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Shelf-life Estimation of Instant *Pempek Rajungan* (*Portunus pelagicus*) Rancidity with the Arrhenius Acceleration Method

Pendugaan Umur Simpan Pempek Rajungan Instan Menggunakan Metode ASLT dengan Pendekatan Model Arrhenius

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Abstract

Rancidity is one of the potential damages to the instant *pempek rajungan (Portunus pelagicus)* during storage. The purpose of this study was to estimate the shelf life of instant *pempek rajungan* packed with polypropylene plastic (PP) based on the relationship between storage temperatures and free fatty acids produced. Estimation of shelf life using the acceleration method based on the Arrhenius model. Samples were stored for 30 days at three different temperatures, 25 ± 2 °C, 35 ± 2 °C, and 45 ± 2 °C. The parameter observed during the storage process was an assessment of the rancidity level based on the free fatty acids formed in the sample. Data were analyzed using linear regression and the equation obtained was used to calculate the shelf life of *pempek rajungan* at room temperature (27 °C). The results showed that the storage time for instant *pempek rajungan* products at temperatures of 25 ± 2 °C, 35 ± 2 °C, and 45 ± 2 °C could be estimated to be 78, 86, and 70 days respectively with an increase in the number of free fatty acids of 85.2018%, 265.3478%, and 253.1427%.
Keywords: Arrhenius, free fatty acid, instant, *pempek rajungan*, shelf life

Abstrak

Ketengikan menjadi salah satu potensi kerusakan pempek rajungan instan selama penyimpanan. Tujuan dari penelitian ini untuk menduga umur simpan pempek rajungan instan yang dikemas dengan plastik polypropylene (PP) berdasarkan hubungan antara suhu penyimpanan terhadap asam lemak bebas yang dihasilkan. Pendugaan umur simpan menggunakan metode akselerasi berdasarkan model Arrhenius. Sampel disimpan selama 30 hari pada tiga suhu yang berbeda, yaitu 25 ± 2 °C, 35 ± 2 °C, dan 45 ± 2 °C. Parameter yang diamati selama proses penyimpanan adalah penilaian tingkat ketengikan berdasarkan asam lemak bebas yang terbentuk pada sampel. Data dianalisis menggunakan regresi linier dan persamaan yang diperoleh digunakan untuk menghitung umur simpan pempek pada suhu ruang (27 °C). Hasil penelitian menunjukkan bahwa, waktu penyimpanan produk pempek instan pada suhu 25 ± 2 °C, 35 ± 2 °C, dan 45 ± 2 °C dapat diduga berturut-turut sampai 78, 86, dan 70 hari dengan peningkatan jumlah asam lemak bebas berturut-turut sebesar 85,2018%, 265,3478%, dan 253,1427%.
Kata kunci: *Arrhenius, asam lemak bebas, instan, pempek rajungan, umur simpan*

INTRODUCTION

Indonesia's territorial waters are 7.81 million km^2, with fishery resource production reaching 6.4 million tons. *Rajungan* (*Portunus pelagicus*) is one of Indonesia's fisheries potentials, mainly in the Java region, Pasir Putih Village on the coast of Karawang, West Java, Indonesia. In accordance with the Indonesian Ministry of Marine Affairs and Fisheries, the average catch of *rajungan* in the village reached 393 tons in 2013 (Kementerian Kelautan dan Perikanan, 2016). *Rajungan* is generally used as an economic field for the people of Pasir Putih Village, Karawang in conventional form, or peeled meat to be sold in export markets, including the United States, Singapore, and Taiwan (Awaludin, 2019).

Rajungan fat or *lemi* is one part of the *rajungan* that is not used or becomes waste. *Lemi* is the material of the *rajungan* body, which is located under the boiled shell. The nutritional content in *lemi* is no less high than rajungan meat, including protein (20.06%), fat (5.80%), water content (67.78%), ash content (2.52%), and carbohydrates (4.11%) (Anggo & Romadhon, 2006). The high protein content in *lemi* is a new potential for diversifying processed food products into instant *pempek rajungan* to have a higher selling value.

Instant food products have many advantages, including longer shelf life, practicality, and fast food so that consumers can save time (Lilly, 2014). Lifestyle changes currently make consumer demand for instant products increase (Srinivasan & Nirmala, 2014). Instant *pempek* is a dry product made through high-temperature processing by boiling and frying.

The deep-frying process of *pempek* with a high temperature of around 165-190 °C can cause mass food transfer, oil and air simultaneously encourage physical and chemical changes so that water loss, oil absorption, color change, flavor development, gelatinization, and oil oxidation will occur (Ma et al., 2016). The oil content in *pempek* is easily oxidized when exposed to oxygen which can cause rancidity (Ketaren, 2008). Rancidity is an unwanted type of damage to *pempek*, so it is necessary to know how long the food product can be stored in a condition that is still acceptable to consumers. Therefore, it is necessary to analyze the estimated shelf life of these products.

Estimation of shelf life aims to ensure that the product reaches consumers in good condition, safe for consumption, the quality of the product remains after the production process, packaging, and storage at certain temperature conditions so that changes in chemical, physical, microbiological, and sensory characteristics can be identified (Haouet et al., 2019). Estimation of shelf life using the Accelerated Shelf Life Test (ASLT) method is an estimation of shelf life by storing products under extreme conditions and checking periodically until the product safety limit is reached then projecting the shelf life under the actual storage conditions (Haouet et al., 2019).

The ASLT method can be used with the Arrhenius model approach and critical water content. The critical water content model is usually used for products that are easily damaged due to water absorption during storage, while the Arrhenius model uses temperature acceleration so that it can accelerate reactions that cause damage to the product. This model is usually used for products that are sensitive to changes in storage temperature. The purpose of this study was to estimate the shelf life of instant *pempek rajungan* packed with polypropylene plastic (PP) based on the relationship between storage temperatures and free fatty acids produced.

METHODS

The research was conducted with a descriptive experimental method on samples of instant *pempek rajungan* stored at three storage temperatures: 25 ± 2 °C, 35 ± 2 °C, 45 ± 2 °C. Estimation of shelf life is carried out using the ASLT method of the Arrhenius acceleration model. Samples were stored in an incubator at three storage temperatures for 30 days. Observations were made on days 0, 5, 10, 15, 20, 25, 30. The main criteria used to estimate shelf life are the method of measuring the acid number or Free Fatty Acid (FFA) (American Oil Chemists' Society, 1998) and several supporting criteria, namely water content (Association of Official Analytical Chemists, 2000) and Total Plate Count (TPC) (Badan Standarisasi Nasional, 2008).

The tools used in this research include autoclave, bulb pipette, bunsen, aluminum plate, petri dish, colony counter, desiccator, fin tip, glass spatula, test tube, burette, Erlenmeyer flask, graduated flask, oven, graduated pipette, static, analytical scales, and a wooden incubator. The materials used in this study were instant *pempek rajungan* produced by Sumber Rizky Pasir Putih Karawang, distilled water, 70% alcohol, 96% ethanol, PP packaging, PP indicators, NaOH, 0.85% Physiological NaCl, and Plate Count Agar (PCA).

RESULTS AND ANALYSIS

Quality Decrease Rate Kinetics of Free Fatty Acid Parameters

Free fatty acid content is a chemical property that describes the amount of free fatty acids in a material expressed in percent. The results of the observation data analysis of the free fatty acid content of instant *pempek rajungan* at three storage temperatures can be seen in Table 1.

Table 1 shows that free fatty acids have been formed in the *pempek rajungan* product after processing or at the beginning of storage. The free fatty acid composition continued to increase after storage until the 6^{th} week. Storage at a temperature of 25 ± 2 °C can increase the formation of fatty acids up to 85.1790%, storage at a temperature of 35 ± 2 °C can increase the formation of fatty acids to 265.3478%, and storage at a temperature of 45 ± 2 °C can increase the formation fatty acids up to 253.1427%. Deep-frying processing can produce three reactions: hydrolytic reactions, oxidation reactions, and reactions due to temperature changes (Table 2). The hydrolytic reaction occurs due to the evaporation of water from the material that changes into the oil phase, causing the formation of free fatty acids, mono and diacylglycerol, and glycerol. The oxidation reaction combines the degradation reaction of oxidation products to form short-chain fatty acids, aldehydes, ketones, hydrocarbons, epoxides, and other products called oxidized triacylglycerol (TAG). Temperature changes produce cyclic compounds, which are intramolecular reactions of dimers with polymers by conjugation of C-C chains in intermolecular reactions (Weisshaar, 2014).

The results (Table 1) show that the more extended storage and the higher the storage temperature will increase the amount of free fatty acids in the instant *pempek rajungan*. Increasing the temperature will multiply the molecules that have kinetic energy. This energy can make the particles involved in the reaction move faster (Table 2) so that the oil hydrolysis reaction will run faster with high-temperature treatment as a catalyst.

The value of the free fatty acids amount from the research results is then plotted so that the regression equation found is used to obtain the reaction order. The selection of the reaction order in determining shelf life is based on a regression equation that has a value of R^2 close to 1. Determination of the quality deterioration rate constant (k) for each storage temperature is carried out after knowing the reaction order used. The k value is obtained from the slope value of each regression equation in reaction order one. The value of k shows the reaction rate of changes in free fatty acids that occur during storage due to the effect of temperature on the reaction rate. The regression equation in determining the reaction order and the k value can be seen in Table 3.

Table 1. Value of free fatty acid change (%) at various storage temperature

Week	Temperature 25 ± 2 °C	Temperature 35 ± 2 °C	Temperature 45 ± 2 °C
0	0.7792	0.6799	0.9164
1	0.8171	1.0621	1.8040
2	0.9325	1.3848	2.0095
3	1.1328	1.8228	2.5598
4	1.2116	2.1567	3.1719
5	1.3939	2.4079	3.2018
6	1.4431	2.4840	3.2362

Table 2. Major compound changes of oil during the deep-frying process

Reaction	Causative Factor	The New Compound Produced
Hydrolytic	Humidity	Free fatty acids Diacylglycerol Monoacylglycerol
Oxidation	Air	Monomers are oxidized Oxidized dimers and polymers Volatile compounds (aldehydes, ketones, hydrocarbons) Oxide sterols
Heat	Temperature	Non-polar dimers and polymers Cyclic monomers Trans isomers and position isomers

(Source: Bordin et al., 2013)

Table 3. Free fatty acid linear regression equation at various storage temperature

Temperature	Regression Equation		R^2		k
	Ordo 0	Ordo 1	Ordo 0	Ordo 1	
25 ± 2 °C	y = -0.0372x + 0.8136	y = -0.0564x + 4.4138	0.977	0.978	-0.0564
35 ± 2 °C	y = -0.0965x + 0.8642	y =-0.2131x + 4.6249	0.975	0.984	-0.3001
45 ± 2 °C	y = -0.1187x + 0.7399	y = -0.7065x + 5.3151	0.911	0.932	-0.7065

Table 3 shows that the selected order is order one because it has a coefficient of determination R^2 greater than that of zero-order (R^2 order 0 < R^2 order 1). R^2 values that are close to 1 have better accuracy (Imamah, Hasbullah, & Nugroho, 2016). The value of k at each temperature has a negative value. This means that there is a decrease in the quality of the instant *pempek rajungan* due to the increase of free fatty acids amount along with the storage time. Determination of the shelf life of instant *pempek rajungan* can be done by plotting the K value of storage temperature in the Arrhenius equation, $\ln k = \ln k_0 - (Ea/R)(1/T)$. The parameters of the change in free fatty acids in ln k and 1/T of instant *pempek rajungan* can be seen in Table 4.

The plot result between 1/T and ln k is the Arrhenius equation: y = 1.2639x - 4.0033 with R^2 = 0.97. The correlation coefficient obtained is close to 1. This means that temperature is very influential on changes in free fatty acids. Rancidity in deep-frying food products is caused by the activity of the lipase enzyme, which reacts with water vapor and temperature. The reaction will be fast if there is an increase in temperature and light. Oxidation reactions can be prevented by storing at low temperatures, removing oxygen in the package, avoiding light in direct contact with the product, or adding anti-oxidants. Oils with a high content of unsaturated fatty acids are more susceptible to oxidation damage, so they need packaging that better protects products from oxygen and light (Oke et al., 2018).

Estimated Shelf Life

Estimation of shelf life is done by calculating the activation energy (Ea) obtained from the Arrhenius equation using the formula Ea = slope x R with a value of R = 1.986 cal/mol. The result of the calculation of the activation energy of this *pempek rajungan* is 10.15 Kcal/mol.K. This Ea value is related to the speed with which the product is damaged. Instant rajungan pempek has activation energy which is considered quite low. The lower the Ea value, the reaction speed for deterioration of quality will run quickly.

The activation energy value of Ea is then substituted into the equation $k_T = k_0 . e^{-Ea/RT}$ so that the k_T value is obtained, which is used to estimate the shelf life of instant *pempek rajungan*. The k_T value is then plotted in the first-order reaction kinetics equation with the formula $\ln k_T = (Ea/R)(1/T) + \ln k_0$. The results of the calculation of the shelf life of instant *pempek rajungan* at various storage temperatures can be seen in Table 5.

Table 5 shows that the shelf life of instant *pempek rajungan* is shorter at higher storage temperatures. This means that the temperature increase causes the reaction rate to speed up, which causes the instant *pempek rajungan* to break down quickly so that the shelf life is getting shorter. The rate of chemical reactions is faster at higher temperatures, which means that the decline in product quality is fast (Palupi et al., 2010)

Increasing the temperature will multiply the molecules that have kinetic energy. This energy can make the particles involved in the reaction move faster. Based on the parameters of free fatty acids, the instant *pempek rajungan* produced by Sumber Rizky has a shelf life of 86 days or two months 26 days at a storage temperature of 35 °C. The oil in the product will undergo oxidation and hydrolytic during storage. The reactions that occur are the same as the deep-frying process, but the reactions that run are slower (Bordin et al., 2013).

Table 4. Arrhenius equation parameters change in free fatty acids during storage

T (K)	1/T	Slope (k)	Ln k
298	0.0034	0.0564	-2.,8753
308	0.0032	0.3001	-1.2036
318	0.0031	0,.7065	-0.3474

Table 5. Prediction of shelf life of instant *pempek rajungan* at various storage temperatures

Storage Temperature	Shelf Life (Days)	Shelf Life
25 ± 2 °C	78.4907	2 months 18 days
35 ± 2 °C	85.9351	2 months 26 days
45 ± 2 °C	69.6680	2 months 10 days

Characteristics of Instant *Pempek Rajungan* Total Plate Count (TPC)

Total Plate Count (TPC) is carried out as a test of supporting parameters. Testing of supporting parameters is carried out at the beginning of storage until the end of storage (day 30). The increase in the microorganisms number in instant *pempek rajungan* can be seen in Figure 1. The number of initial microorganisms at various storage temperatures was 2.5 x 101 CFU/g. The number of microorganisms at the end of storage was respectively 2.3 x 10^4; 2,6x10^4; 1x10^5 CFU/g at 25 ± 2 °C, 35 ± 2 °C, and 45 ± 2 °C. The Indonesian National Standard (SNI) sets the expected value for the number of microorganisms

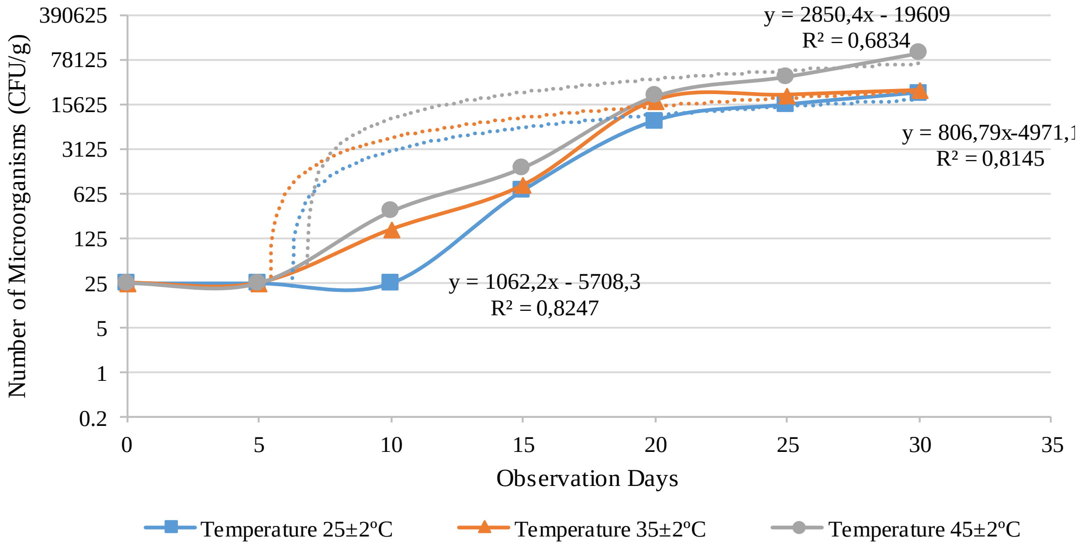

Figure 1. The Growth of Instant *Pempek Rajungan* Microorganisms

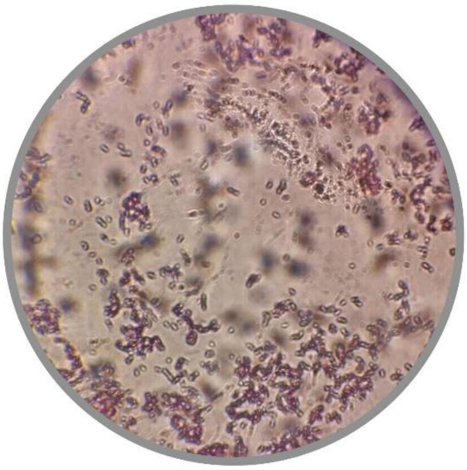

Figure 2. Gram Stain Result, Magnification of 100 x 40

in TPC units. According to SNI 2713: 2009, the maximum TPC limit for fish/mollusk-based dry-processed products is a maximum of $5x10^4$ CFU/g. This shows that the amount of TPC at the beginning of storage with various storage temperatures and at the end of storage at 25 °C, 35 °C still meets the standards, while the amount of TPC at 45 ± 2 °C storage temperature does not meet the standard, which means that instant *pempek rajungan* is not suitable for consumption.

Figure 1 shows that the increase in the number of microbes is found at a temperature of 45 °C. This means that bacteria that grow in instant *pempek rajungan* can grow optimally at that temperature. The bacteria that usually grow at this temperature are thermophilic. Two factors influence the growth of microorganisms, namely extrinsic factors and intrinsic factors. Extrinsic factors include handling, processing, and storage. Intrinsic factors include moisture content in the product. Water is the most critical element in food that can determine the growth of microorganisms. Water activity (a_w) is free water as a growth medium for microorganisms in food products (Sandulachi, 2012). Changes in a_w can cause osmotic pressure, giving rise to a sporulation response to spore-forming microorganisms (Tapia, Alzamora, & Chirife, 2008). The lower the water content in food, the lower the ability of microorganisms to grow and become destructive to foodstuffs (Carter, 2008). The microorganisms found in instant *pempek rajungan* products after storage at critical temperatures have gram-positive morphology and in the cocci form (Figure 2). According to Brooks *et al.* (2012), bacteria that may grow in fish products and their processed products are *Pseudomonas*, *Micrococcus*, and *Microbacterium*.

Water Content

Another supporting parameter tested is moisture content. Water content was tested at the beginning of storage until the end of storage (day 30). The water content of instant *pempek rajungan* before and after storage still meets SNI 2713: 2009 concerning fish-based dry-processed products, which is maximum water content of 12%. Observation data plotted on the graph can be seen in Figure 3.

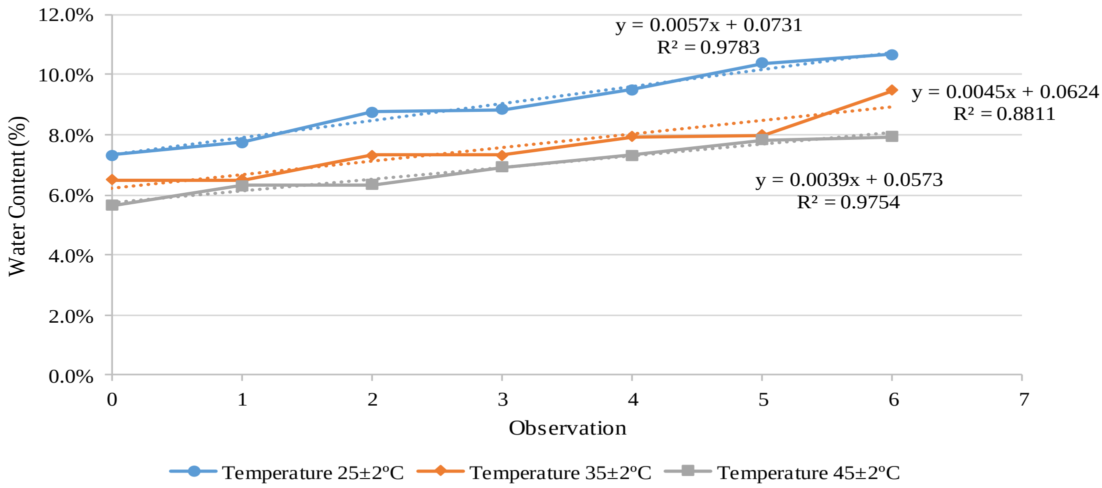

Figure 3. Graph of the Relationship Between Water Content and Storage Time

Figure 3 shows that the water content increase in the instant *pempek rajungan* product occurred at storage temperatures of 25 °C, 35 °C, and 45 °C during storage. The increase in water content can be seen from the positive value of the slope of the equation line. The instant *pempek rajungan* stored at 25 °C had the highest increase in water compared to 35 °C and 45 °C. This is caused by differences in air humidity at various storage temperatures. In accordance with Arizka and Daryatmo (2015), storage temperature is related to the partial pressure of water vapor (RH). The product will absorb moisture from the environment through the packaging if the humidity of the ambient air is high, while the sample will release free water to the environment if the ambient air humidity is low so that the final weight of the sample will increase with the length of storage. Another factor that affects the increase in moisture content is the permeability of the packaging. Instant *pempek rajungan* is packaged using polypropylene plastic (PP). PP packaging has a permeability value of 1.114 g/cm^2/24 hours. The low permeability value has the ability to hold water vapor into the package. The increase in water content of instant *pempek rajungan* was greater at high-temperature storage. This is due to the expansion of the package's pores so that it is easier for water vapor to migrate from the environment to the material (Puspita, Rangga, & Sartika, 2016).

The increase in *pempek rajungan* water content (Figure 3) during storage is directly proportional to the increase in microorganisms' growth (Figure 1) and the increase in the amount of free fatty acids formed in the product. This proves that the increase in water content is caused by the absorption of water vapor during the storage process. The deep-frying process causes the water in the ingredients to diffuse into the oil in the form of steam so that the water content of the product is low, the texture is crunchy, and contains higher fat (Raleng et al., 2019). After the frying process, the *pempek rajungan* will be hygroscopic, so it easily absorbs environmental moisture. The absorption of water vapor in the product greatly affects the growth of microorganisms because of the presence of free water and accelerates the process of forming free fatty acids, which can cause a rancid aroma.

CONCLUSIONS

The ASLT method with the Arrhenius model approach can estimate the shelf life of instant *pempek rajungan* packed with PP plastic with storage temperatures of 25 ± 2 °C, 35 ± 2 °C, and 45 ± 2 °C to 78, 86, and 70 days respectively with an increase in the free fatty acids number, respectively 85.2018%, 265.3478%, and 253.1427%. Storage at 35 ± 2 °C able to maintain the shelf life of instant *pempek rajungan* for 86 days with 2.6 x 10^4 CFU/g of microorganisms and 9.47% moisture content which has met SNI 2713: 2009.

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