 Hardness and pH of pempek during tyndallization at various temperatures

Parameter	Proces temperature (°C)	Control	Heating cycle (time)			Slope
			1	2	3	
Hardness (g.force)	100	3246,7	1535,8	1468,6	1134,3	-640,44
	105	3246,7	862,8	773,4	769,6	-752,07
	110	3246,7	3481,4	858,2	320,2	-1140,3
pH	100	6,75	6,65	6,55	6,53	-0,076
	105	6,75	6,8	6,56	6,53	-0,09
	110	6,75	6,51	6,53	6,48	-0,079

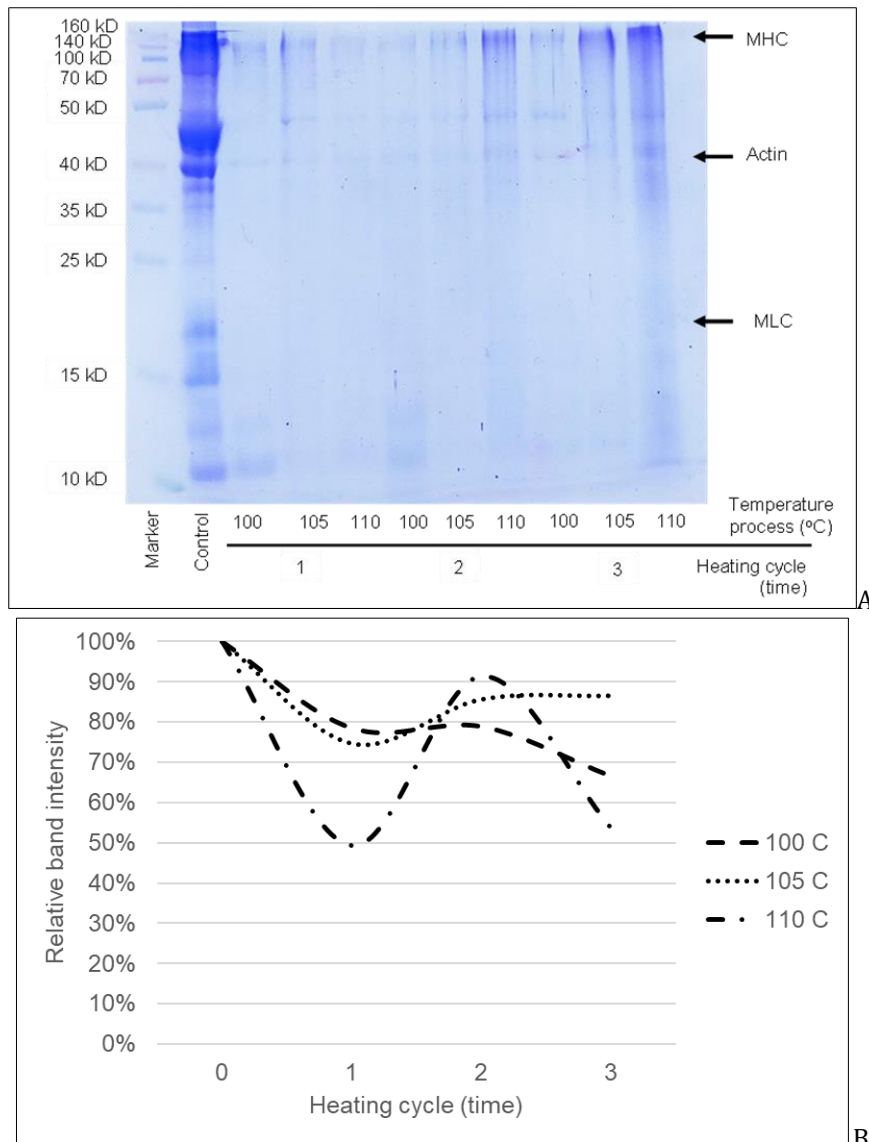
3.4. pH

The pH of pempek ranges between 6.48 and 6.8 (Table 3). No significant differences were observed despite microbiological activity during the incubation period.

3.5. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and quantification of myosin heavy chain and actin

The SDS-PAGE results of pempek after tyndallization at different levels and temperatures can be seen in Figure 2 above. The changes in protein patterns indicated that the relative intensity of MHC bands in the Tyndallized pempek followed a sinusoidal pattern with heating cycles at 100°C, 105°C, and 110°C. Two major protein bands above 150 and 40 kDa were identified, which were assumed to be MHC and actin (14).

However, the overall SDS-PAGE patterns were different. The pattern at 105°C was similar to that at 100°C, but the relative intensity of MHC bands was more significant in the third heating cycle, while at 110°C, the pattern was sharper. At the end of the process, the relative intensity of MHC bands was the lowest. Thus, it can be concluded that the sinusoidal pattern formed was likely due to different protein polymerization mechanisms and recurring cycles and temperatures of tyndallization in composite foods like pempek. Changes in protein polymerization were not linear, as seen in the protein pattern changes in single products like kamaboko (8).



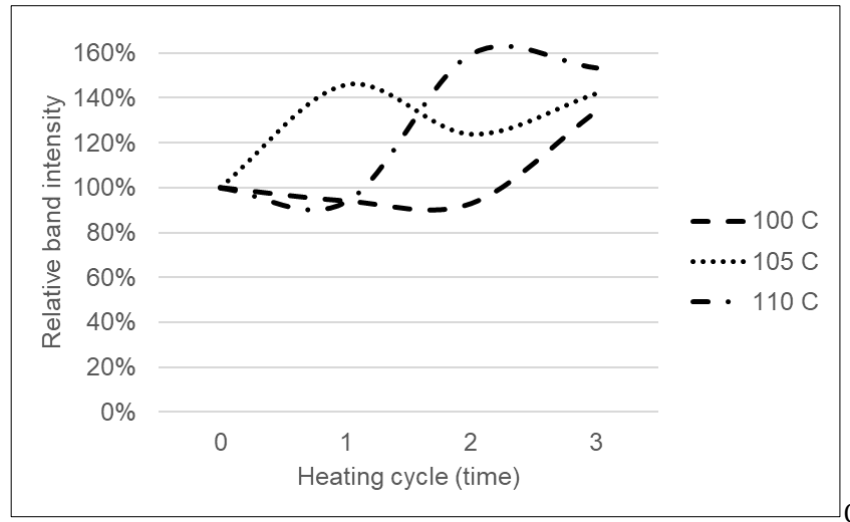


Figure 2 The SDS-PAGE pattern of pempek at different stages of tyndallization (A) and relative band intensity of MHC (b) and actin (c).

Unlike MHC, which exhibited a sinusoidal pattern with a declining trend in relative band intensity towards the end of the process, the actin changes also demonstrated a sinusoidal pattern but with an upward trend in relative band intensity after the process.

MHC, in this case, refers to myosin, an essential factor in gel properties, although not the only one. However, MHC degradation can be an indicator of gel quality (15) because MHC plays a significant role in forming textural properties and water-holding capacity of the gel (16). With a high processing temperature increase, protein polymerization can occur, leading to changes in the gel network (17). Interactions in the polymerization process mainly involve non-disulfide covalent bonds, causing the weakening of the gel network (8).

3.6. Structure secondary proteins

Fourier Transform Infrared (FTIR) is an instrument that can be used to identify functional groups and observe interactions between molecules with the help of electromagnetic radiation originating from the infrared region. In identifying functional groups using FTIR, there is a wavelength range that can identify these groups, which lies between 4000-200 cm⁻¹.

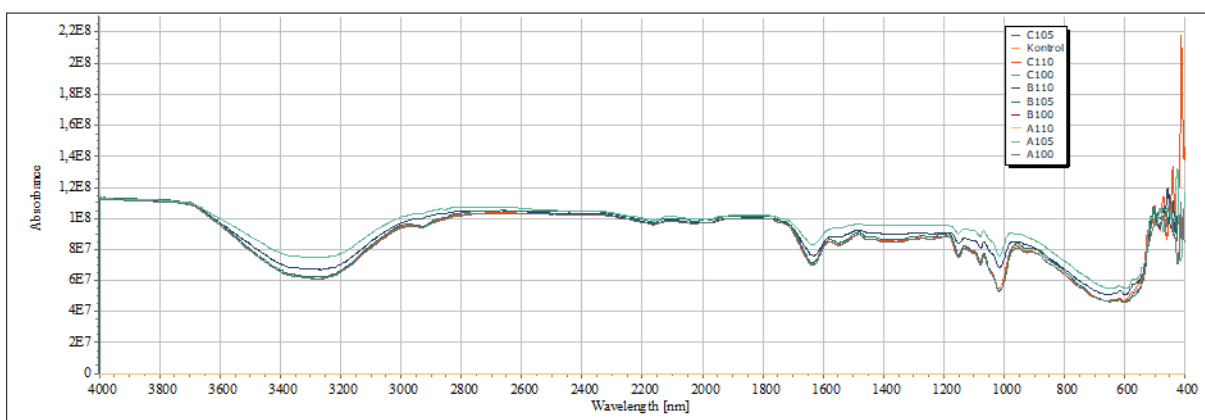


Figure 3 FTIR spectra of pempek at different tyndalization levels and temperatures

Different hydrogen bond vibrations between amino acids in the geometry of amide bonds allow for varying vibrational frequencies associated with secondary structural folding, resulting in distinct spectra. These differences form the basis for dividing the absorption regions of infrared (IR) protein spectra, which are classified into nine regions: amide A (NH stretching, frequency 3300 cm⁻¹), amide B (NH stretching frequency 3100), amide I (80% CO stretching), amide II (60% CN stretching, 40% NH bending frequency 1400-1600), amide III (40% CN stretching, 30% NH bending frequency 1200-

1300), amide IV (OCN bending), amide V (out of plane NH bending), amide VI (out of plane CO bending), and amide VII (skeleton torsion, frequency 200) (18). The differences in spectra between samples are shown in Figure 3.

Amide I is a sensitive spectral region and can be used to study the secondary structure of proteins. Understanding the secondary structure of proteins leads to insight into the components that make up the complete protein. The secondary structure consists of α helix, β sheet, β turn, and random coil. The secondary structure's relevance to forming fish meat gel is the composition of β sheets, β turns, and coils in forming the gel matrix and myosin.

Specifically, studying protein secondary structure in the amide I region is most frequently examined due to its high sensitivity to protein secondary structure. The shifts in the amide I band are minimal compared to the intrinsic bandwidth, resulting in a broad single peak. Mathematical procedures such as deconvolution (inversion) and second derivative can quantitatively resolve overlapping bands to analyse protein secondary structure.

The results of the second derivative of the Amide I spectrum of pempek, influenced by tindalization, are shown in Figure 4 below.

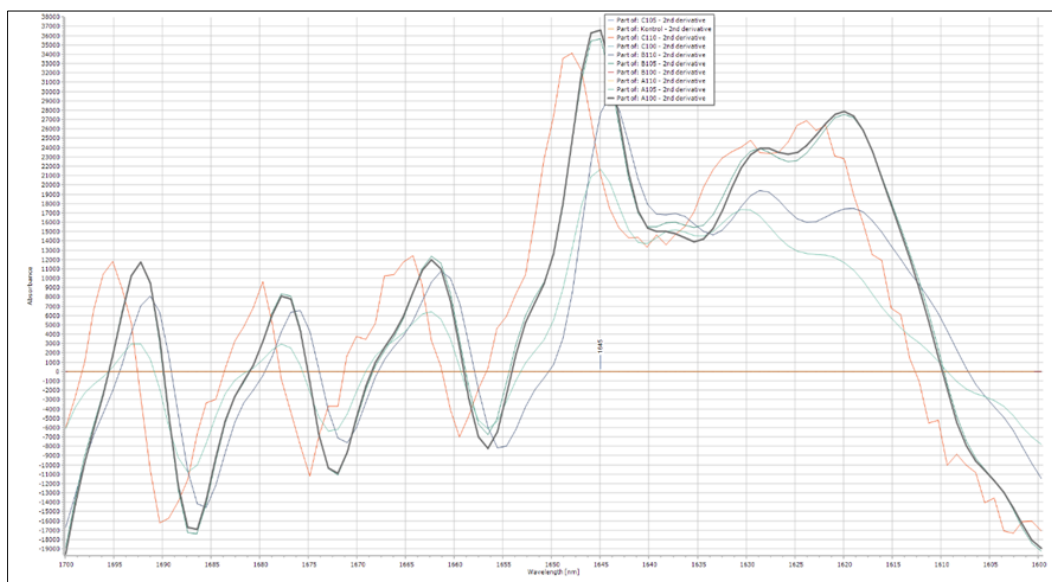


Figure 4 The second derivative of the Amide I spectrum of pempek at various tindalization levels and temperatures.

In Figure 4, it can be observed that there is a change in the peak spectrum of Amide I due to the influence of different tindalization levels and temperatures. The identification results of the FWHM (Full Width at Half Maximum) in the Amide peak spectrum are shown in Table 4 below.

The Amide I region is an area that can be used to verify the conformation of protein secondary structures. This study shows that from the control sample, the identified secondary protein structures are dominated by the β helix structure without any α helix, which is consistent across different levels and temperature changes during tindalization. The β helix protein structure consists of folded formations. Frequency and band intensity changes indicate secondary structure alterations (Table 4).

The change in the amount of β helix protein during the tindalization process appears unique. During tindalization at 100°C, the amount of β helix protein decreases with increasing repetition of tindalization; at 105°C, the amount of β helix protein decreases at the second level and increases again at the third level. In contrast, during tindalization at 110°C, the amount of β helix protein reverses from the tindalization temperature of 105°C. It informs us of a phenomenon similar to the formation of kamaboko, where proteins conform at 105°C and then, with an increase in temperature to 110°C, the protein structures break down.

Table 4 Full Width Half Maximum (FWHM) spectrum peaks Amida I

Process temperature (°C)	Heating cycle (time)	Wavelength (nm)	Functional group	FWHM (nm)
Control	0	1645,6	Amida I (β helix) proteins that have peptide bonds (C=O) stretching	7,741
100	1	1645,3		7,432
	2	1645,5		7,5184
	3	1645,3		7,3988
		1692,4		3,6603
105	1	1645		29,491
	2	1645,4		7,476
	3	1645,3		8,1325
110	1	1645,3		7,782
	2	1643,9		13,237
	3	1647,9		7,765

4. Conclusion

Although vegetative bacterial cells can be easily eliminated at relatively low temperatures, higher temperatures can induce bacterial endospores to germinate, even though the temperature and tyndallization cycles were insufficient to achieve commercial sterilization in pempek. Pempek exhibited unique behaviour as a composite product when subjected to tyndallization treatment. From a quality standpoint, tyndallization caused the colour of the pempek to become more yellowish, yet the texture became softer without a change in pH. Protein polymerization occurred, evidenced by decreased MHC and increased actin as the temperature and tyndallization cycles progressed. The MHC increase and actin decrease followed a sinusoidal pattern dominated by β -sheet proteins. In this study, it is essential to find a balance between the processing temperature and heating cycles to achieve commercial sterility while maintaining the quality of pempek. In this case, additional processing time, lower temperature variation, and heating cycles may be required.

Compliance with ethical standards

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Disclosure of conflict of interest

We, the authors, declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contribution

Agus Supriadi: Conceptualization, Data curation, Formal analysis, Investigation,. Daniel Saputra: Methodology, Writing - original. Gatot Priyanto: Resources, Writing - review and editing. Eka Lidiasari: Methodology, Resources. Sigit Purwanto: Supervision. Siti Nurhasanah: Validation, Writing - review & editing

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