「日本食品工学会誌」, Vol. 4, No. 3, pp. 77 - 82, Sep. 2003

◇◇◇ **Original Paper** ◇◇◇

Effects of Single Food Components on Freeze Concentration by Freezing and Thawing Technique

Phang Lai YEE[1], Minato WAKISAKA, Yoshihito SHIRAI[†] and Mohd Ali HASSAN[2]

Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology, Kitakyushu 808-0196, Japan

[1]*Department of Biochemical Engineering and Science, Faculty of Computer Science and Systems Engineering, Kyushu Institute of Technology, Fukuoka 820-8502, Japan*

[2]*Department of Biotechnology, Faculty of Food Science and Biotechnology, University Putra Malaysia, 43400 UPM Serdang Selangor, Malaysia*

Effects of single food components such as sodium chloride, sugars and milk protein on freeze concentration by freezing and thawing treatment were evaluated in this study. This method has a different concept from traditional freeze concentration whereby complete freezing is essential prior to thawing. The concentrated fractions were separated upon thawing the frozen sample in a chamber at 10°C. Various thawing rates were obtained when thawing the food components of different concentrations. The concentration of the solute affects the freezing point depression of the aqueous solution. The concentration index was used to evaluate the effectiveness of freezing and thawing on concentrating the food components within the range of 1 to 20%. The 1% of sodium chloride, glucose, fructose, lactose and sucrose solutions were concentrated 4-5 times in the first fraction, except dextrin (less than 2.5 times). As the concentration of the food components increased, the concentration effect reduced as shown by the reducing index approaching unity. However, dextrin and milk protein solutions were not effectively concentrated by freezing and thawing due to their smaller freezing point depression.

Key words: Freezing, Thawing, Concentration, Food Components, Freezing Point Depression

1. Introduction

Freeze concentration involves the partial crystallization of water in the aqueous solution, after which the crystals are separated from the concentrate. It possesses several advantages: (a) low chemical deterioration due to low microbiological and enzymatic activity, (b) no loss of volatile aroma components and (c) low product losses [1, 2]. Freeze concentration offers various commercial applications in food industries such as coffee, fruit juice, vinegar and dairy products. The application is not limited to the liquid food industry, but also extended to wastewater treatment field [3,4,5]. The major disadvantages of this process are high capital costs and limited maximum product concentration.

Freezing and thawing is a well-known treatment method for conditioning and dewatering sludge [6]. Recently, we reported that suspended solids (SS) could be removed from POME by freezing and thawing method with up to 70% efficiency at less than 5 mℓ/min thawing rate [7]. The effect of concentration change in thawed POME solution after freezing and thawing treatment was similar to that observed by Shirai and Yoshikawa [8]. They simulated the quality change in frozen food after freezing and thawing by using vitamin B_{12}. Facey and Smith [9] studied the freezing and thawing fundamentals and mechanisms responsible for concentration of high strength effluents derived from Kraft pulp mill.

To our best knowledge, there is no reported work in the literature on the use of freezing and thawing as a concentration method for food components such as sodium chloride, sugars and protein solutions. This method involves crystallization of aqueous solution, which is quite similar to that of freeze concentration technology. The main difference between freezing and thawing and traditional freeze concentration is that the aqueous solution is completely frozen in the former process and then thawed under controlled condition, in particular at low thawing rate. Thus, the objective of this paper is to evaluate the effects of indi-

(Received 14 April 2003: accepted 3 July 2003)

† corresponding author Tel: 81-93-695-6060, Fax: 81-93-695-6005.
E-mail: shirai@life.kyutech.ac.jp

vidual food components on freeze concentration after being treated by freezing and thawing. The relative importance of the initial concentration of liquid food is described with respect to treatment performance.

2. Materials and Methods

2.1 Materials

The following substances and chemicals used in the work were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Sodium chloride, D-glucose, saccharose (sucrose), levulose (fructose) and lactose monohydrate (lactose) were of analytical grade. Dextrin hydrate (dextrin) was practical grade. Aqueous solutions were prepared by dissolving the chemicals in distilled water: 1%, 5%, 10% and 20% (w/w) sodium chloride, 1%, 5%, 10% and 20% (w/w) glucose, 1%, 5%, 10% and 20% (w/w) fructose, 1%, 5%, 10% and 20% (w/w) sucrose, 1%, 5% and 10% (w/w) lactose and 1%, 5% and 10% (w/w) dextrin. Milk protein was separated from the reconstituted milk solution by gel chromatographic separation method as mentioned below. Whole milk powder was commercially available product. Reconstituted milk solution was prepared by dissolving 20 g of whole milk powder per 100 g of distilled water. The whole milk powder contained in (w/w), 12.3% total protein, 27.8% fat, 54.9% lactose, 2.2% minerals and 2.8% moisture.

2.2 Freezing and thawing method

A sample solution of 200 g was placed in an ice cube tray to produce ice cubes by freezing it at −18°C for more than 12 hours in Sanyo home freezer, HF-6M. The size of each ice cube was about 29 mm × 29 mm × 20 mm. The solutions with high concentration such as 20% (w/w) sodium chloride and 20% (w/w) sugars were frozen at −80°C in a freezer, ULT-1490-9JD (REVCO ultra-low freezer, Asheville, NC, USA) to produce completely frozen ice cube. Frozen sample was thawed on a wire mesh (pore size 6 mm × 6 mm) in an environmental chamber KCL-1000 (EYELA, JAPAN) under controlled temperature at 10°C. Low thawing rate could be achieved at this thawing condition as the concentration index increased with low thawing rate reported in previous study [7]. The melted drips were collected into 10 fractions, about 20 mℓ per fraction. The freezing and thawing procedure is shown in Fig. 1.

2.3 Analysis

Sodium chloride concentration (%) was determined by using an electrical conductivity meter (TOA, CM-21P). The sugar content was measured as % Brix° with ATAGO hand refractometers type N-1_E (0 – 32%) and type N-10_E (0 – 10%). Protein concentration (%) was determined by a protein assay rapid kit (Wako, Osaka, Japan).

2.4 Determination of freezing point

A 1 liter glass vessel containing 300 mℓ of sample solution was cooled by the coolant of ethylene glycol solution. The temperature of the ethylene glycol solution in the temperature bath was maintained at −15°C. The sample solution was constantly agitated with a stirrer (impeller diameter of 8.0 cm) at 200 rpm. Figure 2 shows a schematic diagram of the experimental apparatus used. The solution

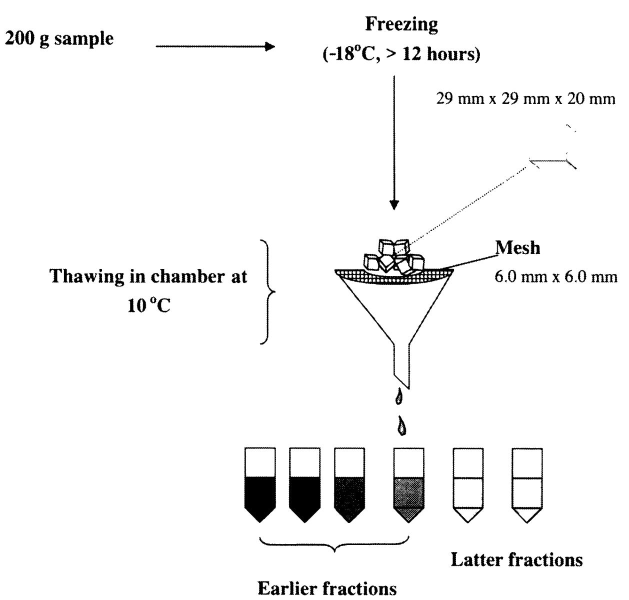

Fig. 1 Experimental procedure for freezing and thawing of individual food components.

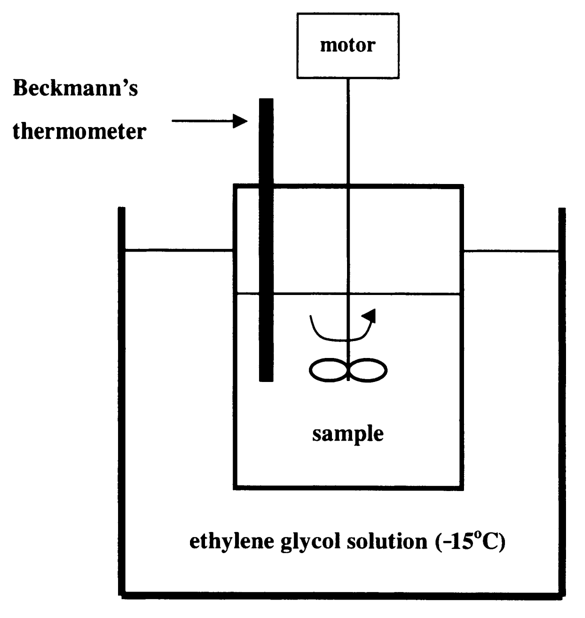

Fig. 2 Experimental apparatus for freezing point measurement.

Table 1 Freezing point depression and thawing rate of sodium chloride, sugars and milk protein at various concentrations.

Solution	Freezing point °C	Thawing Rate ml/min
Salts		
1 % NaCl	− 0.64	1.94
5 % NaCl	− 3.17	2.88
10 % NaCl	− 7.20	3.62
20 % NaCl	−	4.06
Sugars		
a) monomer		
1 % Glucose	− 0.13	1.50
5 % Glucose	− 0.63	1.75
10 % Glucose	− 1.28	2.05
20 % Glucose	− 2.79	2.40
1 % Fructose	− 0.12	1.69
5 % Fructose	− 0.57	1.88
10 % Fructose	− 1.26	2.15
20 % Fructose	− 2.96	3.63
b) dimer		
1 % Sucrose	− 0.07	1.63
5 % Sucrose	− 0.31	1.78
10 % Sucrose	− 0.66	2.20
20 % Sucrose	− 1.63	2.94
1 % Lactose	− 0.06	1.86
5 % Lactose	− 0.34	1.95
10 % Lactose	− 0.67	2.15
c) oligomer		
1 % dextrin	−	0.98
5 % dextrin	−	1.26
10 % dextrin	−	1.80
Protein		
0.03 % milk protein	−	1.30
0.2 % milk protein	−	1.10

temperatures were measured by a Beckmann's thermometer. Temperatures could be measured to within 0.01°C. The thermometer was initially calibrated using distilled water at 0°C. The temperature plateau was recorded from which the freezing point temperature of the sample was deduced. The measured freezing point of the aqueous solutions is shown in Table 1.

2. 5 Determination of thawing rate and concentration index

An example of time course for the cumulative volume of melted solution and concentration for each collected fraction is shown in Fig. 3. All the samples were completely frozen at −18°C or −80°C prior to thawing. The thawing rate was determined by the slope (ΔY/ΔX) of the plot for sample cumulative volume versus time, as shown in Fig. 3. The thawing rate of the food component is shown in Table 1. The concentration index is given as:

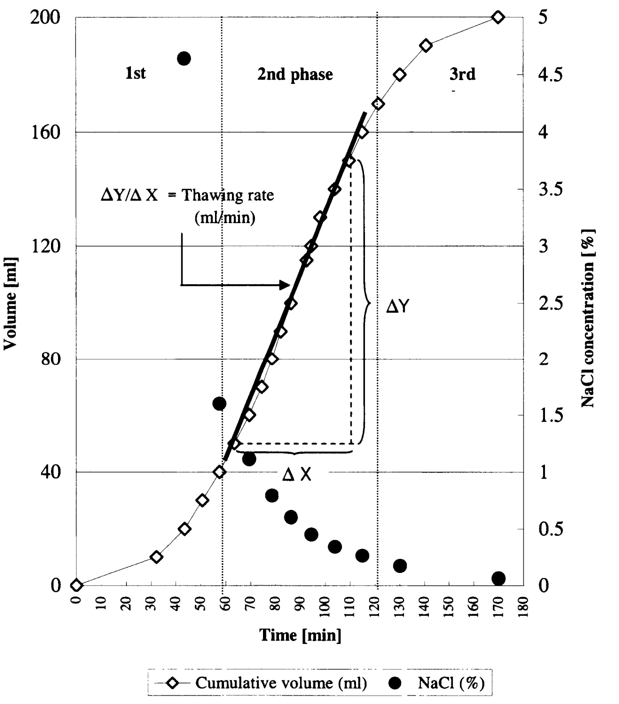

Fig. 3 Time course for the concentration profile and cumulative volume of melted sample solution with 1% NaCl solution during the thawing process.
1st phase = Initial thawing phase, 2nd phase = Constant thawing phase and 3rd phase = Final thawing phase.

$$\text{Concentration Index} = \frac{\text{Concentration of each fraction}}{\text{Concentration of original solution}} \quad (1)$$

2. 6 Desalting of milk protein by gel chromatography

The desalting of milk protein was carried out by the same method as reported in the previous paper [10]. The column used was a glass chromatography column (type ILC-A11-750, KIRIYAMA, Japan) of 1.1 cm in diameter and 75.0 cm in length. Cellulofine GCL-2000c was purchased from SEIKAGAKU Co. (Japan). A 5 mℓ sample was loaded into the column and eluant (distilled water) was fed at a flow rate of 1 mℓ/min. The void fraction was determined as mentioned previously [10] and it was approximately 0.4. The concentration profiles of the elution curves were determined in order to identify the milk protein fractions.

3. Results

3. 1 Concentration of sodium chloride solution

The effect of different concentrations of sodium chloride on freeze concentration is shown in Fig. 4. The concentration index decreased with increasing concentration of sodium chloride. It was found that more than 4.5 times con-

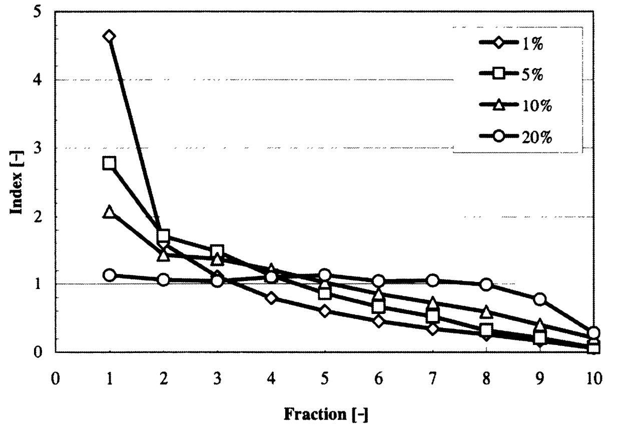

Fig. 4 Concentration profile of NaCl after freezing and thawing.

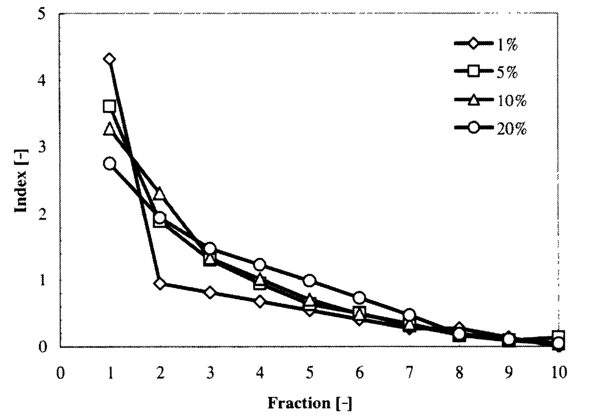

Fig. 5 Concentration profile of glucose after freezing and thawing.

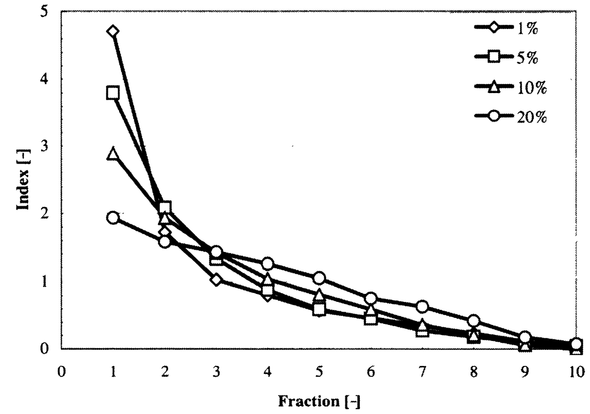

Fig. 6 Concentration profile of fructose after freezing and thawing.

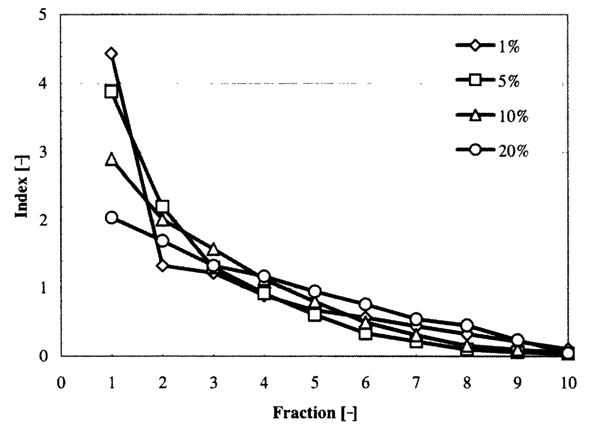

Fig. 7 Concentration profile of sucrose after freezing and thawing.

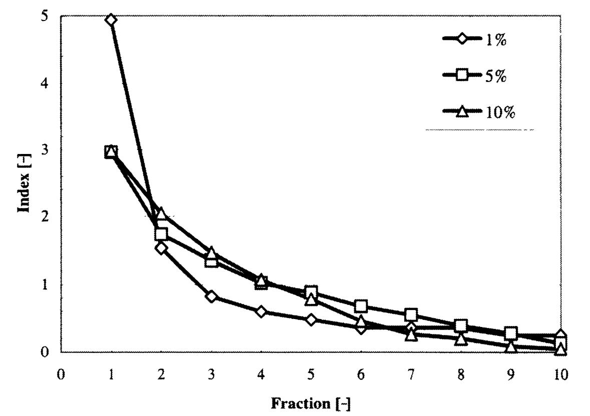

Fig. 8 Concentration profile of lactose after freezing and thawing.

centrated sodium chloride solution could be obtained in the first fraction of 1% solution. A 20% solution was not concentrated as shown by the concentration index approaching unity from fraction no. 1 to no. 8. Similar experiments were carried out at freezing temperature of −80°C and thawing temperature of 17−18°C (room temperature), respectively. It was found that the concentration index was not affected by freezing the sample solution at −18°C or −80°C (data not shown). Thawing the sample at 10°C or room temperature showed similar result to that reported in our previous study [7].

3.2 Concentration of sugars solution

The concentration effects of 1%, 5%, 10% and 20% glucose, fructose, sucrose, lactose and dextrin solutions were evaluated. These sugars could be divided into 3 groups based on their molecular weight or size, i.e. glucose and fructose (M.W = 180.16 g as monomer), sucrose and lactose (M.W = 342.3 g and 360.13 g as dimers respectively), and finally dextrin as oligomer.

The concentration profiles for glucose and fructose are shown in Figs. 5 and 6, respectively. The concentration index decreased with increasing order of sample's concentration. The 1% glucose and fructose solutions were concentrated 4.4 times and 4.7 times, respectively, in the first fraction. Most solutes or soluble substances were concentrated in fraction no. 1 and 2, which is most significant for 1% solution. More than 2 times concentrate could be obtained by freezing and thawing of 20% solution. The melted solutions of low concentration index (less than 1) were obtained in all fractions after fraction no. 3, except 20% solution. For the 1% glucose and fructose solutions, low level of sugar content (about 1%) remained in fraction no. 9 and 10.

Similar concentration effect was observed in sucrose and lactose solutions. Increasing the concentration of the solution

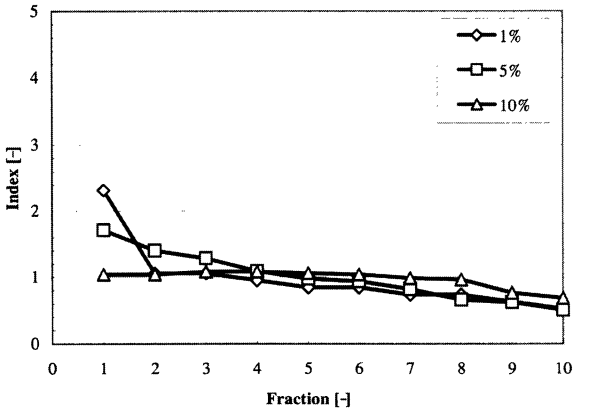

Fig. 9 Concentration profile of dextrin after freezing and thawing.

resulted in lower concentration index, as can be seen in Figs. 7 and 8, respectively. The 1% lactose solution achieved higher concentration index as 5 in the first faction, as compared to the 1% sucrose with concentration index of 4.4. However, the index reduced to 3 for 5% lactose solution, which was lower than that of 5% sucrose solution (concentration index = 3.9).

Dextrin was not concentrated effectively as compared to glucose, fructose, sucrose and lactose by freezing and thawing, as shown by low concentration index in Fig. 9. There was almost no concentration for dextrin solution, except the first fraction of 1% dextrin, which was concentrated by 2.3 times. More than 50% of the dextrin remained in the last fraction, which means low concentration effect on dextrin solution by freezing and thawing.

3.3 Concentration of milk protein

Low concentration index was observed in milk protein by freezing and thawing. Protein concentration increased from first fraction to last fraction, which showed different result as compared to other aqueous solutions. The solution of 0.03 % milk protein was concentrated less than 2 times for the final fraction as shown in Fig. 10.

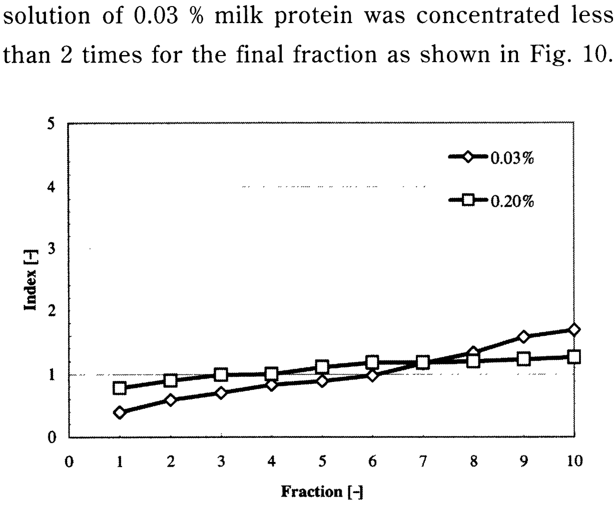

Fig. 10 Concentration profile of milk protein after freezing and thawing.

Furthermore, no concentration was observed for the solution of 0.2 % milk protein.

4. Discussion

The thawing rate increased with increasing concentration of food components, irrespective of the type of food components. Sodium chloride solutions showed higher thawing rate than sugars and protein solutions (Table 1). Sodium chloride solution has greater impact on freezing point as compared to non-electrolytes such as sugars at the same concentration (% in weight), due to its ionic characteristic in water (ion concentration of 1 molar sodium chloride solution = 2 moles). Dextrin and milk proteins are larger particles that have little influence on freezing point. In addition to that, milk protein is slightly soluble in water at freezing temperatures, thus having minimal effect on freezing point. Therefore, the freezing points of dextrin and milk protein solutions are assumed to be near 0°C.

In this study, most of the concentration occurred within the first to third fractions of the tested food components, except 10% (w/w) dextrin and milk protein solutions. As reported by Shirai and Yoshikawa [8], the concentration of vitamin B_{12} decreased from earlier melted fractions towards latter melted fractions after addition of sodium chloride. The concentrated portion was believed to thaw first followed by the ice directly adjacent to it, due to the differences in density of the concentrate and ice, as reported by Facey and Smith [9]. The presence of sodium chloride influenced the mechanism of freezing and thawing by lowering the freezing point of the solution. The freezing point depression increases with increasing sodium chloride and sugars concentration. According to Martel [11], transition from planar to dendritic crystal growth occurs in alum sludge with sodium chloride concentration greater than 100 mg/ℓ (0.01%). Therefore, the growth of dendritic crystal during the freezing of sodium chloride and sugar solutions is expected, as the freezing point becomes lower. These dendritic ice crystals melted upon thawing, to form channels that allow the concentrate to be drained out as earlier fractions. In our assumption, the dendritic crystals grow into more fine and tiny structures as the concentration of the solution increases. This is because more surface area is needed for heat transfer at lower freezing point. These fine and tiny ice crystals do not form channel upon thawing. Conversely, melting of these fine ice crystals has diluted the concentrate. Therefore, low concentration index was obtained at high concentration solution.

In contrast, the concentrated milk protein fractions were obtained at the end of the melting process of the 0.03% milk protein solution (as shown in Fig.10). Milk protein was separated by gel chromatography in order to remove salts and other components which may affect the freezing and thawing mechanism. As mentioned above, milk protein has little affect on freezing point, which means no freezing depression occurs during freezing. Ice crystals grow in planar form, resulting in rejection of milk protein into the center part of the frozen ice cube. Therefore, low concentration milk protein solution drained out as first fraction. This phenomenon was similar to the freezing and thawing of vitamin B_{12} without addition of sodium chloride [8].

5. Conclusions

Sodium chloride and some sugar solutions can be successfully concentrated by freezing and thawing. It has been shown that the initial concentration affected the freeze concentration of the single food component. Most of the concentrated food components were in the fractions no. 1, 2 and 3. The 1% sodium chloride, glucose, lactose, sucrose and fructose solutions achieved concentration index of 4 - 5. Based on the experimental results, it is highly probable that freezing point depression affects the freeze concentration of the aqueous solutions by freezing and thawing. Since dextrin and milk protein solutions have no freezing point depression, they were not effectively concentrated by freezing and thawing. However, it is still difficult to understand the concentration effect in the more complex aqueous solution, such as fruit juices and other beverages. Further study on freezing and thawing of food components have to be extended to more complex food component models such as combination of sodium chloride and sugars or sodium chloride and protein, simulated liquid food models and actual liquid foods, to reveal the mechanism of the freeze concentration with freezing and thawing.

References

1) Niro Process Technology bv; Current large-scale commercial application of freeze concentration in the food industry. Tech. Publ., The Netherlands (1994).
2) Z. Zhang and R. W. Hartel; A multilayer for freeze concentration of liquid milk, J. Food Eng., **29**, 23–38 (1996).
3) J. Rosen; Freeze concentration beats the heat. Mechanical Engineering, December, 46–50 (1990).
4) Y. Shirai, M. Wakisaka, O. Miyawaki and S. Sakashita; Conditions of producing an ice layer with high purity for freeze wastewater treatment. J. Food Eng., **38**, 297–308 (1998).
5) Y. Shirai, M. Wakisaka, O. Miyawaki and S. Sakashita; Effect of seed ice on formation of tube ice with high purity for a freeze wastewater treatment system with a bubble-flow circulator. Wat. Res., **33**, 1325–1329 (1999).
6) R.C. Baskerville; Freezing and thawing as a technique for improving the dewaterability of aqueous suspensions. Filtration & Separation, March/April, 141–144 (1971).
7) L.Y. Phang, M. Wakisaka, Y. Shirai and M.A. Hassan; Freezing and thawing technique for the removal of suspended solids and concentration of palm oil mill effluent (POME). J. Chem. Eng. Japan, **35**, 1017–1019 (2002).
8) Y. Shirai and T. Yoshikawa; Change in components during freezing and thawing for food storage. Nippon Shokuhin Kagaku Kogaku Kaishi, **46**, 447–453 (1999).
9) R.M. Facey, and D.W. Smith; Freeze-thaw treatment of membrane concentrates derived from Kraft pulp mill operations. J. Cold Regions Eng., **15**, 69–90 (2001).
11) K. Nakanishi, S. Yamamoto, R. Matsuno and T. Kamikubo; Operational conditions for desalting by gel chromatography, Agric. Biol. Chem., **43**, 2507–2513 (1979).
11) C.J. Martel; Influence of dissolved solids on the mechanism of freeze-thaw conditioning. Wat. Res., **34**, 657–662 (2000).

「日本食品工学会誌」, Vol. 4, No. 3, pp. 77 - 83, Sep. 2003

◇◇◇ **和文要旨** ◇◇◇

凍結融解法による凍結濃縮に及ぼす食品成分の影響

パンライイー，脇坂　港，白井義人，モハメッドアリハッサン

凍結融解法による凍結濃縮に及ぼす食品成分（NaClや糖類，ミルクタンパク）の影響について本研究では検討した．凍結融解法による凍結濃縮は，溶液をいったん完全に凍結させたのちに融解させるため，溶液を部分的に凍結させて濃縮を達成する従来の凍結濃縮と概念を異にする．凍結させたサンプルを10 ℃のチェンバーで融解させることによって濃縮液画分が得られた．濃度の異なる各食品成分サンプルを融解したときの融解速度はまちまちであった．溶質濃度は，溶液の凝固点降下に影響する．成分濃度1～20 %の各食品成分の凍結融解法による濃縮を評価する指標を導入した．1 %のNaCl，グルコース，フルクトース，ラクトース，スクロース溶液は，最初のフラクションで4～5倍に濃縮された．ただし，デキストランの場合は2.5倍であった．各成分濃度が上昇するに伴い，濃縮効果は低減した．しかし，デキストランとミルクタンパクの場合にはその凝固点降下が小さいことから，高い濃縮効果は得られなかった．