Safety and Quality Characteristics of Freeze-Defrost Cycles in Muscle Foods

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Abstract

Freezing is a reliable method of extending the shelf-life of muscle-based foods but it is associated with quality deteriorations if subjected to temperature fluctuations during the period of storage. The product may undergo freeze-defrost cycles, and this is to be expected in retail outlets, restaurants and homes, in places of inconsistent power supply. This phenomenon of freeze-defrost cycles adversely affects the safety and quality of the final product, and is of great concern to national food safety control authorities and consumers. This paper is a review of the extent of damage to food quality as a result of freeze-defrost operations. Three major aspects of product deteriorations are involved, with one depending on the other to interdependently and synergistically impact the quality of the final product. These include physicochemical characteristics, biochemical quality and microbiological safety. Physicochemically, freeze-defrost cycles disrupt muscle structure and denature muscle proteins affecting water-holding capacity, nutritional value and pH. It is noteworthy that changes in pH can transform food to support the growth of some pathogenic microbes. Some quality characteristics that are influenced by enzyme and/or microbial activity with increased freeze-defrost cycles include discolouration and this is linked to protein oxidation, with corresponding accumulation of thiobarbituric acid reactive substances, a measure of oxidative rancidity. Total volatile basic nitrogen (TVB-N) is also a chemical marker of spoilage in muscle foods that results in changes in odour, flavour and texture, as a result of enzymatic break down of protein and other nitrogenous compounds. The concentration of TVB-N increases with increased enzyme and/or microbial activity, and this is characteristic of freeze-defrost operations. Freeze-defrost cycles are therefore detrimental to product quality. Efforts targeted at sustaining the cold chain in muscle foods will go a long way in maintaining product safety and quality characteristics comparable to that of a fresh product. Should the cold chain be interrupted, refreezing is not recommended because any damage caused is irreversible and also exponential.

Keywords: Freeze-defrost cycles; muscle foods; muscle protein; microbial/enzyme activity; food safety and quality

Abbreviations: TVB-N: Total volatile basic nitrogen; TBARS: Thiobarbutic acid reactive substances

Introduction

Freezing is an important method of preserving both the chemical and microbiological quality of food by arresting enzyme activity and microbial growth. Nonetheless, some quality characteristics like texture, flavour, appearance, colour and nutritional value, of frozen foods can deviate from that of a fresh product if subjected to temperature abuses during storage.

A lot of structural, molecular and biochemical changes occur during freezing and freeze-defrost cycles in muscle-based foods. For example, formation of ice crystals causes disruption and separation of muscle fibre bundles [1,2]. This denatures muscle proteins, and decreases their solubility and water-holding capacity, and also pH, as solute concentration is increased [3]. Upon defrosting, which happens when there is temperature fluctuation, water with soluble nutrients is leached from the muscle tissues. Reformation of ice crystals...
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and melting in a repetitive fashion can therefore adversely affect the physicochemical quality, and this includes the nutritional value, of frozen fish, meat and their products.

It is worth noting that invasion of some microbes like *Clostridium perfringes* in fresh meat permits the growth of even more obligatory anaerobic microorganisms [4], and also, not all pathogens which might have been exposed to food products prior to freezing perish during the freezing process. Some microorganisms like *Salmonella spp* and *Campylobacter spp* are reported to survive the freezing temperature by going into hibernation and coming back viable when favourable conditions are restored [5]. Moreover, the toxins left by some microbes like *Staphylococcus spp* and *Clostridium botulinum* during their proliferation can neither be destroyed by freezing nor heating [5]. Even at lowered pH, which is characteristic of repeated freezing and thawing, some pathogens like *Clostridium botulinum* are reported to produce toxins at pH as low as 4.2 [4]. Ideally, freezers should be operating at temperatures -18°C and below. At warmer temperatures, for example -5 to -10°C which could occur when there is power outage, some psychrotrophic microbes can still grow and deteriorate food [5].

The phenomenon of freeze-defrost cycles is plausibly expected in retail outlets, restaurants and homes, notably in areas of consistent power fluctuations, and this is of major concern to national food safety control authorities and consumers. This paper highlights some safety and quality characteristics of frozen foods when subjected to frequent power cuts. Some suggestions to ensure high quality frozen muscle foods for consumers have also been made.

**Product Quality Deterioration**

Safety and quality characteristics of freeze-defrost cycles in muscle foods may be viewed from the physicochemical, biochemical and microbiological perspectives, where one factor depends on the other and complementarily or synergistically deteriorate the product as depicted in Figure 1.

![Figure 1: Scheme of product deterioration in freeze-defrost cycles.](image)

Reduced pH due to increased solute concentration as a result of reduced water holding capacity emanating from denatured muscle proteins, may permit the growth of specific pathogens, and in turn compromise microbiological safety through the release of microbial toxins. Increased microbial load may influence biochemical pathways of protein oxidation and product discolourisation, and this may impact negatively on the aesthetic value of the product. Moreover, loss of pro-oxidants like haem iron from the formation of some anaerobic microbes also enhances protein oxidation, product discolourisation and the release of of rancid off-flavours (TBARS), which are negative attributes of physico-chemical characteristics. Physico-chemical quality are further impacted through cell membrane rupture from the formation of ice crystals. Repeated ice crystal formation and melting damages cell membranes, oxidizes membrane lipids and denatures muscle proteins. Physically, protein oxidation is linked to reduced juiciness and increased flabbiness or flaccidity of the product. Biochemical and microbiological quality of the product are also impacted through the release of TVB-N, which is a bio-marker of spoilage resulting from increased enzyme and microbial activities due to increased freeze-defrost cycles. Ultimately TVB-N will impact the physico-chemical quality through changes in odour, flavour and texture of the product.

Physicochemical aspects of product quality

Structural changes that may occur during freezing and freeze-defrost operations involve distortion of muscular morphology and protein denaturation. Changes in volume of water in muscles, i.e. increase at freezing temperatures and decrease at 4°C, degrades the hydrophilic and hydrophobic interactions in the myofibrillar protein network and ultimately denatures the muscle proteins during freeze-defrost operations [6]. This results in shrinkage and weakening of muscle fibres as water with soluble nutrients is leached from the muscle tissues and lost as drip, to also increase solute concentration and decrease pH. As ionic strength and pH directly influence the efficiency of myofibrillar proteins of the muscle cells to entrap water [7], ultimately, there is decreased water holding capacity of muscle proteins and this adversely affect the juiciness or the elasticity of the product. This was confirmed in one study where expressible drip was found to be significantly higher in frozen fish samples and even worse in double-frozen ones, compared to fresh samples. This observation was attributed to reduced water-holding capacity from denatured proteins and irreversible damage to muscle structure from freezing and thawing [1].

In a study examining the effects of freeze-thaws on chicken breast colour [6], the authors reported that the original colour of the product was significantly affected by multiple freeze-thaws. These investigators observed increased lightness and attributed it to protein denaturation. The yellowness of the product also decreased with increased number of freeze-thaw cycles, attributing it to the formation of metmyoglobin, an indication of protein oxidation. This mechanism, in which the oxygenated form of ferrous myoglobin (attractive, cherry-red oxymyoglobin) is oxidised in the presence of molecular oxygen to form grey/brown metmyoglobin, is very crucial in meat quality [8]. Further on the study [6], protein oxidation in chicken breast was monitored by measuring the amounts of carbonyl- and sulphydryl-groups, with the former significantly increasing during all the six cycles studied, and the latter decreasing significantly in the last 3 freeze-defrost cycles. High carbonyl and low sulphydryl concentrations are both indicative of freeze-defrost cycle-associated protein oxidation [6]. Physically protein oxidation is linked to reduced tenderness and juiciness, and flavour deterioration [9].

Biochemical and Microbiological aspects of product quality

Comparing changes in fresh fish, frozen fish and double frozen fish (i.e. 2 freeze-defrost cycles), the researchers [1] reported remarkable differences in total volatile basic nitrogen (TVB-N), total viable counts, enterobacteriaceae counts and psychrotrophic bacteria counts. TVB-N is one of the chemical markers of spoilage in fish. Its concentration increases with increase in microbial and/or enzyme activity, resulting in changes in odour, flavour and texture. From the study, significantly higher concentration of TVB-N was reported in the double frozen fish compared to the single frozen and even much more compared to the fresh fish. In another study [10], fish subjected to 3rd freeze-thaw cycle reached unacceptable levels of TVB-N, under refrigeration thawing conditions. Similar to the findings on the effect of freeze-thaw abuse and refrigeration thawing on microbial quality of chevon [2], Popelka and colleagues [1] further reported that microbiological indicators followed the same trend as the TVB-N, with the exception of enterobacteriaceae counts, which was vice versa.

Manheem and colleagues [11] also examined the impacts of multiple freeze-thaw cycles (1, 3 and 5) on enzyme activity (polyphenoloxidase and protease activities) and melanosis in pre-cooked shrimps using different thawing methods (refrigeration temperature and tap water thawing). These researchers reported increase in enzyme activity with increase in freeze-thaws, likewise thawing using running tap water compared to refrigeration (4°C) thawing. Melanosis, which is a cascade of biochemical reactions induced by polyphenoloxidase and proteolytic enzymes, yielding the dark pigment, melanin, was reported to be most pronounced in shrimps defrosted with running tap water and subjected to 5 freeze-thaw cycles and extended storage time. Even though melanosis is an aesthetic issue and may reduce the commercial value of the product, no safety implications have been documented on it.

These findings are similar to that reported on the biochemical and physicochemical changes in catfish subjected to different freeze-thaw cycles under refrigeration thawing conditions [12]. Even though this study [12] did not involve microorganisms, the connection between microbial load and discoulouration in muscle-based foods is well established. According to some authors [13], microbial load is usually the most important cause of discoulouration in muscle-based foods, because it decisively affects the biochemical mechanisms of discoulouration. Antioxidants which may improve colour stability even have little effects in the context of high microbial load, for example, to the tune of 106/g [13]. Further on the results of Benjakul and co-workers [12], there was significant decrease in the content

of haem iron which may be linked to protein denaturation. The growth of anaerobic bacteria generates reducing substances that may also decrease the contents of haem iron [13]. The cumulative effect of these is thus increased accumulation of thiobarbituric acid reactive substances (TBARS), a measure of lipid oxidation or oxidative rancidity, as documented by some authors [12,14]. According to these authors, increased concentration of TBARS with multiple freeze-thaw cycles is partly as a result of loss of pro-oxidants, especially, haem iron, and damage to cell membranes by ice crystals.

Conclusions and Recommendations

Clearly freeze-defrost cycles have detrimental effects on the quality and safety of frozen foods, especially muscle-based foods, which are highly perishable. Efforts targeted at sustaining the cold chain of frozen foods will go a long way in preserving product quality. The safety and quality of the product is also dependent on the microbiological status prior to freezing; efforts should therefore be made to limit fresh and frozen products exposed to air prior to or during freezing or during the process of thawing. In homes, good and readily accessible thawing methods may include refrigerator thawing (4°C) and thawing in sink/bowl completely filled with water (i.e. immersion or cold water thawing) to keep the product chilled in order to retard microbial and enzyme activity. Certainly, thawing at ambient temperature is not recommended because this will give microorganisms’ ample time to proliferate. Even under the supposedly good thawing conditions, refreezing defrosted food product is not a recommended practice. This is because any damage caused, whether physical, chemical or microbiological, in the first freeze-defrost cycle is multiplied exponentially in the subsequent cycles.

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