Journal of Animal & Plant Sciences, 35(6): 2025, Page: 1630-1642
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2025.6.0137

OPTIMIZING FREEZE DRYING CONDITIONS AND ENHANCING QUALITY OF CHEESE POWDER FLAVOURS THROUGH SURFACE AREA TO VOLUME RATIO MANIPULATION

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ABSTRACT

This study aims to optimize the freeze-drying conditions for cheese powder flavor production and to evaluate the effect of cheese type and surface area-to-volume ratio of cheeses on the quality characteristics of the resulting powder flavors. During the first phase, three types of cheese (Mozzarella, Cheddar, and enzyme-modified Cheese) were prepared into three geometric forms (shreds, slices, and cubes), representing different surface area-to-volume ratios. These samples were frozen at four different temperatures (−20 °C, −30 °C, −40 °C, and −50 °C), and then freeze-dried at four shelf temperatures (20 °C, 23 °C, 26 °C, and 29 °C). Moisture content was recorded at several time intervals ranging from 5 to 26 hours to identify the most efficient freeze-drying condition. In the second phase, a factorial experiment (3 × 3) was conducted to investigate the effects of cheese type (Mozzarella, Cheddar, and enzyme-modified Cheese), and surface area to volume ratio of cheeses (shreds, slices, cubes) on the quality characteristics of resulting cheese powder flavors. The experiment was laid out in a completely randomized design with three replications resulting in a total of 9 treatments. The results showed that a longer freeze-drying time reduced the moisture content due to prolonged sublimation, with enzyme-modified Cheese requiring more time due to its dense structure and enzyme activity. Higher freezing temperatures (-50°C) resulted in faster sublimation compared to lower temperatures (-20°C), as the formation of larger ice-crystals at -50 °C facilitated more efficient water loss. The drying temperature also accelerated moisture loss by increasing the kinetic energy of the water molecules. Physicochemical analyses revealed significant differences in fat, protein, moisture, pH, acidity, and ash content between the different cheeses, with enzyme-modified cheese having higher fat and moisture content but lower protein content. The surface area-to-volume ratio had a significant positive effect ($p \leq 0.05$) on moisture content, bulk and tapped density, flowability, cohesiveness, solubility index, and dispersibility. Shredded cheese powders exhibited lower density and dispersibility, but higher solubility, due to their larger surface area. Enzyme-modified Cheese showed better flowability but lower cohesiveness. Mozzarella cheese showed higher hardness and gumminess due to the formation of protein networks, especially in shredded form.

Key words: Cheese, Physicochemical properties, Surface area to volume ratio, Texture.

Published first online September 22, 2025 Published final November 30, 2025

INTRODUCTION

Cheese powder is produced by removing moisture from cheese, typically through a process involving cheese emulsions and drying techniques (Deshwal *et al.*, 2024). It is a good source of important nutrients, such as vitamins, minerals, protein, carbohydrates and fats (da Silva *et al.*, 2018). According to recent market analyses, the amount of cheese consumed as an ingredient is increasing rapidly (Lodianov *et al.*, 2021). Cheese powder, available in flavors like white Cheddar, Parmesan, and sharp Cheddar, is widely used in the food industry as a flavoring and nutritional supplement (Fox *et al.*, 2017). It enhances flavor in various foods, including soups, pasta, sauces, biscuits, crackers, chips, and baby foods (da Silva *et al.*, 2018; Pisecky, 2005). Dry cheese powder is usually added to or sprinkled over baked potatoes, salads, soups, pasta or macaroni dishes, and cooked vegetables. It adds a delicious and useful combination of flavor and functionality. It can be used in place of salt or regular cheese (Guinee and Kilcawley, 2017)

Globally, drying is the most popular and economical technique for extending the shelf life of edible products (Falih *et al.*, 2024). It inhibits microbial reproduction, lowers enzyme activity, and slows down harmful chemical reactions (Dong *et al*., 2024). There are several benefits to turning fresh dairy products into powder, including easy handling, a long shelf life, cost-effective packaging and transportation, and refrigeration-free storage (Sahin *et al.,* 2018; (Atalar and Yazici, 2018). Furthermore, the demand for ready-to-eat and/or ready-to-cook meals, or convenience dairy products, is steadily increasing (Hannon *et al.,* 2006). The choice of drying method for the production of cheese powder flavor depends on the physicochemical characteristics of the cheese and desired end product, and cost of production. In the dairy industry spray drying, drum drying (Kaviani *et al*., 2022) double drum dryer (Jakkamsetty *et al*., 2024) single drum vacuum dryer, two roller vacuum dryers are most commonly used methods. These methods have potential for thermal degradation of heat-sensitive components and losses in flavor or aroma compounds due to the high process temperatures. Snack industries are looking for cheese powder flavors that have pleasant sensory characteristics and healthy nutritional profile after processing and gain consumers preference at the time of purchase (Chandan, 2011)

Freeze-drying is an innovative technique being used for the drying of dairy products. In this technique, dehydration is done at low temperature that helps to maintain the color and aroma of the product and preserve nutritional profile (Silva-Espinoza *et al*., 2021). In the process of "freeze-drying" water is directly transformed from solid (ice) to vapor, eliminating the liquid phase, and then the water is extracted from the dry layer (Oetjen *et al*., 2004).

Selecting the right temperature and pressure are important parameters for cheese drying (Salum *et al.,* 2023 in order to get preferred physicochemical characteristics. To guarantee the dissolution of ice crystals during the freeze-drying process, the heating temperature must be regulated, and the drying phase must not be overheated and denatured. Elevating the heating temperature can expedite the drying process; however, excessive heating can result in the denaturation of chemical compounds (Fox *et al.,* 2015).

The surface area to volume ratio is an important parameter that affects the cooling behavior of any object as a function of size like shreds, cubes or slices (Planinsic and Vollmer, 2008). A balanced surface area to volume is necessary to achieve efficient and uniform drying, and other factors like desired end product quality characteristics and cheese composition.

Nowadays, health-conscious consumers prefer to use healthy natural flavors instead of synthetic, therefore food producers are widening the range of their products that are necessary for the development of natural cheese flavors that not only give flavor but also give healthy nutrients (Junaid *et al.,* 2013). Utilizing biocatalytic and thermal steps allows for the efficient generation of flavors under mild conditions, aligning with consumer preferences for natural products (Bel-Rhlid *et al*., 2018). This necessity has led to the production of natural cheese powder flavors with minimal loss of chemical compounds, as well as the investigation of the challenges encountered and their possible solutions during production.

Therefore, this research provided valuable insights into the production of cheese powder flavors through freeze-drying. It examined the impact of various freeze-drying process variables and the surface area-to-volume ratio (shreds, slices, and cubes) of different cheese types Mozzarella, Cheddar, and enzyme-modified Cheese (EMC) on the quality characteristics of their respective powder flavors.

MATERIALS AND METHODS

Production of cheeses: Buffalo milk was procured from Dairy Training and Development Center, University of Veterinary and Animal Sciences Ravi Campus, Pattoki. Direct Vat Set (DVS) type starter cultures and rennet were procured from Christian Hansen Company, Denmark through local suppliers.

For the preparation of Mozzarella cheese, milk was pasteurized at 65℃ for 30 minutes. The milk was cooled to 37℃ and inoculated with *Streptococcus thermophilus* FD-DVS STI-12 culture at a pH of 6.6. Following inoculation, coagulant (rennet) was added when the pH reached to 6.5. Once the curd was formed, the subsequent processes of cutting, whey removal, cheddaring, milling, and stretching were carried out (Nazish *et al*., 2022). Cheddar and EMC cheeses were made following the procedures of Amelia *et al*. (2013) and Kendirci *et al.* (2020) respectively. In the first phase, three types of cheese (Mozzarella, Cheddar, and enzyme-modified cheese) were prepared into three geometric forms (shreds, slices, and cubes), representing different surface area-to-volume ratios as per dimensions described in table 1. These samples were frozen at four different temperatures (−20 °C, −30 °C, −40 °C, and −50 °C), and then freeze-dried at four shelf temperatures (20 °C, 23 °C, 26 °C, and 29 °C). Moisture content was recorded at several time intervals ranging from 5 to 26 hours to identify the most efficient freeze-drying condition. In the second phase, a 3 × 3 factorial arrangement of treatments was applied under completely randomized design, with each treatment replicated three times. The total of 27 samples were studied to investigate the impact of cheese type and surface area to volume ratio on the quality characteristics of resulting cheese powder flavors as depicted in Table 1.

Table 1. Detail of Treatment plan.

Cheese Types	Treatments	Cheeses samples: (Surface area to volume ratio
Mozzarella	T_1	Mozzarella Shreds 0.47 × 0.67cm^2: 0.47 × 0.67 × 0.85cm^3
	T_2	Mozzarella Slices 0.65 × 3 cm^2: 0.65 × 3 × 4 cm^3
	T_3	Mozzarella Cubes 1×1 cm^2: 1×1×1 cm^3
Cheddar	T_4	Cheddar Shreds 0.47 × 0.67 cm^2: 0.47 × 0.67 × 0.85cm^3
	T_5	Cheddar Slices 0.65 × 3 cm^2: 0.65 × 3 × 4 cm^3
	T_6	Cheddar Cubes 1×1 cm^2: 1×1×1 cm^3
Enzyme-	T_7	EMC Shreds 0.47 × 0.67 cm^2: 0.47 × 0.67 × 0.85cm^3
modified cheese	T_8	EMC Slices 0.65 × 3 cm^2: 0.65 × 3 × 4 cm^3
(EMC)	T_9	EMC Cubes 1×1 cm^2: 1×1×1 cm^3

Optimization of freeze-drying process: For the optimization of freeze-drying conditions, cheese samples were prepared in three geometric forms: shreds, slices, and cubes. The cheese proportions were kept in freeze dryer for subsequent drying operations under controlled conditions in order to optimize the freeze-drying process (Alinovi *et al.,* 2021). These samples were frozen at temperatures of -20 °C, -30 °C, -40 °C, and -50 °C, and then freeze-dried at temperatures of 20 °C, 23 °C, 26 °C and 29 °C. The effects of freezing temperatures (-20 °C, -30 °C, -40 °C and -50 °C), and freeze-drying temperatures (20 °C, 23 °C, 26 °C and 29 °C) on the drying efficiency/time of cheeses in different physical forms (shreds, slices and cubes) were monitored. The moisture behavior of cheeses in different physical forms (Shreds, Slices and Cubes) was studied across various drying durations ranging from 5 to 26 hours, following the AOAC (2016) method.

Physicochemical analysis and water activity of cheese powder flavor: The physicochemical properties (fat, protein, moisture, pH, acidity and ash) of cheese powder flavors were analyzed according to AOAC (2016). The water activity (a_w) of the samples obtained was determined at 25 °C using a water activity meter (Aqualab, Decagon 3TE, Decagon Devices Inc., Pullman, WA). All measurements were performed in triplicate.

Textural behavior of freeze-dried cheese powder flavors with different surface area to volume ratio

Determination of loose and tapped density: The loose density was evaluated by measuring a 100 mL standardized cylinder filled with sample material, balanced without compression, and weighed it again is expressed as follows:

$$Loose\ density = Powder\ weight\ (g) / Powder\ volume\ (cm3)$$

The tapped density was determined on a tube containing the sample that had been lightly squeezed on a tabletop for at least ten minutes, ensuring that there was no visible volume changed between results. The graduated cylinder was used to read the new volume obtained after tapping. The density is expressed as follows:

$$Tapped\ density = powder\ weight(g) / volume\ of\ tapped\ powder\ \text{(cm3)}$$

Flow ability: The flow ability of cheese powder flavors was determined using Carr’s Index/Compaction Index (CI) and Hausner Ratio (HR). Bulk density and tapped density values of the cheese powder flavors were determined and used to calculate Carr's Index and Hausner Ratio. Higher Carr's Index and Hausner Ratio values indicated lower flow ability (Reddy *et al.*, 2014).

$$CI = [(Tapped\ density - Loose\ density) / Tapped\ density] \times 100$$

$$HR = Tapped\ density / Bulk\ density$$

Cohesiveness: The cohesiveness of freeze-dried cheese powder flavor was done with texture analyzer by measuring the mechanical properties of cheese powders. The known quantity of cheese powder was compressed between two flat surfaces and the force required to compress the powder was measured. A more cohesive powder required more force to compress and formed a more compact mass (Konuk Takma *et al.*, 2024).

Solubility Index: One gram of each cheese powder flavor was mixed with 100 mL distilled water and blended in a hand blender. The solution was transferred to 50 mL centrifuge tubes and centrifuged at 3000 rpm for 5 min. It was allowed to settle for 30 min and 25 mL of the supernatant was transferred to pre-weighed petri plates which were oven dried at 105°C for 5 hr. The solubility (%) was calculated as the weight difference (Hougaard *et al*., 2015).

Dispersibility: Distilled water (10 mL at 25 °C) was taken in a 50 mL beaker and 1 g sample was added. The sample was stirred vigorously for 15 sec. making 25 complete movements back and forth across the whole diameter of the beaker. The reconstituted powder was poured through a 212 µm sieve into a reweighed aluminum pan. The pan with a sieved sample was dried at 105 °C for 4 h. The dispersibility was calculated according to the formula given by Ogolla *et al*. (2019) as given in Eq. (5).

$$Dispersibility\ (\%) = (10 + \alpha) \times \%TS / \alpha \times (100 - b) / 100$$

Where, *α* is amount of cheese powder (g) taken, b is moisture content in the powder, and %TS is dry matter in percentage in the reconstituted powder after it has been passed through the sieve.

Determinations of post melt hardness and gumminess: The CT3 Brookfield-Texture analyzer was used to measure post-melt hardness and gumminess after the cheese powders had been reconstituted. Post-melt hardness was measured by applying controlled force to a sample and the force required to compress the sample was observed. Gumminess was evaluated by analyzing the force-displacement curve during compression (Nishinari *et al*., 2018).

Statistical Analysis: The data regarding physicochemical analysis, texture analysis, and post melt hardness and gumminess were analyzed through two-way ANOVA technique using PROC GLM in SAS software (version 9.1.3) under completely randomized design, considering three cheese types (Mozzarella, Cheddar, and enzyme-modified Cheese powders) and three cheese dimensions (surface area to volume ratio) as main factors and their interactive effects were evaluated. In parameters regarding moisture, the interactive effect of cheese type and dimension were evaluated over different drying times. For the comparison of significant treatment means, Tukey's HSD test was applied considering $p \leq 0.05$ as significance level.

RESULTS

Freeze-Drying Optimization of Cheese Powder flavor – Effect of freeze-drying time on the moisture content: The freeze-drying time significantly affected the moisture content of various cheese types (Mozzarella, Cheddar, and enzyme-modified cheese) in different forms (shreds, slices, and cubes) ($p \leq 0.05$). As freeze-drying time increased, moisture content decreased substantially in all cheese forms (Figure 1). Shreds consistently exhibited the lowest moisture content, followed by slices and cubes, with significant variations among cheese types ($p \leq 0.05$).

Specifically, Mozzarella's moisture content decreased from 14.94% (shreds), 16.56% (slices), and 18.62% (cubes) at 5 hours to 2.01% (shreds), 2.22% (slices), and 2.32% (cubes) at 20 hours. Cheddar's moisture content dropped from 16.48% (shreds), 18.47% (slices), and 22.38% (cubes) at 5 hours to 2.56% (shreds), 2.69% (slices), and 2.78% (cubes) at 22 hours. Enzyme-modified cheese had the highest initial moisture content (21.27% (shreds), 23.87% (slices), 24.65% (cubes)), decreasing to 2.84% (shreds), 2.95% (slices), and 3.09% (cubes) after 26 hours

Freeze-Drying Optimization of Cheese Powder flavor – Effect of freezing temperature on drying time: The mean values showed that freezing temperature had a significant effect ($p \leq 0.05$) on the drying time of all cheese types (Mozzarella, Cheddar, and enzyme-modified cheese) in different forms (shreds, slices, and cubes) (Figure 2). A consistent reduction in drying time was observed as the freezing temperature decreased from –20 °C to –50 °C. For Mozzarella cheese, drying times declined from 18.40 ± 0.10 h (shreds), 20.10 ± 0.32 h (slices), and 22.10 ± 0.31 h (cubes) at –20 °C to 12.55 ± 0.26 h, 14.30 ± 0.26 h, and 15.55 ± 0.25 h, respectively, at –50 °C. Cheddar cheese showed a similar trend, with drying times decreasing from 26.45 ± 0.23 h (shreds), 28.15 ± 0.31 h (slices), and 29.58 ± 0.35 h (cubes) at –20 °C to 23.20 ± 0.33 h, 20.35 ± 0.26 h, and 24.45 ± 0.29 h, respectively, at –50 °C. For enzyme-modified cheese, drying times were reduced from 26.90 ± 0.36 h (shreds), 29.25 ± 0.37 h (slices), and 30.10 ± 0.31 h (cubes) at –20 °C to 23.50 ± 0.21 h, 25.55 ± 0.15 h, and 26.55 ± 0.29 h, respectively, at –50 °C.

Freeze-Drying Optimization of Cheese Powder flavor – Effect of drying temperature on moisture: An increase in drying temperature from 20 °C to 29°C resulted significant ($p \leq 0.05$) reduction in the moisture content of all cheese types (Mozzarella, Cheddar and enzyme-modified Cheese) and physical forms (shreds, slices and cubes) (Figure 3). In Mozzarella cheese, the moisture content decreased from 2.36±0.058% (Shreds) and 2.49±0.032% (Cubes) at 20 °C to 2.01±0.027% (Shreds) and 2.32±0.032% (Cubes) at 29 °C. Cheddar cheese showed a similar trend, with moisture content decreased from 2.72±0.035% (Shreds) and 2.95±0.052% (Cubes) at 20 °C to 2.56±0.046% (Shreds) and 2.78±0.047% (Cubes) at 29 °C. Enzyme-modified Cheese showed moisture content dropped from 2.99±0.089% (Shreds) and 3.24±0.052% (Cubes) at 20 °C to 2.84±0.026% (Shreds) and 3.09±0.036% (Cubes) at 29 °C.

Water activity of freeze-dried cheese powder flavors with different surface area to volume ratio: Water activity of freeze-dried cheese powder flavor differed significantly ($p \leq 0.05$) with cheese type (Mozzarella, cheddar and enzyme-modified Cheese) and surface area to volume ratio (shreds, slices and cubes) (Table 2). In Mozzarella cheese powder flavor, the water activity values were observed 0.20±0.03 (Shreds), 0.21±0.02 (Slices), and 0.22±0.06 (Cubes), with cubes showed slightly higher water activity than slices and shreds. In Cheddar cheese powder flavor, the water activity increased from 0.22±0.02 (Shreds) to 0.25±0.03 (Cubes), indicating that cubes retain more water compared to shreds. Similarly, enzyme-modified Cheese exhibited the highest water activity as compared to Cheddar and Mozzarella cheese, with values of 0.24±0.06 (Shreds), 0.25±0.09 (Slices), and 0.27±0.04 (Cubes).

Physicochemical analysis of freeze-dried cheese powder flavors with different surface area to volume ratio: The results regarding physicochemical composition of freeze-dried cheese powder flavors are depicted in Table 2. Mean values indicated that physicochemical composition (fat, protein, moisture, acidity and ash) varied significantly ($p \leq 0.05$) with cheese type. Moreover, surface area to volume ratio (shreds, slices, and cube) also significantly ($p \leq 0.05$) influenced the moisture content of cheese powder flavors. However, it did not affect the fat, protein, pH, acidity and ash content of cheese powder flavors.

Mozzarella cheese showed a fat content ranging from 45.04±0.27% (shreds) to 48.42±0.26% (cube), while enzyme-modified Cheese displayed the highest fat content, ranging from 50.01±0.24% to 50.23±0.28% in shreds and cubes respectively. The protein content in Mozzarella cheese was found higher (36.01±0.13% (shreds) to 36.42±0.09% (cube)), as compared to cheddar (37.47±0.08% (shreds) and 37.82±0.09% cube), and enzyme modified Cheese (33.07±0.12% (shreds) and 33.99±0.13% (cubes). Mozzarella displayed moisture content ranging from 2.02±0.037% (Slices) to 2.32±0.032% (Cubes), while Cheddar cheese moisture ranged from 2.56±0.032% (Slices) to 2.78±0.041% (Cubes). Enzyme modified Cheese exhibited the highest moisture content among the cheese types, with values ranging from 2.85±0.040% (Slices) to 3.9±0.039% (Cubes). Mozzarella had a pH between 4.94±0.04 (Slices) and 4.99±0.01 (Cubes), while Cheddar cheese had a slightly higher pH ranging from 5.31±0.03 (Shreds) to 5.36±0.02 (Cubes). Enzyme modified Cheese showed the highest pH values, from 5.65±0.02 (Slices) to 5.77±0.02 (Cubes), indicating lower acidity. Mozzarella cheese had the highest acidity (0.91±0.01% in Cubes), while enzyme-modified cheese displayed the lowest acidity, with values ranging from 0.054±0.007% (Slices) to 0.061±0.007% (Cubes). The ash content was highest in enzyme-modified Cheese, ranging from 16.35±0.09% (Slices) to 16.63±0.08% (Cubes), indicating a higher mineral content. Mozzarella cheese showed an ash content of 15.06±0.08% (Slices) to 15.39±0.06% (Cubes), while Cheddar cheese had the lowest ash content, ranging from 12.19±0.07% (Slices) to 12.98±0.10% (Cubes).

Texture analysis of freeze-dried cheese powder flavors with different surface area to volume ratio: The texture parameters (loose and tap densities) of freeze-dried cheese powder flavors significantly ($p \leq 0.05$) affected by both cheese type and surface area to volume ratio (Table 3). Among the cheese types, enzyme-modified Cheese exhibited the highest loose and tapped density values, with cubes showing the maximum (0.57±0.005 g/cm³ and 1.11±0.03 g/cm³), respectively. In contrast, Mozzarella showed the lowest values, particularly in shreds (0.20±0.006 g/cm³ for loose density) and 0.47±0.02 g/cm³ for tapped density.

Flowability varied significantly with cheese type and surface area to volume ratio. Enzyme-modified Cheese cubes exhibiting the highest value (6.37±0.12), indicating a more cohesive and less flowable structure while Mozzarella shreds showed the lowest flowability (2.89±0.24). Cohesiveness followed a similar trend, being highest in enzyme-modified Cheese cubes (0.68±0.05 gf/cm), and lowest in Mozzarella shreds (0.59±0.02 gf/cm).

In terms of solubility, Mozzarella shreds showed the highest index (65.61±2.09%), whereas, enzyme-modified Cheese cubes had the lowest (25.14±0.62%). Conversely, dispersibility was greatest in enzyme-modified Cheese cubes (61.25±1.65%), followed by Cheddar cubes (50.01±1.45%), while Mozzarella shreds exhibited the lowest (25.34±0.55%).

Effect of Surface to volume ratio on Post melt hardness and Gumminess of cheese powder flavor: The cheese type and surface area to volume ratio had a significant effect ($p \leq 0.05$) on the post-melt hardness and gumminess of freeze-dried cheese powder flavors (Table 3). Mozzarella exhibited the greatest post-melt hardness, with the shred form recording the highest value (14.92±3.08 mJ), while the cube form showed a markedly lower hardness (7.22±3.21 mJ). In contrast, enzyme-modified Cheese displayed the lowest hardness values overall, particularly in cubes (3.01±1.79 mJ) and slices (3.04±2.07 mJ). Cheddar followed an intermediate trend, with hardness decreasing as the surface area-to-volume ratio declined, from 8.62±2.76 mJ in shreds to 3.06±2.06 mJ in cubes.

A similar pattern was observed for gumminess. Mozzarella shreds exhibited the highest gumminess (1355.40 ± 131.8 gf), whereas cubes showed substantially lower values (755.00±55.52 gf). Cheddar cheese powder showed lower gumminess compared to Mozzarella, with shreds and cubes measuring 466.90±43.98 gf and cubes at 161.00±43.98 gf. Enzyme-modified Cheese presented the least gumminess among all treatments, with cubes recording only 156.00±45.58 gf.

Table 2 Physiochemical analysis of freeze-dried cheese powder flavors with different surface area to volume ratio

Cheese Type	Surface area to volume	Fat %	Protein %	Moisture %	pH	Acidity (%)	Ash (%)	Water Activity (a_w)
Mozzarella		45.27^{c}	36.26^{ab}	2.18^{c}	4.96^{b}	0.88^{a}	15.20^{b}	0.21^{ab}
Cheddar		48.47^{b}	37.64^{a}	2.70^{b}	5.34^{ab}	0.62^{b}	12.47^{c}	0.23^{b}
Enzyme Modified Cheese		51.07^{a}	33.45^{b}	3.23^{a}	5.72^{a}	0.57^{b}	16.52^{a}	0.25^{a}
SEM		1.43	1.12	0.43	0.92	0.02	1.25	0.01
p-value		0.041	0.046	0.021	0.033	<0.0001	0.043	0.039
	SH	49.00	35.63	2.48	5.34	0.68	14.65	0.22
	SL	47.05	35.70	2.64	5.31	0.66	14.53	0.23
	C	47.68	36.01	3.00	5.37	0.72	15.00	0.25
	SEM	1.43	1.12	0.43	0.92	0.02	1.25	0.01
	p-value	0.993	0.968	0.684	0.999	0.262	0.963	0.446
Mozzarella	SH	48.06	36.34	2.02^{d}	4.96	0.87^{a}	15.15	0.2
	SL	45.04	36.01	2.21^{c}	4.94	0.85^{a}	15.06	0.21
	C	45.53	36.42	2.32^{bc}	4.99	0.91^{a}	15.39	0.22
Cheddar	SH	47.34	37.47	2.56^{b}	5.31	0.61^{b}	12.24	0.22
	SL	46.74	37.82	2.76^{ab}	5.35	0.60^{b}	12.19	0.23
	C	47.33	37.62	2.78^{ab}	5.36	0.64^{b}	12.98	0.25
Enzyme Modified Cheese	SH	51.61	33.07	2.85^{b}	5.75	0.56^{b}	16.57	0.24
	SL	49.37	33.28	2.94^{a}	5.65	0.54^{b}	16.35	0.25
	C	50.92	33.99	3.09	5.77	0.61^{b}	16.63	0.27
SEM		2.47	1.93	0.75	1.59	0.04	2.17	0.03
p-value		0.291	0.513	0.023	1.000	0.07	0.694	0.635

SH=Shreds (0.47 × 0.67cm^{2}: 0.47 × 0.67 × 0.85cm^{3}**), SL=Slices (**0.65 × 3 cm^{2}: 0.65 × 3 × 4 cm^{3}**), C=Cube** 1×1 cm^{2} :1×1×1 cm^{3}**;**
$^{a\text{-}b}$ Superscripts on different means within column differ significantly at $p \leq 0.05$.

Table 3 Texture analysis, Post melt hardness and Gumminess of freeze-dried cheese powder flavors with different surface area to volume ratio.

Cheese Type	Surface area to volume	Loose density (g/cm^{3})	Tapped density (g/cm^{3})	Flow ability	Cohesiveness (gf/cm)	Solubility index (%)	Dispersibility (%)	Post melt hardness (mJ)	Gumminess (gf)
M		0.23^{c}	0.49^{c}	3.98	0.61^{b}	63.08^{a}	28.43^{c}	11.05^{a}	976.57^{a}
C		0.40^{b}	0.86^{b}	4.51	0.64^{b}	45.18^{b}	47.11^{b}	5.38^{b}	278.47^{b}
EMC		0.54^{a}	1.05^{a}	5.22	2.65^{a}	29.82^{c}	55.34^{a}	3.97^{b}	215.33^{b}
SEM		0.01	0.02	0.44	0.33	1.52	1.22	0.63	29.44
p-value		<0.0001	<0.0001	0.167	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	SH	0.36	0.76^{b}	3.38^{b}	0.61^{b}	50.17^{a}	40.22^{b}	9.80^{a}	703.10^{a}
	SL	0.39	0.80^{b}	4.52^{ab}	0.63^{b}	45.92^{ab}	42.91^{b}	6.16^{b}	409.93^{b}
	C	0.42	0.85^{a}	5.81^{a}	2.66^{a}	41.99^{b}	47.75^{a}	4.43^{b}	357.33^{b}
	SEM	0.01	0.02	0.44	0.33	1.52	1.22	0.63	29.44
	p-value	0.061	0.003	0.004	<0.0001	0.005	0.001	<0.0001	<0.0001
M	SH	0.20^{c}	0.47^{f}	2.89	0.59^{b}	65.61^{a}	25.34^{e}	14.92^{a}	1355.40^{a}
	SL	0.22^{c}	0.49^{f}	3.91	0.61^{b}	63.41^{a}	27.97^{de}	11.00^{b}	819.30^{b}
	C	0.26^{c}	0.52^{f}	5.13	0.63^{b}	60.21^{a}	31.98^{d}	7.22^{cd}	755.00^{b}
C	SH	0.38^{b}	0.81^{e}	3.29	0.61^{b}	49.80^{b}	45.20^{c}	8.62^{bc}	466.90^{c}
	SL	0.40^{b}	0.85^{de}	4.31	0.64^{b}	45.12^{bc}	46.12^{c}	4.45^{de}	207.50^{d}
	C	0.42^{b}	0.91^{cd}	5.93	0.67^{b}	40.62^{cd}	50.01^{bc}	3.06^{e}	161.00^{d}
EMC	SH	0.51^{a}	0.99^{bc}	3.96	0.62^{b}	35.10^{de}	50.12^{bc}	5.87^{cde}	287.00^{d}
	SL	0.54^{a}	1.05^{ab}	5.34	0.65^{b}	29.23^{ef}	54.64^{b}	3.04^{e}	203.00^{d}
	C	0.57^{a}	1.11^{a}	6.37	6.68^{a}	25.14^{f}	61.25^{a}	3.01^{e}	156.00^{d}
SEM		0.03	0.03	0.77	0.58	2.64	2.11	1.09	50.98
p-value		<0.0001	<0.0001	0.060	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

M=Mozzarella**, C=**Cheddar**, EMC=**Enzyme Modified Cheese**; SH=Shreds (**0.47 × 0.67cm^{2}: 0.47 × 0.67 × 0.85cm^{3}**), SL=Slices (**0.65 × 3 cm^{2}: 0.65 × 3 × 4 cm^{3}**), C=Cube** 1×1 cm^{2} :1×1×1 cm^{3}**;**
$^{a\text{-}f}$ Superscripts on different means within column differ significantly at $p \leq 0.05$.

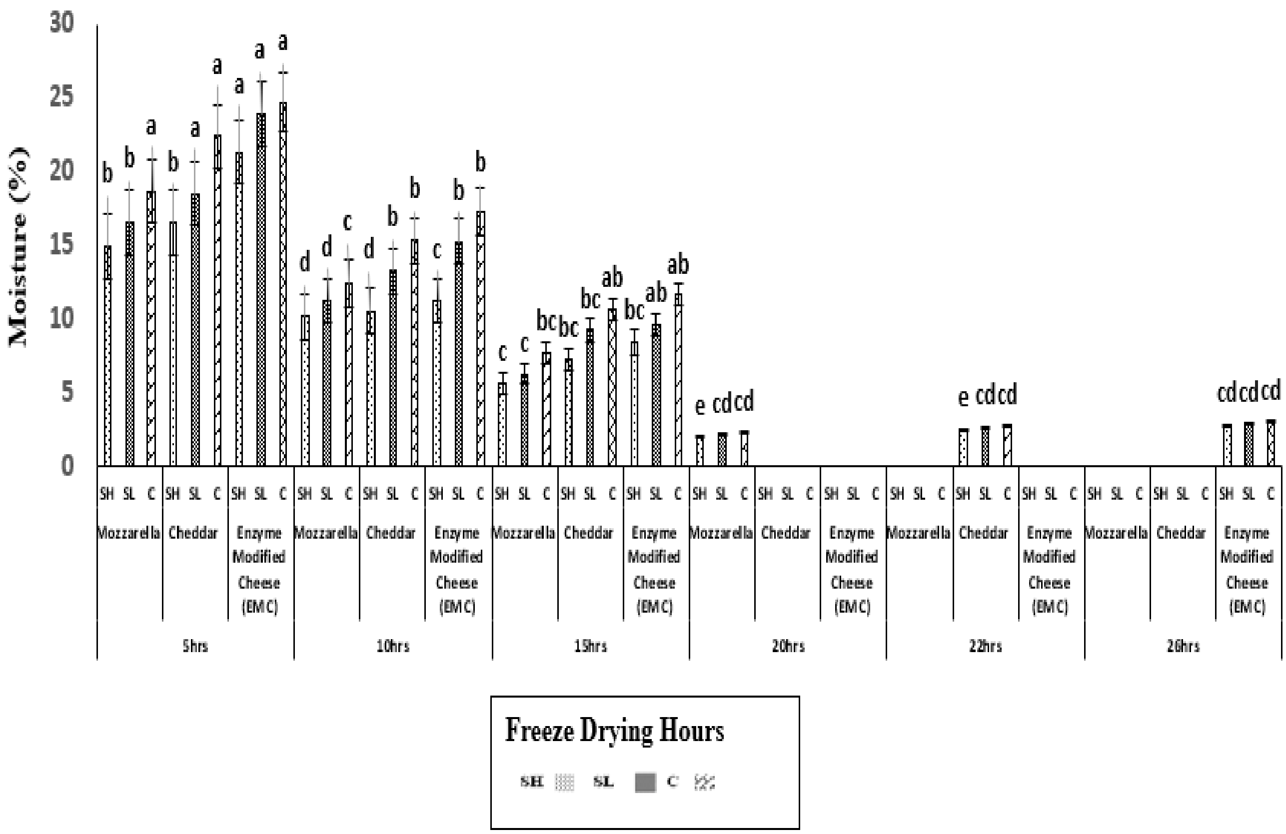

Fig. 1. Effect of Freeze-dryiny time on the moisture content of Cheese powder flavor; SH=Shreds (0.47 × 0.67cm^2: 0.47 × 0.67 × 0.85cm^3**), SL=Slices (**0.65 × 3 cm^2: 0.65 × 3 × 4 cm^3**), C=Cube** 1×1 cm^2 :1×1×1 cm^3

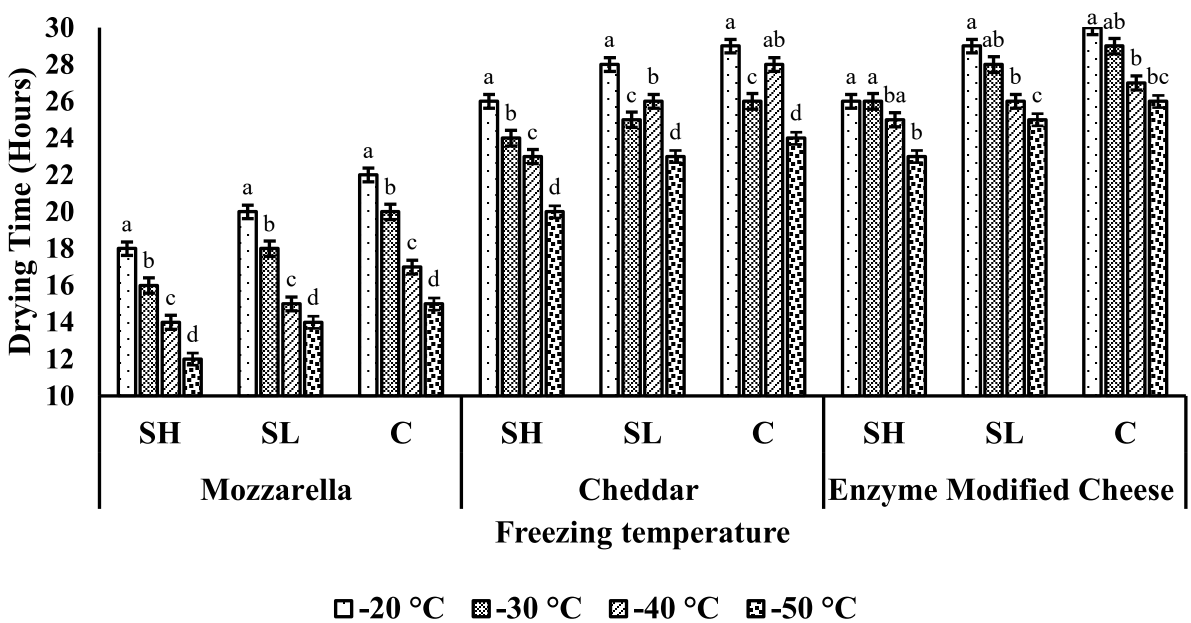

Fig. 2. Effect of Freezing temperature on drying time; SH=Shreds (0.47 × 0.67cm^2: 0.47 × 0.67 × 0.85cm^3**), SL=Slices (**0.65 × 3 cm^2: 0.65 × 3 × 4 cm^3**), C=Cube (**1×1 cm^2 :1×1×1 cm^3**).** Freezing temperatures (-20 °C, -30 °C, -40 °C, -50 °C)

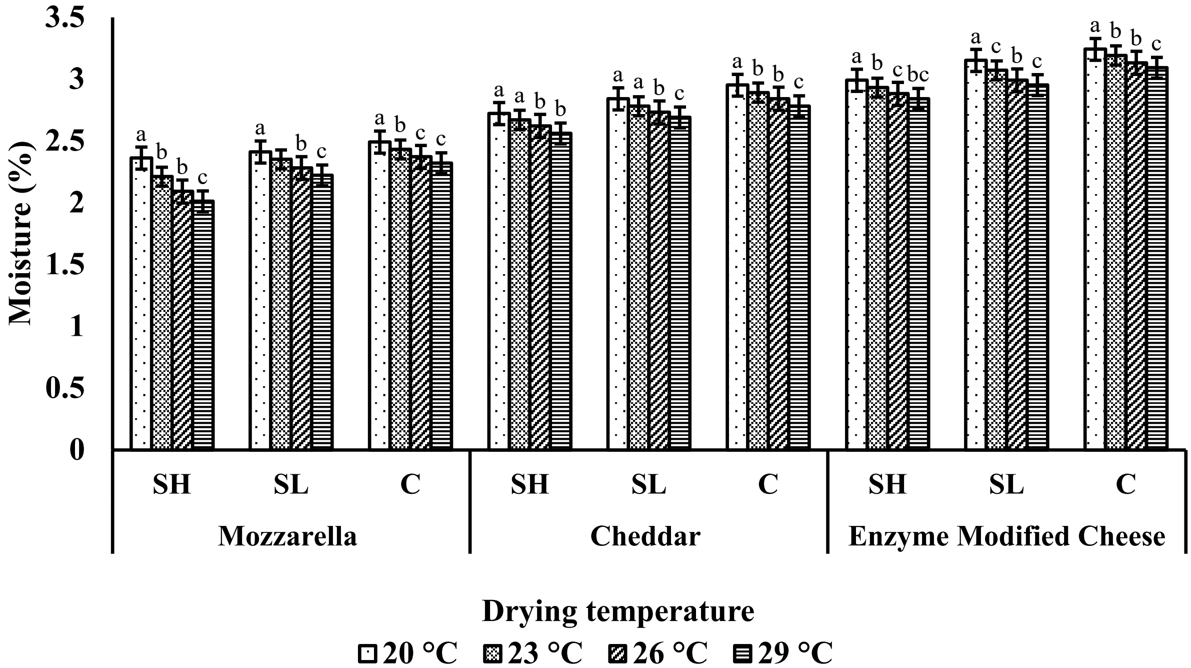

Fig. 3. Effect of Drying temperature on moisture content of Cheese powder flavor; SH=Shreds (0.47 × 0.67cm^2: 0.47 × 0.67 × 0.85cm^3**), SL=Slices (**0.65 × 3 cm^2: 0.65 × 3 × 4 cm^3**), C=Cube (**1×1 cm^2 :1×1×1 cm^3**)**. Freeze-drying temperatures (20 °C, 23 °C, 26 °C, 29 °C).

DISCUSSION

Effect of freeze-drying time on the moisture content of Cheese powder flavor: Mean values indicated that moisture content in cheese powders decreased significantly with increasing freeze-drying time (Figure 1). This occurs because a longer freeze-drying process allows more time for the frozen water molecules to sublimate, leaving behind a drier product (Köprüalan *et al*., 2022).

Enzyme-modified Cheese retained more moisture, due to its higher water-binding capacity, while Mozzarella cheese showed less retention. Among the forms, shreds possessed less moisture content, followed by slices and cubes, due to their higher surface area-to-volume ratio. Mean values further revealed that enzyme-modified cheese required a longer freeze-drying time (Figure 1). This behavior can be explained by the proteolytic enzymes added during its manufacture, which modify the protein structure. and alter the cheese matrix compared with Mozzarella and Cheddar. The proteolytic enzymes break down proteins into smaller peptides and amino acids, which bind water molecules thus potentially increasing the time for the removal of moisture content of the cheese (Bouroutzika *et al*., 2021). Moreover, Enzyme-modified cheese has a more compact and denser structure as compared to Mozzarella and Cheddar cheese. Therefore, the sublimation of frozen water molecules takes more time, and the drying of product from the surface and interior of the cheese is prolonged. The stretching step in Mozzarella cheese produces a smooth texture that facilitates the freezing and sublimation process (Lawrence *et al*., 2012).

Effect of freezing temperature on drying time: Freezing temperature during the freeze-drying process significantly influenced the drying time of cheeses in different forms (Shreds, Slices and Shreds) (Figure 2). The mean values for Mozzarella, Cheddar and enzyme-modified Cheese at varying surface area to volume ratios (Shreds, Slices, Cubes) indicated that lower freezing temperature consistently reduced sublimation (drying) time. Among the forms, shreds dried faster as compared to slice and cube. It is because the smaller pieces or shreds have more exposed surface area for freezing of water facilitating quicker drying (Pax *et al*., 2021). The Mozzarella cheese shreds that were frozen at -20°C required 18.40±0.10 h for drying, whereas shreds frozen at -50°C required only 12.55±0.26 h. Similarly, in enzyme-modified cheese, shreds frozen at -50°C sublimated more rapidly than those frozen at -20°C. The underlying mechanism is related to ice crystal morphology at different freezing temperatures. Higher freezing temperature promotes the formation of larger size ice crystals of residual water present in cheese (shreds/slice/cube) as compared to low freezing temperature, leading to greater structural damage and development of porous channels that facilitate water larger ice crystals have a greater surface area that increases sublimation resulting in faster drying. Higher freezing temperatures also increased water mobility within the cheese structure (Shreds, Slice, and cube) which increased the movement of water molecules to the surface, from where sublimation becomes more rapid (Digvijay *et al*., 2025).

Effect of drying temperature on drying time: The increase in sublimation temperature significantly reduced the drying time of cheeses with varying surface area-to-volume ratios (Figure 3). This reduction can be attributed to the increased energy provided to the frozen moisture within the cheese particles, which enhanced the kinetic energy of water molecules and facilitated their transition from solid to gas phase. As a result, the vapor pressure of ice increased, promoting the sublimation of ice crystals (Alinovi *et al*., 2021). Furthermore, the higher drying temperature expedited the diffusion of water molecules from regions of high to low concentration, thereby accelerating water vapor removal from the cheese samples.

The drying time of various cheese forms was significantly impacted by sublimation temperature, with cubes consistently requiring longer drying times compared to slices and shreds at each temperature. This difference can be attributed to the lower surface area-to-volume ratio of cubes, which limited the exposure of internal moisture to the drying environment and increased drying time (Bintsis and Papademas, 2017). In contrast, slices and shreds, with their greater surface area, were more exposed to the drying environment, resulting in reduced drying times.

Water activity of freeze-dried cheese powder flavors: The water activity of freeze-dried cheese powder flavors differed significantly ($p \leq 0.05$) among cheese types (Mozzarella, Cheddar, and enzyme-modified cheese) and geometric forms (shreds, slices, and cubes) (Table 2). Mozzarella cheese powder exhibited the lowest water activity, indicating effective moisture removal and enhanced stability against microbial growth compared to Cheddar and enzyme-modified cheese powders. In contrast, enzyme-modified cheese powder flavor had the highest water activity, likely due to its composition and higher moisture-binding capacity (Jin *et al*., 2019; Hardy *et al*., 2002). Among the geometric forms, shredded cheese powders consistently showed lower water activity than slice and cube powders, regardless of cheese type.

Physico-chemical analysis of cheese powder flavor: Physicochemical composition (Fat, Protein, Moisture, Acidity and Ash contents) varied significantly with cheese type, while surface area to volume ratio (shreds, slices, and cube) influenced only the moisture content of cheese powder flavors (Table 2). The fat content in enzyme-modified cheese powder was found to be marginally higher (51.07.37±0.28%) than that of Cheddar and Mozzarella cheese powder flavors (48.47±0.32% and 45.27±0.26%, respectively). Protein content in Cheddar cheese found higher (37.62±0.14%) than Mozzarella (36.42±0.09% and enzyme-modified cheese (33.92±0.13%). Moisture content was consistently higher in enzyme-modified Cheese powder than in Cheddar and Mozzarella. The removal of moisture content was found difficult in enzyme-modified cheese as compared to Cheddar and Mozzarella cheese. This may be the reason of high moisture content in enzyme-modified cheese powder flavor. Surface area to volume ratio of cheeses impacted the moisture contents of cheese powder flavors. Cube form of cheeses retained more moisture due to their compact structure, which restricts evaporation during freeze-drying consequently resulted more moisture in powder flavor (Koca *et al.,* 2015). The pH values of Mozzarella cheese powder flavors were observed significantly ($P<0.05$) lower than that of Cheddar and Enzyme-Modified cheese powder flavors (Li *et al*., 2023). However, acidity of Mozzarella cheese powder flavor was noted high as compared to other cheese powder flavors. This is due to lower milling pH of Mozzarella cheese as compared to Cheddar and Enzyme-modified cheese (Salum *et al*., 2022). Ash content was greatest in enzyme-modified cheese powders, reflecting compositional changes introduced during enzymatic modification used to enhance flavor and texture (Ashraf *et al*., 2023).

Texture analysis of freeze-dried cheese powder flavors with different surface area to volume ratio

Loose density: Loose and tapped densities of cheese powder flavors showed a significant ($p<0.05$) variation with cheese type and surface area to volume ratio (Table 3). The mean values indicated that the Mozzarella cheese had less loose and tapped density values as compared to Cheddar and enzyme-modified cheese. This difference may be attributed to the soft texture of Mozzarella cheese, which leads to a more porous structure after freeze-drying (Reid and Yan, 2004). Conversely, denser and compact structures of Cheddar and enzyme-modified Cheese contributed to their higher bulk densities. Among the different forms, powder flavors prepared from shreds showed lower loose and tapped densities, whereas cube form exhibited the highest values. The loose densities of shredded Mozzarella, Cheddar, and enzyme-modified Cheese were 0.36 g/cm^3, 0.49 g/cm^3, and 0.51 g/cm^3 respectively, while the cubed form measured 0.32 g/cm^3, 0.38 g/cm^3, 0.44 g/cm^3 respectively. The tapped densities of shredded Mozzarella, Cheddar and Enzyme-modified cheese were 0.47±0.02 g/cm^3, 0.81±0.02 g/cm^3, and 0.99±0.02 g/cm^3 respectively. The difference in loose densities is due to the air that is created inside the particles that contribute to the space between the powder particles. Shredded forms increased surface area and more open structure, which reduced particle packing during tapping (Pugliese *et al.,* 2017).

Flow ability and cohesiveness: Flow ability and cohesiveness of cheese powder flavors significantly ($p<0.05$) changed with cheese type. Enzyme-modified cheese exhibited higher flowability compared to Cheddar and Mozzarella, whereas its cohesiveness was lower. This may be attributed to stronger cohesive forces between particles of Mozzarella cheese that caused them to stick together resulting in reduced flow ability and increased cohesiveness (Banville *at al.,* 2013). Surface area to volume ratio had a marked influence on the flow ability and cohesiveness of cheeses (Mozzarella, Cheddar and enzyme-modified cheese) powder flavors. The shredded cheese powder flavors showed less flowability (Mozzarella: 2.89± 0.24, Cheddar: 3.29± 0.11 and enzyme-modified Cheese: 3.96± 0.16) as compared to cubed cheese powder flavors (Mozzarella: 5.13± 0.21, Cheddar: 5.93± 0.12 and enzyme modified Cheese 6.37± 0.12). Also, the cohesiveness of shredded cheese powder was found more in shredded samples as compared to cubed powder flavors. This is due to more void spaces between shredded powder flavor particles that decreased flow ability and increased cohesiveness. However, cubed powder flavors had greater bulk densities therefore increased flowability and less cohesiveness (Amighi *et al*., 2016). Suhag *et al*. (2024) studied that flowability of powder depends on the size of particles and decreases when the size of particle is less than 0.2 mm.

Solubility Index: The solubility index of cheese powder flavors differed significantly among cheese types (Table 3). Mozzarella cheese showed greater solubility index as compared to Cheddar and Mozzarella cheese. The greater solubility of Mozzarella powder flavor might be due to low calcium content, as higher calcium level tends to reduced solubility as observed in Cheddar and enzyme-modified Cheese powder flavors (Guinee *et al*., 2002). Surface area-to-volume ratio also played a decisive role in solubility behavior. The shredded powder flavors (Mozzarella, Cheddar, and enzyme modified Cheese) are more soluble than the slice and cube powder flavors. The enhanced solubility of shredded powders is likely due to their smaller particle size and larger surface area, which provides more active sites for interaction with water that facilitate dissolution (da Silva *et al*., 2018)

Dispersibility: The mean values regarding dispersibility of powder cheese flavors indicated that the Mozzarella cheese had less dispersibility as compared to Cheddar and enzyme-modified cheese (Table 3). This is due to processing treatments like stretching that decreased the dispersibility. In contrast, the higher dispersibility of Cheddar and enzyme-modified cheese powders is likely linked to protein denaturation, which facilitates better interaction with water.

Surface area-to-volume ratio also had a significant ($P<0.05$) effect on dispersibility. The shredded powders of Mozzarella, Cheddar, and Enzyme-modified cheese exhibited lower dispersibility values (25.34±0.55, 45.20±0.90, and 50.12±1.40 respectively) compared to cubed powders (31.98±0.73, 50.01±1.45, 61.25±1.65 respectively). This is due to smaller size particles of shreds that have higher surface area-to-volume ratio allowing them to disperse more readily in the water as compared to slices and cubes (Guinee *et al.,* 2017). Additionally, compositional factors also influence the dispersibility. Enzyme-modified Cheese powder flavors had more fat content that acted as lubricant and helped to reduce friction between particles and promote flow and dispersibility (Chen *et al*., 2014).

Texture analysis

Post-melt hardness and gumminess: The mean values showed that post-melt hardness and gumminess of cheese powder flavors differed significantly ($P<0.05$) with cheese type and surface area to volume ratio. Among the varieties, Mozzarella cheese powder flavor exhibited more post-melt hardness and gumminess as compared to Cheddar and enzyme-modified cheese powders. This can be attributed to the strong tendency of Mozzarella to form networks upon melting, leading to increased viscosity and firmness. This is due to stretching or kneading step during Mozzarella cheese production, which affected the protein structure and texture of the final cheese product (Guinee, 2016).

Surface area to volume ratio also influenced texture, with shredded samples showing higher hardness and gumminess than slices and cubes. The order of hardness and gumminess in the cheese powder flavors was slice > shred > cube. This might be due to increased surface area of shredded cheese powder flavor that facilitated efficient moisture redistribution during melting. As moisture is released and redistributed within the melted cheese, it facilitated protein-protein interactions and the formation of a denser network structure, resulting in higher post-melt hardness and gumminess. Additionally, mechanical disruption of the protein in shredded powders, increases surface roughness and exposure of protein surfaces. This promoted the protein aggregation and network formation during melting, contributing to higher post-melt hardness and gumminess (Ardö, 2017). Loudiyi *et al*. (2019) reported that the textural profile of food is typically related to species, constituent composition and water-holding capacity, and water loss from oxidation may reduce the water-holding capacity and thus affecting and gumminess.

Conclusion: The study demonstrated that freeze-drying time, freezing temperature, and drying temperature significantly influenced the moisture content, drying efficiency, and physico-chemical properties of cheese powder flavors. Among the cheese type, enzyme-modified cheese required longer freeze-drying time (≈26 hours), due to its dense structure and water-binding capacity, while Cheddar and Mozzarella required 22 hours and 20 hours respectively. Shredded powders dried more efficiently than slices and cubes and exhibited different density, solubility, and dispersibility characteristics owing to their higher surface area to volume ratio. Mozzarella cheese showed higher post-melt hardness and gumminess, influenced by its unique protein structure. The shredded form demonstrated the highest drying efficiency due to its optimal surface area-to-volume ratio.

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