

# THE EFFECTS OF INULIN ON THE TEXTURAL, THERMAL, AND MICROSTRUCTURAL PROPERTIES OF REDUCED-FAT CHEESE

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## Abstract

This experimental study investigates the effects of inulin on the physicochemical properties of reduced-fat cheese and its potential as a partial fat replacer. In the study, the compositional, textural, thermal, and microstructural properties of inulin-containing reduced-fat cheese were compared with those of full-fat cheese (control) and inulin-free reduced-fat cheese. The findings revealed that the addition of inulin induced fat and protein recovery in the inulin-containing reduced-fat cheese. Furthermore, the use of inulin significantly reduced the hardness of the reduced-fat cheese, particularly after 7 days of refrigerated storage, but minimally altered the cheese's springiness and cohesiveness. The microstructure of the inulin-containing reduced-fat cheese was noticeably less dense and resembled that of the full-fat cheese. Moreover, the fat melting enthalpy and the fat melting profile of the inulin-containing reduced-fat cheese closely resembled those of the full-fat cheese. Overall, the addition of inulin contributed to the improved compositional, textural, thermal, and microstructural characteristics of the reduced-fat cheese, rendering it comparable to those of the full-fat cheese. Thus, inulin is an effective partial fat replacer in a reduced-fat cheese product.

**Keywords:** Reduced-fat cheese, inulin, texture, fat melting profile, microstructure

## Introduction

With the prevalence of health information, modern consumers are more health conscious and attach greater importance to the health benefits and risks of their dietary intake. There exists extensive evidence on the association between saturated animal fats and the

incidences of chronic diseases, including cardiovascular disease, diabetes, and certain types of cancer. A reduction in animal fat consumption is thus widely recommended (USDA and HHS, 2010).

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Among the healthy diet alternatives, especially for those concerned with the dietary fat intake, are reduced- and low-fat foods. Nevertheless, due to the major role of fats in the overall physical characteristics of many foods, the removal of fats often deteriorates the appearance and palatability of the finished products (McClements and Decker, 2008). Specifically, the reduced fat content in cheese products directly influences their texture, flavor profile, functional properties, and overall acceptability (Gunasekaran and Ak, 2003; Johnson, 2011; O'Conner and O'Brien, 2011). To mitigate the problem, previous studies have proposed the use of dietary fibers, such as inulin, as the fat replacer (Drake and Swanson, 1995; Franck, 2002; Karimi *et al.*, 2015).

The fat content in cheese is characterized as the percent fat on either a wet weight basis or a dry matter basis, which becomes a basis for the classification of cheese products by fat levels: the full-fat, reduced-fat, and low-fat cheeses. In addition, the Codex Commission on International Trade has specified a maximum limit of 50% fat reduction (on a dry matter basis) from a referenced variety for a cheese to be labeled as a reduced-fat cheese. Meanwhile, in the United States, a reduced-fat cheese requires a minimum 25% reduction in fat from the normal fat level of a referenced variety (Gunasekaran and Ak, 2003; Fuquay *et al.*, 2011).

Inulin is a carbohydrate polymer consisting of fructose units known as fructans. Inulin can be safely used without specific limitations in a wide variety of food products, especially in dairy products as a food ingredient, a soluble dietary fiber, and a fat replacer (Franck, 2002; Chaito *et al.*, 2016). Inulin, which is low in calories, possesses many health benefits, especially the prebiotics that stimulate the growth of beneficial intestinal bacteria (Paul and Coussement, 1999; Karimi *et al.*, 2015). In addition, inulin has been effectively used to achieve textural modification and organoleptic improvement in food products (Karimi *et al.*, 2015). The recommended amount of 2-10% inulin in low-fat dairy products offers a better-balanced round flavor and a creamier mouthfeel (Frank, 2002).

Existing research has investigated the effects, on the physicochemical and sensorial

properties of dairy products, of inulin as the prebiotic, fat replacer, and texturizer (Meyer *et al.*, 2011; Karimi *et al.*, 2015; Chaito *et al.*, 2016). Guggisbert *et al.* (2009) documented that the introduction of inulin considerably improved the consistency of set yogurt. Juan *et al.* (2013) successfully produced a reduced-fat fresh cheese containing 3% inulin as the partial fat replacer and compared the sensory profiles with conventional fresh cheese. Tiwari *et al.* (2015) examined the effects of variable inulin concentrations (i.e. 2, 4, and 6%) as the fat replacer on the quality of low-fat ice cream.

Previous research studies focused on the effects of inulin on the quality of imitation cheese (Hennelly *et al.*, 2006), full-fat and low-fat cheese analogues (Liu *et al.*, 2008), fresh cheese made from caprine milk (Salvatore *et al.*, 2014) and the acid casein processed cheese analogues (Solowiej *et al.*, 2015). However, studies involving the effects of inulin on reduced-fat fresh cheese, particularly on the physicochemical properties, are very limited. This experimental study thus aims to investigate the effects of inulin, as the partial fat replacer, on the compositional, textural, thermal, and structural properties of reduced-fat fresh cheese. The experimental results are then compared with those of the full-fat cheese and the inulin-free reduced-fat cheese.

## Materials and Methods

### Materials

In this study, pasteurized full-fat milk and reduced-fat milk were obtained from Suranaree University of Technology (Nakhon Ratchasima, Thailand), and the inulin (Fibruline® S20) was from Cosucar-Groupe Warcoing SA, Pecq, Belgium). In addition, rennet (CHY-MAX® Power Extra NB, Chr. Hansen, A/S, Horsholm, Denmark) was used as the milk coagulant following the manufacturer's instructions (0.1 g L<sup>-1</sup> milk).

### Cheese Preparation

Prior to the experiments, 3 types of laboratory-scale fresh cheese were produced: the full-fat cheese with 4% milk fat (control); the reduced-fat cheese with 2.2% milk fat (sample 1), and the reduced-fat cheese with 2.2% milk fat and 4% w/w inulin (sample 2).

In the cheese preparation, the pasteurized milk was first heated to 32°C and the rennet was then added before being allowed to set for 45 min without a starter culture. The curd was cut into 1 cm cubes and left to settle for another 5 min before re-heating to 46°C to facilitate whey expulsion. The mixture was then poured through a cheesecloth-lined colander and allowed to drain for 5 min. The curd was squeezed and then 1 wt% salt added before transferring to an aluminum mold to drain at 4°C for 3 h. The experimental cheeses (i.e. the control, and samples 1 and 2) were refrigerated at 4°C for subsequent analysis on days 1, 4, and 7 of storage.

### Compositional Analysis

The fat and protein contents of the experimental cheeses after 1 day (day 1) of refrigeration were individually analyzed in triplicate using a Milko Scan FT2 (Foss Analytical A/S, Hillerod, Denmark). The fat in dry matter (FDM) was calculated as per Schenkel *et al.* (2013), and the inulin content in the cheese was determined by the extraction and hydrolysis of fructooligosaccharide (Verspreet *et al.*, 2012). The quantification of fructose was carried out using high performance anion exchange chromatography. The analyses were performed in triplicate.

### Textural Analysis

The texture profile analysis was undertaken using a Texture Analyzer (TA. XTPlus, Texture Technologies Corp., Scarsdale, NY, USA and Stable Micro Systems, Ltd., Godalming, UK) equipped with a 100 mm diameter aluminum cylindrical probe. Prior to the analysis, the refrigerated cheeses (25 mm in diameter and 20 mm in height) were tempered at 20°C for 30 min. In the analysis, the pre-test, test, and post-test compression speeds were 1, 5, and 5 mm s<sup>-1</sup>, respectively, while the compression distance, the two-bite time interval, and the trigger force were respectively 10 mm, 5 s, and 5 g. The analyses were carried out in triplicate. The cheeses' hardness, springiness, and cohesiveness were determined using the Texture Exponent Software (version 6.1.7.0, Texture Technologies Corp., Scarsdale, NY, USA and Stable Micro Systems, Ltd., Godalming, UK).

### Thermal Analysis

The thermal analysis was carried out using a differential scanning calorimeter (DSC) (NETZSCH DSC 204 F1 Phoenix®, Erich Netzsch GmbH & Co. Holding KG, Selb, Germany) following the procedure in Lopez *et al.* (2006) with some modifications. In the analysis, 10 mg each of the experimental cheeses after 4 days (day 4) of refrigeration were placed in separate aluminum pans. The pans were then sealed and placed in an automatic sample changer. The cheeses were analyzed according to the following program: (1) heating to 70°C at a rate of 10°C min<sup>-1</sup>, (2) cooling to -30°C at a rate of 5°C min<sup>-1</sup>, and (3) re-heating from -30°C to 70°C at a rate of 5°C min<sup>-1</sup>. The onset temperature (T<sub>o</sub>), peak temperature (T<sub>p</sub>), conclusion temperature (T<sub>c</sub>), and fat melting enthalpy were determined using the NETZSCH Proteus® software. The analyses were performed in duplicate.

### Microstructural Analysis

The microstructural analysis was conducted after 4 days (day 4) of refrigeration using a Quanta 450 environmental scanning electron microscope (ESEM) (FEI Co., Hillsboro, OR, USA). Prior to the analysis, the cheeses were cut into small cuboidal shapes of 1 × 3 × 10 mm in dimension using a scalpel, and cryofixed by immersing in liquid nitrogen. The specimens were then transferred into the microscope chamber where the micrographs were rendered at -5°C, with a 10 kV accelerating voltage, 30% relative humidity, and 1600x to 3000x magnification. The most representative images were selected.

### Statistical Analysis

In this study, all analyses were undertaken either in duplicate or triplicate, and the results expressed as the means ± standard deviations. A one-way analysis of variance (ANOVA) was applied to assess the effects of inulin on the physicochemical properties of the reduced-fat cheese. The Tukey-Kramer multiple comparison was used to compare the mean values given that the significant differences at  $p < 0.05$  were observed. The statistical analysis was carried out using RStudio version 3.2.2 RStudio, Inc., Boston, MA, USA).

## Results and Discussion

### Compositional Analysis

Table 1 tabulates the chemical composition of the experimental cheeses. The total solids, protein, and fat contents were in the ranges of 48.83 - 59.34 wt%, 14.40 - 16.50 wt%, and 17.73 - 31.05 wt%, respectively. Samples 1 and 2 exhibited at least a 25% fat reduction relative to the full-fat cheese (control). Thus, samples 1 and 2 could be classified as reduced-fat cheese according to the U.S. standards (Gunasekaran and Ak, 2003). By comparison, the total solids and fat contents of the control (the full-fat cheese) were significantly higher than those of samples 1 and 2, while the protein content was significantly lower. The results are consistent with Oliveira *et al.* (2011), who documented that the increase in the cheese fat content led to the decrease in the moisture and protein contents.

The analysis results also revealed that the introduction of 4% inulin into the reduced-fat milk prior to coagulation contributed to a retention of 2.4% inulin in the reduced-fat

cheese (sample 2). Furthermore, the addition of inulin increased the total solids and fat content of the reduced-fat cheese, consistent with Juan *et al.* (2013). In Table 2, the fat and protein recovery values of the inulin-containing reduced-fat cheese (sample 2) were significantly higher than those of the inulin-free reduced-fat cheese (sample 1). This is probably due to the formation of a gel network of inulin that helps to retain the fat and protein in the cheese matrix (Franck, 2002).

### Textural Analysis

Table 3 tabulates the hardness, springiness, and cohesiveness of the experimental cheeses on days 1, 4, and 7 of refrigerated storage (4°C). The hardness is a measurement of the maximum force during the first compression, and the springiness is the recovery of deformation after compression. Meanwhile, the cohesiveness refers to the strength of internal bonds, e.g. the protein-protein interactions (Gunasekaran and Ak, 2003).

The analysis revealed that the hardness values of the experimental cheeses after 1 day

**Table 1. Chemical composition of the experimental cheeses after 1 day (day 1) of refrigeration**

Treatments <sup>1</sup>	Chemical compositions (wt%) <sup>2</sup>				
	Total solids	Fat	FDM <sup>3</sup>	Protein	Inulin
Control	59.34 ± 1.19 <sup>b</sup>	31.05 ± 0.30 <sup>c</sup>	52.32 ± 0.50 <sup>c</sup>	14.40 ± 0.26 <sup>a</sup>	ND <sup>4</sup>
Sample 1	48.83 ± 3.27 <sup>a</sup>	17.73 ± 0.04 <sup>a</sup>	36.31 ± 0.09 <sup>a</sup>	16.50 ± 0.04 <sup>b</sup>	ND
Sample 2	50.71 ± 1.50 <sup>a</sup>	19.62 ± 0.09 <sup>b</sup>	38.69 ± 0.17 <sup>b</sup>	16.08 ± 0.09 <sup>b</sup>	2.42 ± 0.0

<sup>1</sup> Control denotes the full-fat cheese; Sample 1 denotes the reduced-fat cheese (inulin-free); Sample 2 denotes the 2.4%-inulin-containing reduced-fat cheese.

<sup>2</sup> The values represent the means of triplicate ± standard deviations. The different letters in the same column represent the difference among treatments at  $p < 0.05$ .

<sup>3</sup> FDM denotes fat in dry matter.

<sup>4</sup> ND denotes not detected.

**Table 2. Recovery of fat and protein in the cheeses based on the chemical composition after 1 day (day 1) of refrigerated storage**

Treatments <sup>1</sup>	Recovery (wt%) <sup>2</sup>	
	Fat	Protein
Control	96.61 ± 0.92 <sup>b</sup>	63.11 ± 1.12 <sup>a</sup>
Sample 1	91.37 ± 0.22 <sup>a</sup>	63.34 ± 0.04 <sup>a</sup>
Sample 2	98.51 ± 0.43 <sup>b</sup>	69.44 ± 0.37 <sup>b</sup>

<sup>1</sup> Control denotes the full-fat cheese; Sample 1 denotes the reduced-fat cheese (inulin-free); Sample 2 denotes the 2.4%-inulin-containing reduced-fat cheese.

<sup>2</sup> The values represent the means of triplicate ± standard deviations. The different letters in the same column represent the difference among treatments at  $p < 0.05$ .

of refrigeration (day 1) were not significantly different. In general, the fat content is inversely correlated to the cheeses' hardness because fats break up the protein structure, resulting in a softer texture. However, the increased moisture content lowered the hardness of the reduced-fat cheese due to the increased plasticization induced by the protein hydration (Gunasekaran and Ak, 2003; Hennelly *et al.*, 2006; Juan *et al.*, 2013). Thus, the lower hardness values of the reduced-fat cheeses (samples 1 and 2) relative to that of the full-fat cheese (control) were attributable to the moisture content (Table 1). Nevertheless, the inulin-free reduced-fat cheese (sample 1) exhibited a significantly stronger texture than did the control, after 4 and 7 days (days 4 and 7) of refrigeration.

Importantly, the introduction of inulin into the reduced-fat cheese (sample 2) restored the hardness to that of the control, indicating the water absorbability of inulin and the subsequent increase in the moisture content. The finding is consistent with the experiments

with reduced-fat fresh cheese (Juan *et al.*, 2013) and Karish cheese (Alnemr *et al.*, 2013). On the other hand, the replacement of fat with inulin gel in the imitation cheese increased the hardness of the finished product (Hennelly *et al.*, 2006).

The springiness and cohesiveness of the experimental cheeses were in the ranges of 0.57-0.81 and 0.73-0.93, respectively. The findings indicated that the introduction of inulin had no impact on the cheeses' springiness and cohesiveness during storage. Specifically, the addition of inulin improved the textural properties of the reduced-fat cheese by restoring the hardness of the finished cheese product to that of the full-fat cheese.

### Thermal Analysis

The thermal profiles of the milk fat in the experimental cheeses were determined using DSC. The cheeses after 4 days (day 4) of refrigeration were first heated to 70°C to melt the milk fat and to eliminate their thermal

**Table 3. The hardness, springiness, and cohesiveness values of the experimental cheeses**

	Days	Control <sup>1</sup>	Sample 1 <sup>2</sup>	Sample 2 <sup>3</sup>
Hardness (N)	1	32.08 ± 3.30	35.51 ± 1.30	33.88 ± 5.69
	4	34.78 ± 1.19 <sup>a</sup>	42.78 ± 2.10 <sup>b</sup>	36.55 ± 3.61 <sup>ab</sup>
	7	39.82 ± 4.41 <sup>a</sup>	53.09 ± 5.37 <sup>b</sup>	40.60 ± 6.15 <sup>a</sup>
Springiness (-)	1	0.57 ± 0.08	0.70 ± 0.10	0.60 ± 0.07
	4	0.71 ± 0.10	0.74 ± 0.06	0.65 ± 0.04
	7	0.76 ± 0.03	0.81 ± 0.10	0.70 ± 0.07
Cohesiveness (-)	1	0.73 ± 0.08	0.84 ± 0.05	0.78 ± 0.10
	4	0.73 ± 0.07	0.85 ± 0.03	0.87 ± 0.02
	7	0.73 ± 0.07 <sup>a</sup>	0.93 ± 0.01 <sup>b</sup>	0.87 ± 0.05 <sup>ab</sup>

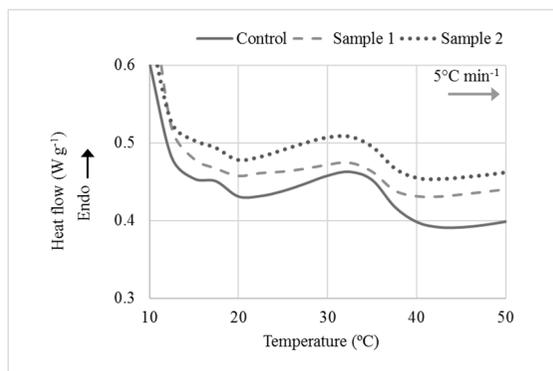
<sup>1,2,3</sup> Control denotes the full-fat cheese; Sample 1 denotes the reduced-fat cheese (inulin-free); Sample 2 denotes the 2.4%-inulin-containing reduced-fat cheese. The values represent the means of triplicate ± standard deviations. The different letters in the same row represent the difference among treatments at  $p < 0.05$ .

**Table 4. Thermal parameters of the milk fat in the experimental cheeses after 4 days (day 4) of refrigerated storage**

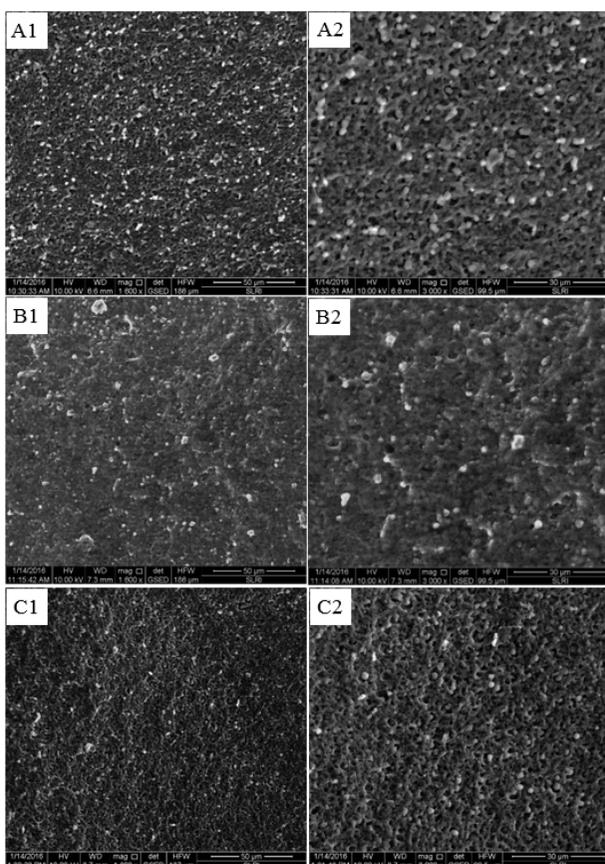
Treatments <sup>1</sup>	Fat melting enthalpy <sup>2</sup> ( $\Delta H$ , J g <sup>-1</sup> )	Conclusion temperature <sup>3</sup> (T <sub>c</sub> , °C)
Control	2.74 <sup>b</sup> ± 0.42	37.80 ± 0.42
Sample 1	1.74 <sup>a</sup> ± 0.22	36.65 ± 0.92
Sample 2	2.36 <sup>b</sup> ± 0.82	37.55 ± 0.07

<sup>1</sup> Control denotes the full-fat cheese; Sample 1 denotes the reduced-fat cheese (inulin-free); Sample 2 denotes the 2.4%-inulin-containing reduced-fat cheese.

<sup>2,3</sup> The values represent the means of triplicate ± standard deviations. The different letters in the same column represent the difference among treatments at  $p < 0.05$ .



**Figure 1.** The DSC thermograms of the full-fat cheese (control), the inulin-free reduced-fat cheese (sample1), and the 2.4%-inulin-containing reduced-fat cheese (sample 2) after 4 days of storage



**Figure 2.** The ESEM images of: (A) the full-fat cheese (control), (B) the inulin-free reduced-fat cheese (sample 1), (C) the 2.4%-inulin-containing reduced-fat cheese (sample 2) after 4 days of storage, where the white specks are the fat globules and the dark area represents the protein network. The magnification and scale bars are 1,600x and 50 µm in length for A1, B1, and C1; and 3000x and 30 µm in length for A2, B2, and C2

history as per the procedure in Lopez *et al.* (2006). The cheeses were then cooled down to  $-30^{\circ}\text{C}$  and re-heated to  $70^{\circ}\text{C}$  at  $5^{\circ}\text{C min}^{-1}$  to obtain the thermal parameters and the fat melting curves, as respectively presented in Table 4 and Figure 1.

In Table 4, no significant differences in the fat melting enthalpy and the conclusion temperature ( $T_c$ ) between the control and sample 2 were observed. In Figure 1, the melting profiles of both cheeses (the control and sample 2) were similar, with the formation of a small shoulder in the temperature range of  $14^{\circ}\text{C} - 20^{\circ}\text{C}$  before the main endothermic peak ( $20^{\circ}\text{C} - 38^{\circ}\text{C}$ ). The observations are agreeable with Lopez *et al.* (2006), who studied the milk fat properties in Emmental cheese and found a small endothermic peak from  $15^{\circ}\text{C}$  to  $20^{\circ}\text{C}$  and a broad endothermic event from  $20^{\circ}\text{C}$  to  $40^{\circ}\text{C}$ . In addition, Tunick and Malin (1997) investigated the cow-milk fat melting profiles in fresh mozzarella cheese and reported a low-temperature melting curve between  $-30^{\circ}\text{C} - 11^{\circ}\text{C}$ , a medium-temperature melting peak at  $17^{\circ}\text{C}$ , and a high melting region from  $21^{\circ}\text{C}$  to  $38^{\circ}\text{C}$ .

However, this experimental study could identify only 1 broad endothermic peak from  $22^{\circ}\text{C}$  to  $37^{\circ}\text{C}$  for sample 1, with a significantly lower fat melting enthalpy than those of the control and sample 2. According to Tunick and Malin (1997), the melting temperature of the milk fat containing a low molecular weight (MW) and unsaturated fatty acids was lower than that with high MW and saturated fatty acids. It could thus be inferred from the experimental results that the reduction in the fat content (sample 1) probably contributed to the variations in the fat composition. In addition, the introduction of inulin into the reduced-fat cheese (sample 2) restored the fat composition to resemble that of the full-fat cheese.

### Microstructural Analysis

The microstructural analysis was carried out after 4 days (day 4) of refrigeration using environmental scanning electron microscopy (ESEM). Figures 2(a1)-(a2) illustrate the micrographs of the control (the full-fat cheese) in which numerous fat globules were distributed within a protein matrix network. In Figures 2(b1)-(b2), the reduction in the fat content

(sample 1) resulted in the decrease in the fat globules. In addition, the protein structure was very compact and showed a less porous matrix compared to the control. The analysis results are consistent with Gunasekaran and Ak (2003), who reported that the reduced fat content altered the microstructure of cheeses. The lower fat globules in the reduced-fat cheese enhanced the formation of cross-links between the protein networks, resulting in a denser structural matrix.

In Figures 2(c1)-(c2), the inulin-containing reduced-fat cheese (sample 2) exhibited a less dense protein structure with numerous pores compared to the inulin-free reduced-fat cheese (sample 1), while the microstructure of sample 2 closely resembled that of the control. The findings are consistent with Meyer *et al.* (2011), who reported that inulin contributed to the increased porosity in the protein matrix network; and with Salvatore *et al.* (2014), who noted that the addition of inulin into the fresh cheese disrupted the formation of protein networks, leading to a more open structural matrix.

### Conclusions

This study has investigated the effects of inulin on the physicochemical properties of reduced-fat cheese and its potential as a partial fat replacer. To this end, the compositional, textural, thermal, and microstructural properties of the inulin-containing reduced-fat cheese were compared with those of the full-fat cheese (control) and the inulin-free reduced-fat cheese. The experimental findings revealed that the introduction of 4% inulin into the reduced-fat milk before coagulation resulted in a retention of 2.4% inulin in the reduced-fat cheese. In addition, the inulin induced the fat and protein recovery in the reduced-fat cheese. The inulin-containing reduced-fat cheese exhibited lower hardness values than the inulin-free reduced-fat cheese but was similar to that of the control. Moreover, the thermal profiles and the microstructural characteristics of the inulin-containing reduced-fat cheese closely resembled those of the full-fat cheese. Thus, the use of inulin as the partial fat replacer contributed to the improved compositional, textural, thermal, and microstructural properties of the reduced-

fat cheese, rendering it comparable to those of the full-fat cheese.

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