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Effect of packaging type and storage time of frozen food fish cake using bacteriological tests

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Abstract Fish cakes are one of the famous fish-based products in Indonesia. Improper handling causes processed products to be easily contaminated by spoilage bacteria. While plastic packaging has revolutionized food preservation, it is not a foolproof solution against bacterial contamination. In the case of fish cakes, it can promote bacterial growth. Banana leaves, a common household item in many regions, have long been used for food preservation. This practice is rooted in the leaves' innate antibacterial properties, primarily attributed to the presence of lactic acid bacteria. The results showed that the type of packaging affected the moisture content but was not affected total plate count (TPC) and lactic acid bacteria (LAB). Banana leaf and plastic packaging had a moisture content of 62,18%, while plastic packaging has 62,98%. Storage time had affected to TPC, LAB, and moisture content. The longer storage time reduced the amount of TPC, LAB, and moisture content, TPC from $14,8\times10^4$ CFU/g to $1,6\times10^4$ CFU/g, LAB from $8,4\times10^4$ CFU/g to $1,3\times10^4$ CFU/g, and moisture content from 63,66% to 61,44%. The packaging type and storage length interaction affected LAB but was not affected TPC and moisture content. The type of banana leaf and plastic packaging had the highest amount of LAB at two weeks of storage, while the type of plastic packaging was at 0 weeks of storage. In conclusion, combination of banana leaf and packaging demonstrates promising potential as a biopreservative due to the presence of lactic acid bacteria, which exhibit significant antibacterial properties.

Keywords: Fish cake, Lactic acid bacteria, Total plate count, Moisture content

Introduction

Fish is one of the high-quality animal protein sources, which has omega 3 [(n-3)] *Polyunsaturated Fatty Acids* (Tacon and Metian, 2013), especially

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Eicosapentaenoic Acid (EPA) and *Docosahexaenoic Acid* (DHA) (Pandiangan *et al.,* 2019). EPA and DHA play an essential role in fetal development, especially in nerve, retinal, and immune function (Swanson *et al*., 2012). However, in 2017, the level of fish consumption in Indonesia is lower than in other Asian countries, such as Japan, Myanmar, South Korea, Malaysia, and Hong Kong, with consumption levels of 45.49; 47.32; 54.97; 57.62; and 70.75 kg/capita/year respectively, compared to Indonesia which only reached 44.67 kg/capita/year (Food and Agricultural Organization, 2020). Therefore, the variety of fishery products is a way to increase and optimize the utilization of marine products (Alam *et al*., 2021) to increase public interest in consumption. Fish cakes, also called fish pastes, are made from rocking eye fish (*Priacanthus tayenus*), commonly known as purple-spotted bigeye, one of the marines caught in Cirebon city, Indonesia. The rocking eye fish is a demersal reef fish with unique characteristics of pink color, has large eyes, and on the pelvic fins, there are blackish purple spots (Firmansyah *et al*., 2016).

Improper handling causes processed products to be easily contaminated by spoilage bacteria. Sartika *et al*. (2019) reported that fish cakes packaged using plastic in the traditional markets in Bandar Lampung City were contaminated with pathogenic bacteria, such as *Escherichia coli*, *Salmonella* sp., and *Staphylococcus aureus*. The plastic packaging used as packaging material for fish cakes has not been fully protected from bacteria contamination. Using banana leaves as the central primary packaging and then in plastic packaging is expected to reduce bacteria contamination because banana leaves contain lactic acid bacteria (LAB), which can inhibit the growth of harmful microorganisms.

Yang *et al*. (2016) reported that banana leaves contain LAB as much as 4.5±0.8 log CFU/g. Lactic acid bacteria are generally recognized as safe microorganisms with the ability to produce natural antimicrobial compounds, such as bacteriocins, lactic acid, hydrogen peroxide, diacetyl, and carbon dioxide (Syaputri *et al.,* 2023). Therefore, lactic acid bacteria can inhibit the growth of harmful microorganisms (Vieco-Saiz *et al*., 2019). The shelf life of seafood products can be effectively extended by lowering the storage temperature and reducing water activity by freezing at least -15°C (Syaputri and Iwahashi, 2020). This study aimed to determine the effect of the type of packaging, storage time, and the interaction of the two on the total colony, LAB, and moisture content.

Materials and methods

Fish cakes preparation and processing

All the samples were collected and processed in Cirebon City, Indonesia. The primary raw material, namely 80% mashed minced of rocking eye fish was mixed with 15% tapioca flour and 5% spices: salt, sugar, pepper, garlic, and shrimp. All the ingredients were steamed at 100°C for 10-15 minutes. The packaging was carried out in two ways, first using banana leaves which are then covered with plastic polyethylene, and the second using plastic directly. The fish cakes were stored at -20°C for six weeks, and samples were taken every two weeks for analysis; for 0 weeks of storage, the sample was stored for (24 h) in the freezer.

Analysis of total plate count (TPC) in fish cake

The twenty-five-gram sample was mixed with 225 mL of Butterfield's Phosphate Buffered (BFP) solution (Merck, Germany) and homogenized for 2 min. Plate Count Agar (PCA) (Merck, Germany) was sterilized and added with 0.5% Triphenyl Tetrazolium Chloride (TTC) (Merck, Germany). Ten mL of the PCA+TTC solution was mixed into 90 ml of the BFP solution and diluted until 10^{-10}. For isolation of bacteria, 1 mL of suspension from each dilution was inserted into sterile petri dishes in duplicate, then 20 mL PCA was poured into petri dishes and incubated at 35°C for 48±2 hours (Sulasih and Mudawaroch, 2013). Plates with a total of 25–250 colonies were calculated using the following formula:

$$N = \frac{\Sigma C}{[(1 \times n_1) + (0{,}1 \times n_2)] \times d}$$

N : number of colonies, expressed in CFU/ml or CFU/g
ΣC : the number of colonies on all plates counted
n_1 : number of plates in the first dilution calculated
n_2 : number of plates in the second calculated dilution
d : the first dilution used

Analysis of lactic acid bacteria (LAB) colony number in fish cake

Ten-gram sample was mixed into 90 ml of 0.85% NaCl solution. One-milliliter suspension pours into petri dishes, then 15 mL De Man Rogosa Sharpe agar (MRS Agar) was poured into petri dishes. The petri dishes were incubated at 34°C for 48 hours. The plates with 25–250 colonies were counted using the TPC calculation formula (Miranti *et al.,* 2022).

Moisture analysis in fish cake

The procedure followed by the National Standardization Agency Indonesia; Moisture Testing on Fishery Products (2015), the empty porcelain cup was placed in an oven at 105–110°C for 2 hours, then transfer the empty cup to a desiccator for ±30 minutes and the mass (A) was weighed. Two grams of sample were put into the cup (B). Then, put the filled cup into the oven for 16–24 hours, transferred it to a desiccator for ±30 minutes, and weighed (C). Calculations were carried out using the following formula:

$$Moisture\ contet\ = \frac{B - C}{B - A} \times 100\%$$

A : weight of empty cup (g),
B : weight of cup + initial sample (g),
C : weight of cup + dry sample (g)

Statistical analysis

All the experiments were repeated 3-12 times to make sure the data was suitable for statistical analysis. The data displayed contains the mean, standard deviation and standard error. The data were analyzed using a Two-Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT), for the type of packaging factor, followed by the Paired T-Test. Data analysis was carried out using the Statistical Package for the Social Science (SPSS) version 26 suitable for Windows 10. $p<0.05$ was considered as significantly different.

Results

Analysis of TPC, LAB, and moisture content on raw material

The analysis of TPC, LAB, and the moisture content of the raw material for fish cakes from rocking eye fish were shown in Table 1. The average TPC, LAB, and moisture content of the raw material for fish cakes were 12.7×10^7 CFU/g, 15.3×10^4 CFU/g, and 78.40%, respectively. The results showed that the average colony in raw material exceeded the standard for fresh fish which was 5×10^5 CFU/g. However, for LAB and moisture content, National Standardization Agency did not have criteria for raw materials.

Table 1. TPC, LAB, and moisture content of fish cakes raw material

Fish Cakes Raw Material (Rocking Eye Fish)					
Parameters	Average	n	Std. Error	Std. Deviation	SNI 2729:2013
TPC (CFU/g)	12.7×10^7	3	0.56×10^7	0.97×10^7	5.0×10^5
LAB (CFU/g)	15.3×10^4	3	1.6×10^4	2.8×10^4	-
Moisture content (%)	78.40	3	0.16	0.28	-

Analysis of TPC, LAB, and moisture content in fish cakes dough

The analysis of TPC, LAB, and the moisture content of the dough before and after steaming were shown in Table 2. Result showed that the fish cake dough before steaming had a higher amount of TPC, LAB, and moisture content than after steaming (Table 2). The fish cake dough before steaming had an average of TPC 15.8×10^6 CFU/g, however after steaming the average TPC was decreased to 13.0×10^4 CFU/g. The colony of BAL before steaming was 51.8×10^4 CFU/g and after steaming became 1.5×10^4 CFU/g, and the moisture content before steaming was 65.39% and after steaming was 63.32%. This difference was tested using the Paired T-Test, which resulted in a significant difference for each of the measured parameters. These showed that the steaming process had affected on the TPC, LAB, and moisture content tests.

Table 2. TPC, BAL, and moisture content of fish cakes dough

Fish Cakes Dough						
Parameters	Dough	Average	Std. Error	Std. Deviation	Sig. (T-Test)	SNI 7757:2013
TPC (CFU/g)	Before Steaming	15.8×10^6	0.58×10^6	1.0×10^6	0.001*	5.0×10^4
	After Steaming	13.0×10^4	0.65×10^4	1.1×10^4		
LAB (CFU/g)	Before Steaming	51.8×10^4	3.6×10^4	6.2×10^4	0.005*	-
	After Steaming	1.5×10^4	0.21×10^4	0.36×10^4		
Moisture content (%)	Before Steaming	65.39	0.08	0.13	0.010*	60.00
	After Steaming	63.32	0.17	0.30		

n=3; the sign (*) indicates that the significance different $p<0.05$

The effect of types of packaging on TPC, LAB, and moisture content

The combine packaging using banana leaf and plastic, and direct plastic were the treatment factor of these analyses, with parameters were moisture content, TPC and BAL. The Two-Way ANOVA test showed a significant level of moisture content and LAB but was not significant on colony number. Therefore, a Paired T-Test was carried out on the parameters of moisture content and LAB to determine whether there was a difference in the average between the two samples that were related to each other. The data analysis of the Paired T-Test was significantly affected the moisture content but did not give a substantial impact on LAB. The effect of the types of packaging on the moisture content was shown in Table 3.

Table 3. Effect of types of packaging on moisture content

Types of Packaging						
Parameters: Moisture Content (%)						
Types of Packaging	Average	n	Std. Error	Std. Deviation	Sig. (T-Test)	SNI 7757:2013
Banana leaf and plastic (A_1)	62.18	12	0.39	1.36	0.000*	60.00
Plastic (A_2)	62.98	12	0.29	1.01		
Parameters: TPC (CFU/g)						
Banana leaf and plastic (A_1)	6×10^4	12	2×10^4	7×10^4	0.000*	5.0×10^4
Plastic (A_2)	5.3×10^4	12	1.3×10^4	4.5×10^4		
Parameters: LAB (CFU/g)						
Banana leaf and plastic (A_1)	1×10^5	12	5.3×10^4	1.8×10^5	0.000*	-
Plastic (A_2)	6.7×10^4	12	1.9×10^4	6.6×10^4		

The sign (*) indicates that the significance different $p<0.05$.

Result showed that the average moisture content in banana leaf and plastic packaging was 62.18%, while plastic packaging was 62.98%, TPC were 6×10^4 and 5.3×10^4; and BAL were 1×10^5 and 6.7×10^4, respectively (Table 3). It showed that the average moisture content and TPC of the fish cakes, both with the combine type of banana leaf and plastic packaging and plastic packaging directly exceeded the standard for fish cakes (SNI 7757:2013).

The effect of storage times on TPC, LAB, and moisture content

The effect of storage times on TPC, LAB, and moisture content was treated for 0, 2, 4, and 6 weeks. The Two-Way ANOVA ($p<0.05$) showed a significant level of TPC, LAB, and moisture content. Therefore, it was continued with DMRT to determine which treatments were significantly different. The effect of storage time on TPC was shown in Table 4.

Table 4. Effect of storage times on TPC

Storage Times					
Parameters: TPC (CFU/g)					
Storage Times	Average	n	Std. Error	Std. Deviation	SNI 7757:2013
0 Weeks (B_0)	$14.8 \times 10^{4\ d}$	6	1.6×10^4	3.9×10^4	5.0×10^4
2 Weeks (B_2)	$3.6 \times 10^{4\ c}$	6	0.49×10^4	1.2×10^4	
4 Weeks (B_4)	$2.5 \times 10^{4\ b}$	6	0.39×10^4	0.96×10^4	
6 Weeks (B_6)	$1.6 \times 10^{4\ a}$	6	0.18×10^4	0.43×10^4	

The numbers followed by different lowercase superscripts showed significant differences *p<0.05.* $^{a\text{-}d}$Significantly different between the storage time and total plate count

Result showed that the average TPC of fish cakes, with a storage period of 0 weeks (B_0) was 14.8×10^4 CFU/g exceeded the standard of fish cakes (SNI 7757:2013), which was a maximum of 5×10^4 CFU/g. However, after two weeks (B_2), four weeks (B_4), and six weeks (B_6) storage, the colony showed 3.6×10^4 CFU/g, 2.5×10^4 CFU/g, 1.6×10^4 CFU/g, respectively. The average value of TPC complied with the National Standardization Agency.

Table 5. Effect of storage times on LAB

Storage Times				
Parameters: LAB (CFU/g)				
Storage Times	Average	n	Std. Error	Std. Deviation
0 Weeks (B_0)	$8.4 \times 10^{4\ a}$	6	3.8×10^4	9.5×10^4
2 Weeks (B_2)	$21.5 \times 10^{4\ c}$	6	8.8×10^4	2.2×10^5
4 Weeks (B_4)	$2.9 \times 10^{4\ b}$	6	0.71×10^4	1.7×10^4
6 Weeks (B_6)	$1.3 \times 10^{4\ a}$	6	0.77×10^3	1.9×10^3

The numbers followed by different lowercase superscripts showed significant differences *p<0.05.* $^{a\text{-}c}$Significantly different between the storage time and LAB

The effect of storage times on LAB was shown in Table 5. It showed that the average LAB with a storage period of 0 weeks (B_0) (8.4×10^4 CFU/g) was not significantly differed with a storage period of 6 weeks (B_6) (1.3×10^4 CFU/g). However, 2 weeks (B_2) storage times (21.5×10^4 CFU/g) and 4 weeks (B_4) (2.9×10^4 CFU/g) had a significant difference in the length of storage. LAB growth developed rapidly at 2 weeks of storage, then decreased to 6 weeks of storage.

Table 6. Effect of storage times on moisture content

Storage Times					
Parameters: Moisture Content (%)					
Storage Times	Average	n	Std. Error	Std. Deviation	SNI 7757:2013
0 Weeks (B_0)	63.66^b	6	0.12	0.30	60.00
2 Weeks (B_2)	63.56^b	6	0.23	0.56	
4 Weeks (B_4)	61.66^a	6	0.35	0.86	
6 Weeks (B_6)	61.44^a	6	0.36	0.87	

The numbers followed by different lowercase superscripts showed significant differences *p<0.05.* $^{a\text{-}b}$Significantly different between the storage time and moisture content

The effect of storage times on moisture content was shown in Table 6. The average moisture content with a storage time of 0 weeks (B_0) (63.66%) was not significantly differed with a storage time of 2 weeks (B_2) (63.56%), however, both had significantly differed with storage time 4 weeks (B_4) (61.66%) and 6 weeks (B_6) (61.44%). The average moisture content of fish cakes with the highest value at 0 weeks of treatment was 63.66% and the lowest value at six weeks of treatment was 61.44%. This shows that more extended storage at freezing temperatures can reduce the moisture content in fish cakes.

Effect of interaction between types of packaging and storage times on TPC, LAB, and moisture content

The results of the Two-Way ANOVA test analysis showed that there was an interaction between the types of packaging and the storage times on the LAB parameters, however, there was no interaction with the TPC parameters and moisture content. Therefore, the LAB parameter was continued with the DMRT test to find out which interaction treatments were significantly different.

Table 7. Interaction of types of packaging and storage times on LAB (CFU/g)

Types of Packaging	Storage Times (Weeks)			
	B_0	B_2	B_4	B_6
Banana leaf and plastic (A_1)	$2.4 \times 10^{3\ a}$	$38.2 \times 10^{4\ e}$	$1.7 \times 10^{4\ b}$	$1.4 \times 10^{4\ b}$
Std. Error	0.42×10^3	10.0×10^4	0.14×10^4	0.49×10^3
Std. Deviation	0.72×10^3	18×10^4	0.24×10^4	0.85×10^3
Plastic (A_2)	$17.0 \times 10^{4\ d}$	$4.8 \times 10^{4\ c}$	$4.0 \times 10^{4\ c}$	$1.2 \times 10^{4\ b}$
Std. Error	2.7×10^4	1.0×10^4	1.1×10^4	0.15×10^4
Std. Deviation	4.7×10^4	1.8×10^4	1.8×10^4	0.25×10^4

n=3; The numbers followed by different lowercase superscripts showed significant differences according to the DMRT test at the 5% level. $^{a\text{-}d}$The interaction of types of packaging and storage times on LAB

The interaction effect of packaging types and storage times on LAB was shown in Table 7. The average of LAB A_1B_0, A_1B_2, and A_2B_0 were 2.4×10^3 CFU/g, 38.2×10^4 CFU/g, and 17.0×10^4 CFU/g, respectively, had significant differences from other treatments. A_1B_4 treatment (1.7×10^4 CFU/g), A_1B_6 (1.4×10^4 CFU/g), and A_2B_6 (1.2×10^4 CFU/g) had no significant differences, but all three had substantial differences from other treatments. A_2B_2 treatment (4.8×10^4 CFU/g) and A_2B_4 (4.0×10^4 CFU/g) had no significant difference, but all three had essential differences from other treatments. Overall, the results of the analysis show that the survival of LAB depends on the type of packaging and storage time. The type of banana leaf and plastic packaging had the highest amount of LAB at two weeks of storage, while the type of plastic packaging had the highest amount of LAB at 0 weeks of storage.

Discussion

Fish cake is one of the popular processed fish foods in Indonesia. In Cirebon City, rocking eye fish was used as the main product to make a fish cake. This research showed that the number of colonies in rocking eye fish exceeded the standard for fresh fish. This was presumably due to the thawing process of fish meat for too long at room temperature. Frozen meat will return fresh after thawing (Vera *et al.,* 2021). In addition, the process of thawing fish meat can cause a decrease in the quality of fish meat. If the thawing method is carried out incorrectly, it can reduce the chemical quality of the essence due to the presence of water-soluble nutrients that can be lost along with the liquid that comes out called drip (Fahruzaky *et al.,* 2020). Meat quality during freezing does not change, but there will be changes in meat quality during thawing (Diana *et al.,* 2018). Frozen meat that has been thawed and has been at room temperature for 2 hours or more must be discarded and cannot be reused (Food and Drug Supervisory Agency, 2021).

The next process in manufacturing to make the fish cake was to make the dough. The results showed the number of colonies, LAB, and moisture content before and after steaming. The steaming process reduced the moisture content. Puntillo *et al*. (2020) reported that the higher the temperature and the longer the cooking time, the moisture content will decrease. Reducing moisture content can eliminate the risk of toxigenic bacteria, such as *S. aureus*, *Clostridium botulinum*, and *Bacillus cereus* (Sun, 2012). Therefore, the total number of bacteria will decrease if there is a decrease in moisture content. Kiwak *et al.* (2018) reported that the higher the moisture content value, the bacterial contamination will increase. High steaming temperatures will damage proteins that support bacterial life and cause bacteria to die (Utama and Christiyanto, 2021). Still, it cannot eliminate microbes (Handayani *et al.,* 2017). The longer the steaming time, the lower the total microbes in the product (Syahfitri *et al.,* 2018).

In addition, one of the changes in moisture content in foodstuffs is influenced by the permeability of the packaging materials used. Packaging materials have different permeability properties depending on their thickness and density; the thicker it is, the smaller the permeability value (Susanti *et al.,* 2020). Banana leaf packaging which covered with plastic has a lower water content than plastic packaging only because the banana leaf will retain the water vapor from the environment that diffuses through the plastic wall before it enters the food product. Pudjiastuti *et al.* (2013) reported that the packaging material's density caused water vapor to diffuse. The high density of the packaging material made water vapor difficult to penetrate the walls of the packaging material, so the value of the water vapor transmission rate is lower. The lower the rate of water vapor transmission that passes through the walls of the packaging material, the food packaged in it will have a longer shelf life (Pudjiastuti *et al.,* 2013).

The results of length storage with the number of colonies showed that the longer storage at freezing temperature, the colony will decrease. Storage at frozen temperatures can reduce the number of colonies in fish cakes and extend the shelf life to 6 weeks. Based on the results, six weeks of storage was the most effective treatment to give a death effect on polluting bacteria compared to other treatments. Sangadji (2013) reported that the length of storage influences the total growth of bacterial colonies. Bacteria will die at cold and freezing temperatures; the longer the warehouse, the less the total number of bacterial colonies (Sangadji, 2013). Freezer storage can reduce the bacterial content of the product (Sangadji *et al.,* 2019). At the freezer temperature, bacteria did not grow because the liquid will freeze so the bacteria did not grow. The food spoilage bacteria were mesophilic bacteria whose growth temperature was 15–55ºC, with optimal growth at 25–40ºC (Sulasih and Mudawaroch, 2013). Frozen temperature storage can improve food safety microbiologically and extend shelf life (Sari *et al.,* 2017).

In addition, the freezing temperatures were not optimum for LAB growth, the more extended storage at freezing temperatures, the less the LAB growth. The optimal temperature for the growth of lactic acid bacteria was from 30–40°C (Syaputri *et al.,* 2021). Low temperatures will result in bacteria being dormant and not growing (Utama and Christiyanto, 2021).

O'Brien *et al.,* (2016) reported that fermented goat's milk stored at a freezing temperature (-8ºC to -20ºC) for 0, 7, 14, and 30 days decreased the total amount of LAB. During freezing, LAB cells were subjected to cold stress resulting in membrane hardening, cell dehydration, and volume reduction (Fonseca *et al.,* 2016). The freezing process caused cell membrane damage, protein and enzyme denaturation, and DNA damage (Kandil and El Soda, 2015). Frozen storage caused changes in membrane fluidity which will affect nutrient transport, decrease enzyme activity, and stability of RNA structure which will affect the translation process so that it affects bacterial viability (Liu *et al.,* 2020).

Moreover, Sun (2012) reported that freezing can reduce water availability (Sun, 2012). During the storage process at freezing temperatures, free water in foodstuffs turns into ice crystals (Nakazawa and Okazaki, 2020). The formation of ice crystals will reduce the liquid in the food so that the cells were dehydrated (Chen *et al.,* 2021). Therefore, extended storage at freezing temperatures will reduce the moisture content, thus inhibiting the growth of microorganisms (Sakti *et al.,* 2016) from extending the shelf life (Zhu *et al.,* 2021). The moisture content will change during storage, so the moisture content was affected in determining the durability of food material (Susanti *et al.,* 2020).

In plastic packaging, the number of LAB with a storage period of 0 weeks was higher than that of banana leaf and plastic packaging. Because of, in banana leaves, there were also pathogenic bacteria that will affect the growth of LAB. Yang *et al.* (2016) reported that banana leaves contain coliform and aerobic bacteria and lactic acid bacteria. Mubarok *et al.* (2020) reported that LAB growth was influenced by the presence of early pathogenic microbes, environmental temperature, chemical composition, substrate availability, free moisture content (a_w), and the presence of oxygen. If bacteria can survive at the initial freezing temperature, they can still live for a long time at freezing temperatures. The decrease in the amount of LAB that occurred was due to bacteria experiencing cold shock, resulting in high LAB mortality (Satria *et al.,* 2017). Lactic acid bacteria can still grow in frozen storage because of the availability of nutrients in these foodstuffs.

This research concluded that the types of fish cake packaging had significant affect moisture content but no significant on TPC and LAB. Banana leaf and plastic packaging has a lower moisture content value than packaging that only uses plastic. The length of storage of fish cakes affects TPC, LAB, and moisture content. The interaction between the types of packaging and storage times affects the LAB parameters but did not affect the TPC parameters and moisture content.

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