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Initial freezing point of Mozzarella cheese

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Abstract

The initial freezing point in unsalted fresh Mozzarella cheese was measured. The influence of the water-soluble solids on the freezing point depression of unsalted Mozzarella cheese was analyzed. Central temperature profiles and the initial freezing point of unsalted cylindrical cheese samples (1 cm radius; 3 cm height) were determined. A lyophilized aqueous extract of the soluble solids at pH 4.6 was obtained from the unsalted cheese. Initial freezing points of the solutions, prepared with a concentration of soluble solids similar to the one occluded in unsalted cheese matrix and different NaCl concentrations, were determined. Initial freezing points of the unsalted cheese were in the range of −1.2 to −2.4 °C, while a value of −1.7 ± 0.1 °C was observed in the solution of soluble solids without NaCl. Moreover, the initial freezing points of the solutions of soluble solids and NaCl showed an additional depression of approximately −1.7 °C compared to the initial freezing point of NaCl solutions. Therefore, it was clearly shown that not only NaCl but also the other soluble solids present in the aqueous phase significantly contribute to the freezing point depression of Mozzarella cheese.

Keywords: Freezing point; Mozzarella cheese; Measurement

1. Introduction

Most foods contain large quantities of water. When such foods are cooled below 0 °C, a temperature is reached at which water begins to convert into ice. Unlike pure water, all the water in food does not change to ice at the same temperature, due to the presence of different solutes. The initial freezing point of a food is the highest temperature at which ice may exist in that food in thermal equilibrium (Miles, 1991). This is one of the most important thermodynamic properties required for the prediction of thermal and physical properties in a frozen food. Values of the initial freezing point can be used to calculate properties such as effective molecular weight, water activity, bound, free, and frozen water, and enthalpy below freezing (Rahman et al., 2002). Therefore, the knowledge of the initial freezing point is very important to analyze freezing and thawing of foods.

High moisture cheeses have short storage lives at refrigerated temperatures. Freezing is one of the methods used to extend cheese shelf-life during marketing. Particularly, the freezing of Mozzarella cheese has been studied considering different methodologies and analyzing the effect of different operating conditions on the main characteristics of the cheese (Bertola, Califano, Bevilacqua, & Zaritzky, 1996a, 1996b; Cervantes, Lund, & Olson, 1983; Chaves, Viotto, & Grosso, 1999; Graiver, Zaritzky, & Califano, 2004; Kuo & Gunasekaran, 2003; Lück, 1977; Oberg, Merril, Brown, & Richardson, 1992; Tunick, Mackey, Smith, & Holsinger, 1991). In this case, the initial freezing point is one of the thermodynamic properties useful for studying the freezing process. Unfortunately, data of initial freezing point in cheese are scarce in literature (Esteban, Marcos, & Fernández-Salguero, 1987; Miles, Mayer, Morley, & Houška, 1997; Pham, 1996; Polley, Snyder, & Kotnour, 1980).

The freezing point is affected mainly by the concentration of solutes of low molecular weight. Esteban et al. (1987) related the initial freezing points of aqueous cheese

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extracts to the water activity of cheeses. The authors pointed out that in fresh cheeses the depression of water vapor pressure is determined almost solely by the presence of sodium chloride. However, in samples of unsalted cheeses, a freezing point depression can be observed, probably due to the presence of soluble solids associated with the products of the initial protein degradation. Therefore, our objectives were to measure the initial freezing point and to study the contribution of the water-soluble solids to the freezing point depression of unsalted fresh Mozzarella cheese.

2. Materials and methods

2.1. Samples

Unsalted fresh Mozzarella cheese (3500 g weight, $30 \times 10 \times 10$ cm^3 size) was brought from a local factory to our laboratory. The initial composition was: $50.6 \pm 0.1\%$ (w/w) moisture, $26.3 \pm 0.3\%$ (w/w) protein, $17.00 \pm 0.01\%$ (w/w) fat, non-detected NaCl.

2.2. Central temperature profiles in Mozzarella cheese

Cylindrical samples of Mozzarella cheese of 3 cm height and 1 cm radius were used. A thermocouple (type T, 30 AWG, OMEGA Engineering, Inc., Stanford, USA) was inserted in the geometric center of the cylindrical sample. Samples were frozen by immersion in a solution of NaCl 20% w/w, cooled by a cooling system HAAKE EK45 (Thermo Haake International, Karlsruhe, Germany). Data of temperature were obtained through a data adquisition system Data Shuttle DS-16-8-TC (OMEGA Engineering Inc., Stanford, USA) at 1 s^{-1}. Central temperature profiles of 6 samples were obtained.

2.3. Preparation of the fraction of soluble solids

Water-soluble fraction extraction was performed with a modified procedure developed by Kuchroo and Fox (1982). Grated cheese (20 g) mixed with 30 mL water was homogenized using an ULTRA-TURRAX T25 (Ika Werke, Janke & Kunkel GmbH & Co KG, Staufen, Germany) homogenizer for 2 min. The homogenate was held at 40 °C for 1 h, pH was adjusted to 4.60 ± 0.05, and the suspension was centrifuged at 4800 rpm and 5 °C for 30 min (Biofuge 28RS; Heraeus Sepatech, Osterode, Germany). After centrifugation, the upper layer of fat was removed. The supernatant, after being filtered through Whatman No. 42 paper, was lyophilized in a Freeze Dryer FD 2.5 (Heto Lab Equipment A/S, Allerød, Denmark), constituting the fraction SS.

Nitrogen determination was carried out using the micro-Kjeldahl method with an automatic digestor model 430 and distillation unit model 322 (Büchi, Flawil, Switzerland) and a DL40RC titrator (Mettler Instrument AG, Greifensee, Switzerland).

2.4. Determination of the initial freezing point from time–temperature plots

When an aqueous solution is cooled without stirring to below the freezing point (supercooling), the solution temperature initially reduces to induce nucleation and then reaches a point when a sufficient number (or size) of nuclei become available to start an auto-crystallization. From this point, the temperature increases instantly from supercooling temperature to a plateau of relatively constant temperature that corresponds to the freezing point of the sample. Mechanical vibration during nucleation can also induce an auto-crystallization (Chen, Chen, & Free, 1996). The method used in this study to determine the initial freezing point is based on this principle.

A schematic diagram of the experimental set up used for measurement of the initial freezing point is shown in Fig. 1. The solution container was made of 2-mm glass and it was placed in 4.5 L of a solution of NaCl 23% w/w, cooled to -15 ± 1 °C with a cooling system HAAKE EK45 (Thermo Haake International, Karsruhe, Germany). Twenty milliliters of the sample was used for each determination. The sample temperature was measured with 3 thermocouples (type T, 30 AWG, OMEGA Engineering Inc., Stanford, USA). Temperature data were obtained through a data adquisition system Data Shuttle DS-16-8-TC (OMEGA Engineering Inc., Stanford, USA) at 1 s^{-1}. The experimental set up allowed obtaining a cooling rate of 1 °C per min, which is recommended to ensure an adequate visualization of the cooling curve (Fennema, Powrie, & Marth, 1973).

To determine the initial freezing point of a solution, the sample is cooled slightly below its initial freezing point (usually 2 °C below the initial freezing point of the sample) and crystallized by vibration of the wire. The temperature of the sample immediately rises. Temperature data are collected for approximately 3 min. The extrapolation of the temperature plateau to the cooling curve was used to

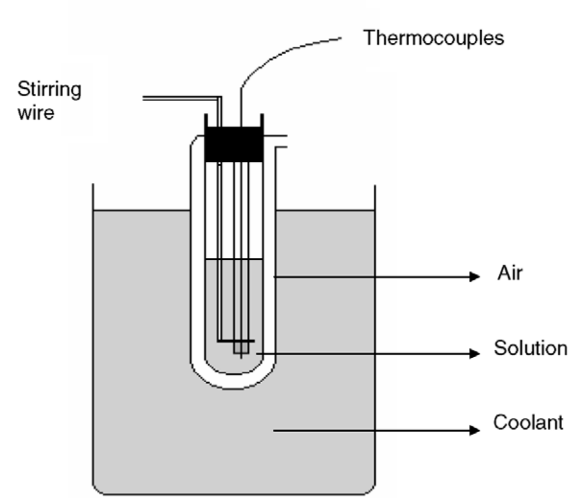

Fig. 1. Schematic diagram of the experimental set up used for the determination of freezing point.

obtain the initial freezing point as shown in Fig. 2 (Fennema et al., 1973).

2.5. Determination of the initial freezing point of sodium chloride solutions

The initial freezing point of NaCl solutions was determined in a range of 0–4% w/w. The aqueous solutions were obtained using NaCl of analytical grade and bi-distillated water. Determinations were carried out in quadruplicate.

2.6. Determination of the initial freezing point of solutions of NaCl and soluble solids

In order to prepare a solution with similar characteristics of the occluded solution in the cheese, the nitrogen content of the fraction of soluble solids of cheese was determined. A value of 0.093 ± 0.008 meq N/g cheese or 0.18 ± 0.02 meq N/g water was determined. The nitrogen content of the lyophilized fraction SS was also determined. Solutions were prepared as shown in Table 1 and the nitrogen content of solutions were determined. The initial freezing point determination of the prepared solutions was carried out in duplicate.

3. Results and discussion

3.1. Central temperature profiles in Mozzarella cheese samples

Central temperature profiles of unsalted fresh Mozzarella cheese samples are shown in Fig. 3. Initial freezing points between −1.2 and −2.4 °C were observed.

3.2. Initial freezing point of NaCl solutions

The values of initial freezing point obtained for the NaCl solutions are shown in Table 2. The small standard deviation of the initial freezing points justified the validity of the method used. The standard deviations were in the order of those obtained from other methods used for the determination of freezing point of foods (James, Lejay, Tortosa, Aizpurua, & James, 2005; Rahman et al., 2002). Moreover, it is a simple and low cost method, and allows obtaining the initial freezing point in a short experimental time.

The results were compared with data available in literature (Rahman, 1995). The differences between measured and published data were small (Fig. 4).

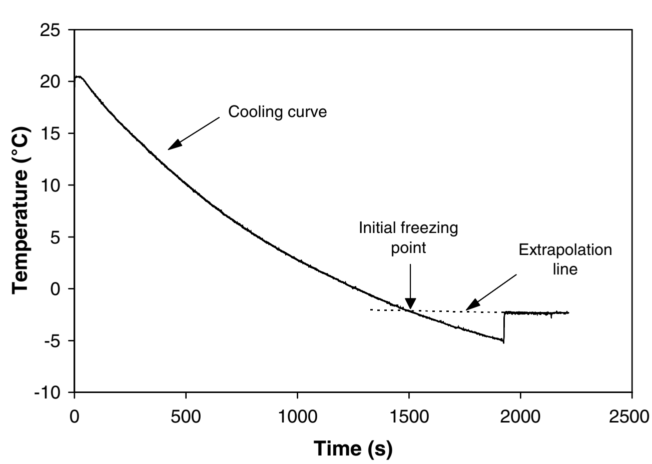

Fig. 2. Typical cooling curve of a NaCl 4% w/w solution. Temperature values correspond to the reading of 3 thermocouples.

Table 1
Concentrations of solutions of NaCl and SS

Solution	NaCl concentration (%w/w)	SS concentration (%w/w)
1	0	8.515
2	1.007	8.616
3	2.033	8.782
4	3.024	8.708
5	4.005	8.761

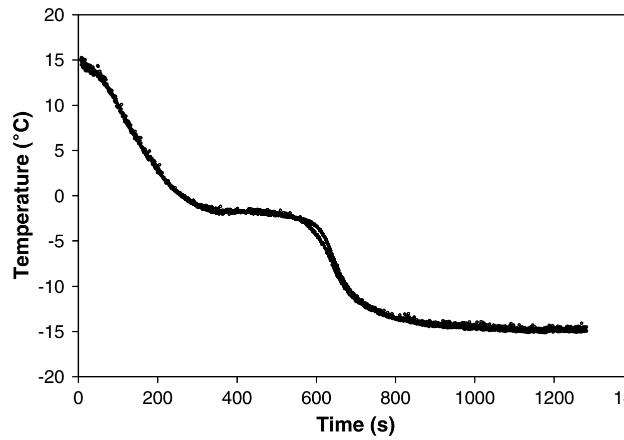

Fig. 3. Central temperature profiles of 3 cylinders of Mozzarella cheese.

Table 2
Initial freezing point values of NaCl solutions

NaCl concentration (% w/w)	Measured freezing point (°C)	Freezing point from literature[a] (°C)
0.000	-0.01 ± 0.04	0
0.982	-0.5 ± 0.2	−0.583
1.988	-1.3 ± 0.4	−1.186
2.922	-2.1 ± 0.3	−1.752
3.884	-2.4 ± 0.2	−2.345

[a] Rahman (1995).

3.3. Initial freezing point of the solutions of NaCl and soluble solids

When no salt is added to cheese, the aqueous phase of fresh cheese may mainly contain amino acids, peptides, lactic acid, and lactose. In this case, compounds based on N were selected as key compounds to reconstitute the occluded solution because the analytical determination of

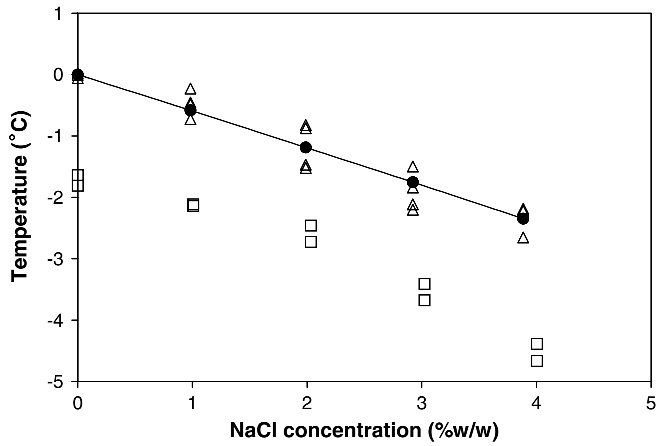

Fig. 4. Initial freezing point values of solutions of NaCl (measured: Δ; from literature: –●–) and of solutions of NaCl and SS (□).

N content is easier and more accurate than the determination of the other compounds.

The nitrogen content and the initial freezing points of the solutions of NaCl and the lyophilized soluble solids are shown in Table 3. The nitrogen contents of the prepared solutions were in the order of the values determined in the cheese extract. It can be clearly observed that the soluble solids in the aqueous phase of unsalted cheese contribute to the initial freezing point observed in Mozzarella cheese. That is, the initial freezing points of unsalted fresh Mozzarella cheese were in the range of −1.2 to −2.4 °C, which are close to the initial freezing point of −1.7 °C, determined for the solution of soluble solids prepared with a N content similar to the one observed in the cheese extract and without NaCl. Moreover, the additional depression of approximately −1.7 °C remains when the solutions of soluble solids and NaCl are prepared with different NaCl concentrations (Fig. 4).

Models for initial freezing point prediction of foods are based on the relation of Clausius-Clapeyron and Raoult's law (Miles et al., 1997; Pham, 1996; Van der Sman & Boer, 2005), which state that the initial freezing point is related to the mole fraction of free water. In an attempt to check the validity of the experimental initial freezing point determined in this work, we used the generalized equation proposed by Miles et al. (1997) for estimating the initial freezing point.

Table 3
Nitrogen content, initial freezing point values and predicted molecular weight of soluble solids using Eq. (1) for the solutions of NaCl and SS

Solution	N content (meq N/ml sol.)	Measured freezing point (°C)	Predicted molecular weight for SS (g/gmol)
1	0.20 ± 0.01	−1.7 ± 0.1	100.1
2	0.191 ± 0.009	−2.13 ± 0.02	120.8
3	0.185 ± 0.005	−2.6 ± 0.2	150.3
4	0.20 ± 0.02	−3.5 ± 0.2	127.0
5	0.21 ± 0.01	−4.5 ± 0.2	104.6

$$\Delta T = \frac{RT_0^2 \sum \frac{\tau_i x_i}{M_i}}{\Delta H_{\text{melt}} \left[\frac{x_w}{M_w} + \sum \frac{\tau_i x_i}{M_i} \right]} \quad (1)$$

where ΔT is the depression of the freezing point of an ideal solution, R is the gas constant, T_0 is the temperature of fusion of pure ice in pure water, ΔH_{melt} is the molar enthalpy of ice fusion, x_i is the mass fraction of the ith component and M_i is its molecular mass, and x_w is the mass fraction of freezable water and M_w is its molecular mass. Factors τ_i allow including molecular dissociation and other non-ideal behavior. A value of 1 was assigned to each τ_i as suggested by Miles et al. (1997). It was considered that the ions related to NaCl were completely dissociated.

Using Eq. (1), an average molecular weight for soluble solids of 121 ± 20 g/gmol was obtained (Table 3). It can be considered that the molecular weight remains almost constant because the dispersion observed may be related to the experimental error. These results reinforce the goodness of the experimental method used for determining the initial freezing point. Moreover, it is important to point out that the contribution of components with high molecular masses to the freezing point depression is negligible (Miles et al., 1997) and therefore in this case, the molecular weight determined may be related to amino acids and other small components expected in the fraction of soluble solids of cheeses (Dave, Sharma, & McMahon, 2003; Feeney, Guinee, & Fox, 2002; Oommen, McMahon, Oberg, Broadbent, & Strickland, 2002).

4. Conclusions

It was shown that the soluble solids at pH 4.6 contribute to the initial freezing point observed during the freezing of unsalted Mozzarella cheese. The initial freezing point of unsalted fresh Mozzarella cheese were in the range of −1.2 to −2.4 °C, while the initial freezing point of the solution prepared with lyophilized soluble solids in a similar concentration to the one occluded in the unsalted cheese matrix, was approximately −1.7 °C. Moreover, the initial freezing points of the solutions of soluble solids and NaCl showed an additional depression of approximately −1.7 °C compared to the initial freezing point of NaCl solutions. Therefore, it was clearly shown that not only NaCl but also the other soluble solids present in the aqueous phase contribute to the freezing point depression of Mozzarella cheese. Molecular weight of soluble solids present in Mozzarella cheese determined using the generalized equation proposed by Miles et al. (1997), was in the order of molecular weights of expected soluble solutes in cheeses.

The method proposed to measure the freezing point was appropriate for this study due to its accuracy and simplicity. Cheese samples generally have variability in composition due to milk composition, coagulant type, manufacturing, seasonal variations, etc. Therefore, it is not necessary to use a method more accurate, which may result in a higher cost.

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