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Estimation of shelf life for Ready-To-Eat sardines (RTE) using the Accelerated Shelf-Life Testing (ASLT) method

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Abstract. Ready-to-eat sardines (RTE) are ready-to-eat foods designed to satisfy the needs of people who don't have time to cook. In order to maintain the quality of RTE products and have a longer shelf life, efforts should be made to maintain the quality of RTE products, one of which is the use of cold storage methods and packaging. The purpose of this study was to estimate the shelf life of RTE sardines using the accelerated method combined with the Arrhenius method. This study employs a completely randomised Design with two factors: packaging type (vacuum and non-vacuum packaging) and storage temperature (cold storage and freezing temperature). The parameters tested in this research include TPC (total plate count), organoleptic properties, fat content, and protein content. The estimated shelf life of ready-to-eat sardines (RTE) in this study shows that RTE fish has a shelf life of up to 558.96 days if stored at a freezing temperature and vacuum packed, and 313.29 days if stored at a refrigerated temperature and vacuum packed. While ready-to-eat sardines (RTE) have a shelf life if stored using pouch packaging of 514.83 days at freezing and 2945.13 days at refrigerated temperatures with the Arrhenius equation obtained, namely ln k = (13.436 – 3034.6) (1/T).

1 Introduction

Ready to Eat (RTE) food is a convenient option for people who lead hectic lives and have little time to cook. Grand View Research (2022) research data reveals an intriguing trend: the ready-to-eat food business has grown significantly. This market expanded by 5.5% in 2020, and this trend is likely to continue through 2027. The rise in demand for RTE food is mostly due to the fact that food manufacturers are increasingly focused on two critical aspects: first,

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delivering ready-to-eat food that does not require cooking, and second, ensuring that these meals still include essential nutrients required by the consumer's body. However, despite the growing popularity of RTE food, some people still have trouble obtaining it due to its high price.

The pricing gap provides an opportunity for small and medium-sized businesses to make ready-to-eat items at lower costs, such as ready-to-eat sardines packaged in pouches. RTE sardines are derived from sardines found in Indonesian waters. Sardines are popular not only because of their abundance, but also because of their complete nutritional composition, which allows them to be utilized as a source of meeting nutritional needs, especially protein. According to [1], fresh sardines contain 72.7 percent water, 19.9 percent protein, 1.8% fat, and 95 milligrams of calcium per 100 grams.

To ensure that ready-to-eat sardine products remain of good quality and have a longer shelf life, an effort is required that can maintain the quality of ready-to-eat sardine products, namely by adopting vacuum packaging techniques. Vacuum packaging is a packaging technology that uses a pressure of 1 atm to remove oxygen from a product.

Vacuum packaging techniques can preserve and prevent food spoiling by restricting the introduction of oxygen and bacteria. [2] discovered that vacuum packaging applications can reduce microbial growth rates (1.5 x 10 CFU/g) for up to 14 days of yellow pindang fish storage. The lack of oxygen in the package can also inhibit chemical reactions such as oxidation, reducing damage to ready-to-eat sardine items and perhaps increasing product shelf life.

As a result of projecting a shorter shelf life, (1) greater production expenses will be incurred, and (2) the product will be wasted because it must be destroyed even though it is still fairly good. Thus, the process of calculating shelf life and expiry limit must be carried out using reliable scientific principles, one of which is the Arrhenius approach's Accelerated Shelf-Life Testing (ASLT) method [3]. In general, this method investigates the quality characteristics of products, what factors influence the pace of change in product quality, and what quality conditions are rejected by consumers or have reached their expiration date. With this information, an experimental design can be carried out to expedite the process of quality damage to the final product and then calculate how long the ready-to-eat product can be stored. On the basis of this description, research will be done to estimate the shelf life of ready-to-eat sardines using the Accelerated Shelf-Life Testing (ASLT) method, which is packaged utilizing vacuum techniques. [4] conducted research on determining shelf life on nori fruit using the Arrhenius model ASLT method by increasing storage temperature, especially by employing temperatures above room temperature: 25°C, 35°C, and 45°C. The findings of Arrhenius computations are chosen using texture properties as essential parameters. Fruit nori has a shelf life of 31 days 13 hours 40 minutes 48 seconds if stored at 25 degrees Celsius, but unpackaged items have a shelf life of 51 days 16 hours 33 minutes 36 seconds. Furthermore, the shelf life of smoked Roa fish was determined using the ASLT method with an Arrhenius approach, with observations based on organoleptic parameters, total microorganisms, water content, and pH. The results revealed that smoked roa fish had a shelf life of 2 months 8 days at 300 degrees Celsius, 4 months 3 days at 400 degrees Celsius, and 7 months 4 days at 500 degrees Celsius [5].

Furthermore, [6] used the Arrhenius model to calculate the shelf life of wood-dried cooked madidihang fish in PP plastic vacuum packaging. The findings of a shelf-life analysis at room temperature (250C) based on the Total plate count (ALT) parameter revealed that madidihang fish can be stored for 15 days. Based on the findings of this study, it is recommended that products be stored at low temperatures to maintain quality for a longer period of time. In addition to processed fish goods, shelf-life analysis was performed on instant liquid formula food products produced from catfish flour and leaves [7]. The ASLT method was used to assess the shelf life of instant liquid formula foods after 42 days of storage at varied storage temperatures. Quality alterations were assessed using key criteria (water content and rancidity) as well as supportive factors (proximate, calcium, calcium bioavailability, and protein digestibility). The results revealed a change in quality during storage with a shelf life of 70 days, showing that the quality of instant liquid formula food can survive based on KA characteristics. The goal of this study was to assess the effect of vacuum packing techniques on the fall in quality of RTE sardine products, as well as the shelf life of RTE sardine goods packaged with vacuum techniques.

2 Research methods

The experimental method employed is a Complete Randomized Design (RAL) with two treatments. The first is packaging techniques (vacuum and non-vacuum packaging), and the second is sardine storage temperature (room temperature, cold temperature, and freezing temperature). The Arrhenius methodology was used to calculate the shelf life of sardine products using the Acceleration method. [3] used the Arrhenius equation to determine the reaction sequence and calculate the shelf life.

$$ln\, k = ln\, k0 - \frac{Ea}{R}\left(\frac{1}{T}\right) \qquad (1)$$

K = rate of deterioration
K0 = konstanta (frequency factor that does not depend on temperature)
Ea = Energy activation
R = Gas regularity
T = temperature (in kelvin)
The Shelflife formula:

$$t = \frac{t_s - t_0}{k} \qquad (2)$$

t = shelf life
ts = Product Quality Limit Value
t0 = Initial Value of Product Quality
k = rate of deterioration

Ready-to-eat sardine products sourced from MSMEs under the name Dapur Q in Pekanbaru, Riau Province, and plastic bag packaging are the primary raw materials used. H_2SO_4, aquades, MRS A, MRS B, and aquades are among the compounds employed. Muffle furnaces, ovens, analyte scales, vacuum packers, and glassware are among the tools employed.

The investigation began with sample preparation by weighing 30 g of material, which was subsequently packaged utilizing vacuum and nonvacuum packing procedures. Each of these samples is then held at different temperatures, namely room temperature, cold temperature, and freezing temperature.

3 Research parameters

3.1 Total plate count

The plate count and dilution method were utilized to calculate total plate count (TPC) in this investigation. TPC is calculated according to the National Standardization Agency (2014), namely the number of colonies on petri dishes. The stages of work in determining this total plate count are as follows: sterilization of equipment, preparation of physiological salt solutions (diluents), preparation of PCA medium, and calculation of total plate counts.

Equipment sterilization is referred to by [8]. Glass equipment (test tubes, petri dishes, erlenmeyer, drip pipettes, volume pipettes, spatulas, measuring cups, and beaker glass) is first washed with detergent until clean, then dried and protected from dust and other debris. After drying, all glass utensils were placed in 1,000 mL of HDPE plastic and sterilized in an autoclave at 121oC for 15 minutes at a pressure of 1 atm. Hockey sticks are disinfected by burning them on bunsen, leaving them for a time, then using them for each use. National Standardization Agency (2014) defines media creation, PCA media weighed 22.5 g and was placed in an Erlenmeyer flask and dissolved in water till the amount reached 1,000 mL. The media is heated with a hot plate while being stirred with a magnetic stirrer until it boils and homogeneously.

The media was sterilized in an autoclave at 121°C for 15 minutes. The media is then cooled to 45°C and put onto petri dishes, with up to 15 mL each petri dish. The substrate is poured in the laminar flow cabinet. The media is hardened in the laminar flow cabinet before being employed as microbial growth media. Media creation is defined by the National Standardization Agency [9]). PCA media weighed 22.5 g and was dissolved in water in an Erlenmeyer flask until the volume reached 1,000 mL. The media is heated with a hot plate and swirled with a magnetic stirrer until it boils uniformly. For 15 minutes, the medium was sterilized in an autoclave at 121°C. The media is then chilled to 45°C and placed on petri dishes, with up to 15 mL in each. In the laminar flow cabinet, the substrate is poured. Before being used as microbial growth media, the media is hardened in a laminar flow cabinet.

The scatter method is used to calculate total plate count (TPC) [8]. The sample was weighed at 5 g and crushed with a crusher and pestle before being placed in an erlenmeyer flask with 45 mL of diluent solution. After homogenizing the sample solution with a vortex, a 1 mL solution is taken and placed in a test tube containing 9 mL of diluent solution, yielding a dilution of 10-1 to 10-5. The diluted sample solution was taken up to 0.1 mL with a micropipette, then inoculated on frozen PCA medium and spread evenly with sterile hockey sticks.

$$TPC\ \left(log\frac{CFU}{mL}\right) = \sum koloni\ \times 1/Fp\ \ (3)$$

3.2 Organoleptik

Sensory assessment is defined as employing a descriptive test [10]. There were 30 descriptive test panelists, and sample testing was done on day 1; 5, 10, 15, 20, 25, and 30 to 30 samples stored at room, cold, and freezing temperatures.

4 Result and disscussion

Total plate count is a method for determining the amount of bacteria in food originating from animals and processed products using agar plate count (PCA) media. The number of bacteria recovered from the sample represents the population. The total plate count microbiological test is used to analyze bacterial growth during storage [11]. Table 1 shows the quantity of microorganisms per total plate count included in RTE sardines packaged utilizing vacuum and pouch packing.

Table 1. Displays the total number of plates. Sardine product data (CFU/gram).

No	Treatments	Days				
		0	5	10	15	20
1	Vacum refrigerating	266.5	603	707.5	741	974,5
2	Vacum freezing	266.5	442	509.5	442	702
3	Pouch refrigerating	266.5	683.5	735	811	1073
4	Pouch freezing	266.5	500	589.5	500	785

According to Table 1, the total plate count grows with the amount of storage days. The total plate counts of vacuum packaged RTE sardines and pouches stored in cold circumstances were higher (974.5 and 1073 CFU/gram) on the 20th day of storage than vacuum packaged RTE sardines and pouches held at freezing temperatures (702 and 785 CFU/gram). Because of the temperature utilized for storage, the total plate count of RTE sardines held at freezing conditions is smaller than those stored at cold temperatures.

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4.1 Shelf life estimation

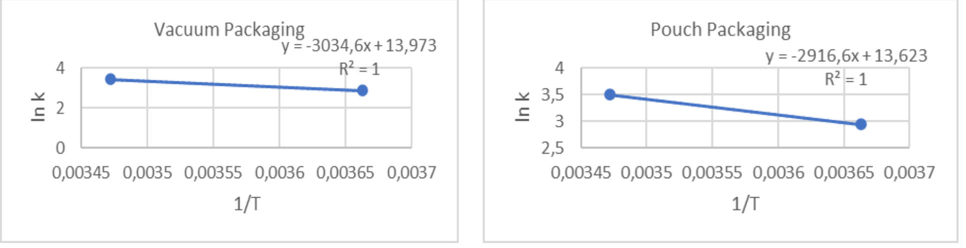
Food products will suffer chemical, biological, and physical changes during the storage process. These changes are induced by reactions that occur during the storage period and cause harm, resulting in product quality decline. The degradation in product quality will render the product unfit for consumption. The kinetics of damage refers to the rate at which quality deteriorates.

RTE sardines that are vacuum packed and pouched and stored in cold and freezing temperatures will likewise degrade in quality over the storage time. The reaction order 0 is followed by the rate of damage that happens linearly or constantly, whereas the reaction order 1 is followed by the rate of damage that occurs inconstantly. The reaction order is determined by the highest determination value (R) shown in Table 2. Based on Table 2, the maximum determination value is discovered on order 0, hence the data utilized to determine the Arrhenius equation are data on order 0.

Table 2. Order of reaction.

Treatments	R^2 ordo 0	R^2 ordo 1
Vacum refrigerating	0.8925	0.7738
Vacum freezing	0.7795	0.749
Pouch refrigerating	0.9045	0.82
Pouch freezing	0.7607	0.7588

Ready-to-eat sardines are processed fish that are readily destroyed. Sardines have a high-water content as well as complete nutrients like as protein and fat, which are ideal for the growth of microorganisms. As a result, the presence of bacteria in sardine products to some extent is one of the symptoms of damage. The total plate count can be used to calculate the population of microorganisms growing on food. As a result, the total plate count can be used to predict damage to ready-to-eat sardine products. Damage rate reaction sequence was determined using regression data between total plate counts on RTE sardines throughout storage. Table 2 shows that the value of R2 on order 0 is greater than that on order 1. As a result, the rate of degradation in RTE sardine products during the reaction is nil. According to [3], the reaction of order 0 causes a general decline in the quality of frozen food products.



(a) (b)

Fig. 1. Relationship graph Ln K and 1/T packaged RTE sardines a). Vacuum Packaging and b). Pouch Packaging.

Table 3. Recapitulation of Quality Deterioration Rate, Energy activation (Ea) and Shelf Life of RTE Sardine Products

Type of Packaging	Arrhenius Equation	Ea (kal/mol k)	Deterioration rate (k)	Shelf-life (days)
Vakum dingin	Ln k= 13.973 – 3034.6 (1/T)	6026.72	31.07	313.29
Vakum beku		6026.72	17.41	558.96
Pouch dingin	Ln k= 13.623 – 2916.6(1/T)	5792.37	32.90	295.13
Pouch beku		5792.37	18.91	514.83

4.2 Shelf life estimation

The Arrhenius equation can be used to calculate the projected shelf life of vacuum-packed RTE sardines and pouches stored at cold and freezing conditions. By advancing the values of ln k and 1/T (temperature in kelvins) shown in Figure 1, the Arrhenius equation can be produced. The slope (gradient) and intercept values of the Arrhenius equation will be derived and utilized to calculate the energy activation and rate of deterioration (k) of RTE sardines. According to Figure 1, the Arrhenius equation for RTE sardines packaged in vacuum packing is k = 12.416 - 2588.6 (1/T), while the Arrhenius equation for pouch packaging is k = 13.623 - 2916.6 (1/T). The rate of quality degradation, energy activation, and shelf life can be calculated using the Arrhenius equation, as shown in Table 3.

According to Table 3, the rate of quality deterioration in cold vacuum packaged RTE sardines is higher (30.81) than in frozen vacuum packaged (18.80). As a result, the shelf life of cold vacuum packed RTE sardines is 315.93 days, whereas frozen vacuum-packed ones can last up to 517.69 days.

Furthermore, RTE sardines stored in cold pouches deteriorate at a faster pace than those packed in frozen pouches. Thus, RTE sardines packaged in cold pouches have a shelf life of 295.13 days, whereas those packaged in frozen pouches have a shelf life of 514.83 days. The findings of this study align with the results reported by [13], who used the ASLT method to predict the shelf life of sausages and chicken nuggets and found that sausages and chicken nuggets stored at freezing temperatures have a shelf life of 476.72 days.

The energy activation (Ea) influences the pace of deterioration (Table 3). The energy necessary to start a reaction is known as energy activation. The energy activation of vacuum packaged RTE sardines was higher (6026.72 cal/mol K) than that of pouch packaged RTE sardines (5792.37 cal/mol K). The rate of quality degradation increases as the energy activation value decreases. The energy activation of bacterial growth in vacuum-packed RTE sardines is 6026.72 cal/mol K, which indicates that it takes 6026.72 cal/mol K to commence bacterial growth in RTE sardines.

According to [14], the energy activation connection is inversely related to the rate of quality degradation, with a small energy activation value indicating a high rate of quality degradation and vice versa. [15] define energy activation as the amount of energy required by chemical molecules to initiate a reaction. Furthermore, Arpah defines energy activation as the amount of energy required for molecules to move, implying that energy activation can provide an overview of how temperature affects the events that occur. According to Arpah, energy activation is classified into three categories: low (200 - 15,000 cal/mol K), medium, and high, with the energy activation in this study falling into the low category.

5 Conclusion

RTE sardines packed under vacuum conditions and stored at cold and freezing temperatures have a longer shelf life of 313.29 days and 558.96 days, respectively, compared to cold and freezing temperature pouch packing with a shelf life of 295.13 and 514.83 days. RTE sardines with the longest shelf life are vacuum packed frozen with the equation Arrhenius Ln k = 13.973 - 3034.6 (1/T) with an Ea value of 6026.72 cal / mol K and a quality reduction of 31.07 units per day.

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