

Effect of Frozen Storage on the Quality of Frozen Foods—A Review

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Abstract: This paper reviews the quality changes that occur towards the food products during the frozen storage period. This research aims to determine the significant physical and chemical changes that had occurred onto the frozen foods. Indeed, refrigerated storage is the easiest way to preserve food within short handling time. Based on the observation, it was discovered that the kinetics reactions help to establish computer-aided quality, prediction models. In certain food products, the negative impact of temperature indicated the need for a thorough evaluation of the quality parameters of individual's kinetics reaction. In general, a reasonable temperature to store food is $-18\text{ }^{\circ}\text{C}$ for most frozen foods, and this will retain the quality of commercial storage.

Key words: Storage, Frozen Foods, food allergen

1. Introduction

The quality and shelf life of seafood are important due to the rise of demand for fishery products. However, the quality of the fishery products in frozen storage starts to deteriorate at a steady rate. Makarios Laham and Lee [1] reported that when the cod and haddock minces were placed in frozen storage, there was an increase in the toughening rate and other changes that had occurred to the texture of the products. Species of fish with high production rates of dimethylamine (DMA) and formaldehyde (FA) such as red hake, were reported to go through deterioration in texture during frozen storage than those with low DMA and FA production rates. This can be concluded that the contact of FA with myofibrillar proteins may be the possible mechanism that causes the textural changes in gadoids. According to Ref. [1], there has not been any research yet with a complete explanation regarding the deterioration process of frozen shrimp during the storage period [2].

The deterioration in the quality of fresh seafood throughout the storage period is mainly because of the microorganisms' actions [3]. Fish, as well as other seafood, are commonly associated with rapid microbial spoilage because of the high soluble nitrogen compounds in their muscles. However, only certain bacterial species are accountable for the damage [4]. While refrigerating the seafood, it was discovered that certain psychrophilic bacteria are present and already grown at $0\text{ }^{\circ}\text{C}$, which causes the deterioration rate to become faster. In general, organisms that are Gram-negative are reported to be the biggest organisms in cold-water fish, whereas organisms that are Gram-positive are found to be abundant in fish of tropical water [2].

The deterioration of seafood quality results from the action of enzymes that derived from the tissues of fish and shellfish or from contaminating microorganisms such as *P. roteases* cause causing undesirable changes in seafood products. In shrimp, the endogenous enzymatic activity causes quality deterioration. Pornrat et al. [5] reported that significant quality

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changes in fresh shrimp and prawn were due to the protease activity. Crapo et al. [6] reported that the texture of the fish flesh was soft during the heating period. During the observation, the shear force value had decreased due to protein's hydrolysis during the alkaline protease activity. Ref. [7] discovered that at 5 °C, the fish actomyosin from a protease that had recovered from the obligately psychrophilic organism, P7, had decreased.

The seafood was refrigerated and kept in frozen storage to prolong its shelf life and to prevent microbial and enzymatic activities that can cause decay or degradation of the product's quality. The deterioration in the product's qualities, such as undesirable changes of texture and appearance during the storage period, is considered to be a significant deficiency in their application. However, there was still vague explanation for the deterioration of quality in fish and seafood products during the refrigerated and frozen storage period. Careche et al. [8] reported the visible changes in the texture of cod and haddock minces during frozen storage. The texture was toughened due to the action of the enzymes that had been released from the muscle cells during mincing. De Koning and Mol [9] found a highly significant correlation between the textures of hake's fillets and hake's mince that had been kept at -18 °C as well as the ratio between proteins' soluble in aqueous salt solution to the total of protein. Based on their discovery, they presented a formula to texture rating for frozen fillets and frozen mince based on the soluble and total proteins.

Singh and Wang [10] reported the characteristics of composites by differentiating their units and significances to determine the unit's level of acceptability and the quality of the foods. The characteristics of the component can give the overall quality of the food as each of the component's characteristics can be measured and controlled independently. The food's quality, such as the flavour, colour, and texture, can be preserved by delaying the changes in the chemical components, hindering the

enzyme's actions and removing the microbial growth. However, the issue of the quality changes towards the food during the frozen storage period remains unsolved due to insufficient information related to the factors of deterioration. The information is essential to predict the property or rate of deterioration. This has led to the necessity of studying the ways to maintain the quality of the frozen food with the collaboration of food engineer, food technologist, chemist, and bacteriologist. Ever since then, past studies had highlighted the quality changes onto the frozen products. In this paper, the ultimate aim is to review the changes that occur to the food during the frozen storage period.

One of the ways to preserve the fish muscle for a long period is to keep it inside frozen storage. However, quality changes, such as its chemical and structural changes, will occur [11]. The changes to the fish muscles during the frozen storage period can be in many forms such as the protein will undergo partial dehydration during freezing period, the protein's environmental changes occur due to freezing of inorganic salts, the reaction between the protein and the lipids, fatty acids, and lipid oxidation products, and the action of the trimethylamine oxide demethylase (TMAOase). According to Ref. [12] the last factor was reported to be crucial, particularly to the fish associated with the gadoid family.

1.1 Storage

Cold storage method can be utilized at any level of the chain between the stages of production up to the consumption. However, to fulfil the demand of the consumer, the production plant will store the products in smaller batches before transferring the frozen products to large bulk stores and nearby or smaller retailers that are geographically convenient to pick up the order. Regardless of whichever the level of cold storage is at, it is crucial to adhere to the temperature and able to identify the different production batch. Furthermore, each level of the cold chain will start and end with the transferring of products through

refrigerated transport. The handlers must know the requirement to move the products to and from the vehicle before going into the main chamber. Buffer zones were said to be large storage that can hold the refrigerated container load and it functions as the medium to bridge the necessity to release the vehicle and to allow the temperature checking of the product before accepting it. Besides, in the buffer zone, the load of the outward-bound products can be assembled and this is advantageous to the mixed loads. Generally, the loss of cold air due to the movement of the products from the main chamber can be reduced. In the frozen food product industry, a standardized one-tone pallet for racking and transporting was used. However, the choice of storage system depends on the retrieval policy. A high volume of multi-pallet load-out system can also be used for simple cubic storage whereby the handlers need to choose the individual pallets that require identification of full pallet and a reliable location for the operation [13].

1.2 Shelf Life

Based on in-depth testing and experience, frozen storage is proven to be the best way to preserve various kinds of food products. Frozen food that is being processed and handled correctly through various level of cold chain tends to have the higher quality that the foods that had degenerated their shelf-life while being transported to markets, shops, and to the consumer without any preservation to the low temperature. However, it is important to note that not all food products are suitable to be kept in the frozen storage such as the salad as it is suitable to be eaten raw and it will lose its crispiness once being defrosted. In general, Pollack [13] highlighted that there is a significant connection between the texture of the defrosted frozen product and the water content in a fresh vegetable.

1.3 Product Quality

Storing the food products in the frozen and cold

storage is known to be among the preservation techniques. The changes will occur and can be noticed once the product had been stored in frozen or cold storage. In simpler words, if the quality of the food in the frozen stage was poor, then the quality of the food will further deteriorate when it is being defrosted [13].

1.4 Refrigerants

Artificial refrigeration is required by the commercial food to produce temperature below 0 °C. Therefore, the design of the refrigeration system needs to be compromised with the performance and the demand of the economy with a lower temperature that can lead to faster freezing and smaller freezer will result in better quality and longer shelf-life. However, the cost will be higher. *Montreal Protocol* had agreed to the dramatic changes to enhance the surrounding by reducing the CFC refrigerants as a replacement for the CFC refrigerant is difficult to be attained. However, HCFC refrigerant such as R22 is attainable throughout Europe. Nevertheless, new devices should not use R22 without sufficient knowledge before changing the refrigerant. In major chemical companies, other initiatives are introduced as a replacement. Hydrocarbon, for instance, is one of the alternatives that are needed with consideration to the flammability aspects before installation. Any modification requires different kinds of lubrication and seeking guidance from the professional is necessary. Careful evaluation is also needed to replace the refrigerants in the existing system. A rise in the system's pressure can affect the safety in the pressure vessels and the compressor. Kennedy also noted the standard specification for the new systems are BS4434-1995 and EN 378 Part 4: 2000.

1.5 Consumers of Frozen Foods Are in Increase—Markets

In the 20th century, the demand for frozen food continues to increase due to the changes in the lifestyle and the current demography, whereby the

numbers of one-parent families and both working-parents families are increasing. Thus, the availability of technology to produce and store frozen food will increase too. In 1998, the market value for the frozen foods in the US was \$64.3 billion. In total \$25.7 billion was from the retail store, which is equivalent to 18% of the retail food sale, and \$38.6 billion was in the food-service sector. In the UK, the market value of frozen food was £4.96 billion in 1998 and continued to grow with 3.5% per annum. Table 1 presents the breakdown sale of frozen foods as the reason for the dramatic growth. In general, frozen foods were related to almost all categories of food products. It can be predicted that there will be an increase in the western lifestyle and technology as the amount of consumption will increase due to its convenience and availability. More consumers preferred to have more food consumption outside their home, which can benefit the industry to grow further [14, 15].

1.6 *Quality Changes to the Food during the Frozen Storage*

Freezing hinders the agents to be accountable for the damage at room temperature without leading to significant changes to the appearance, odour, and flavour of the product. However, it is crucial to mention that freezing does not enhance the quality of certain product and it cannot preserve the quality of the product completely as some deterioration will take place even if the product is in the frozen storage [16]. Singh and Wang [10] mentioned that a vegetable that had been blanched, frozen and packed such as green beans, cauliflower, and spinach, can be stored at -29 °C for five years without any changes to the colour, chemical compositions, flavour, and physical features.

During normal commercial storage, the food materials will not be completely frozen. However, the deterioration in the chemical and physical changes may occur gradually as the storage temperature and

Table 1 Frozen foods in supermarket sales.

Appetisers/snacks	\$463 million
Baked goods	\$372 million
Breakfast foods	\$886 million
Desserts/toppings	\$731 million
Dinners/entrees	\$4,800 million
Fruit	\$178 million
Meat	\$754 million
Novelties	\$1,800 million
Pasta	\$252 million
Pies	\$367 million
Pizza	\$2,100 million
Plain vegetables	\$1,700 million
Pot pies	\$291 million
Potatoes/onions	\$842 million
Poultry	\$1,400 million
Prepared vegetables	\$109 million
Seafood	\$877 million
Side dishes	\$236 million

the type of product control the changing rate. In general, when the storage temperature is lowered, the deterioration rate will decrease. The significant changes to the deterioration rate can be seen due to the type of the products being stored in the frozen storage [17]. In a few past researches, an increase in the rate of deterioration happens when the temperature is low. These reactions will be further elaborated in this paper.

At -80 °C, the biological changes are essential to stop. Yet, in many commercial storage facilities, this temperature can be changed, and the low temperature may not give the advantage of maintaining the quality of the product during normal commercial storage. A recommended temperature to store the food at frozen storage is -18 °C [17]. Certain foods, such as fatty fish and ice creams, are recommended to be kept at a temperature between -23 °C to -29 °C.

A few physical mechanisms may lead to the deterioration of the product's quality during the freezing and frozen storage. The concentration of the food's component while slowly freezing can lead to denaturation of protein in certain frozen fish. For instance, without enzymatic oxidative, the formation of rancid leads to off-flavour in frozen meat and the

loss of ascorbic acid in fruits and vegetables. In localized food products, dehydration can cause denaturation to occur more vigorously. In this reaction, hydrolysis may affect the vegetables resulted in the changing of the chlorophyll to pheophytin, which can be visible based on the changing of colour from bright green to yellow. As for sugar in frozen storage, crystallization will take place causing the mouldy surface to appear whereas, for ice cream, a sandy texture will appear on the surface. Another mechanism that can change the texture of a fish in the frozen storage is desiccation. Therefore, further investigation should be done onto these mechanisms for further understanding [17].

1.7 Physical Changes

As mentioned previously, several mechanisms cause different impacts on the type of products. Crystallization occurs due to the increment of the volume, whereas desiccation commonly occurs on the surface of the frozen foods [18]. This indicated that the spread of the ice crystals in frozen tissue is vital as slow freezing may lead to the development of crystals in the extracellular area. Fennema and Powrie [16] mentioned that these changes can also be noticed to plants and animals' tissue. In contrast, rapid freezing to products with low temperature may consequently lead to the presence of small ice crystals in both intracellular and extracellular areas. Two reasons to clarify the existence of extracellular crystallization during low temperature than the intracellular crystallization are: (1) the freezing point of the products may be higher than the intracellular ones, or (2) the material of the intracellular items may be deficient in heterogeneous nucleation areas.

Among all the mechanisms mentioned above, desiccation was reported to be a significant problem that will occur during the frozen storage period due to the loss of moisture. Kramer [19] reported the issue of weight loss when handling retail size cartons of frozen vegetables that were packed in paperboard cartons to

different levels of protection. It was discovered that frozen products will be highly moisture due to a good barrier of water vapour and were kept under frozen storage for a certain period. A direct connection between the weight of the waxed paper and the rate of moisture loss was presented by Kramer and Twigg [20]. It was reported that heavy waxed paper helped to reduce the rate of moisture loss.

Another factor that influences the weight loss and quality of frozen foods is fluctuations in storage temperature, whereby the appearance of the product changes gradually. This situation is similar to desiccation and also known as "freezer bum". In the case of fish being stored in frozen storage, desiccation will give an impact to the surface of the fish to become dry and fibrous, and sometimes can change the colour of the fish's skin too. Furthermore, desiccation and fluctuations can also result in flavour and colour loss as well as contributing to greater development of rancidity [21].

In certain fish, such as frozen tuna and swordfish, the colour will change to green and brown while being cooked and this will cause the consumers to reject the fish. The browning colour often indicates the presence of metmyoglobin in the fish's muscle, whereas greening colour is associated to the pigments coming from the hemochrome's oxidation when the meat of the product is exposed to the oxidative surroundings while and after being cooked [22, 21]. Indeed, freezing storage will toughen the muscle of the fish, and it requires progressive procedures in the next storage. Heen and Karsti [23] developed a method to measure the muscle's toughness of an uncooked fish.

Overall, the deterioration of the quality of frozen foods has been associated with several factors. Another factor is known as mechanical stress, which happens due to the changes in the volume related to the liquid-solid transformation such as from the dislocation of water that goes along the slow freezing process from the gradients of temperature in the product. When low temperature collaborates with

rapid freezing, a severe cracking will occur to the food that has high percentage of water. This is proven when Brown and Dolev [24] observed the effect of frozen green beans in liquid nitrogen whereby the cracking may be due to no standardize contraction after solidification. Love and Mackay [25] recommended the Langham-Mason effect of which the outer part of the frozen shell will rupture when the internal part freezes and enlarges. Fennema and Powrie [16] highlighted the impact of rapid freezing and suggested the way to overcome is to minimize the gas loss from the inner spaces of the plant tissues. Yet, further investigation must be done onto these postulates.

1.8 Chemical Changes

There are several types of chemical changes that may occur during the frozen storage period such as enzymatic browning, lipid oxidation, degradation of chlorophyll, protein insolubilization, and other pigments and vitamins [16].

The nutritive value of the frozen food has become a major concern by both consumer and manufacturer as it can be retained well during the freezing storage period. Yet, it can also lose its nutrients if the consumer takes one or two steps between the moment the product is being produced and the moment the product is about to be used [26].

Based on the aforementioned factors, the temperature and the duration of the products being stored in the frozen storage area cause significant impacts onto the nutritive values. To protect the nutrients of frozen foods, the presence of ascorbic acid is essential. However, food products, such as frozen fruits or vegetable, can preserve their nutrients if they are stored below $-18\text{ }^{\circ}\text{C}$ or being kept and protected by other matters while it is being transmitted from the wholesale to the retailer's storage for distribution to the consumer [26]. This calls for the oxidative reactions, which the residual enzyme activity will take place in the frozen vegetable [27]. The physical and chemical changes that related to the oxidation can be

prevented with the use of other technical methods such as exposing to the gaseous surrounding that had been modified, packaging using vacuum, and antioxidant's treatment [28]. By applying these methods, the surface of the frozen products can be protected from the oxygen and may not inhibit the changes in the quality of the products. Other products that can be maintained its quality are the nitrogen packaging for pork, vacuum packaging for beef, and ascorbic acid treatment for fish. These products are best to be kept and stored at $-30\text{ }^{\circ}\text{C}$ storage to enhance its quality during frozen storage.

Meanwhile, Piskarev et al. [29] asserted that packaging with carbon dioxide helps to prevent the establishment of rancidity in bacon. Normally, the bacon will rancid after four months if it is stored in the air at $-10\text{ }^{\circ}\text{C}$. However, with the presence of carbon dioxide, the bacon will not rancid after seven months and will only begin to rancid about after 12 months. Meanwhile, Gibbons [30] determined that vacuum packaging was the best packaging method for pre-cooked and pre-fried chicken. Other studies [31, 32] investigated the impact of different kinds of packaging methods on the quality of frozen fish and broilers, respectively.

Bogh-Sorensen [33] mentioned that oil and fats are significant in preserving the fish during freezing storage because it hinders oxidative deterioration. Since fish are sufficient with lipids, the deteriorative reaction is known to be autoxidation towards unsaturated fatty acids. As a result, fatty fish are vulnerable to oxidative changes. Nevertheless, Lane [34] highlighted that the enzyme system in the tissue will function as the pro-oxidants and be vital for oxidation, which can result in rancidity.

Heat treatment before freezing such as blanching in steam or boiling can control the enzyme reaction, which can fasten the oxidative deterioration. During oxidation reaction, chemical changes may occur, causing loss of ascorbic acids, off-flavours, and rancidity [35]. The destruction of ascorbic acids to

function was studied by Lee and Labwza [36]. Meanwhile, Mudhafar et al. [37] presented the decomposition of ascorbic acids from the peppers that had been frozen. In general, it can be concluded that the loss of ascorbic acids may occur with unfrozen water.

Overall, the researcher believes that further investigation should be done to examine the formation of off-flavour chemical in frozen fruits and vegetables. In 1977, Burnette had conducted an in-depth study on the relationship between peroxidase and the quality of the food flavour. Awad et al. and Enser [38, 39] studied the common off-flavour in meat and fish. Awad et al. [38] discovered that the amount of lipid in the muscle of whitefish remained the same when it was being stored at $-10\text{ }^{\circ}\text{C}$ for 16 weeks. However, as the unsaturated fatty acid degraded, the TBA numbers increased.

2. Reaction Rate in Frozen Food

The stability of the quality changes will increase as long as the reaction is limited to one with the low temperature. Further explanation can be obtained from the kinetic theory. It is best to be understood as it has a fundamental explanation of various changes that occur to the common procedures, and it explains the reaction rates in various processes during the processing up to the storing periods. The reaction rate is mentioned as the changing rate of a concentration with the time at a constant temperature.

2.1 Negative Effect of Temperature

Recently, it has been proven that certain chemical reactions such as enzymatic actions can cause the reaction to be faster when the temperature is below the freezing temperature. Several studies had led to the probability of reducing stability by decreasing the temperature in frozen storage.

In certain reactions, the decrease in temperature may lead to increment in the rate of a chemical reaction [16]. Lindelov [40] mentioned that a product

may be fully solid when it is below the eutectic point to indicate that frozen state will occur when the temperature ranges below a freezing point and above the eutectic point. illustrates this phenomenon using a product in the form of solid-liquid equilibrium. In general, the reaction rate will increase when the temperature is below the freezing point and will reach the maximum near the eutectic point. When the temperature is below the eutectic point, the water in the protein maintains unfrozen, and the reaction rate will decline again, causing the rate to be around the freezing point. Thus, it is crucial to highlight that a maximum rate of reaction does not correspond with the final eutectic points in the biological system. Furthermore, the solutes will saturate fully during the freezing storage to ensure that the liquid is attached to the protein.

A study of a cod muscle by Anderson and Raven [41] discovered that the rate of reaction of free fatty acids with protein was higher at $-29\text{ }^{\circ}\text{C}$ than a few temperatures above $0\text{ }^{\circ}\text{C}$. In another study by Pincock and Lin [42], the denaturation of α -chymotrypsin in a frozen solution was observed extensively. It was discovered that the rate of denaturation had increased as the temperature was reduced from $-5\text{ }^{\circ}\text{C}$, $-10\text{ }^{\circ}\text{C}$ to $-12\text{ }^{\circ}\text{C}$ and $-16\text{ }^{\circ}\text{C}$. However, the denaturation rate was lower when it was at $-16\text{ }^{\circ}\text{C}$.

For many years, the process of glycolysis in frozen muscle had been studied. One study done by Behnke et al. [43] discovered that the adenosine triphosphate (ATP)'s rates of depletion and accumulation of lactate in chicken and meat were rapid at the temperature of $-3\text{ }^{\circ}\text{C}$ compared to $10\text{ }^{\circ}\text{C}$ or $0\text{ }^{\circ}\text{C}$.

In general, incorrect storage temperature can cause critical loss to the quality of frozen products. Poulsen and Lindelov [44] mention that low storage temperatures can cause more peroxide oxygen to gather in bacon due to microbiological activity that impacted the quality at low temperatures. Thompson and Fennema [45] conducted a study to determine the storage life of sliced bacon packed in 70 μ polyethylene

bags without vacuum. It was discovered that the sliced bacon had an unusual temperature between 0 °C and -40 °C. They summarized that the temperature of -30 °C will lead to shorter shelf-life compared to the storage temperature of -18 °C for the sliced bacon.

A study on the oxidation rate of L-ascorbic acid in acetate buffer solution was conducted by Mcweeny [46]. The samples were firstly diluted to enable them to dissolve an ample amount of oxygen. A product that has pH values of 5.5 will show the oxidation rate of ascorbic acids during freezing storage. However, if the concentration is higher, and the pH value is 4.6, the oxidation rate of ascorbic acid will decrease during freezing.

Another study that examines the reaction rate between myosin and malonaldehyde was conducted by Alsailawi and Rosmilah [47]. Fig. in previous study presents the rate of reaction whereby the reaction rate will decrease when the temperature was lessened from 45 °C to 0 °C. However, when the mixture is frozen, the reaction rate rises and the maximum rate of reaction occurs at -24 °C. A similar finding was found by Tomasyan [32] whereby the autoxidation stability of beef and tuna solution will occur at the temperature range from -5 °C to -10 °C.

Lindelov [40] also investigated the reaction rate onto the smoked bacon, liver paste, and chopped herring fillets. It was discovered that there was a negative impact of the temperature ranges on these foods. The addition of salt causes a complex connection between temperature and storage life. Therefore, it can be concluded that low temperature does not lead to preserving the quality of frozen food [48].

2.2 Meats

As mentioned previously, freezer burns can cause desiccation that may impact the quality of the frozen meats. A recommendation was made to have a water-vapour proof package to fit the product tightly. Furthermore, it can avoid air pocket in the package.

The meat can be covered with an antioxidant film that contains lard, glycerol, or breeding process. Generally, if the product is covered well, and the desiccation surface is avoided, the changes in the storage temperature will not cause serious defects to the quality of frozen food. Traditionally, the appropriate and practical temperature to store meat is at -18 °C. Poultry meat is best to be kept at -18 °C or below to have a longer storage life. However, a poorly packed poultry meat at -18 °C will last for at least six months, and the well-packed ones will last up to 2 years [48].

2.3 Seafood

When seafood is stored at relatively low humidity, the significant change will be the weight of the product, indicating that the humidity must be high in the frozen storage and should be maintained. Normally, the regular and small fluctuations in the storage temperature may not give any impact to the quality of the seafood that had been stored at frozen storage. In contrast, larger fluctuation may impact the quality of the seafood severely. Past studies had reported that the temperature fluctuation often occurs between -18 °C and -10 °C, which may lead to significant deteriorative impact as more water fluctuates between -15 °C and 20 °C [49].

Different seafood will have different storage life, and it will still be acceptable with slight changes in quality. However, when there are major or significant changes to the seafood product, there is a possibility that the product will not be accepted and this happens due to a longer period of storage. Variation in the storage time will have an impact on the fish before being kept freezing. The use of vacuum packaging is an alternative to facilitate the extension of the shelf life of the seafood product [49].

3. Impact of Storage Conditions on the Protein Profiles

Fig. in previous study shows the use of SDS-PAGE and IgE immunoblotting to examine the

changes in the protein and allergen profile. Based on the figure, a molecular mass of a protein is 15 kDa. It was discovered that Der p2, is the strongest allergen and the second strongest allergen is the 42 kDa protein, which depicted faster degradation at 4 °C, compared to the 15 kDa allergen [50].

4. Effects of Storage Conditions on IgE Reactivity

At week 52 at RT, the extracts reformed itself in 50% glycerol solution and retained 89.9% of glycerol only, and 93.1% of glycerol and HSA to observe the IgE reactivity. In contrast, the extract that reformed without glycerol exhibited 33.3% of DW and 36.6% of HSA only, as shown in previous study Overall, the extracts that are stored at 4 °C did not show any major reduction in the overall allergenicity, even during the buffering condition, and the IgE reactivity remained constant under these circumstances. The researcher believes that it is essential to investigate the mite's extract's stability with the presence of several protease inhibitors to maximize their shelf-life. The presence of protease inhibitors has proven that it can increase the shelf life of a cockroach extract, which is rich with protease. Meanwhile, although HDM contains less protease, yet the structural integrity of the protein allergen maintains itself, and it is a vital factor for stability [50].

5. Conclusion

In general, this study concluded that the condition of the refrigerated storage is crucial to sustain and preserve the allergenicity of HDM extracts. HSA and glycerol can also raise the shelf-life of extracts at RT, and repetitive use of RT may lead to major degradation.

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