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Title: The use of supercooling for fresh foods: a review

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Abstract: Supercooling is a food processing technique which has the potential to significantly increase the shelf life of foods and to reduce wastage of food products from the production and retail sectors. The process uses storage temperatures below the initial freezing point of the food without the product freezing, which maintains the quality attributes associated with fresh foods. The removal of the freezing process leads to shorter processing times from harvest to delivery to retail as well as lower energy consumption (no latent heat removal) and so lower emissions during manufacture when compared to standard frozen food production.

Cover Letter

Professor Judith Evans
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21 July 2014

Dear Professor Singh,

Please find enclosed a revised copy of the manuscript entitled "The use of supercooling for fresh foods: a review".

We hope that we have managed to successfully answer the points raised by reviewers and would like to thank the reviewers for their helpful comments.

Yours sincerely,

J. A. Evans

Judith Evans (Professor)

***Cover Letter**

JFOODENG-S-14-00484-4: The use of supercooling for fresh foods: a review

Reviewers comments are listed below in black, responses are listed in red.

Reviewer1

The paper reads well even though very descriptive

Many thanks.

HOWEVER,

A clearer definition of supercooling is done, some other process should be mentioned

We are not totally clear what the reviewer means by 'some other processes should be mentioned'. The paper covers supercooling and so in our view other processes are not relevant. If the editor disagrees, and could help us by clarifying this point, we are more than happy to amend the paper according to this point.

In clear, it seems that

Supercooling = as described in the paper even though for some authors, the temperature is between 0°C and IFP (initial freezing point), Chilling is between 0°C and IFP (initial freezing point), Superchilling is below IFP In France, the ministry of agriculture has issued a not on the topic:

NOTE DE SERVICE DGAL/SDSSA/N2014-281 Date: 9 avril 2014 They accept only storage below 0°C but above the initial freezing point ; this is supported by EU regulations.

Although this is a useful point we are not sure it is totally relevant. The aim of the paper is to review supercooling, how it is achieved and where it has been used in the past. It can be argued that when we supercool that we are actually still above the initial freezing point as we do not have any ice.

Several papers are missing. In particular, the anteriority of Le Danois is hard to skip (ref below), Le Danois, E., 1920, Nouvelle méthode de frigorification du poisson, French Patent No. 506.296.

The patent from Le Danois refers to superchilling and not supercooling and this is why we have ignored it. We could not access the original patent but this point is clearly is clearly stated in:

Advances in superchilling of food – Process characteristics and product quality. Ola M. Magnussen, Anders Haugland, Anne K. Torstveit Hemmingsen, Solfrid Johansen,Tom S.

Nordtvedt. Trends in Food Science & Technology. Central European Congress on Food – CEFood. Volume 19, Issue 8, August 2008, Pages 418–424.

In this paper the authors state:

'The process of superchilling was described as early as by Le Danois (1920), even though he did not actually use the terms ''superchilling'' or ''partial ice formation''.'

L10 : is it below the initial freezing point or the initial freezing point of water ?

Below the initial freezing point of the product which will be affected by the constituents of the food. We think this is clear within the existing text.

L 50 - 69. It would be worth to put all the names in a table and to indicate what is prefered for example by IIR ?

To us this seems unnecessary. However, if the editor disagrees we are more than happy to comply.

Very often, authors put to the fore the fact that supercooling permits to lower the water activity.

The slide below reminds what the water activity of ice at low temperature is. In freezing or supercooling conditions, the water activity of a biological substance is equal to the water activity of ice. There is a myth to break here, as the reduction in aw is not so big…

The extension of shelf life comes mainly from reduction of kinetics of reaction of microbial activity

Thanks this is a useful point. We are not claiming anywhere in the article that supercooling lowers the water activity of food, we always claim that the extension in shelf life is related to temperature.

L 195 - paragraph on fish

A mass of papers are missing there ; impossible to skip the numerous papers on the topic of fish ... see below

Thanks for all these references. However, they refer to superchilling and supercooling. We have highlighted in red this issue in the list of papers. We are reporting on supercooling and not superchilling. There are already some excellent review articles on superchilling and we do not wish to repeat this work!

L 196; suoerchilling appears here... Please use adapted wording.

This is correct; we are reporting the wording of the authors. We believe that they use this term incorrectly.

1.Bahuaud, D., et al., Effects of -1.5°C Super-chilling on quality of Atlantic salmon (Salmo salar) pre-rigor Fillets: Cathepsin activity, muscle histology, texture and liquid leakage. Food Chemistry, 2008. 111(2): p. 329-339.

2.Beaufort, A., et al., The effects of superchilled storage at -2°C on the microbiological and organoleptic properties of cold-smoked salmon before retail display. International Journal of Refrigeration, 2009. 32(7): p. 1850-1857.

3.Claussen, I.C., Superchilling Concepts Enabling Safe, High Quality and Long Term Storage of Foods. Procedia Food Science, 2011. 1(0): p. 1907-1909.

4.Erikson, U., E. Misimi, and L. Gallart-Jornet, Superchilling of rested Atlantic salmon: Different chilling strategies and effects on fish and fillet quality. Food Chemistry, 2011. 127(4): p. 1427-1437.

5.Fernandez, K., E. Aspe, and M. Roeckel, Shelf-life extension on fillets of Atlantic Salmon (Salmo salar) using natural additives, superchilling and modified atmosphere packaging. Food Control, 2009. 20(11): p. 1036-1042.

6.Fernandez, K., E. Aspo, and M. Roeckel, Scaling up parameters for shelf-life extension of Atlantic Salmon (Salmo salar) fillets using superchilling and modified atmosphere packaging. Food Control, 2010. 21(6): p. 857-862.

7.Gaarder, M.O., et al., Relevance of calpain and calpastatin activity for texture in super-chilled and ice-stored Atlantic salmon (Salmo salar L.) fillets. Food Chemistry, 2012. 132(1): p. 9-17.

8.Gallart-Jornet, L., et al., Effect of superchilled storage on the freshness and salting behaviour of Atlantic salmon (Salmo salar) fillets. Food Chemistry, 2007. 103(4): p. 1268-1281.

9.Kaale, L.D. and T.M. Eikevik, A histological study of the microstructure sizes of the red and white muscles of Atlantic salmon (Salmo salar) fillets during superchilling process and storage. Journal of Food Engineering, 2013. 114(2): p. 242-248.

10.Kaale, L.D., et al., A study of the ice crystals in vacuum-packed salmon fillets (Salmon salar) during superchilling process and following storage. Journal of Food Engineering, 2012(0).

11.Kaale, L.D., et al., The effect of cooling rates on the ice crystal growth inÂ air-packed salmon fillets during superchilling andÂ superchilled storage. International Journal of Refrigeration, 2012(0).

12.Stevik, A.M., et al., Ice fraction assessment by near-infrared spectroscopy enhancing automated superchilling process lines. Journal of Food Engineering, 2010. 100(1): p. 169-177.

13. Duun A.S. & T. Rustad, Quality of superchilled vacuum packed Atlantic salmon (Salmo salar) fillets stored at - 1.4 and - 3.6 °C, 2008 Food Chemistry 106 (2008) 122-131

Kaale, L.D. and T.M. Eikevik, A histological study of the microstructure sizes of the red and white muscles of Atlantic salmon (Salmo salar) fillets during superchilling process and storage. Journal of Food Engineering, 2013. 114(2): p. 242-248.

Le Danois, E., 1920, Nouvelle méthode de frigorification du poisson, French Patent No. 506.296.

Kaale, L.D., T.M. Eikevik, T. Rustad, and K. Kolsaker, Superchilling of food: A review. Journal of Food Engineering, 2011. 107(2): p. 141-146.

Line 205-206, exponent are not well located;

Agreed. Changed to:

The lower temperature was found to slow bacterial growth on the fillets. The fillets stored in air at 1°C had a total bacterial count of 108 CFU/g at day 16, whilst those in air at -1°C had approximately 107 CFU/g at day 20. The combination of MAP and low temperature gave bacterial counts of 104 CFU/g at day 23 of storage.

Reviewer #2:

This is a useful review, but the assumption at line 136 means that many reported results may not be relevant because superchilled products may be reported as supercooled. The standard of writing is poor, with many typos, punctuation, grammatical and spelling errors (e.g. "been" instead of "being"). It is rather surprising considering that the paper comes from an UK institution.

We have tried to highlight where there is some lack of clarity in the papers. However, this is a review and we can only critically review what authors have presented. We have also removed table 3 as explained below.

Apologies, the reviewer is correct, we should have checked the paper more thoroughly before submission. We have checked the paper for grammar and spelling errors and those changes are highlighted in the revision submitted.

45: "supercooling (sub zero cooling)": "supercooling (cooling below freezing point without phase change)"

Change applied: Recent research into food refrigeration and storage technologies has proposed alternative methods of extending the shelf life of fresh foods, including the use of superchilling (partial freezing) and supercooling (cooling below the freezing point without phase change).

109: Lan & Farid, (2004) stated that the range in which meat freezes is large and covers temperatures between -2 and -15°C: This is very different from other works. Please explain or comment.

Lan and Farid are referring to salted meat. We agree that this is not really relevant and so have removed this reference.

124: "Because of this natural variation, some authors may report that supercooling occurred while others found superchilling (nucleation) occurred at the same temperature": the more logical consequence of natural variation in freezing point would be that some authors may report that supercooling occurred while the product was actually above its freezing point, i.e. neither supercooled nor superchilled.

We believe that this is the exact point that we are making in the paper.

136: At storage temperatures where nucleation could be expected to occur but the authors did not report nucleation, then supercooling is assumed for the purpose of this review paper: this is a dangerous assumption as nucleation cannot always be easily detected at temperature just below freezing.

We agree, but in this situation the ice fraction (if there was ice) would be very low and so the samples would be close to a supercooled situation. We have removed table 3 in response to this point as the data referred to papers where there was a question as to whether the samples were actually supercooled.

289: Not all reports on the use of supercooling have shown positive results.The following workers reported that subzero storage temperatures damaged the condition of food: but was the food supercooled?

We believe from the information provided that in these situations the food was actually supercooled. We have tried to outline some of the reasons for these phenomena.

310: Though these authors quote the freezing point of smoked-salted salmon to be about -4°C, by storing the product at -2°C they are exceeding the freezing point of water so may be that the water in the product did not nucleate due to a freezing point depression caused by the introduction of the salt: In that case this study should be discarded as it was not "supercooling". Also why include this work under "Some negative reports from the use of supercooling on foods"?

We agree, this is a fair point. We have removed this paragraph.

342-349: Very long and poorly constructed sentence.

Agreed, changed to:

It is believed that supercooling as a practical processing technique for meat (and perhaps fish) would be best used in terms of monetary returns for the most perishable and high value products (per unit weight). Supercooling of lower value products will probably not be a viable technique compared to conventional chilling due to the extra processing costs of energy and equipment needed for maintaining the supercooled storage.

357: Such a distribution system would demand either retrofitting current distribution vehicles with spaces and refrigeration equipment with much better thermal and shock controls or designing new vehicles: I think it would be unrealistic to maintain supercooling during transport due to the inevitable shocks and vibrations and poor temperature distribution that would cause nucleation.

We think that is what we are already indicating. However, we have added a sentence stating that supercooling is probably only practical in storage.

Table 1: What is "Air control"?

It is the control sample which was stored in air. This has been clarified in the revision.

Table 2: Why are "Air (no packaging)", "Air (overwrapped)" etc. are not "types of refrigeration".

Changed to ‘Storage method’ in the revision.

"To reduce temperature fluctuations": not clear what these words are doing in this table. Is this a new heading?

Apologies, should have been removed in the initial submission. Now removed in revision.

The tables are badly designed and illogically organised. Headings are introduced at random, one column overflows into another, most cells are blank and contents don't always agree with headings.

We have completely revised the tables and attempted to make them more ‘user friendly’.

***Highlights (for review)**

Highlights:

1. Supercooling of food is reviewed comprehensively.
2. Supercooling of a large range of food types is considered.
3. The impact of supercooling on food quality and safety is described.
4. The advantages and disadvantages of supercooling are discussed.

The use of supercooling for fresh foods: a review

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Abstract

Supercooling is a food processing technique which has the potential to significantly increase the shelf life of foods and to reduce wastage of food products from the production and retail sectors of the food cold chain. The process uses storage temperatures below the initial freezing point of the food without the product freezing, which maintains the quality attributes associated with fresh foods. The removal of the freezing process leads to shorter processing times from harvest to delivery to retail as well as lower energy consumption (no latent heat removal) and so lower carbon emissions during manufacture when compared to standard frozen food production.

Keywords: Supercooling, food, temperature control, refrigeration, quality, storage life

1.1. Introduction

The majority of foods are perishable (e.g. meat, dairy and marine foods) and so require refrigeration in order to achieve an acceptable length of shelf life and to minimise the risk of food borne illnesses. Such is the concern over the health implications of foods that this is the predominant factor which governs the legislative requirements on food producers. Despite the potential hazards of not maintaining foods at the correct temperature it is estimated that worldwide only about one tenth of total perishable food production is refrigerated, with developing countries much more likely to ignore this requirement than developed ones. It is estimated that as much as 25-30% of perishable food production is wasted and most of this wastage could be saved during post-harvest storage by the correct use of refrigeration

(Coulomb, 2008 and IIR, 2009). Frozen storage in particular has been greatly influential on the design and operation of the modern large-scale food production business. Despite the positives of frozen foods, chilled products are still viewed as being more favourable by many consumers, and so chilled foods can demand higher prices at the point of sale. This consumer perception is partially based on the belief that chilled foods are fresher, less processed and more convenient for cooking than are frozen foods.

Temperature control is an essential part of food production, delivery and storage in the modern food distribution network. Temperature control is used to increase the length of acceptable shelf life of products by reducing the rates of degradation from microbial sources (spoilage and pathogenic) and from intrinsic factors such as lipid oxidation and enzymatic proteolysis. At all stages of the food chain, temperature of the product must be monitored and controlled for both maximum product quality and to comply with the strict food legislation in effect in the EU for foods of animal origin (meat/fish). The use of chilled or frozen storage allows for not only a greater diversity of products to the consumer but also increases the length of available seasonality and distribution area for foods which would be otherwise unavailable away from the production area.

Recent research into food refrigeration and storage technologies has proposed alternative methods of extending the shelf life of fresh foods, including the use of superchilling (partial freezing) and supercooling (cooling below the freezing point without phase change). A review of superchilling was published by Kaale, Eikevik, Kolsaker and Stevik (2013) while this review will focus on the application of supercooling and its effects on food quality in the scientific literature.

1.2. Definition of supercooling

Supercooling is the process of lowering the temperature of a product below its usual freezing point without the phase change solidification (formation of ice crystals) occurring. This process has several terms such as supercooled or undercooled (Charoenrein & Preechathammawong, 2010; James *et al*, 2009; Li & Lee, 2008 and Watson & Leighton, 1926), subcooled (Lucas, 1954), freezing point depression (Chen & Chen, 1997 and Griffith & Ewart, 1995) or Hyo-On (in Japanese papers), (Ando *et al, 2007*). Material can be maintained in this state at atmospheric pressure with accurate temperature control without ice crystal formation occurring when there is an absence of a crystal nucleus (Aparicio *et al*, 2008, Bedecarrats *et al*, 2009; James *et al*, 2009; Sanz *et al,* 1999 and Yin *et al,* 2005;). Theoretically according to Bedecarrats *et al,* (2009); Cox & Moore, (1997) and Fukuma *et al*, (2012) a supercooled product is unstable and spontaneous nucleation can occur at any moment. Bedecarrats *et al*, (2009) describe that the process of ice crystallisation is changeable, as this process may not occur at the same time or temperature in what appear to be identical samples due to slight compositional changes between samples, whereas Fukuma *et al*, (2012) reported finding sections within the same sample which were both frozen and fresh. Yin *et al*, (2005) reported that it is very difficult to create supercooling in a solid food, as either the structure of the food provides surfaces for ice crystal growth, or it inhibits heat conduction from the product in comparison to a liquid, however they did report that this is not always the case, as orange juice behaves like a solid food during freezing and shows little supercooling compared to liquids such as milk.

1.3. Types of food product that supercooling has been used on

The use of supercooling in food refrigeration processes is still relatively new, although it has been used to improve shelf life duration for a variety of foods including vegetables (Fuller & Wisneiwski, 2008 and James *et al*, 2009), fish (Ando *et al,* 2007; Agustini *et al,* 2001; Beaufort *et al*, 2009; Fukuma *et al*, 2012 and Gallart-Jonet *et al*, 2007), and meat (Jeremiah & Gibson, 1997; Jeremiah & Gibson, 2001 and Lawrence *et al*, 2010). In the case of fruit, only a few studies are referred to in the literature for quality preservation, with the majority of these focusing on the ability of the plant to adapt to the cold shock of winter conditions, rather than of supercooling the fruit during storage. Some examples of the application of this technique for the preservation of fruit were cited in James *et al*, (2009), who reported that apples can be supercooled by as much as 4°C (Diehl & Wright, 1924), these authors also cited Lucas, (1954) who published that studies on grapes, navel oranges and lemons showed that supercooling was possible in these fruits when chilled in air, and in alcohol for lemons. The application of supercooling on tomatoes was reported in a patent application by Cox & Moore, (1997) as cited in James *et al,* (2009).

1.4. Supercooling process

In the literature, supercooling can be achieved through a diverse array of equipment which includes: near static air (Beaufort *et al*, 2009; Charoenrein & Preechathammawong, 2010 and James *et al*, 2009) immersion in a water bath with brine or ice slurry (Pineiro *et al*, 2004 and Rodriquez *et al*, 2005) and immersion in alcohol (Lucas, 1954). Fukuma *et al,* (2012) examined the effects of two slow chilling regimes on different types of fish meat. They found that when the temperature was reduced by 1.0°C per day all samples were frozen upon reaching -3.5°C, whereas when chilled with a 0.5°C per day reduction, the fish muscle could be taken as low as -5.0°C in a supercooled state. Nucleation was found to occur in a supercooled product when the meat was subjected to either vibration or a temperature fluctuation. This observation could explain some of the overlap seen in published work on supercooling/superchilling where different outcomes were reported at the same storage temperatures. Cox & Moore, (1997) stated that a very rapid temperature decrease is required in order to create the supercooled state in a food, though a rapid temperature drop would not be associated with near static air or low air flow as used in these other works. In contrast to this statement by Cox & Moore, (1997), it is more likely that superchilling would be induced through rapid chilling rather than supercooling, as shown by numerous workers in the field of superchilling.

The level of the supercooling achieved has been tested on a variety of foodstuffs such as vegetables, fish and meat. The degree of supercooling achieved (amount of freezing point depression) is highly food sensitive and related to the type of food and its constituents (Gabas *et al,* 2003)**.** The degree of freezing point depression increases as solute concentration increases (Goral & Khaza, 2002). Sanz *et al*, reported that meat froze at between -0.6 to -1.6°C, (1999), James *et al*, (1983) and Small *et al*, (2011) reported a freezing point of -1.5°C and - Lowry & Gill, (1984) a freezing point of -2°C. In fish the range was reported to be -0.6 to -2.0°C by Chen & Pan, (1997) and Magnussen *et al*, (2008) and -1.0 to -2.2°C by Silvertsvtig *et al*, (2002), whereas in other foods a range of -0.5 to -1.1°C was reported in milk by Beavers *et al*, (2003) and Boonsupthip & Heldman, (2007). Due to the intra and inter species variation in composition, freezing points (based on water and fat contents) will vary slightly for each experiment. Farouk *et al*, (2013) found that the freezing point of beef muscle was related to the pH of the muscle, with higher pH giving a higher freezing point temperature and lower than normal pH a lower temperature. They stated that these differences were due to the interactions of muscle pH to water holding capacity and so to calculate the freezing point for any muscle the pH would need to be known. Therefore supercooling might be achieved at slightly different temepratures in similar food types and so it is not possible to give an exact value of the freezing point to cover all samples within that specie. Because of this natural variation, some authors may report that supercooling occurred while others found superchilling (nucleation) occurred at the same temperature. With this in mind, it is not always clear which of these processes was reported, or indeed whether the product was actually at a temperature below its freezing point (supercooled) as many studies use a temperature approximately that of the expected freezing point of the food (-1 to -3°C). Chen & Carter, (1986) stated in a report on chilling of citrus fruits that due to natural variation within foods the process of supercooling is complex and unpredictable in terms of maximum temperature decrease achievable and length of supercooling prior to nucleation.

1.5. Storage life

Some examples from the literature of uses of the supercooling preservation method are given in the following sections. At storage temperatures where nucleation could be expected to occur but the authors did not report nucleation, then supercooling is assumed for the purpose of this review paper. Chilled and potentially supercooled stored foods will increase the achievable shelf life of foods, with the lower temperatures used in supercooling giving longer shelf lives than those achieved in the same products in conventional chilled storage (Artes, 2004; IIR, 2009; James & James, 2010 and Tassou *et al*, 2010).

1.5.1. The use of supercooling on meats

The use of supercooling for meat was reported by Jeremiah & Gibson, (1997) who looked at the effects of different storage times, (0-28 days) temperatures (5, 2 and -1.5°C) and packaging methods on the length of achievable storage and display (0-30 hr) in pork cuts. They reported that the lower storage temperature increased the length of acceptable retail appearance and had a lower number of unacceptable sample scores at all display periods compared to the samples stored at 2 and 5°C. After 24 hours of retail display approximately 15% of the cuts from the -1.5°C group were regarded as unacceptable, while at the higher storage temperatures around 30% of the samples had been rated as unacceptable throughout the whole display period. The -1.5°C pork had significantly lower off odours both at the start and end of the 30 hour display period, than either the 2 or 5°C samples. Jeremiah & Gibson, (2001) conducted a similar experiment (based on storage period rather than retail period) and stored beef steaks at 5, 2 and -1.5°C for between 0 and 24 weeks, under either vacuum or controlled atmosphere packaging. There were no significant changes in either muscle pH or colour due to storage temperature prior to retail display, though after 24 weeks the steaks from the -1.5°C trial had slightly higher pH and lower colour values. The steaks stored at -1.5°C had the highest levels of oxymyoglobin while those from the 5°C group had the lowest values, meaning that the colder steaks would have appeared redder in colour during display, so being more visually acceptable. After extended storage and retail display, the data showed that the steaks stored at the lower temperature were significantly less discoloured and had the greatest oxymyoglobin and least metmyoglobin values than those at 5°C. The -1.5°C steaks had the lowest level of detectable off odours compared to the other temperatures after storage and display. After 24 weeks storage and at 2 hours of retail display, only those steaks from the -1.5°C treatment had acceptable retail appearance scores, which were significantly greater than from the other temperatures, which was in agreement with the findings of Gill & McGinnis, (1995) and Jeremiah & Gibson, (1997). Storage temperature and time had major impacts on the length of retail acceptance, with 24 hours of retail display only achievable up to 18, 10 and 8 weeks storage times at -1.5, 2 and 5°C respectively. At the maximum quoted storage period the steaks could be on retail display for 6, 1 and 0 hours respectively before being classified as unacceptable. As no freezing was described by these authors at the storage temperature of -1.5°C (which is approximately the freezing point of meat) it would suggest that the meat samples in these two trials were supercooled not superchilled/frozen.

Gill & McGinnis, (1995) looked at the effects of different concentrations of O_2 and N_2, vacuum packaging and different storage temperatures ranging from 5°C to -1.5°C and from 0 to 48 hours of storage on beef *Longissimus dorsi* (LD) and *Psoas major* muscles. Storage of LD at -1.5°C under N_2 had no significant changes on colour deterioration, metmyoglobin content or discolouration up to 8 hours of storage, and up to 48 hours in concentrations of O_2 up to 400ppm. When the LD samples were stored at 5 and 1°C, they showed similar though significantly higher rates of colour instability (degradation) at the same concentrations of pack atmospheric O_2, whereas at 0°C the rates of colour degradation were much more reduced, though not as much as seen at -1.5°C. These trends were not repeated in the *Psoas* samples, as colour deteriorations were seen at all combinations of storage temperature, time and atmosphere. The authors attributed this to the structure of this muscle and of this structure having a lower colour stability than that of the *Longissimus*, when packaged a short time post mortem and thus if allowed to mature for longer before cutting may have better colour stability at low oxygen concentrations, so having the potential to extend the acceptable visual shelf life in this muscle. Another reason for the differences in colour stability between these muscles would be that they do different amounts of work in the physiological state, so would have different compositions of fast and slow twitch muscle fibre types.

1.5.2. The use of supercooling on fish

Cyprian *et al*, (2012) reported on the use of superchilling (-1°C) and conventional storage (1°C) on tilapia fillets, with or without modified atmosphere packaging. These authors describe the work as superchilling but do not mention freezing occurring. At such a temperature freezing would not be expected, so for this review it has been considered as supercooling. The superchilled samples packed in air were found to have a longer shelf life than air packed conventional samples with values of 20 and 13-15 days for -1 and 1°C respectively. There was no difference between storage temperatures in the MAP samples, with both achieving 23 days of storage. The lower temperature was found to slow bacterial growth on the fillets. The fillets stored in air at 1°C had a total bacterial count of 10^8 CFU/g at day 16, whilst those in air at -1°C had approximately 10^7 CFU/g at day 20. The combination of MAP and low temperature gave bacterial counts of 10^4 CFU/g at day 23 of storage. The lower temperature reduced the amount of drip loss compared to the conventional chilling for both packaging methods, while air storage produced less drip than MAP fillets. The MAP fillets had a shorter period of colour stability with differences noticeable at 2 days of storage. The authors concluded that overall the lower storage temperature was the best for this type of fish.

1.5.3. The use of supercooling on vegetables

The application of a supercooling procedure has been used on foods other than those from an animal origin. One such example was published by James *et al*, (2009) who reported on the use of supercooling for the preservation of garlic and the formation of ice crystals once the limit of supercooling was exceeded. They stated that the freezing point of garlic was -2.9°C, yet were able to store peeled cloves at temperatures down to -9.3°C for up to 69 hours with no detected freezing and no visual alterations compared to chilled garlic. They also reported that there was no correlation between rate of cooling or thawing on the determined freezing point. When the garlic was stored at lower temperatures they reported a mean nucleation point of -13°C. At a storage temperature of -6.6°C the garlic was maintained undamaged and unfrozen for one week.

James *et al*, (2011) reported on supercooling of several types of vegeables to examine the differences in visual appearance between fresh, frozen and supercooled storage temperatures. These authors wanted to show if it was possible to supercool these foods and what the maximum degree of freezing point depression was for each before nucleation. The vegetables studies were broccoli, carrot, cauliflower, garlic, leek, parsnip and shallot. They reported that all of these vegetable varieties could be supercooled, though with the prevalence of supercooling before nucleastion different between the samples. Supercooling was found to occur in all replicates of garlic and shallot while only 40% of the parsnip samples showed supercooling. In the other vegetables the majority supercooled before nucleation. As would be expected the freezing point temperature and maximum degree of supercooling were different for each vegetable type, with the garlic being able to maintain the greatest freezing point depression. The shallots followed a similar pattern to that of the garlic cloves. Unpeeled shallots were held at temperatures as low as -6°C for 24 hours without nucleation, while when stored at -7°C some freezing was recorded.

Hruschka *et al*, (1961) studied the effects of different temperature storage conditions on the feasability and crop production of seed potatoes prior to planting. They stored the potatoes at ambient, (40°F, 4.4°C), chilled (30°F, -1.1°C), supercooled (25°F, -3.9°C) or frozen. It was found that supercooled storage did not have negative effects on the potatoes as no significant differences were found between the proportion which germinated/grew, the length of growth time or the total yield per acre between the ambient, chilled and supercooled samples. Freezing of the potatoes did have negative effects on all measurements which were found to be significantly lower than the other treatments. The authors noted that a supercooled product was unstable and vibration could initiate nucleation leading to freezing related damage of the potatoes. Based on this, they reccommended that once a product is suspected of been in a supercooled state (such as during storage in cold weather) they should not be moved/transported until warmed to avoid spontaneous nucleation.

1.5.4. The use of supercooling on fruits

Workmaster *et al*, (1999) reported on the ability of both the cranberry plant and its fruit to supercool. To test the ability of this plant to maintain a supercooled state the workers stored the cranberry at sub zero temperatures and initiated ice propagation at different sections of the plant/fruit through the introduction of a solution innoculated with ice-nucleating bacteria. They found that the stem would quickly freeze upon introduction of the solution and the movement of the ice front could be measured using infra-red video monitoring. The ice was found to propagate into the leaves but not into the attached fruit, with the fruit been able to hold a supercooled temperature as low as -8°C for an hour after the nucleation of the solution, the trial was stopped at this time. The ripeness of the attached fruit (mature or unripe) was found to have no effect on ice propagation, though the authors believed that ripeness may have been more influential due to changes in structural properties with ripeness. A similar test was conducted on harvested cranberries, with the innoculated solution applied to different parts of the berry surface. It was found that the less ripe berries were not able to supercool for as long or to as low a temperature as measured in the ripe fruit. Twelve ripe (red) and twelve unripe (white) berries were used and it was found that when nucleation occurred it was from the apex (calyx) end of the berry and not from the stem (pedicel) end of the fruit. Ninety-two percent of the white berries and 8% of the red berries had nucleated after twenty minutes of supercooling, After 1 hour of supercooling only 33% of the red berries had nucleated.

Chen & Carter, (1986) studied the effects of storage temperature on degree of supercooling and freezing damage in citrus fruits. The oranges and grapefruit were stored outside during cold weather to simulate damage to pre-harvested fruit. Other fruit from the same harvest had been tested for sugar concentration with groups at either 10 or 20 brix (sucrose % in juice) this was done to resemble the variance in fruit types found in that region. In the first test, fruit were stored in glycol at -4°F (-20°C), at this temperature the 20 brix oranges were found to supercool to 3.6°F (-15.7°C) below the freezing point, though supercooling was not maintained and the fruit nucleated during storage. In the outdoor storage trial the small diameter oranges nucleated at 22.8°F (-5.1°C) while the larger diameter grapefruit nucleated at 30.4°F (-0.9°C) and 4 hours sooner than in the oranges, it was not clear why size of the fruit made such a difference. The brix values for these fruit were 10.5 for oranges and 9.5 in the grapefruit and that they supercooled by 7 and 2°F (-13.9 and -16.6°C) respectively. The ambient temperature fell as low as 20.4°F (-6.4°C) during this trial. They acknowledged that the values measured were representative of a small reduction to the freezing point (supercool) but would be important for avoidance of freezing in growing fruits in the normal weather conditions found in that region. Other examples of shelf life extension for supercooled compared to conventional chilled foods are presented in Tables 1 to 2.

1.6. Some negative reports from the use of supercooling on foods

Not all reports on the use of supercooling have shown positive results. The following workers reported that subzero storage temperatures damaged the condition of food so reducing the achievable shelf life and product quality. Examples from studies on vegetables include: James *et al*, (2009); McColloch, (1953) and Neefs *et al*, (2000), for fruit: Martins & Lopes, (2005) and McColloch, (1953); in meat: Beaufort *et al*, (2009), and in fish: Ando *et al,* (2007). Ando *et al*, (2007) stored yellowtail (mackerel) at temperatures between 10°C and -1.5°C and measured the effect of storage temperature on muscle structure (firmness). This study reported both beneficial and negative effects on muscle structure at sub zero storage. Storage at -0.5°C increased deterioration in the muscle structure compared to storage at 4°C, while at -1.5°C firmness was less impacted than at -0.5°C. Storage at 10°C and -1.5°C produced the least deterioration in muscle firmness after 24 hours storage, however, at this colder temperature cold shortening was observed and the sample shrank significantly. At -1.5 and 10°C, after 24 hours of storage these samples showed development of the smallest extracellular spaces and there was less collagen breakdown compared to storage at 4°C.

Table 1 here

Table 2 here

1.7. Conclusions

The use of supercooled storage has been demonstrated to be beneficial in extending the shelf life of foods in many of the published examples in the scientific literature cited. If supercooling is applied and maintained correctly this process could reduce the amount of product wastage from spoilage at all stages of the food cold chain. Mixed results have been reported for the application of this technique on food quality, with mostly positive results. The variances reported on quality attributes may be because of a lack of understanding of this chilling method leading to use of chilling parameters not suitable for that particular product. In some of these studies; it could be hypothesised that slightly different parameters would have been more favourable, such as where the final storage temperature initiated nucleation leading to superchilling rather than supercooling, a greater understanding of how food composition influences the freezing point may have been beneficial.

Another beneficial aspect of supercooled storage is in the lower energy requirements of this technique compared to frozen storage or super chilling (IIR, 2009). As there is no latent heat from freezing to be removed so less energy is needed to cool the product to the final storage temperature and processing times will be faster. The energy requirement for supercooling will be greater than for conventional chilled storage due to the lower temperatures used but such temperatures will allow for longer display periods and less wastage than chilled storage which may make the process as economical, or more so, than conventional storage methods overall. It is believed that supercooling as a practical processing technique for meat (and perhaps fish) would be best used in terms of monetary returns for the most perishable and high value products (per unit weight). Supercooling of lower value products will probably not be a viable technique compared to conventional chilling due to the extra processing costs of energy and equipment needed for maintaining the supercooled storage. However supercooling may be of use to the cheaper products (such as mince) which conventionally have a much shorter shelf life than whole meat (e.g. steaks) due to increased microbial growth (greater exposure of oxygen-reactive compounds and nutrients) and so would potentially give a greater proportional increase in shelf life compared to that gained by a whole meat product such as a leg or loin, by extending the shelf life of a product such as mince would therefore reduce wastage and so reduce the environmental impact for disposal but also make the product have a higher profitability.

Maintaining the supercooled state demands that the product is subjected to a minimum of external influences such as fluctuations in temperature or physical vibration. Such environmental variants can act as stimuli for the onset of nucleation of ice crystals within the supercooled product so leading to a state with low ice fraction content (superchilling rather than supercooling). To maintain the conditions required for supercooling (close temperature and stability control) throughout the cold chain would allow the benefits of supercooling to be extended further along the cold chain, with the aim that the product would reach the consumer still in a supercooled state. Such a distribution system would demand either retrofitting current distribution vehicles with spaces and refrigeration equipment with much better thermal and shock controls or designing new vehicles. Not only this but the distribution facilities and retailers (maybe also consumers) would require altered/new refrigeration systems capable of maintaining such close temperature controls. It would also be necessary to easily identify such products so that operations personnel (and consumers) would know which refrigeration method would need to be used. A new distribution chain of this manner would be exceedingly expensive and difficult to introduce and might lead to increased equipment disposal as newer equipment would be introduced to replace some of the current facilities. Pragmatically supercooled products may only be suited to cold stores where these conditions can be maintained.

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1.8. Bibliography

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Table

Table 1 Examples of the use of supercooling in lamb preservation (McGeehin et al, 1999).

Type of process	Temps used	Effect of process
Control sample stored in air	4°C at 0.2m/s for 5 days	Had intermediate weight loss during chilling at 1.48%. Shear force and panel toughness score increased with aging.
Intermediate cooling (supercooling)	-2°C at 2.5m/s for 24 hours, then 4 days in control.	The meat reached a temperature of -1°C within 3 hours on the surface and around 8 and 12 hours in the loin and leg respectively. No differences were found in tenderness to controls, though sarcomere length was significantly shorter than control at day 2. Ando *et al*, (2007) also reported cold shortening in sub zero storage, which may have been related to sarcomere size. Shear force and panel toughness scores increased with aging. No freezing occurred showing that the meat supercooled not superchilled. Had the greatest proportion of weight loss during chilling at 2.21%, which the authors put down to the high air velocity and no humidity control in the chiller leading to surface drying.
Ultra-rapid (superchilling)	-20°C at 1.5m/s for 3.5 hours, then 4 days and 20.5 hours in control.	Had the lowest proportion of weight loss during chilling at 0.57%, in agreement with (Redmond, et al., 2001). The lower weight loss during rapid chilling suggested that these samples were superchilled giving crust freezing (some ice fraction). No differences were found in tenderness, though sarcomere length was significantly shorter than control at day 5. Shear force and panel toughness score increased with aging. Wet appearance up to 48 hours in the control chiller due to condensation onto the carcasses, could be reduced by lower humidity in the second chiller.

Table 2 Long term storage of supercooled beef and lamb (Eustace & Bill, 1988).

Storage method	Temps used	Effect of process
Air (no packaging)	-2.2 to -2.6°C	All joints were frozen within 3 days.
Air (overwrapped)	-2.2 to -2.6°C	All joints were frozen within 3 days. Ice crystals were visible between the meat surface and overwrapping before meat began to freeze and the authors thought these crystals had facilitated nucleation in the meat.
Air (vacuum packed and stored in cartons)	-2.2 to -2.6°C	Not as successful at reducing temperature fluctuations as brine immersion, though better than air storage alone, as 24 of the 47 packs had frozen at week 2, with only 10 packs unfrozen at 3 months and 8 packs of beef at 1 year of storage were still unfrozen. The lamb joints (n=16) were assessed at 10 weeks of storage with no signs of freezing in any pack.
Brine immersion (vacuum packed)	-2.2 to -2.6°C	In beef only 5 of the 28 joints were frozen after 2 weeks storage, by 3 months storage 19 joints were still not deemed to be frozen. Of these 19, only 1 joint was frozen after 1 year of storage.
Vacuum packed and stored in either carton or brine		On opening the bags after 15 weeks of storage, beef was found to still bloom readily, retained colour for 3 days in retail display and had no putrid odour, with packaging odour assessed as the same as meat stored at 0°C for 4-6 weeks. On cooking, meat had an excellent flavour, the same was found for lamb after 10 weeks storage. Topsides opened after 1 year of storage, still had a good red colour after blooming, with only scattered brown areas. After 24 hours of retail display the surfaces were entirely brown. Odour on opening was assessed as similar to that of beef vacuum packed for 12 weeks at 0°C. Microbial assessment showed variable results but in general growth was very slow in vacuum packed beef at this temperature. The flora predominantly being lactic acid bacteria, as is often the case with vacuum packing. After 14 weeks storage, bacterial counts averaged values around $2x10^3$ and ranged from $1x10^3$ to $1x10^8$ CFU/cm^2 after 1 year of storage, while lamb after 10 weeks had values of $3x10^6$ to 1.1x107 CFU/cm^2. The cooked flavour of the topsides was described as 'liver-like', which is usually associated with protein degradation.