Effect of Storage of Skim Milk Powder, Nonfat Dry Milk and Milk Protein Concentrate on Functional Properties

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Abstract: The physicochemical changes during the storage of high protein powders, such as skim milk powder (SMP), nonfat dry milk (NDM), and milk protein concentrates (MPC), can result in a variation in the functional properties of the powders. The objective of this study was to evaluate the effects of the storage of various milk powders (SMP, NDM, MPC40, and MPC70) on their functional properties. Three different lots of the powders were collected from US manufacturers and were analyzed for functional properties after 3, 9, and 15 months of storage at 25 °C. Additionally, this study also evaluated the effects of seasonal variation on the functionality of SMP and NDM. Functional properties, such as solubility, emulsification ability index (EAI), foaming, and surface hydrophobicity index (SHI), were evaluated at each storage time point. The solubility of MPC70 and the foam overrun of SMP, MPC40, and MPC70 decreased significantly (p < 0.05) with an increase in the storage time. The emulsification properties of MPC70 were significantly higher than other powders. Except for foam drainage, there was no effect of the season on the SMP and NDM functional properties. The storage of milk powders has an impact on some functional properties, and a proper selection of powders based on end-use is recommended.

Keywords: skim milk powder (SMP); nonfat dry milk (NDM); milk protein concentrate (MPC); storage; functionality

1. Introduction

Milk proteins are valuable and provide various functionality to the products in which they are used. High protein powders, such as skim milk powder (SMP), nonfat dry milk (NDM), and milk protein concentrate (MPC), provide varying levels of protein content. The protein contents of SMP and NDM vary between 32% and 37%, whereas MPC may provide protein content between 35% and 85%. When SMP, NDM, or MPC is used for formulation, the ratio of casein and whey proteins is similar (80% casein to 20% whey protein) to the skim milk from which it is manufactured. However, other components of skim milk, such as lactose and minerals, will change, especially in MPC, depending upon the diafiltration water used [1]. Diafiltration is a process of adding water during MPC manufacture to remove soluble constituents, such as lactose and minerals, and to increase the protein content of the finished product. All these changes affect the functionality, hence the usage.

NDM and SMP are manufactured by removing water from the skim milk with the combination of concentrating and drying (generally spray drying). Even though SMP and NDM have less than 5% moisture and less than 1.5% by weight and are similar in composition, such as fat, lactose, and minerals, there is a significant difference in how the protein content is defined. The SMP defined by CODEX Alimentarius has a minimum 34% milk protein requirement, which allows for protein standardization by adding other milk
protein sources, such as MPC. The U.S. Food and Drug Administration defines NDM and has no requirement for percent protein standardization hence supplementation with other milk ingredients is not done.

Milk protein powders produced by the membrane processes can be an alternative for fortification in dairy products. The application of a membrane process, such as the ultrafiltration (UF) of milk, can increase protein:TS and maintain the same casein to serum protein ratio as compared to unconcentrated milk [1]. During UF, the milk is filtered through a membrane (primarily 10 kDa), where the high molecular weight components, such as proteins and fat, are retained in the retentate, and low molecular weight components, such as lactose and minerals, pass through the membrane as permeate [1–4]. The MPC is classified based on the protein content; therefore, products may contain various amounts of protein. Various grades of MPC can be produced with the use of diafiltration to remove soluble constituents and increase milk protein content [1]. The protein content of an MPC powder is usually indicated by the figure following the designation MPC, e.g., MPC40 and MPC70 will have protein contents of 40% and 70%, respectively.

The effects of fortification of MPC on the textural properties of dairy products have been studied extensively [4–8]. While the fortification of milk with MPC improves product functionalities, alternate methods of fortification include either a complete or a partial replacement of SMP or NDM. Because of the increase in the protein amount of the MPC, the replacement of both SMP and NDM with MPC will result in an increase in the percent protein in product formulations. As a result of this increase, MPC fortified products can bind more water, thus contributing to the viscosity of the resultant product.

In summary, MPC fortification can provide similar or improved textural properties as compared to SMP or NDM fortification. Additionally, MPC fortification can also improve sensory attributes as compared to SMP or NDM fortified dairy products [9,10]. As a result of the improved functional properties, MPC can be considered a suitable alternative to other dried ingredients for fortification. However, some research notes that the solubility of MPC is affected negatively due to various factors, such as the quality of the raw material, the degree of protein concentration, and the conditions of the final product’s storage [11–14]. Typically, the food industry uses MPC for its gelling, foaming or emulsifying characteristics [3,11,12,15]. The MPC used as a food ingredient can either be freshly made or stored at room temperature until needed. However, storing MPC leads to several physicochemical and biochemical changes (lactose crystallization, Maillard reaction, and oxidation) that can adversely affect its functional properties.

The solubility of MPC is a critical functional property, as the powder does not fully express its functional properties if it remains insoluble [16]. In other words, reduced solubility may prevent MPC from reaching its full market potential. The use of MPC as an ingredient in a product formulation requires its complete dissolution in water typically at room temperature with moderate agitation. However, stored MPC can exhibit poor solubility [3,11–13,17–19]. At a given temperature, the solubility of MPC decreases with an increase in storage time [11]. In another study, it was reported that a sample of MPC85 when stored for 24 months at 20 had 32% solubility as compared to 53% solubility when stored for 2 days at 20 °C [3]. The loss of solubility during storage of MPC has been attributed to the formation of a skin on the surface of the particle [11], or, in other words, the conformational modification of the protein molecules [17]. Several authors also reported insoluble material consisting of large particles (~100 µm) that were composed of casein micelles fused together via hydrophobic interactions [3,13]. Similarly, the atomic force microscopy of both fresh (stored at 4 °C) and aged (stored at 25 °C for 30 days) MPC85 powder indicated a higher concentration of hydrophobic material in aged powders as compared to fresh powder [20].

In brief, the storage of MPC results in the reduced solubility of powders. The reduced solubility of powders may also affect functional properties, such as foaming, emulsification, and hydrophobicity. Similarly, this reduction in the solubility of stored MPC powder may also affect the characteristics of the product in which it is used. However, limited information is available regarding the effects of such reduced solubilities of MPC in terms
of either the functional properties or the textural properties of a product in which it is used. The objective of this study was to evaluate the functional properties of SMP, NDM, MPC40, and MPC70 stored for 3, 9, and 15 months. Additionally, the seasonal effect of SMP and NDM on the functional properties was also studied. The functional properties of powders included powder solubility, surface hydrophobicity index (SHI), foam overrun (FO), foam drainage (FD), and emulsification activity index (EAI).

2. Materials and Methods

2.1. Experimental Design

Three replicates of four different milk powders (low heat SMP, NDM, MPC40, and MPC70) manufactured in the summer season (May–September) were procured from US manufacturers. Additionally, three replicates of SMP and NDM manufactured in the winter months (November–February) were procured from commercial US manufacturers. Each replicate of powder was analyzed in duplicate at 3, 9, and 15 months for functional properties, including solubility, EAI, SHI, FO, and FD.

2.2. Storage of Powders

Each replicate of milk powder (SMP, NDM, MPC40, and MPC70) was divided into three portions. The powder samples were sealed and stored in specimen containers Ziplock bags (SC Johnson, City, MI, USA) at 25 °C for 3, 9, and 15 months (from the date of manufacture) for analysis.

2.3. Chemical Analyses of Powders

The moisture content of the powder was determined using a vacuum oven method as described in the American Dairy Products Institute [21]. The total protein content of each powder sample was analyzed by the Kjeldahl block digester method as described by Hooi et al. (2004) [22]. The ash content of powders was obtained from the certificate of analysis provided by the manufacturers.

2.4. Functional Properties of Powders

2.4.1. Solubility

The solubility method described by Havea (2006) was used with some modifications [3]. Each powder replicate was dissolved in distilled water separately to achieve a 5% protein solution. The 5% protein solution (100 mL) was stirred for 30 min at 20 °C using magnetic stirrers on a stirrer plate. The stirrers used had the same configuration and were run at a controlled speed (300 rpm) on the stirrer plates. After stirring, the protein solution was stored for overnight hydration at 4 °C. After overnight hydration, the protein solution was again stirred in identical conditions as mentioned above. The final pH was adjusted to 7.0 ± 0.05 using 0.1 M NaOH. Aliquots of the solution (45 mL) were centrifuged (Jouan Inc., CR4-12, Winchester, VA, USA) at 700 g for 10 min (at 20 °C). Both the samples of 5% protein solution (before centrifugation) and the supernatant (after centrifugation) were taken for TS determination. The TS was determined by oven drying (Fisher Scientific, Waltham, MA, USA) at 105 °C the pre-weighed samples for 24 h. The solubility of powder was calculated as the TS of the supernatant, expressed as a percentage of the TS of the 5% protein solution prior to centrifugation.

2.4.2. Emulsification Ability Index

The turbidimetric method described by Casper et al (1999) was used with some modifications to determine the EAI of each powder replicate [23]. A total of 40 mL of 1% protein solution (w/v) was prepared from SMP, NDM, MPC40, and MPC70, using 0.1 M phosphate buffer (pH 8.0), and hydrated overnight at 4 °C. For analysis, 28 mL of 1% protein solution and 12 mL of vegetable oil (Pure Wesson, ConAgra Foods Inc., Omaha, NE, USA) were emulsified using a hand-held homogenizer (Biohomogenizer M 133/1281-0, Biospec Products Inc., Bartlesville, OK, USA) at a high-power setting of “2”, for
4 min while cooling in an ice-water bath. The emulsion was diluted using 60 mL of 0.1 M phosphate buffer (pH 8.0), followed by second emulsification at a low power setting of “1” for 30 s. Forty microliters of the resultant emulsion was added into 10 mL of a 0.1 M phosphate buffer (pH 8.0). The solution was vortexed for 5–10 s, and the absorbance value was measured at a wavelength of 500 nm using a UV visible spectrophotometer (Cary 50 Bio UV visible spectrophotometer, Lake Forest, CA, USA). Absorbance values were reported as EAI.

2.4.3. Foaming Properties

The FO and FD are good indicators of foaming properties and were measured by the method described by Phillips et al. (1987) with some modifications [24]. To produce foam, 150 mL of 5% (w/v) aqueous protein solution was prepared and hydrated overnight at 4 °C. The pH was adjusted to 7.0 ± 0.05 using 0.1 N NaOH. The solution was poured into the kitchen-mixer bowl (Sunbeam Deluxe Mixmaster Mixer, Sunbeam Appliance Company, Downers Grove, IL). To produce foam, the protein dispersion was whipped at a mixer speed of “3” (blend) for 3 min and continued at “10” (desserts) for a total of 10 min. The foam generated was used to analyze FO and FD.

Foam Overrun

Immediately after foam formation, the foam was gently transferred to tared weighing boats (WB, Fischer Scientific, USA), followed by the removal of excess foam on the top of the WB using a rubber spatula. The weight of WB was measured using distilled water before starting the experiment. The foam overrun (FO) was calculated as % FO (as shown in the equation below).

\[ \% \text{FO} = \left( \frac{\text{weight of water in WB} - \text{weight of the foam in WB}}{\text{weight of the foam in WB}} \right) \times 100 \]

Foam Drainage

Foam was scooped gently into plastic funnels. The foam was then allowed to drip into tared WB for 20 min. Weights of the drippings were recorded after 20 min and expressed as FD in grams.

2.4.4. Surface Hydrophobicity Index

The method described by Lee et al. (2006) was utilized to measure SHI with some modifications [25]. A protein solution of 0.03% (w/v) from SMP, NDM, MPC40, and MPC70 was prepared using 0.1 M phosphate buffer (pH 7.0) and hydrated overnight at 4 °C. After bringing the solution to room temperature, subsequent protein solutions of 0.02%, 0.01% and 0.005% (w/w) were prepared by diluting 0.03% protein solution with 0.1 M phosphate buffer (pH 7.0). Thirty microliters of 8.0 mM l-anilino-naphthalene-8-sulfonate (ANS, molecular weight 299.34, Invitrogen Corporation, Carlsbad, CA, USA) fluorescence probe was added to 3 mL of each milk protein concentration. The dye was prepared with restricted exposure to sunlight by dissolving 119 mg of dye powder into 50 mL of HPLC grade methanol (Fisher Scientific, Waltham, MA, USA). Hydrophobic sites on the protein surface exhibited hydrophobic affinity for the ANS fluorescence probe observed at the excitation and emission wavelengths of 390 and 470 nm, respectively. Fluorescence intensity was determined using Aminco Bowman II Luminescence Spectrometer (Thermo Electron Corporation, Madison, WI, USA). The net relative fluorescence intensity (RFI) at each protein concentration was determined by subtracting the fluorescence intensity of the protein solution containing no ANS from the fluorescence intensity of the corresponding protein solution containing ANS. The initial slope of RFI versus protein concentration was calculated as linear regression analysis and designated as the SHI. For each powder replicate, two duplicates were analyzed.
2.5. Statistical Analyses

A 4 × 3 factorial design consisting of four different powder types (SMP, NDM, MPC40, and MPC70) and three different storage times (3, 9, and 15 months) with three replications was used for statistical analysis, and changes in functional properties powders (solubility, EAI, FO, FD, and SHI) were analyzed using a split-plot design. The PROC mixed procedure of SAS, which involved four factors (powder type, replicate, season, and storage time) as class variables, was used for the data analysis [26].

3. Results and Discussion

3.1. Chemical Analyses of Powders

The chemical composition of powders is shown in Table 1. As shown in Table 1, the average protein of SMP, NDM, MPC40, and MPC70 was 32.3, 33.5, 39.8, and 68.6 respectively. Based on the moisture content, the average calculated total solids of SMP, NDM, MPC40, and MPC70 were 96.3, 96.2, 96.1, and 95.4, respectively. The %Ash of SMP, NDM, MPC40, and MPC70 were 8.03, 7.83, 7.77, and 7.14 respectively as per the specification provided by the powder manufacturers.

Table 1. Average (n = 3) protein, total solids and ash of SMP 1, NDM 2, MPC40 3 and MPC70 4.

<table>
<thead>
<tr>
<th>Powder Types</th>
<th>Protein, %</th>
<th>Total Solids, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMP</td>
<td>32.3 ± 0.21</td>
<td>96.3 ± 0.03</td>
</tr>
<tr>
<td>NDM</td>
<td>33.5 ± 0.25</td>
<td>96.2 ± 0.25</td>
</tr>
<tr>
<td>MPC40</td>
<td>39.8 ± 0.03</td>
<td>96.1 ± 0.05</td>
</tr>
<tr>
<td>MPC70</td>
<td>68.6 ± 0.62</td>
<td>95.4 ± 0.61</td>
</tr>
</tbody>
</table>

1 SMP = skimmed milk powder; 2 NDM = non-fat milk powder; 3 MPC40 = milk protein concentrate powder with 40% protein; 4 MPC70 = milk protein concentrate powder with 70% protein.

3.2. Functional Properties of Powders

3.2.1. Solubility

The MS and probabilities (in parentheses) of powder solubility are shown in Table 2. The solubility of the powders was significantly (p < 0.05) affected by the powder type, storage time, and the interaction effect of storage time × powder type. The average solubility results of SMP, NDM, MPC40, and MPC70 at 3, 9, and 15 months of storage are shown in Table 3. The mean solubility of SMP, NDM, and MPC40 was not significantly different (p > 0.05) at 3, 9, and 15 months of storage (Table 3). Similar results were reported by McKenna(2000), where the author reported 98% solubility of SMP stored at 20 °C for 6 months [13]. In contrast, the mean solubility of MPC70 was significantly different (p < 0.05) at each time point, and the solubility of MPC70 decreased from 76% at 3 months to 70% and 60% at 9 and 15 months of storage, respectively. These results were in accordance with previous studies where the authors reported a decrease in solubility of MPC upon storage [3,11,12]. A study by McKenna (2000) reported the presence of a large insoluble fraction consisting of fused casein micelles via hydrophobic interactions while studying the insoluble fraction of stored powders using microstructure techniques [13]. The author also suggested an increase in the protein–protein interaction and formation of insoluble aggregates as the protein content and storage time of MPC increases. The loss in solubility of MPC70 in this study can be attributed to these insoluble protein–protein hydrophobic interactions at the surface of powder particles preventing its dispersion in water [3,11,12]

Additionally, Anema (2006) studied the effect of different storage temperatures on the solubility of MPC85 and reported that the degree of insoluble protein–protein interaction increases with the increase in storage temperature during the storage of powders [11]. In summary, as the protein content of MPC increases, their solubility decreases during storage at room temperature.
Table 2. Mean squares and probabilities (in parentheses) of the functional properties (solubility, EAI, FO, FD, SHI) of SMP, NDM, MPC40, and MPC70 stored for 3, 9, and 15 months at room temperature.

<table>
<thead>
<tr>
<th>Factors</th>
<th>df</th>
<th>Solubility</th>
<th>EAI</th>
<th>FO</th>
<th>FD</th>
<th>SHI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powder Type</td>
<td>3</td>
<td>2113 *</td>
<td>0.019 *</td>
<td>33.255 *</td>
<td>4.29 *</td>
<td>13.047 *</td>
</tr>
<tr>
<td>Replicates</td>
<td>2</td>
<td>0.111</td>
<td>0.0002 *</td>
<td>3741 *</td>
<td>0.513 *</td>
<td>4.36 *</td>
</tr>
<tr>
<td>Time</td>
<td>2</td>
<td>48.09 *</td>
<td>0.0009</td>
<td>4682 *</td>
<td>0.511 *</td>
<td>150.69</td>
</tr>
<tr>
<td>Powder Type</td>
<td>6</td>
<td>0.60</td>
<td>0.0005</td>
<td>3323 *</td>
<td>0.187</td>
<td>793 *</td>
</tr>
<tr>
<td>Replicates Time</td>
<td>16</td>
<td>1.4062</td>
<td>0.0002</td>
<td>278</td>
<td>0.085</td>
<td>195</td>
</tr>
</tbody>
</table>

* Statistically significant (p < 0.05); 1 EAI = emulsification ability index; 2 FO = foam overrun; 3 FD = foam drainage; 4 SHI = surface hydrophobicity index; 5 SMP = skimmed milk powder; 6 NDM = non-fat milk powder; 7 MPC40 = milk protein concentrate powder with 40% protein; 8 MPC70 = milk protein concentrate powder with 70% protein.

Table 3. Mean (n = 3) functional properties (solubility, EAI, FO, FD, SHI) of SMP, NDM, MPC40, and MPC70 at 3, 9, and 15 months of storage.

<table>
<thead>
<tr>
<th>Storage Time</th>
<th>SMP</th>
<th>NDM</th>
<th>MPC40</th>
<th>MPC70</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 months</td>
<td>99.83 ± 0.75 aA</td>
<td>99.17 ± 0.75 aA</td>
<td>99.50 ± 0.84 aA</td>
<td>76.00 ± 1.55 bA</td>
</tr>
<tr>
<td>9 months</td>
<td>99.67 ± 1.03 aA</td>
<td>99.50 ± 0.55 aA</td>
<td>99.33 ± 0.82 aA</td>
<td>70.20 ± 1.17 bB</td>
</tr>
<tr>
<td>15 months</td>
<td>99.67 ± 0.52 aA</td>
<td>99.67 ± 0.52 aA</td>
<td>99.00 ± 0.89 aA</td>
<td>60.30 ± 2.66 bC</td>
</tr>
<tr>
<td>EAI</td>
<td>0.3867 ± 0.032 b</td>
<td>0.3862 ± 0.011 b</td>
<td>0.3936 ± 0.022 b</td>
<td>0.4696 ± 0.031 a</td>
</tr>
<tr>
<td>3 months</td>
<td>0.3906 ± 0.011 b</td>
<td>0.4015 ± 0.028 b</td>
<td>0.4039 ± 0.010 b</td>
<td>0.5068 ± 0.029 a</td>
</tr>
<tr>
<td>9 months</td>
<td>0.3726 ± 0.037 c</td>
<td>0.3948 ± 0.016 b</td>
<td>0.4042 ± 0.004 b</td>
<td>0.4741 ± 0.025 a</td>
</tr>
<tr>
<td>15 months</td>
<td>500.0 ± 9.9 cA</td>
<td>583.0 ± 32.9 bA</td>
<td>625.8 ± 18.2 aA</td>
<td>624.0 ± 50.1 aA</td>
</tr>
<tr>
<td>3 months</td>
<td>461.7 ± 11.9 bB</td>
<td>583.5 ± 32.0 aA</td>
<td>611.7 ± 8.6 aAB</td>
<td>606.8 ± 75.9 aAB</td>
</tr>
<tr>
<td>9 months</td>
<td>464.2 ± 17.9 cB</td>
<td>557.2 ± 18.6 bA</td>
<td>588.2 ± 16.0 ab</td>
<td>565.7 ± 40.6 ac</td>
</tr>
<tr>
<td>15 months</td>
<td>2.53 ± 0.36 aA</td>
<td>1.77 ± 0.33 bA</td>
<td>1.68 ± 0.26 bA</td>
<td>1.37 ± 0.33 bB</td>
</tr>
<tr>
<td>3 months</td>
<td>3.32 ± 0.23 abA</td>
<td>1.78 ± 0.47 bA</td>
<td>1.88 ± 0.44 bA</td>
<td>0.97 ± 0.27 cA</td>
</tr>
<tr>
<td>9 months</td>
<td>3.07 ± 0.63 abB</td>
<td>2.25 ± 0.45 bA</td>
<td>2.05 ± 0.25 bCA</td>
<td>1.62 ± 0.50 bB</td>
</tr>
<tr>
<td>15 months</td>
<td>353.0 ± 15.3 b</td>
<td>408.5 ± 14.5 a</td>
<td>351.3 ± 16.5 b</td>
<td>325.3 ± 34.5 c</td>
</tr>
<tr>
<td>3 months</td>
<td>344.7 ± 19.0 b</td>
<td>412.0 ± 20.9 a</td>
<td>343.5 ± 31.8 b</td>
<td>316.2 ± 5.6 c</td>
</tr>
<tr>
<td>9 months</td>
<td>342.8 ± 22.2 b</td>
<td>409.3 ± 22.3 a</td>
<td>336.7 ± 19.6 b</td>
<td>322.3 ± 15.4 b</td>
</tr>
<tr>
<td>15 months</td>
<td>356.7 ± 22.0 b</td>
<td>412.0 ± 20.9 a</td>
<td>343.5 ± 31.8 b</td>
<td>325.3 ± 34.5 c</td>
</tr>
</tbody>
</table>

* Means within the same row not sharing common subscripts are significantly different (p < 0.05); 1 EAI = emulsification ability index; 2 FO = foam overrun; 3 FD = foam drainage; 4 SHI = surface hydrophobicity index; 5 SMP = skimmed milk powder; 6 NDM = non-fat milk powder; 7 MPC40 = milk protein concentrate powder with 40% protein; 8 MPC70 = milk protein concentrate powder with 70% protein.

3.2.2. Emulsifying Ability Index

The emulsifying activity of milk protein is very important during the manufacturing of several food products, such as coffee, soups, salad dressing, meat products, sausages, etc. Protein molecules can form emulsions, where the molecules diffuse at the oil–water interface and form a cohesive continuous film around the oil droplets [27]. The EAI is an indicator to evaluate emulsifying ability of the proteins, which can be defined as the area of the oil–water interface stabilized per unit weight of protein. The ability of protein molecules...
to form emulsion depends on their ability to diffuse to the interface, adsorb, unfold, and interact with oil at the oil–water interface [28]. An increase in the EAI indicates an increase in the ability of protein molecules to reduce the surface tension at the oil–water interface, thereby improving the emulsifying ability of the protein solution. The MS and probabilities of powder EAI are shown in Table 2. The EAI of the powders was significantly affected ($p < 0.05$) by the type of powder and replicates. The storage time of the powders did not have a significant effect ($p \geq 0.05$) on the EAI of the powders. Although, there were some significant ($p < 0.05$) differences between the mean EAI values of SMP, NDM, and MPC40, the values were in close proximity, ranging from 0.3726 to 0.3906, 0.3862 to 0.4015, and 0.3936 to 0.4042, respectively (Table 3). In contrast, the mean EAI of MPC70 (range: 0.4696 to 0.5068) was significantly higher ($p < 0.05$) as compared to SMP, NDM, and MPC40 at each time point (Table 3).

The increased EAI of MPC70 as compared to SMP, NDM, and MPC40 can possibly be explained by the increased adsorption of non-micellar caseins at the oil–water interface. The application of UF during MPC manufacture involves the removal of lactose, non-protein nitrogen compounds, and soluble salts, such as calcium and potassium, to pass through the membrane [14]. As a result of the loss of soluble calcium from UF retentate, the colloidal calcium phosphate (CCP) from the casein micelle becomes solubilized to equilibrate reduced calcium in the retentate [29–31]. Consequently, the loss of CCP from the casein micelle structure results in a partial disintegration of the casein micelles and the release of non-micellar caseins into serum [14,30]. Both the application and the extent of DF during MPC manufacture further aggravate the loss of CCP and increase the possibility of the release of non-micellar caseins in serum. As a result, the presence of increased non-micellar caseins in MPC70 would increase the number of protein molecules that are available for adsorption at the oil–water interface during emulsion formation as compared to SMP, NDM, and MPC40. In addition, it can be assumed that the release of non-micellar caseins would be considerably less during MPC40 manufacture and therefore the EAI of MPC40 is in close proximity to SMP and NDM.

In summary, the storage of powders did not have a significant impact on the EAI of powders in this study. Additionally, MPC may provide similar or improved EAI as compared to SMP and NDM, depending on the application of UF and DF during MPC manufacture. Nevertheless, it is important to note that the evaluation of emulsifying properties is not a standardized procedure and results may vary from one experiment to another. The emulsifying properties of protein solution depend on several other parameters, such as the type of protein (such as casein, whey protein, and caseinates), the calcium concentration of the powder, the solubility of the powder, the concentration of the protein solution for emulsion, the pH of the solution, and the conditions of emulsion (time, temperature, volume, and homogenization) [32,33]. Thus, the result of the EAI of powders may differ from one study to another and therefore cannot be compared.

3.2.3. Foam Overrun and Foam Drainage

Foam Overrun

Form formation occurs because of the unfolding of the hydrophobic regions of proteins and their interaction with air. The whipping process during foam formation initiates the unfolding of protein molecules and the arrangement of proteins around air bubbles to decrease the surface tension between the air and water interface [34]. Table 2 depicts MS and the probabilities (in parentheses) of the mean foam overrun of the powders at each time point. The FO of the powders was significantly affected ($p < 0.05$) by the powder type, replicates, storage time and the interaction effect of the powder type × replicates. The results of the mean FO of SMP, NDM, MPC40, and MPC70 are shown in Table 3. Although the storage time had a significant effect ($p < 0.05$) on the FO values, the values were in close proximity, ranging from 465% to 500%, 557% to 583%, 588% to 626%, and 566% to 624% for SMP, NDM, MPC40, and MPC70, respectively. Although the mean FO of MPC40 and MPC70 at 3 and 15 months of storage was significantly higher ($p < 0.05$) as compared to
NDM, the values were not significant \((p > 0.05)\) at 9 months of storage. The relatively lower mean FO of SMP at 9 months in the statistical model may have resulted in no significant difference in FO of MPC40 and MPC70 as compared to NDM. Additionally, the FO value of SMP (500\%) was significantly lower \((p < 0.05)\) as compared to NDM, MPC40, and MPC70 at 3 months of storage. Similar results were recorded for SMP FO values at 9 and 15 months of storage. These results were in agreement with previous study [35], where the authors reported an increase in FO of MPC foams as compared to SMP foams. Although the author did not discuss the possible reasons for the increased FO of MPC foams, it can be argued that the presence of non-micellar caseins and their rapid adsorption on the air surfaces may have resulted in increasing the overrun of the MPC foams. The non-micellar caseins are released into serum as the casein micelle structure disintegrates because of the loss of CCP [14,30], and the DF (application and the extent) may aggravate the loss of CCP and increase the possibility of the release of non-micellar caseins in serums.

**Foam Drainage**

The stability of foam depends on the strength of the interfacial layer between air and water, which is formed by protein molecules [34]. With an increase in time after foam formation, the water between air cells (foam) drains, and the air cells approach each other. An increase in the quantity of water drained indicates a decrease in the FD of the protein solution. The foam stability results in this study indicate the amount of foam drained \((g)\) in 10 min immediately after foam formation. The MS and probabilities (in parentheses) of FD are shown in Table 2. The powder type, replicates, storage time and the interaction effects of the powder type \(\times\) replicates, storage time \(\times\) powder type, and storage time \(\times\) powder type \(\times\) replicates have a significant effect \((p < 0.05)\) on the FD values. Although, the storage time had a significant effect \((p < 0.05)\) on the foam drainage values, a clear pattern could not be observed between the FD values of SMP, NDM, MPC40, and MPC70, and thus the effect of storage time on FD could not be established (Table 3).

The foam drainage values of SMP were significantly lower \((p < 0.05)\) as compared to NDM, MPC40, and MPC70 at each time point. Although some of the mean FD of NDM and MPC40 powders were significantly different \((p < 0.05)\), the mean FD were in close proximity, ranging from 1.77 g to 2.25 g and 1.68 g to 2.05 g for NDM and MPC40, respectively. Although the mean FD of MPC70 at 9 months of storage was significantly higher \((p < 0.05)\) as compared to SMP, NDM, and MPC40, the values were not significant \((p > 0.05)\) at 3 and 15 months of storage. The relatively lower mean FD values of SMP at 3 and 15 months in the statistical model may have resulted in a similar FD of MPC70 as compared to NDM and MPC40.

The decreased FD values of MPC70 can possibly be explained by the increased adsorption of non-micellar caseins and their increased ability to hold air cells in MPC70 as compared to SMP, NDM, and MPC40. Additionally, the presence of additional amounts of foam depressants, such as calcium and lactose in SMP, NDM, and MPC40 foams may have resulted in the additional drainage of foam as compared to MPC70 foams [35].

In summary, MPC may provide similar or improved foaming properties as compared to SMP and NDM. However, it is important to note that the evaluation of foaming properties is not a standardized procedure, and results may vary from one experiment to another. The FO and FD values of a protein solution depend on several experimental parameters, such as the type of protein, the protein concentration of the solution, the pH of the solution, the whipping conditions (time, temperature, volume, bowl configuration, blade arrangement, and speed of whipping), sampling time, and time for FD measurement. Apart from the experimental parameters, processing conditions during the manufacture of powders, such as the composition of the product, heat treatment, and pH during the manufacturing process, also affect the foam formation and FD values of a protein solution.
3.2.4. Surface Hydrophobicity Index

Hydrophobic interaction in food proteins has a major contribution to the functional properties of food. The structure of a milk protein molecule is driven by hydrophobic, electrostatic, and steric parameters. In a food system, interactions such as protein–protein or protein–lipid are greatly influenced by the surface hydrophobicity of a protein molecule [36]. Thus, the quantification of the surface hydrophobicity of protein is vital in assessing its contribution toward functionality.

The MS and probabilities (in parentheses) of the SHI are given in Table 2. The SHI of the powders is significantly affected \( (p < 0.05) \) by the type of powder, replicates and the interaction effect of the type of powder and replicates (Table 2). Storage time did not have a significant effect \( (p > 0.05) \) on the SHI of the powders. The mean SHI of the powders at each time point is shown in Table 3. As shown in Table 3, the mean SHI of NDM was significantly higher \( (p < 0.05) \) as compared to SMP, MPC40, and MPC70 at each time point. Although there were some significant \( (p < 0.05) \) differences between the SHI of SMP, MPC40, and MPC70, their SHI were in close proximity, ranging from 344.7 to 353, 336.7 to 351.3, and 316.2 to 325.3, respectively.

Although the exact reason for the lower SHI of SMP as compared to NDM was not known, it can be theorized that the presence of additional lactose in SMP as compared to NDM may have interfered with the hydrophobic interactions of proteins. Additionally, limited information is available on the comparison of SHI of MPC to SMP or NDM; it can be theorized that the lower SHI of MPC may have resulted because of the fusion of the casein micelle. Several researchers have reported the presence of either tightly packed protein molecules or the fusion of casein micelles in MPC [3,11,13,20]. The extent of fusion depends on the protein content of the powders, where an increase in the protein content will increase the fusion of casein micelles [13]. Several authors also reported the fusion of casein micelles in MPC, where the reason for fusion is believed to be a combination of both hydrophobic interactions and electrostatic forces between the casein micelles [11,13,20]. Thus, it can be theorized that because of these hydrophobic interactions between casein micelles, the availability of hydrophobic sites for ANS dye-binding may be less as compared to SMP and NDM. Additionally, it was not known if the reduced solubility of MPC70 as compared to SMP or NDM was also contributing to the lower mean value of SHI of MPC70. It is important to note that MPC40 had 99% solubility at each time point as compared to the solubility of MPC70, ranging from 60% to 76%. Additionally, the fusion of casein micelles will be relatively less in MPC40, which may explain its SHI in close proximity with SMP and NDM as compared to MPC70.

3.2.5. Seasonal Comparison of SMP and NDM Functional Properties

The SMP and NDM powders manufactured in summer (May to September) and winter (November to February) were compared for the functional properties at 3, 9, and 15 months of storage time. The mean squares and probabilities (in parentheses) of functional properties of powders manufactured in the summer and winter seasons are shown in Table S1. As shown in Table S1, the powder type and replicates had a significant \( (p < 0.05) \) effect on the FO, FD, and SHI of powders. The storage time had a significant effect \( (p < 0.05) \) on FO, FD, and SHI. The EAI of powders was significantly \( (p < 0.05) \) affected by the powder type. The interaction effect of the product type and replicates had a significant effect \( (p < 0.05) \) on FO and SHI. However, it is interesting to note that the effect of season was not significant \( (p > 0.05) \) on the solubility, EAI, FO, and SHI. The effect of season was significant \( (p < 0.05) \) only for the FD of powders. The higher FD value of 15 months stored NDM-S (2.25 g) as compared to the FD of 15 months stored NDM-W (1.72 g) may have resulted in a significant \( (p < 0.05) \) effect of the season in the statistical model (Table 4). However, the exact reason for the drastic change in FD of the 15 months stored NDM-S was not understood. The FD value of SMP-S, SMP-W, NDM-S, and NDM-W ranged from 2.53 g to 3.07 g, 2.77 g to 2.98 g, 1.77 g to 2.25 g, and 1.47 g to 1.72 g, respectively. Seasonal variation and its impact on the composition of milk and gelation properties have been studied by several
authors [37–40]. The authors have reported a variation in the Ca\(^{2+}\) (ionic calcium) activity, amount of whey proteins, whey protein denaturation, and milk fat amongst others for the variation in functionality. Although these properties have been shown to impact acid gelation properties, they may not impact the functional properties of powders, such as solubility, EAI, FO, FD, and SHI. A study by Augustin et al. (2008) reported increased foaming properties as a result of an increase in the citrate content of the milk [41]. As the citrate contents of the powders were not evaluated in this study, a direct comparison could not be established.

Table 4. Mean (\(n = 3\)) functional properties (solubility, EAI \(^1\), FO \(^2\), FD \(^3\), SHI \(^4\)) of SMP \(^5\), NDM \(^6\), manufactured in summer (May to September) and winter (November to February) seasons at 3, 9, and 15 months of storage.

<table>
<thead>
<tr>
<th>Storage Time</th>
<th>SMP</th>
<th>Winter</th>
<th>SMP</th>
<th>NDM</th>
<th>Winter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Summer</td>
<td>Winter</td>
<td>Summer</td>
<td>Winter</td>
<td></td>
</tr>
<tr>
<td>Solubility, %</td>
<td>100</td>
<td>99</td>
<td>99</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>101</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>9 months</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>15 months</td>
<td>100</td>
<td>99</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EAI</td>
<td>0.3867 (^a)</td>
<td>0.3826 (^a)</td>
<td>0.3862 (^a)</td>
<td>0.4046 (^a)</td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>0.3906 (^{ab})</td>
<td>0.3736 (^b)</td>
<td>0.4015 (^{ab})</td>
<td>0.4045 (^a)</td>
<td></td>
</tr>
<tr>
<td>9 months</td>
<td>0.3726 (^a)</td>
<td>0.3719 (^b)</td>
<td>0.3948 (^a)</td>
<td>0.4002 (^a)</td>
<td></td>
</tr>
<tr>
<td>15 months</td>
<td>0.3702 (^c)</td>
<td>0.3678 (^b)</td>
<td>0.3901 (^a)</td>
<td>0.3946 (^a)</td>
<td></td>
</tr>
<tr>
<td>FO, %</td>
<td>500 (^b)</td>
<td>483 (^a)</td>
<td>583 (^a)</td>
<td>577 (^a)</td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>462 (^b)</td>
<td>483 (^b)</td>
<td>584 (^a)</td>
<td>570 (^a)</td>
<td></td>
</tr>
<tr>
<td>9 months</td>
<td>464 (^b)</td>
<td>476 (^b)</td>
<td>557 (^a)</td>
<td>564 (^a)</td>
<td></td>
</tr>
<tr>
<td>15 months</td>
<td>3.25 (^a)</td>
<td>2.92 (^a)</td>
<td>2.25 (^b)</td>
<td>1.72 (^c)</td>
<td></td>
</tr>
<tr>
<td>FD, g</td>
<td>3.32 (^a)</td>
<td>2.92 (^a)</td>
<td>2.25 (^b)</td>
<td>1.72 (^c)</td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>3.07 (^a)</td>
<td>2.92 (^a)</td>
<td>2.25 (^b)</td>
<td>1.72 (^c)</td>
<td></td>
</tr>
<tr>
<td>9 months</td>
<td>3.35 (^b)</td>
<td>337 (^b)</td>
<td>412 (^a)</td>
<td>402 (^a)</td>
<td></td>
</tr>
<tr>
<td>15 months</td>
<td>343 (^b)</td>
<td>325 (^b)</td>
<td>410 (^a)</td>
<td>403 (^a)</td>
<td></td>
</tr>
</tbody>
</table>

\(^{ab}\) Means within the same row not sharing common subscripts are significantly different (\(p < 0.05\)); \(^1\) EAI = emulsification ability index; \(^2\) FO = foam overrun; \(^3\) FD = foam drainage; \(^4\) SHI = surface hydrophobicity index; \(^5\) SMP = skimmed milk powder; \(^6\) NDM = non-fat milk powder.

In summary, no seasonal variation was found in other functional properties studied (solubility, EAI, FO, and SHI) in SMP and NDM.

4. Conclusions

High protein powders (SMP, NDM, MPC40, and MPC70) functionality is affected by the storage. Storage time had a significant effect (\(p < 0.05\)) on solubility and foaming properties. The solubility of MPC70 and foam overrun of SMP, MPC40, and MPC70 decreased significantly (\(p < 0.05\)) with an increase in the storage time. The emulsification properties of MPC70 were significantly higher (\(p < 0.05\)) than SMP, NDM, and MPC40. Even though the solubility of MPC70 decreased with storage time, the powder may still be preferred for its emulsification properties, which did not change. There was not much difference in the functionality of SMP and NDM powders produced in the summer (May to September) or winter (November to February) seasons. Except for the foam drainage property, no effect of season was observed in SMP and NDM, and there were small but non-significant differences in their functional properties, such as solubility, EAI, and hydrophobicity. This indicates that the manufacturers can purchase these SMP and NDM across any season without worrying about functionality. In conclusion, the storage of milk powders has an impact on some of their functional properties, and the proper selection of powders based on end use is recommended.
Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/dairy3030040/s1, Table S1: Mean squares and probabilities (in parentheses) of the functional properties (solubility, EAI1, F02, FD3, SHI3) of SMP5, NDM6 (Nov’09 to Feb’10) stored for 3, 9, and 15 months at room temperature.

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