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RESEARCH PAPER

The utilization of spices to improve the quality and safety of traditional dried sardine fish

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Abstract

Pedetan is a traditional spiced dried fish dish processed by the community in the Jembrana Regency, Bali. Pedetan is made from lemuru fish, this product utilizes boneless lemuru meat and a head that is seasoned and then shaped into a flat shape resembling jerky (sweet, dried meat/fish). Traditional fish processing has prospects of being developed as an alternative to equal distribution between producing and consumer areas. Damage to dried fish is often caused by enzymes and microorganisms such as bacteria and moulds. Spices applied to dried fish can be used as an inhibitor of spoilage. This study tested a new seasoning composition based on the ratio of the community's spices and seven compositions of pedetan sardine fish seasoning formula and was stored for 12 weeks. The results showed that the water content of pedetan increased, while the protein and fat levels decreased during storage until the 12th week. However, the ash content was insoluble in acid and salt. Microbiological analysis met the Indonesian National Standard 8273-2016 (SNI) requirements as the dried salted fish had a maximum of 10^5 CFU, while sensory analysis showed a value of 7.93 following the SNI. Seasoning II composition consisted of garlic, coriander, *Kaempferia galangal*, *Alpinia galangal*, ginger, vinegar, salt, and extended sardine fish shelf life up to the 12th week. According to the standards set for food made from raw fish, seasoning II has the best quality and sensory value.

1. Introduction

Traditional fish processing has better development prospects and opportunities. This is very complex and primarily based on conceptions passed down from one generation to another. Pedetan is one of the traditional Balinese food products processed by the community in Jembrana Regency, Bali Province, Indonesia, made from sardine fish (*Sardinella lemuru*), salt and spices, sun-dried for two to three days, then stored in room temperature (Singapurwa *et al*., 2014). Good and correct processing makes fish durable and allows it to be distributed between regions. Traditional fish processing such as fumigation and drying has the prospect of being developed as an alternative to equal distribution between producing and consumer areas.

Dried fish damage occurs during storage and distribution in marketing, including the ones caused by microorganisms, such as bacteria and moulds. The seasoning used in sardine fish production is applied to other processed dried fish. The microbial growth inhibitory effect of a type of spice is unique, and each antimicrobial compound has a specific inhibitory ability for a particular type of microbe. The spices known to have strong antimicrobial activity include shallots, garlic, red chillies, ginger, turmeric, and galangal (Rahayu, 2000).

The various procedures of pedetan processing in each village make this product a distinctive feature of the producing villages. This also affects food safety and the existence of different qualities. Therefore, the products obtained are not uniform quantitatively or qualitatively (Singapurwa *et al*., 2014), with varying durability for only 2-4 weeks, making it difficult to standardize. The manufacturer with several seasoning compositions carries out dried sardine fish processing. Generally, people use a mixture of different spices depending on the local community's taste. The spices that are always used in making pedetan are coriander and garlic. Several

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studies showed garlic has an antimicrobial effect against Gram-positive and Gram-negative bacteria, fungi, and parasites (Biradar *et al., 2014)*. The microbes that contaminate sardine fish are *Aspergillus flavus, A. aculeatus, A. niger,* and *A. tubingensis* (Singapurwa *et al*., 2018).

Allicin and organosulphur compounds in garlic inhibit the growth of *Staphylococcus*, *Salmonella*, *Vibrio*, *Mycobacteria*, *Proteus* sp. and *Helicobacter pylori* bacteria as well as function as anti-parasite, anti-fungal, and anti-viral (Biradar *et al*., 2014; Gupta *et al*., 2015). Garlic oil nano-emulsion is more effective against *S. aureus* than *E. coli* (Hassan dan Mujtaba, 2019). Also, *Kaempferia galangal, Allium sativum, Zingiber officinale, Curcuma longa,* and *Alpinia galangal* inhibit *Fusarium* sp. mycelia growth (Sanit, 2016). Turmeric (*Curcuma longa*) is commonly used as a food additive in cooking. As the main compound extracted from *C. longa*, Curcumin serves various therapeutic, preventive, and antiparasitic purposes (Cheraghipour *et al.,* 2018). Coriander also has an inhibitory power against *Candida* spp. growth (Freires *et al*., 2014), and contains a phenolic acid of 357.36 mg/100 g wet weight (Opara and Chohan, 2014), a bioactive compound. *Kaempferia galangal* rhizome inhibits pathogenic bacteria such as *Staphylococcus aureus, Streptococcus faecalis, Bacillus cereus, B. subtilis, Enterobacter aerogenes, Salmonella enterica* serovar Typhi*, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Vibrio cholerae,* as well as four mould species, namely *A. niger, A. flavus, A. fumigatus* and *Candida albicans* (Kochuthressia *et al.,* 2012). Ginger with sulfites suggests that ginger may partially reduce the observed oxidative damage in the rats after exposure to sulfite (Shekarforoush *et al*., 2020). Curcumin and thyroxine supplementation could inhibit the liver oxidative damage of catfish by reducing liver malondialdehyde levels and serum concentrations of serum glutamic pyruvic transaminase, so it can protect the liver and increase vitellogenin synthesis as indicated by the increased RNA concentrations in the liver (Rawung *et al*., 2021).

Based on the foregoing, this study aimed to determine the spice composition's effectiveness, which inhibits microbial contaminant growth in pedetan, and the composition of new pedetan spices in extending pedetan shelf life. The results provided scientific evidence on the effectiveness of several types of spices developed by the community to date and provide more effective and safer alternative compositions.

2. Materials and methods

2.1 New seasoning composition testing

There are several spices used by various community groups in processing shredded sardines. The most commonly used spices consist of garlic, coriander, *Kaempferia galangal*, and salt. A new seasoning composition was prepared, and its activity was tested for nutritional value and inhibition of microbial growth. The comparison of the new spice composition can be seen in Table 1. Dried sardines with new composition spices were packaged in mica plastic and stored for 12 weeks at room temperature of 25 -30°C with 48-50% humidity, then tests were carried out every two weeks. Quality and safety were based on SNI 8273:2016 (Badan Standarisasi Nasional (BSN), 2016) quality and safety requirements and sensory assessment of dried salted fish.

2.2 Determination of water content

Water content determination was based on SNI 2354.2:2015 (BSN, 2015a). A non-vacuum oven was

Table 1. Comparison of seasoning composition.

No.	Seasonings	Composition (g)						
		Seasoning I	Seasoning II	Seasoning III	Seasoning IV	Seasoning V	Seasoning VI	Seasoning VII*
1	Garlic	25	25	25	25	25	25	25
2	Coriander	10	10	10	10	10	10	10
3	*K. galangal*	12	12	12	12	12	12	12
4	Salt	15	15	15	15	15	15	15
5	Vinegar	20	20					
6	*A. galangal*	6	6	6	10	10	10	
7	Chili					5	5	
8	Turmeric	4		4				
9	Ginger		3		3			
10	Acid			2		2		
11	Sugar			13				
12	Brown sugar				10			

* Source of the composition : Seasoning VII = Spices composition that the community has used.

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used for the Gravimetric Procedure which was conditioned at 105°C until the temperature stabilized. The empty plates were placed in the oven for at least 2 hrs and then transferred to a desiccator for about 30 mins until they reached room temperature and weighed afterwards. Approximately 2 g of the sample that had been prepared in another dish was weighed before being placed in a non-vacuum oven at 105°C for 16 hrs. They were then transferred into a desiccator for 30 mins using tweezers and then weighed.

2.3 Determination of protein levels

The determination of protein content was based on SNI 01-2354.3-2006 (BSN, 2006), in which samples of dried sardines were mashed until homogeneous and put in a clean and closed plastic or glass container. After that, about 2 g was weighed on a weighing paper, folded, and put into the digestion flask. Then, two catalyst tablets and some boiling stones were added. Next, 15 mL of concentrated H_2SO_4 (95-97%) and 3 mL of H_2O_2 were added slowly and allowed to stand for 10 mins in the acid chamber. In the next process, it was digested at 410°C for 2 hrs or until the solution became clear, then it was allowed to stand until it reached room temperature plus 50-75 mL of distilled water.

Next, 25 mL of 4% H_2BO_3 solution was prepared in an Erlenmeyer flask containing an indicator as a distillate reservoir. The flask containing the digestion products was mounted on a steam distillation apparatus and added with 50-75 mL of sodium thiosulphate hydroxide solution. The distillate obtained was stored in an Erlenmeyer until the volume reached at least 150 mL and turned yellow. It was then titrated with standard 0.2 N HCl until the colour changed from green to neutral grey.

2.4 Fat content determination

The determination of fat content was based on SNI 01-2354.3-2006 (BSN, 2006). Dried sardine samples were mashed until homogeneous and put in a clean and closed plastic or glass container. They were then conditioned at room temperature and ensured to remain homogeneous before weighing. At the time of separation between the liquid and the sample, stirring was done with a blender before carrying out the analysis. They were then weighed and 2 g of the homogenate sample was put into an empty round bottom flask in the fat sleeve. Furthermore, 150 mL of chloroform was put into a round bottom flask, the fat sleeve was also put into the Soxhlet extractor, and the Soxhlet circuit had to be made well installed. After that extraction at 60°C was carried out for 8 hrs. The mixture of fat and chloroform was evaporated to dryness in a round bottom flask which then was placed in an oven at 105°C in order to remove the remaining chloroform and moisture. The flask and fat content were then cooled in a desiccator for 30 mins, while the constant weight was measured.

2.5 Determination of total plate count

Determination of the total plate count (TPC) was carried out based on SNI 2332.1: 2015 (BSN, 2015b), where the dried sardine samples were taken randomly and cut into small pieces. A total of 25 g of solid sample was weighed and put into a sterile container or plastic, then added with 225 mL of buffered phosphate field solution and homogenized for 2 mins. This homogenate was a 10^{-1} dilution solution which was then taken 10 mL with a sterile pipette and put into 90 mL of Buffer solution to obtain a 10^{-2} dilution. Subsequent dilutions (10^{-3}) were carried out by taking 10 mL of sample from the 10^{-2} dilution and putting it into 90 mL of Buffer solution. At each dilution, mixing was done at least 25 times, and 12-15 mL of PCA was added to each cup containing the sample. In order for the sample and PCA media to be completely mixed, the plate was rotated from side to side and was incubated upside down at 35°C.

2.6 Determination of Escherichia coli

The determination of *E. coli* bacteria was carried out based on SNI 2332.1: 2015 (BSN, 2015b). Sample preparation was carried out by weighing 25 g of dried sardines and then placing them in a sterile container or plastic, and adding 225 mL of butter field phosphate buffer solution. It was then homogenized for 2 mins, and the homogenate was a solution with a dilution of 10^{-1}, while the *E. coli* test was carried out in several stages. The suspected coliform test was carried out by preparing a 10^{-2} dilution by dissolving 1 mL of 10^{-1} solution in 9 mL of Butterfield's phosphate buffer diluent. In addition, an *E. coli* detection test was performed by inoculating each positive LTB tube into an EC broth tube containing a Durham tube, using an Ose needle. The confirmation test for *E. coli* was carried out by inoculating a positive EC broth tube using a loop needle and streaking it on Levine's eosin methylene blue (L-EMB), which was then incubated for 24 hrs at 35°C.

The morphological tests were carried out using a microscope with gram staining on each suspected *E. coli* colony. Cultures were taken from PCA which had been incubated for 24 hrs. All of those that appear as Gram-negative were short rods that would need to be biochemically tested and reinoculated into LTB medium to confirm gas build-up. The biochemical test was carried out in several stages, namely indole production, Proskauer Voges test, methyl red test, citrate test and gas production from lactose. The Proskauer Voges test was carried out by inoculating one loop of PCA slant into the Broth MRVP which was incubated for 48 hrs at 35°C, on the other hand, methyl red was incubated again at the same period and temperature.

2.7 Determination of insoluble ash content in acids

The determination of acid insoluble ash content was based on SNI-2354.1:2010 (2010). First, the dried sardine sample was mashed until homogeneous and put in a clean and closed plastic or glass container. Next, 2 g of the homogenized sample was put into a porcelain dish and put in the oven at 100°C for 24 hrs. Then the plate was transferred to the ashing furnace and the temperature was increased gradually to 550°C. It was then maintained for 24 hrs until white ash was obtained. Next, the temperature was lowered to around 40°C. After that, the ash was slowly moistened with distilled water, dried on a hot plate, and then ignited again at 550°C to a constant weight. Then the product was dissolved with 25 mL of 10% HCl and boiled for 5 mins. The solution was then filtered using Whatman 41 filter paper and washed several times with distilled water until free of chloride/acid. Dry filter paper containing acid ash does not dissolve in an oven at 100°C. After that, the porcelain plate was again transferred to the ashing furnace, and the temperature was gradually raised to 550°C for 16 to 24 hrs. When finished, the temperature was lowered to around 40°C, while the plate was removed using tongs and placed in a desiccator for approximately 30 mins.

2.8 Determination of salt levels

The determination of salt content was based on SNI 01-2359-1991 (1991). A sample of 2 g of dried sardines was weighed and put into a 250 mL Erlenmeyer flask. In addition, 25-50 mL of 0.1 N $AgNO_3$ was pipetted and put into a flask, then added with 20 mL concentrated HNO_3 and boiled slowly using a hotplate in an acid chamber, where all solids dissolved, except AgCl. Next, 50 mL of halogen-free water was added before cooling to room temperature; 3 mL of ferric indicator was also added and titrated with 0.1 N NH_4CNS until the solution became permanently light brown. The volume of 0.1 N NH_4CNS used for titration was recorded and the salt content (%) was determined in the brine solution. A total of 2 g of sample was weighed and put into an Erlenmeyer, titrated with 0.1 N $AgNO_3$ until a light brown color was reached at the endpoint; Potassium chromate was used as an indicator in this process.

2.9 Sensory analysis

The sensory assessment of dried sardines was based on SNI 8273: 2016 and was carried out every two weeks by 15 untrained panellists. This was done from 10.30 to 23.30 local time when the panellists were between 20 and 55 years old. In addition, the specifications considered included appearance, smell, taste, texture, and mould. The procedure used followed the organoleptic testing procedure. At the same time, each panellist was given the sample being tested, and for each change of sample, the panellist needed to neutralize their sense of taste with plain bread and a drink of warm water. Sampling was repeated every 2 weeks by placing the remaining samples back for further analysis (repeated sampling). Each sampling was repeated three times. The data obtained was analyzed by ANOVA with the SPSS application.

3. Results and discussion

3.1 Observation results of water content

According to Figure 1, dried sardines water content increased during storage until week 12. Immaculate *et al.* (2012) reported that traditional sun drying of *Sardinella fimbriata* reduced the fish water content by up to 11.6%. It was also reported that the moisture content of small mackerel fish is 7.7% when being dried under the sunlight and 7.5% when dried artificially (Abraha *et al.*, 2017). Kumar *et al.* (2013) reported that the moisture content of dried *Labeo gonius* fillets stored for three months was 11.50% which increased to 15.07% after six months. The water content of unstored dried chela (*Laubuka dadiburjori*) fish was 5.88%, which became 10.83% when stored for 30 days at room temperature and 14.90% after 60 days (Reza *et al.*, 2015). Meanwhile, Sam *et al.* (2015) reported that dried fish moisture content was initially 8.5-17% to 19-33% in Indian Tuticorin increased during storage for six months. Bioactive compounds in seasoning can inhibit microbial growth to extend the shelf life of fish.

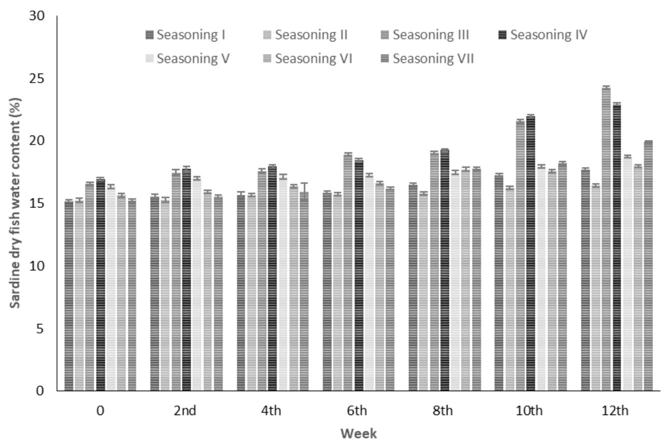

Figure 1. Sardine dry fish water content (%) with different shelf times.

The water content of fish fillets (*Oreochromis niloticus, Pylodictis olivares* and *Cyprinus carpio*) which was sun-dried and treated with garlic and ginger was 7.91% and 8.24%, as well as significantly different from

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the control (10.18%). After three months of storage, these treatments were 9.66% and 9.98% significantly different from the control (11.9%) (Tarle *et al*., 2016). Meanwhile, Farid *et al*. (2014) reported that the water content of sun-dried shoal fish which was treated with salt and turmeric was 30.92%, which became 35.26% after storage for 165 days, and 36.98% after 180 days. The water content increases because during storage the dried fish absorbs moisture from the air to fulfill the law of humidity equilibrium.

3.2 Observation results of protein

The protein content in this study generally decreased during storage until 12 weeks (Figure 2). The decrease in protein occurred because the protein had been degraded by microorganisms and due to enzyme activity, usually indicated by an increase in the value of TVB (total volatile base) (Pravakar *et al*., 2013; Reza *et al*., 2015). The high or low measured protein value can be affected by the amount of water lost (dehydration) from the product.

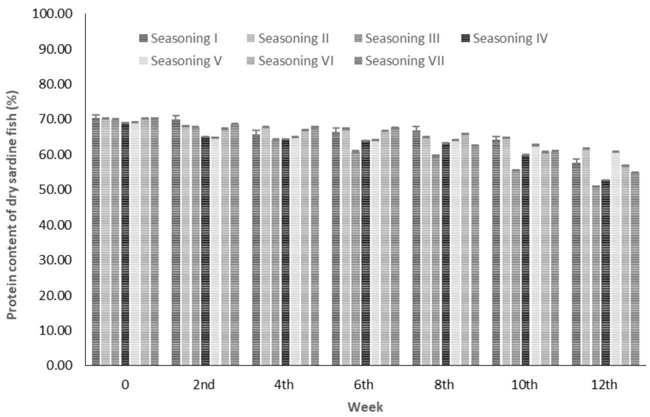

Figure 2. The protein content of dry sardine fish (%) with different shelf times.

The dried sardine fish protein content of 75.25% was lower than the value reported by Abraha *et al*. (2017) for sun-dried small pelagic fish, which had 78.82%. Meanwhile, Suvitha *et al*. (2015) obtained a protein content of 26.03% in sardine fish (*Sadinella longiceps*) and 32.56% in catfish (*Plotosus lineatus*). Tarle *et al*. (2016) reported that the protein content of fish filets (*Oreochromis niloticus*, *Pyloditis olivaris* and *Cyprinus carpio*) which were dried in the sun and treated with garlic and ginger was 74.84% and 76.12%; it was significantly different from the control which was 75.10%. After three months of storage, the protein content of fish fillets with the treatments raised to 75.13% and 76.43% and it's not significantly different from the control (75.39%). Meanwhile, Farid *et al*. (2014) reported that the protein content of sun-dried shoal fish which was treated with salt and turmeric was 41.0%, and it became 38.62% after storage for 165 days and became 38.01% after 180 days. Kumar *et al*. (2013) reported that the dry *Labeo gonius* fillet's protein content stored for three months was 48.08%, which decreased to 46.33% after six months. Reza *et al*. (2015) reported that the protein content of unstored dried Chela (*Laubuka dadiburjori*) fish was 62.80%, which became 60.12% when stored for 30 days at room temperature and raised to 57.34% after 60 days.

3.3 Observation results of fat content

The difference in seasoning composition causes differences in fat content. This occurs because phenolic compounds denature tissue proteins at a level that causes decreased protein emulsification properties and certain fat-content effects (Berkel *et al*., 2004). According to Figure 3, the fat content decreased during storage until week 12. This decrease probably occurred due to free fatty acid breakdown, which indicated rancidity in the product. This was in line with Abraha *et al*. (2017), who showed an increase in free fatty acids (FFA) from 1.33% to 1.74% during five days of the fish drying process. Sardine fish is known as a type of small mackerel fish that contains a lot of unsaturated Omega 3 and Omega 6 fatty acids (Mahrus, 2008; Arifan and Wikanta, 2011; Mahrus *et al.,* 2012).

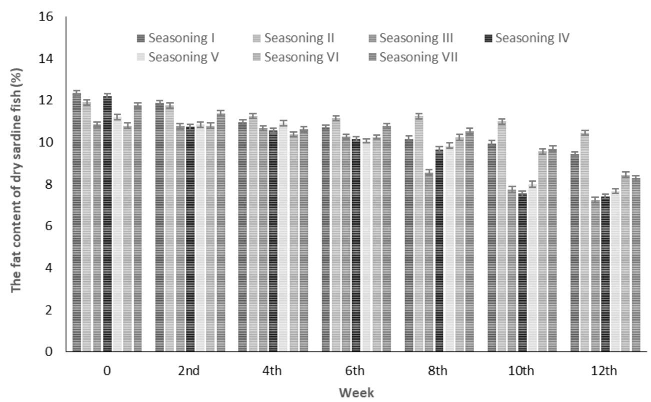

Figure 3. The fat content of dry sardine fish (%) with different shelf times.

Fat content decreased during storage, possibly due to tissue cell damage and heating effect during the drying process and polyunsaturated fatty acids oxidation in the product and synthesis of peroxides, aldehydes, ketones, and free fatty acids (Daramola *et al*., 2007). According to Immaculate *et al.* (2012), traditional sun drying on *Sardinella fimbriata* fish reduces the fat content by up to 6.3%. Research by Abraha *et al*. (2017) for small mackerel fish dried in the sun showed a fat content of 3.32% while those dried with an artificial dryer were 3.74%. This happens because the artificial dryer is able to maintain the fat content in the fish.

Tarle *et al*. (2016) reported that the fat content of the sun-dried fish fillets (*Oreochromis niloticus*, *Pylodictis olivaris,* and *Cyprinus carpio*) which were treated with

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garlic and ginger was 9.75% and 8.88%, which were not significantly different from the control (9.71%). After three months of storage, the fat content increased to 9.70% and 8.80%, significantly different from the control (9.66%). On the other hand, Farid *et al*. (2014) reported that the fat content of sun-dried shoal fish which was treated with salt and turmeric decreased from 4.79% after storage for 165 days to 3.81% and 3.62% after 180 days. Kumar *et al.* (2013) stated that the fat content of dried *Labeo gonius* fillet stored for three months was 7.35% which decreased to 5.78% after six months. Meanwhile, Reza *et al.* (2015) reported that the fat content of unstored dried Chela (*Laubuka dadiburjori*) fish was 14.00%, but became 13.80% when stored for 30 days at room temperature and went down to 11.33% after 60 days.

3.4 Observation results of insoluble ash content in acid

Food ash content is the minerals or organic substances that do not evaporate or are known as combustion product residue. The more the spices' salt and mineral concentrations are not burned in the ashing process, the higher the ash content is (Suryaningrum *et al*., 2013). This pedetan product has met SNI 8273-2016 requirements regarding dried salted fish, stating that the insoluble ash content in acid needs to be below 0.3 (Figure 4). The insoluble ash content in acid was observed to determine metals such as Hg, Pb, and Cd, which tend to experience precipitation when given an HCl solution. Furthermore, the ash content is an analysis of the control compounds which are the total minerals contained in the food samples tested.

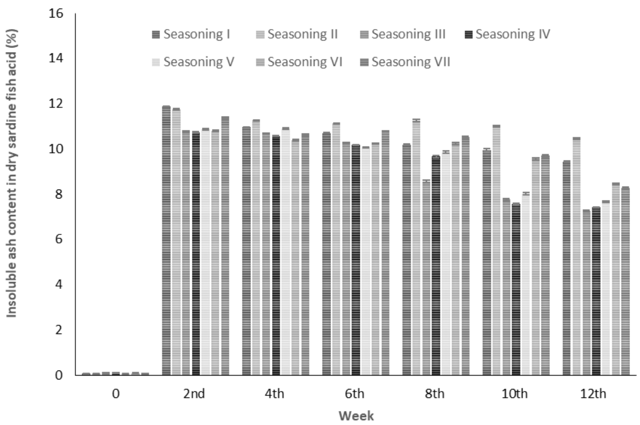

Figure 4. Insoluble ash content in dry sardine fish acid (%) with different storage times.

Immaculate *et al.* (2012) reported that 0.04% of ash content was obtained from traditional sun drying of *Sardinella fim*briata fish. Abraha *et al.* (2017) reported 9.2% ash content for sun-dried small mackerel fish and 9.9% for those dried artificially. Meanwhile, Suvitha *et al*. (2014) obtained an ash content of 5.67% in sardine fish (*Sadinella longiceps*) and 3.46% in catfish (*Plotosus lineatus*). In addition, Tarle *et al*., 2016 reported that the ash content of the sun-dried fish fillets (*Oreochromis niloticus, Pylodictis olivaris,* and *Cyprinus carpio*) which were treated with garlic and ginger was 7.36% and 6.56%, which were not significantly different from the control (7.31%). After three months of storage, the treatments became 7.03% and 6.40%, significantly different from the control (7.17%).

3.5 Observation results of salt levels

This product met SNI 8273:2016 (2016) requirements regarding dried salted fish, stating the required salt content needs to be below 20%. This study's salt content was lower than that required in SNI because dried fish were not considered salted due to less salt utilization than salted fish (Figure 5). Salt levels are also influenced by enzymes in spices which will cause water withdrawal in fish, so the salt levels in calves will be influenced by the compounds contained in spices. The salt content in this study is lower than that required by SNI because calf fish is not salted fish because the use of salt is much less than salted fish. Decreased salt levels are associated with water vapor absorption during storage due to salt's hydrostatic nature (Dewi *et al.,* 2011). In addition, Reza *et al.* (2015) stated that dried fish salt content decreased during storage for 60 days, from 17.54% on day 0 to 16.96% on day 30 and 15.67 on day 60.

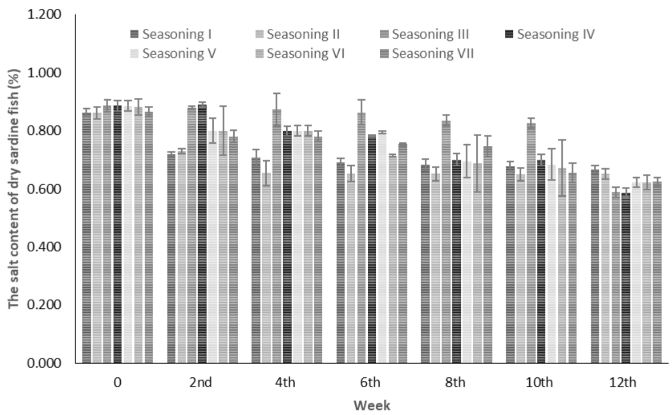

Figure 5. The salt content of dry sardine fish (%) with different shelf times.

3.6 Observation results of microbial populations

The pedetan product has met SNI 8273:2016 (2016) requirements regarding dried salted fish, stating the total plate value needs to be below 10^5 CFU/g (Figure 6). Total microbes in the traditional dried fish of Kejeik in Sudan were 3.4×10^6 - 7.8×10^8 CFU/g, while *Bacillus cereus* was 1.0×10^4 - 5.0×10^5 CFU/g, and halophilic bacteria were 2.3×10^5 - 8.5×10^5 CFU/g (Sulieman *et al.,* 2014). Abraha *et al.* (2017) reported a 5.7×10^3 CFU/g total microbe for sun-dried small pelagic fish, and those dried artificially had 3.2×10^3 CFU/g.

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In Ogongo and Odote's (2015) study, total microbes in sun-dried fish were 4.2×10^3 CFU/g and 2.6×10^3 CFU/g in artificially dried fish. Meanwhile, (Reza *et al.,* 2015) reported that the total microbes in unstored dried Chela fish (*Laubuka dadiburjori*) were 2.0×10^4 CFU/g, which became 5.2×10^4 CFU/g when stored for 30 days at room temperature and became 7.3×10^4 CFU/g after 60 days. Microbial growth occurred because of water's presence in the substrate. Food moisture content is not always proportional to water activity (a_w). Some dried foods with 0.7 a_w have different water content for each food ingredient. Food with an a_w of 0.7 is considered sufficiently resistant during storage. Microbial populations are consistently positively correlated with water and TVB activity (Mohamed and Ahmed, 2016; Abraha *et al.,* 2017). The product's a_w was between 0.663 to 0.0667. It is resistant to microbial growth during storage.

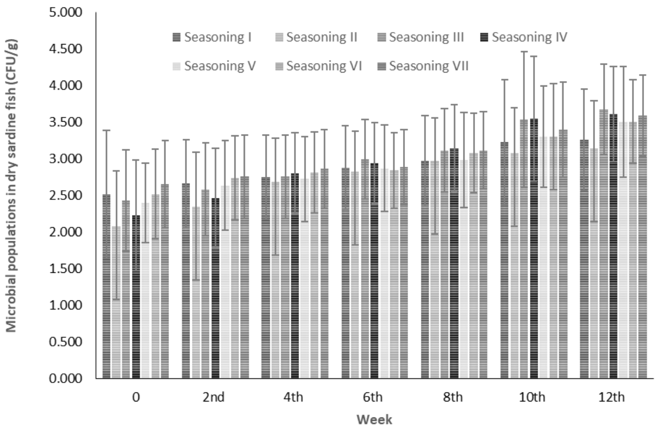

Figure 6. Microbial populations in dry sardine fish (CFU/g) with different storage times.

The lowest microbial population was found in products with seasonings I and II because both of these spices used vinegar. This acetic acid affects pH and has an inhibitory power against microbial growth. The pH value (degree of acidity) of sardine sheds increased from 6.1 to 6.6 during storage. The pH value increase was related to the production of basic components caused by microbial growth. Most microorganisms grow well at a pH of 6.6-7.5. The microbial population in pedetan was low because of spices utilization and the application of food processing basic feasibility with Good Manufacturing Practice (GMP) and Sanitation Standard Operating Procedures (SSOP) during dried fish production. This ensures that contaminant microbes are reduced in sardine fish (Singapurwa and Semariyani, 2017; Singapurwa *et al*., 2017). The spices in this pedetan contain bioactive compounds that inhibit microbial growth. *Allium sativum* extract inhibits the growth of various bacteria, including multidrug-resistant strains with bactericidal or bacteriostatic effects, possibly garlic is used as a supplement during antibiotic therapy, which may increase the effectiveness of gentamicin and ciprofloxacin (Magryś *et al.,* 2021). Garlic has the potential as an antibacterial against *B. cereus* bacteria (Athaillah and Lestari, 2020).

3.7 Observation results of Escherichia coli

The results showed no *E. coli* was detected (observation <3) in pedetan in all the treatments up to 12 weeks of storage. The pathogenic bacteria in traditional Kejeik dried fish in Sudan were, for example, Enterobacteriaceae of 1.7×10^3 - 3.5×10^5 CFU/g, and Coliform of 0.7×10^4 - 3.6×10^5 CFU/g (Sulieman *et al.,* 2014). Ogongo and Odote (2015) reported that there was no *E. coli,* coliform*,* and *Salmonella* sp. in sun-dried fish, but smoked fish contained *E. coli* of 8.0×10^1 CFU/g and coliform of 2.4×10^2 CFU/g.

In this study, there was also no growth of *V. cholerae*, *Salmonella* sp. and *S. aureus* compared to Singapurwa and Semariyani (2017) who reported negative results for the growth of these contaminant microbes. This was because the process of making the pedetan involved the use of spices and following food processing basic processing such as the GMP and SSOP to reduce contaminant microbes in dried sardine fish (Singapurwa and Semariyani, 2017; Singapurwa *et al*., 2017). The spices used during the manufacturing process contained bioactive compounds that inhibit pathogenic microbial growth. In some of the dried fish contaminated with heavy metals (Pb, Cd, Cr and others), pesticide residues (dichlorodiphe nyltrichloroethane, heptachlor, endrin, aldrin, dieldrin), higher levels of lipid oxidation, excess total plate count and the presence of *E. coli* highly pathogenic, *Salmonella* sp., *Vibrio* sp. was found in some dried fish that can cause a substantial human health hazard after consumption. The possible factors of these hazards are traditional drying, use of illegal pesticides with higher doses, lack of hygiene, sanitation, packaging, storage, distribution, and water pollution (Rasul *et al*., 2020). Garlic extract, vitamin C, vitamin E-selenium can be considered active compounds to reduce the hepatotoxicity caused by lead acetate but cannot be considered heavy metal chelators suitable to overcome lead bioaccumulation in vital organs (Shraideh *et al*., 2021).

3.8 Observation results of mould growth

The fungi in the product grew at the 10th week of storage for all treatments, except for seasoning II which did not grow until the 12th week (Table 2). This indicated that seasoning II compositions inhibited the growth of all mould types that tended to contaminate the product. The spices found in pedetan contain bioactive compounds that inhibit mould growth. The total mould in traditional Kejeik dried fish in Sudan was 0.9×10^4 - 1.6×10^4 CFU/g

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Table 2. Mould on dry sardine fish (CFU/g) with different storage times.

Treatment	Week						
	0	2nd	4th	6th	8th	10th	12th
Seasoning I	nd	nd	nd	nd	nd	$3.2\times10^0\pm2.00$	$8.3\times10^0\pm4.00$
Seasoning II	nd	nd	nd	nd	nd	nd	nd
Seasoning III	nd	nd	nd	nd	nd	$6.2\times10^0\pm8.00$	$1.7\times10^2\pm10.00$
Seasoning IV	nd	nd	nd	nd	nd	$5.3\times10^0\pm1.00$	$1.9\times10^2\pm8.00$
Seasoning V	nd	nd	nd	nd	nd	$4.6\times10^0\pm6.00$	$1.3\times10^2\pm20.00$
Seasoning VI	nd	nd	nd	nd	nd	$4.3\times10^0\pm3.00$	$1.2\times10^2\pm25.00$
Seasoning VII	nd	nd	nd	nd	nd	$5.5\times10^0\pm4.00$	$1.4\times10^2\pm30.00$

nd: not detected.

(Sulieman *et al.,* 2014). Research by Bawinto *et al.* (2015) showed that mould was found in packaged smoked tuna at $1.4\text{x}10^3$ CFU/g, while the one in the unpackaged one was 1.6×10^3 CFU/g. Also, the mould found in dried tuna was *Aspergillus* sp. and *Penicilium* sp.

Reza *et al*. (2015) reported that the mould in unstored dried Chela (*Laubuka dadiburjori*) fish was 1.8×10^2 CFU/g, which became 4.3×10^2 CFU/g when stored at room temperature for 30 days and 6.8×10^2 CFU/g after 60 days. These moulds include *A. niger, A. flavus, Rhizopus niger, Penicillium* spp., *Mucor,* and *Archaean halococcus*. Job *et al.* (2016) reported 2.0×10^3 CFU/g to 3.1×10^4 CFU/g moulds in smoked fish, and the positive mould that produced aflatoxins was *A. flavus*. This quantity increment occurred due to an increase in the a_w value and humidity. Mould growth during storage occurred at a product moisture content of 15% and an a_w value below 0.75. The dominant mould that grows on fish is *Aspergillus* sp. and *Penicillium*. Although mycotoxins' presence in dried fish products has not been reported, it is still necessary to pay attention to the traditional processing and preservation of fish (Gandotra *et al*., 2012; Reza *et al.,* 2015).

3.9 Results of sensory observations

The glossy appearance and specific taste are also influenced by the phenol content in the seasoned fish products. This product has met the SNI 8273-2016 requirements regarding dried salted fish, which states that the appearance sensory value needs to be above a score of 7 and the average at week 12th of storage needs to be 7.88 (Figure 7). Reza *et al*. (2015) reported an appearance sensory value of 6.67 for sun-dried small mackerel fish and 7.55 for those dried artificially. Generally, drying causes a browning reaction due to seasoning and fat oxidation. The intensity of browning is influenced by free amino acids, sugar reduction, proteolytic enzymes, and fat oxidation stages. Spice microparticles can efficiently reduce nitrite in sodium nitrite solution (nitrite clearance percentage: 36-100%) and high nitrite Tilapia muscle model (nitrite clearance percentage: 11-42%). In addition, the scavenging ability of nitrite depends on the type of spice and the microparticle size of the spice. Seasoning microparticles have other potential roles besides adding flavour when applied in fish and meat preservation processes (Wu *et al*., 2020).

Spice accumulation influences the aroma of fish. Furthermore, spices' volatile oils represent the aromatic aspect and contribute significantly to flavour. The non-volatile oil (oleoresin) is a flavour component that gives a sharp, hot, sweet, or bitter impression (Brown, 2009). This dry product has met the SNI 8273-2016 requirements regarding dried salted fish, which state that the required odour sensory value needs to be above a score of 7 and the average at week 12 of storage needs to be 7.85. Abraha *et al*. (2017) reported 7.00 for sun-dried small pelagic fish and 7.22 for those dried artificially.

The pedetan product has met SNI 8273-2016 requirements regarding dried salted fish, which state that the taste sensory value needs to be above a score of 7 and the average at week 12 of storage needs to be 7.73 (Figure 7). Abraha *et al.* (2017) reported an overall sensory acceptance of 6.78 for sun-dried small pelagic fish and 7.44 for those dried artificially. This calorie product has met the SNI 8273-2016 requirements regarding dried salted fish, stating that texture sensory value needs to be above a score of 7 and the average at week 12 of storage needs to be 7.48 (Figure 7). Abraha *et al.* (2017) reported an overall sensory acceptance of

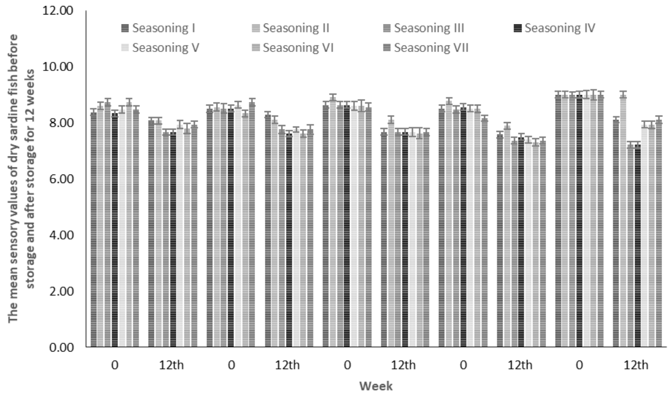

Figure 7. The mean sensory values of dry sardine fish before storage and after storage for 12 weeks.

7.33 for sun-dried small pelagic fish and 7.78 for those dried artificially.

There was no fungal growth in the seasoning II treatment until the 12th week of storage. This dried product has met the SNI 8273-2016 requirements regarding dried salted fish, stating that the fungal sensory value needs to be above a score of 7 and the average needs to be 7.93 in the 12th week (Figure 7).

4. Conclusion

The water content of pedetan increased during storage until the 12th week. The levels of protein and fat in this study generally decreased during storage until the 12th week. Ash content was insoluble in acid and salt content, microbiological analysis had met the requirements of the Indonesian National Standard 8273-2016 regarding dried salted fish. The sensory analysis shows a value of 7.93 and by the SNI. The composition of seasoning II which consists of garlic, coriander, *Kaempferia galangal*, *Alpinia galangal*, ginger, vinegar, and salt, is effective in extending the shelf life of sardine fish up to 12th weeks, has the best sensory and quality according to the standards set for food made from fish raw.

Conflict of interest

The authors declare no conflict of interest.

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