CHAPTER-5

Lactose free yogurt with enzymatically modified pea peel dietary fiber: its effect on texture, microstructure and rheological properties

5.1 Introduction

Pea peel constitutes up to 40% of the fresh fruit weight and considered as a by-product. Currently, this by-product used as animal feed, in the incineration process, or in landfills. This causes negative environmental effects by producing methane gas or other strong greenhouse gases [26]. However, pea peel contains a significant amount of dietary fiber [17] which has high functional properties. Early research has shown the successful incorporation of pea peel fiber into bakery products as a flour or sugar substitute and in dairy products to modify their structural properties and enhance their fiber composition and sensorial attributes [13, 20]. Pea peel fiber has also been utilised to enhance or change the microstructure and sensory properties of a variety of food compositions, including dairy products, extruded snacks, and meat supplements [20, 27]. Despite this, studies on the influence of pea peel dietary fiber on the gelation property of milk protein and its functionality in yogurt have not been much explored. Yogurt is one of the famous and highly consumed dairy products. Set-type yogurt is a fermented dairy product with a firm gel texture that is spoonable and slimy. The firmness of set-type yogurt is due to lactic acid bacteria cause the casein micelles in the milk to stick together and form a gel-like structure [26]. Studies have shown that yogurt can work better when it contains dietary fiber and other beneficial substances, which can improve the texture and storage capabilities of the yogurt [15, 19]. Dietary fiber have been used to interact with the casein matrix, which helps to alter the gelation properties of milk. Many studies have focused on the interaction of polysaccharides with casein during fermentation. However, the effect of modified dietary fiber (MDF) on yogurt have not been explored extensively. Previous research has demonstrated the effect of dietary fiber in dairy industries gave huge scope for fiber incorporation. Abdel-Hamid et al. [1] have reported that probiotic yogurt with 2% *Siraitia grosvenorii* fruit extract showed the highest antioxidant property, ACE-I, and antibacterial activities; however, 1% conferred better sensory attributes. Karaca et al. [15] found that adding 1% persimmon and apple fiber to low-fat, full-fat, and fat-free probiotic yogurts improved acidity, water holding capacity, whey separation, viscosity index, color, and promoted probiotic bacteria growth. Likewise, Bertolino et al. [4] reported 6% of hazelnut skin had a significant effect on functional ability when incorporated with yogurt but decreased sensory preference, whereas 3% of hazelnut had maximum consumer acceptance. Espírito-Santo et al. [11] conferred that 1% of passion fruit fiber was incorporated in the preparation of yogurt and divided into four groups according to different probiotic strains had a significant effect on its rheological behaviour. At the end of cold storage, the apparent viscosity in fiber yogurts co-fermented by *lactobacilli* was substantially greater than in control. de Campo et al. [6] also reported the yogurt formulation with zeaxanthin nanoparticles (Zea-NP) and zeaxanthin nanoemulsion (Zea-NE) where, at 28 days of storage zeaxanthin retention was higher in Y-NP (22.31 ± 2.53 %) than in Y-NE (16.84 ± 0.53 %). However, the effect of modified pea peel dietary fiber on yogurt has not been studied yet. Yogurt has numerous health benefits besides the fact that it contains about 30% less lactose than milk, which could still harm lactose-intolerant people [2]. Lactose intolerance caused by a genetic deficiency in the enzyme -galactosidase, which converts lactose to glucose and galactose monomers. Approximately 57% of those who have been tested currently have verified cases worldwide, compared to 65% of those who have estimated cases. [22]. However, there was scanty of research on modified dietary fiber incorporated lactose-free yogurt.

Therefore, the aim of the study was to illustrate the effect of modified dietary fiber on lactose-free yogurt. It was assumed that dietary fiber might interact with milk proteins, thus enhancing the capacity to gel and other functional qualities. This interaction may stabilise yogurt by interacting with milk protein molecules. Therefore, the objective of this research was to characterize the functionality of lactose-free set-type yogurt enriched with enzymatically modified pea peel dietary fiber. The effect of fiber on texture, rheology, and microstructure were investigated. The sensorial and storage stability of developed yogurt were also investigated.

5.2 Materials and methods

5.2.1 Materials

Pea peels and lactose-free skim milk was obtained from Tezpur local market, Assam, India. All other chemicals were purchased from Sigma Aldrich, USA. *Streptococcus thermophilus* ASCC 1275 (*S. thermophilus*) and *Lactobacillus bulgaricus* Lb1466 (*L.*

bulgaricus) was purchased from Sigma Aldrich, USA. Strains were stored at -80°C in MRS broth with 40% glycerol and used to make starter cultures.

5.2.2 Modification of dietary fiber

Pea peel dietary fiber (DF) was extracted using an ultrasound-assisted alkaline extraction method and the condition were NaOH 1.2%, extraction time 30 min, solid to liquid ratio 1: 30, and ultrasonic amplitude 30%. The extracted fiber was modified using cellulase (300 U/g) and xylanase (2500 U/g) enzymes [17, 18]. The extracted modified dietary fiber was dried in a hot air oven for 4 h and further stored at -4°C for analysis.

5.2.3 Lactose-free yogurt

The preparation of set-type yogurt was done according to Mary et al. [19] with slight modifications. The lactose-free milk yogurt were prepared by reconstituting different ratios of modified dietary fiber such as 1%, 1.5%, 2% and 2.5%. For the comparison, yogurt without fiber (C) and yogurt with unmodified fiber (C_{UM}) were prepared. The milk homogenized and subjected to heat treatment at 85°C and cooled down followed by incubation with 1% (v/v) of each *S. thermophilus* ASCC 1275 and *L. bulgaricus* Lb1466 monocultures. The mixes were poured into polystyrene cups and incubated at 42°C until pH of 4.8± 0.06 was achieved. Thereafter, the yogurts were immediately cooled and stored at 4°C for 28 days. Furthermore, it was also taken out at regular 7 days intervals till 28 days and physicochemical analysis was performed.

5.2.4 Prebiotic effects on *L. bulgaricus* and *S. thermophiles*

The pour plate method and serial dilutions in normal saline (0.9% NaCl) was used to cultivate *L. bulgaricus* and *S. thermophiles*. MRS agar (pH 5.4) was used to count *L. bulgaricus*, and the plates were anaerobically incubated at 37°C for 24 h, whereas *S. thermophilus* was counted on M17 agar (pH 7.2), which was aerobically incubated at 37°C for 24 h. A 100 micro litre aliquot was pipetted onto a plate, which was then incubated for 48 h at 37°C. To calculate the cell concentration in CFU/ml, the number of cells was counted [1].

5.2.5 pH, titratable acidity and syneresis

A sample of 10 g yogurt was dissolved in 100 ml of distilled water and keep for 3 min to reach equilibrium at room temperature (25±0°C). Later, pH was determined at 1^{st}, 7^{th}, 14th, 21st, and 28th day during storage at 4°C by pH meter (Model # pH700, EUTECH Intrument, Make # Singapore).

For analysing titratable acidity, the samples were diluted two times with water. Then the diluted yogurts were titrated using 0.1 M NaOH. The data were presented as g of lactic acid/100 g of yogurt and calculated by the following formula:

$$Titratable\ Acidity\ (\%) = \frac{(f \times V \times 0.9)}{W} \quad (1)$$

Where, W is the weights of initial yogurt, f is molarity of NaOH, V is volume of 0.1 mol/L NaOH and 0.9 is scaling parameter for lactic acid.

The syneresis value was determined according to the method described by Dong et al. [10] where syneresis was calculated as supernatant (whey) generated from the gel matrix. A 25 g yogurt sample was centrifuged at 3500 × g for 10 minutes at 4°C at low speed and weight of sediment was measured. The syneresis was calculated as the weight % ratio of sediment mass to total weight of yogurt before centrifugation and expressed as

$$Syneresi(\%) = \frac{Ws}{Wy} \times 100 \quad (2)$$

Where, Ws is the weight of control/supplemented yogurts and Wy is the weight of the supernatant.

5.2.6 Degree of hydrolysis

The o-phthalaldehyde method was used to determine the degree of hydrolysis of the yogurt as described by Abdel-Hamid et al. [1]. Briefly, 5 g of yogurt was mixed with 10 mL of trichloroacetic acid solution (0.75%). After centrifugation at 3000 x g for 15 min at 4°C, the upper layer was filtered through a 0.45 µm syringe filter (Model # Millex GP, Merck Millipore Ltd. Make # Ireland). The filtrate (30 µL) was mixed with 1 mL of o-phthalaldehyde reagent and kept at 24°C for 2 min. The absorbance was measured at 340 nm using a UV/Vis spectrophotometer (Model # Metash UV-800, Make # Shanghai, China). The degree of hydrolysis was expressed as the absorbance of the free NH_3 groups at 340 nm.

5.2.7 Color

The color of the yogurt samples were determined in term $L*$ (lightness), $a*$ (red-green), and $b*$ (yellow-blue) by using a colorimeter (Model # UltraScan VIS, Make # Hunter Lab). The chroma (C*, brightness) is calculated using Eq. (3) and hue angle using Eq. (4).

$$C^* = (a^{*^2} + b^{*^2})^{\frac{1}{2}} \tag{3}$$

$$h = tan^{-1}(\frac{b^*}{a^*}) \tag{4}$$

5.2.8 Texture analysis

The texture of set-type yogurt samples were measured using a texture analyser (Model # TA.HD plus; Make # Stable Micro Systems, UK) with a 25 kg load cell and flat-ended aluminium cylinder probe (SMS P/20). The probe moved down at a pre-test speed of 1 mm/s with a surface trigger force of 5 g. The test speed was 1 mm/s into the yogurt sample for a penetration depth of 10 mm and moved up a post-test speed of 10 mm/s. The firmness was measured from the force vs time graph and expressed as gram (g) [24].

5.2.9 Flow behavior

The flow properties of the yogurt was carried out to study the flow behaviour of the product using a controlled-stress rheometer (Model # Physical MCR 301; Make # Antron Paar) at 25± 0.1°C. The plate and plate geometry having 50 mm in diameter with 1 mm gap was used to measure the properties. The ratio of shear stress (σ) and shear rate data was fitted with Power law (Eq. 5) model.

$$\tau = K.(\,\gamma)^n \tag{5}$$

Where, τ is parameters shear stress, n is flow behaviour index, and K is consistency coefficient were used to characterize the flow behaviour of yogurt samples.

5.2.10 Dynamic rheology

To oscillatory behaviour of the yogurt was study using a controlled-stress rheometer (Model # Physical MCR 301; Make # Antron Paar) at 25± 0.1°C. A probe with 50 mm dia was used for the analysis. The sample was placed between the plates having a gap of 1mm, and edges of samples were cautiously trimmed out with a spatula.

Frequency sweep experiments (mechanical spectra) between 0.1 and 30Hz were performed to obtain 100 data sets. The data sets comprised storage modulus (G′), loss modulus (G″). Each experiment was repeated thrice.

5.2.11 Microstructure

The microstructure of yogurt was studied using a scanning electron microscope. Briefly, yogurt was lyophilized using a freeze dryer (Model # JSM-6390LV, Make # JEOL, Japan). The dried powder mounted on an aluminum stub with a double-sided adhesive carbon tape and sputtered with gold (up to 15 nm). The image were captured at 1000 magnification.

5.2.12 Sensory properties

The organoleptic attributes of the yogurt was assessed by 30 trained panellists from Tezpur University, Assam, India. The panellists were asked to rate the yogurt samples based on flavor, appearance, texture, and overall acceptability on a scale of 1 to 9, where 1 representing the least acceptability of product and 9 representing the most acceptability of product. All the samples were in random order in polystyrene plastic cups.

5.2.13 Statistical analysis

The Origin 8.5 (Origin Lab Corporation, Northampton, USA) software was used for graphs. Duncan's test was performed using SPSS Statistics 17.0 (IBM, Chicago, USA) software after the triplicate experiment data was collected. The significant at $p \leq 0.05$ was expressed by separate letters in superscript.

5.3 Results and discussion

5.3.1 Incubation time, viable count and degree of hydrolysis

The incubation time of yogurt was decreased due to the addition of modified pea peel fiber. The yogurt containing 2.5% of pea peel fiber (Y_4) took 5 h and 10 min to reach pH 3.94 followed by a 2% pea peel fiber (Y_3) containing sample that took 5 h and 75 min to reach pH 3.94. Likewise, 1.5% pea peel fiber (Y_2) took 6 h and 80 min to reach pH 3.75. It was observed that incubation time was gradually decreased and the pH increased by the addition of modified pea peel fiber concentration, whereas the control sample showed 7 h and 55 min incubation time to reach pH 3.96. The decrease in incubation time is due to higher amount of availability of simple sugars from modified dietary fiber. This result likely due to pea peel fiber may provide additional source of carbohydrates, proteins, phytochemicals, vitamins, and minerals that help the growth of yogurt cultures in acid production. A similar observation was reported by Abdel-Hamid et al. [1] that the addition of fiber to yogurt accelerated the fermentation process. This could be due to a link between nutritional compounds such as fiber, protein, fats and minerals found in food by-products. Due to faster rate of acidification, colloidal calcium phosphates are released from casein micelles more quickly, which promotes the early formation of the casein network. This causes rapid protein aggregation, which results in the formation of those few protein-protein interactions and significant rearrangement of the particles/clusters. As a result, a weak gel with wide pores which enhanced whey separation.

The viable counts for *L. bulgaricus* and *S. thermophilus* were performed after 1 day of storage, as shown in Table 5.1. The pea peel fiber concentration showed no significant effect on *S. thermophiles* counts ($p \geq 0.05$) whereas the *L. bulgaricus*, count steadily increased with increase in fiber concentration ($p \leq 0.05$). The *L. bulgaricus* and *S. thermophiles* count was satisfactory because lactic acid bacteria count must be of > 7 log CFU/g in yogurt to ensure health benefit [5]. Pea peel fiber indicating higher survival of this probiotic in yogurt could be due to related to metabolization of free sugars and soluble fiber ingredients by added probiotic culture with stimulation of its growth over time [17]. The recommended dose is at least 8–9 log CFU/g or mL, which can be achieved by consuming at least 100 g of a food with counts between 6 and 7 log CFU/g or mL [25]. Similar result was found by Sah et al. [24] where pineapple peel powder had no significant ($p \leq 0.05$) effect on *S. thermophiles* count. The *L. bulgaricus* count ranged from 9.01 to 9.93 log CFU/g, whereas the *S. thermophiles* count decreased from 10.60 to 10.50 after Day 1 (Table 1), indicating that *L. bulgaricus* was somewhat reduced to *S. thermophilus*. The 1% fiber has no significant ($p \geq 0.05$) effect on *L. bulgaricus* where as 2% to 2.5% showed stimulated growth of *L. bulgaricus* in probiotic yogurt. These findings suggested that the bacterial strain and supplement had an impact on the prebiotic effect of supplements on the bacterial count of probiotic yogurts.

It can be seen in Table 5.1, degree of hydrolysis values significantly ($p \leq 0.05$) increased by increasing the amount of dietary fiber. This observation may be attributed to the pea peel fiber-induced growth rates of *L. bulgaricus* and *S. thermophilus*, which can hydrolyze milk protein. These findings are in agreement with Abdel-Hamid et al. [1], who observed a significant increase in DH when 2% SGF extract was supplemented in probiotic yogurt.

Table 5.1 Viable count, degree of hydrolysis and incubation time of lactose-free yogurt with fiber during refrigerated storage

Sample	Prebiotic (Pea peel dietary fiber)	Culture combinations of lactose-free set-type yogurt	Viable count log CFU/g		Degree of Hydrolysis (At 340 nm)	Incubation time (h)
			L. bulgaricus	*S. thermophiles*		
C	Without fiber	*S. thermophilus* + *L. bulgaricus*	9.01±0.1[a]	10.60±0.19[a]	0.26±0.003[a]	7.55±0.24[e]
C_{UM}	UnMDF (1.0% w/v)	*S. thermophilus* + *L. bulgaricus*	9.03±0.10[a]	10.50±0.12[a]	0.34±0.001[b]	7.01±0.05[d]
Y_1	MDF (1.0% w/v)	*S. thermophilus* + *L. bulgaricus*	9.05±0.12[a]	10.55±0.19[a]	0.48±0.006[c]	7.05±0.21[d]
Y_2	MDF (1.5% w/v)	*S. thermophilus* + *L. bulgaricus*	9.42±0.18[b]	10.54±0.18[a]	0.57±0.006[d]	6.80±0.19[c]
Y_3	MDF (2.0% w/v)	*S. thermophilus* + *L. bulgaricus*	9.85±0.15[c]	10.54±0.17[a]	0.81±0.007[e]	5.75±0.15[b]
Y_4	MDF (2.5% w/v)	*S. thermophilus* + *L. bulgaricus*	9.93±0.13[c]	10.53±0.15[a]	0.89±0.007[f]	5.10±0.14[a]

Results are represented as mean ± standard deviation (n = 3). Means of the same parameter in the same column with different lowercase superscript letters indicate significant differences ($P \leq 0.05$).

C: lactose free yogurt without added fiber; C_{UM}: lactose free yogurt with 1.0% (w/v) unmodified fiber; Y_1: lactose free yogurt with 1.0% (w/v) modified fiber; Y_2: lactose free yogurt with 1.5% (w/v) modified fiber; Y_3: lactose free yogurt with 2.0% (w/v) modified fiber, Y_4: lactose free yogurt with 2.5% (w/v) modified fiber.

Table 5.2 pH and TA (%) in control and fiber enriched lactose free yogurts over the storage period of 28 days at 4℃

Yogurt types	pH					TA (%)				
	Day 1	Day 7	Day 14	Day 21	Day 28	Day 1	Day 7	Day 14	Day 21	Day 28
C	3.81±0.01^{a}	3.72±0.01^{a}	3.67±0.01^{a}	3.53±0.01^{a}	3.47±0.01^{a}	1.31±0.05^{c}	1.37±0.02^{c}	1.42±0.03^{e}	1.98±0.05^{e}	1.95±0.04^{d}
C_{UM}	4.27±0.04^{d}	4.23±0.04^{d}	4.16±0.03^{d}	4.09±0.03^{d}	4.01±0.03^{d}	1.28±0.04^{b}	1.29±0.01^{b}	1.32±0.02^{b}	1.35±0.01^{b}	1.40±0.01^{b}
Y_1	3.83±0.02^{a}	3.79±0.01^{b}	3.72±0.01^{b}	3.67±0.01^{b}	3.60±0.01^{b}	1.27±0.05^{b}	1.36±0.02^{c}	1.39±0.04^{d}	1.41±0.03^{c}	1.49±0.02^{c}
Y_2	3.85±0.01^{a}	3.80±0.02^{b}	3.75±0.01^{b}	3.68±0.02^{b}	3.61±0.01^{b}	1.25±0.02^{b}	1.29±0.03^{b}	1.36±0.04^{c}	1.48±0.03^{d}	1.50±0.03^{b}
Y_3	3.88±0.02^{b}	3.81±0.02^{b}	3.76±0.02^{b}	3.70±0.02^{b}	3.63±0.02^{b}	1.17±0.03^{a}	1.24±0.02^{b}	1.31±0.02^{b}	1.35±0.02^{b}	1.37±0.02^{a}
Y_4	3.92±0.03^{c}	3.89±0.03^{c}	3.82±0.03^{c}	3.77±0.01^{c}	3.71±0.02^{c}	1.15±0.01^{a}	1.19±0.04^{a}	1.24±0.01^{a}	1.32±0.01^{a}	1.36±0.02^{a}

Results are represented as mean ± standard deviation (n = 3). Means of the same parameter in the same column with different lowercase superscript letters indicate significant differences ($P \leq 0.05$).

C: lactose free yogurt without added fiber; C_{UM}: lactose free yogurt with 1.0% (w/v) unmodified fiber; Y_1: lactose free yogurt with 1.0% (w/v) modified fiber; Y_2: lactose free yogurt with 1.5% (w/v) modified fiber; Y_3: lactose free yogurt with 2.0% (w/v) modified fiber, Y_4: lactose free

5.3.2 pH, syneresis and titration acidity

During the storage, the pH of yogurts varied from 3.47 to 4.27 as observed in Table 5.2. The pH of the lactose-free yogurt with modified fiber was higher than the control sample. In general, all the yogurt formulations showed decrease in pH value ($p \leq 0.05$) during the 28 days of storage. These findings should be associated with the bacterial metabolism that breaks down simple sugars during yogurt fermentation and storage to produce lactic acid and other organic acids [24, 25]. The decrease in pH in yogurts over time should also be related to the production of organic acids by yogurt starter culture, but especially by probiotic culture added [1].

Syneresis is a mechanism to reflect the shrinkage of the gel and the extent of whey separation from yogurt, which is due to the weakening of protein gel network made up entirely of casein aggregates in the fermentation of yogurt [19]. On day 1 after fermention, the syneresis for the control sample was found to be 2.89%. In presence of modified dietary fiber, the syneresis decline significantly ($p \leq 0.05$) as compared to control yogurt (Table 5.3). This may be owing due to the action of insoluble fiber fractions in immobilizing whey inside the casein network and delaying syneresis. However, yogurt with unmodified fiber reported higher syneresis as compared to other yogurt samples with modified fiber. At day 21, all samples except the control exhibited a decrease in syneresis, but on day 28, all samples released more whey, increasing the syneresis score. This might be because all of the yogurt gels shrank during storage (from day 7 to day 28). Faster acidification induces the colloidal calcium phosphate release from casein micelles which causes early casein network with rapid protein aggregation thus rearrangement of clusters promote weak gel with wide pores hence cause more syneresis in the presence of pea peel fiber. The Y_2 sample demonstrated improved attributes by lowering syneresis over time might be due to 1.5% modified fiber would be the optimum concentration to show less seneresis value, which may be used commercially.

The TA of all types of yogurt increased with a similar tendency as the decline in pH value ($p \leq 0.05$) with an increase in storage. The addition of different ratios of modified fiber significantly affects the TA of yogurts compared with control yogurt at the same storage condition. The TA was detected as 1.31% for the control sample C whereas it decreased with the addition of pea peel dietary fiber (Table 5.2). Yogurt enriched with

1% unmodified DF (C_{UM}) reported 1.27% TA. It started decreasing as the concentration of fiber increased by 2.5% dietary fiber (Y_4). The highest titration acidity was detected on day 28 (1.95±0.04%) for the control sample and lowest for Y_4 (1.36±0.02). A similar kind of data was reported by Karaca et al. [15] for probiotic set-type yogurts enriched with fiber-rich persimmon and apple powders and Basiri et al. [3] for mucilage-free flaxseed powder rich stirred type yogurt. The decline in pH and increase of TA during the storage period was attributed to the continuous growth and production of lactic acid by lactic acid bacteria. Increasing concentration of insoluble dietary fiber and available nutrients present in pea peel fiber can promote lactic acid bacteria growth and acid production [3].

Table 5.3 Syneresis (%) in control and fiber enriched lactose free yogurts over the storage period of 28 days at 4°C

Yogurt types	Syneresis (%)				
	Day 1	**Day 7**	**Day 14**	**Day 21**	**Day 28**
C	$2.89±0.04^d$	$2.76±0.04^e$	$2.14±0.02^e$	$2.13±0.02^e$	$2.54±0.03^e$
C_{UM}	$1.92±0.02^c$	$1.88±0.02^d$	$1.82±0.04^d$	$1.85±0.03^d$	$1.90±0.02^d$
Y_1	$1.91±0.06^c$	$1.89±0.02^d$	$1.80±0.02^c$	$1.78±0.02^c$	$1.87±0.04^c$
Y_2	$1.89±0.06^b$	$1.84±0.01^c$	$1.79±0.04^b$	$1.75±0.02^b$	$1.78±0.03^a$
Y_3	$1.87±0.02^a$	$1.82±0.01^b$	$1.79±0.01^b$	$1.72±0.02^a$	$1.79±0.03^a$
Y_4	$1.85±0.02^a$	$1.76±0.01^a$	$1.70±0.02^a$	$1.72±0.01^a$	$1.81±0.01^b$

C: lactose free yogurt without added fiber; C_{UM}: lactose free yogurt with 1.0% (w/v) unmodified fiber; Y_1: lactose free yogurt with 1.0% (w/v) modified fiber; Y_2: lactose free yogurt with 1.5% (w/v) modified fiber; Y_3: lactose free yogurt with 2.0% (w/v) modified fiber, Y_4: lactose free yogurt with 2.5% (w/v) modified fiber.

5.3.3 Color

Color is an essential element in food since it is the first thing that consumers notice and consequently determines their preferences. Lightness (L*), chroma (C*) and hue angle (h) of control and yogurts with dietary fiber were presented in Table 4, stored at 4°C for 28 days. The color parameters significantly ($p \leq 0.05$) differed with the addition of pea peel dietary fiber, with different concentrations because of its pigmentation. Throughout the storage period, the L* values of control yogurt were considerably greater than yogurts with dietary fiber. Incorporation of lactose free milk and pea peel dietary fiber increased the color intensity (C*), color perception (h) shifted towards yellow over the period of time might be due to carotenoid pigment in lactose free milk and rapid fermentation due to pea peel fiber which promotes lactic acid bacterial count. The Y_1 showed no substantial effect on L*, C* value because very less mount of fiber was added compared to control sample, which has the white color and partially blended with the yogurt color. All the yogurt samples prepared with lactose free milk showed slight yellowish color that could be due to carotenoid present in the milk thus resulted in higher b* values.

Table 5.4 Color parameters of lactose free yogurt samples enriched with different ratio of pea peel fiber

	Color Values				
Day	L	a*	b*	Chroma (C*)	h
C					
1	86.64± 0.23^{b}	3.77± 0.05^{c}	10.85± 0.13^{a}	11.48± 0.17^{a}	70.83± 0.21^{a}
7	86.02± 0.21^{b}	3.78± 0.03^{c}	10.91± 0.06^{a}	11.54± 0.18^{a}	70.89± 0.25^{a}
14	85.34± 0.32^{a}	3.63± 0.02^{b}	10.98± 0.04^{b}	11.56± 0.18^{a}	71.70± 0.21^{b}
21	85.11± 0.21^{a}	3.65± 0.02^{b}	11.27± 0.08^{c}	11.84± 0.07^{b}	72.05±0.23^{c}
28	85.03± 0.15^{a}	3.56± 0.02^{a}	11.43± 0.08^{d}	11.97± 0.08^{c}	72.70± 0.22^{c}
C_{UM}					
1	81.87± 0.72^{b}	3.26± 0.01^{c}	18.23± 0.18^{a}	18.51± 0.16^{a}	79.86± 0.71^{a}
7	81.05± 0.72^{b}	3.21± 0.01^{c}	18.34± 0.15^{b}	18.61± 0.14^{b}	80.07± 0.72^{b}
14	79.88± 0.71^{a}	3.15± 0.01^{b}	18.44± 0.17^{c}	18.70± 0.17^{c}	80.30± 0.71^{c}
21	79.76± 0.71^{a}	3.12± 0.02^{b}	18.58± 0.15^{d}	18.84± 0.17^{d}	80.46± 0.70^{d}
28	79.65± 0.70^{a}	3.05± 0.01^{a}	18.65± 0.15^{e}	18.89± 0.17^{d}	80.71± 0.71^{e}
Y_1					
1	85.62± 0.15^{b}	3.08± 0.02^{c}	11.92± 0.12^{a}	12.31± 0.21^{a}	75.51± 0.25^{a}
7	85.98± 0.23^{b}	2.96± 0.02^{b}	11.98± 0.11^{a}	12.34± 0.20^{b}	76.12± 0.21^{b}
14	84.61± 1.62^{a}	2.93± 0.02^{b}	12.13± 0.13^{b}	12.47± 0.18^{c}	76.42± 0.21^{b}
21	84.23± 1.24^{a}	2.85± 0.01^{a}	12.34± 0.13^{c}	12.66± 0.18^{d}	76.99± 0.20^{b}
28	84.01±0.15^{a}	2.81± 0.01^{a}	12.56± 0.14^{d}	12.87± 0.19^{e}	77.38± 0.20^{c}
Y_2					
1	81.27± 0.75^{b}	3.15± 0.02^{c}	16.83± 0.16^{a}	17.12± 0.17^{a}	79.39± 0.71^{a}
7	81.10± 0.62^{b}	3.01± 0.02^{c}	17.04± 0.17^{b}	17.30± 0.10^{a}	79.98± 0.72^{b}
14	80.87± 0.71^{a}	2.88± 0.01^{b}	17.32± 0.17^{c}	17.55± 0.12^{b}	80.55± 0.75^{c}
21	80.65± 0.71^{a}	2.85± 0.01^{b}	17.76± 0.18^{d}	17.98± 0.15^{c}	80.88± 0.75^{c}
28	80.32± 0.70^{a}	2.74±0.01^{a}	17.90± 0.18^{e}	18.10± 0.15^{d}	81.29± 0.71^{d}
Y_3					
1	80.05± 0.70^{a}	2.93± 0.02^{d}	15.55± 0.15^{a}	15.82± 0.09^{a}	79.32± 0.70^{a}
7	80.34± 0.71^{a}	2.86± 0.02^{c}	15.98± 0.12^{a}	16.23± 0.12^{b}	79.85± 0.71^{a}
14	80.98± 0.71^{b}	2.85± 0.02^{c}	16.23± 0.16^{b}	16.47± 0.12^{b}	80.04± 0.71^{b}
21	81.34± 0.73^{b}	2.63± 0.01^{b}	16.56± 0.11^{b}	16.76± 0.13^{c}	80.97± 0.70^{c}
28	81.56± 0.72^{b}	1.03± 0.01^{a}	16.87± 0.12^{c}	16.90± 0.11^{d}	86.50± 0.72^{d}
Y_4					
1	79.68± 0.70^{a}	3.55± 0.01^{c}	18.71± 0.17^{a}	19.04± 0.17^{a}	79.25± 0.75^{a}
7	79.89± 0.69^{a}	3.51± 0.01^{c}	18.98± 0.17^{a}	19.30± 0.17^{b}	79.52± 0.77^{b}
14	80.32± 0.71^{b}	3.47± 0.03^{b}	19.14± 0.17^{b}	19.45± 0.18^{c}	79.72± 0.76^{c}
21	80.43± 0.72^{b}	3.45± 0.02^{b}	19.24± 0.15^{c}	19.54± 0.18^{d}	79.83± 0.75^{d}
28	80.59± 0.75^{b}	3.40± 0.01^{a}	19.09± 0.17^{b}	19.39± 0.17^{c}	79.90± 0.71^{e}

C: lactose free yogurt without added fiber; C_{UM}: lactose free yogurt with 1.0% (w/v) unmodified fiber; Y_1: lactose free yogurt with 1% (w/v) modified fiber; Y_2: lactose free yogurt with 1.5% (w/v) modified fiber; Y_3: lactose free yogurt with 2% (w/v) modified fiber, Y_4: lactose free yogurt with 2.5% (w/v) modified fiber.

5.3.4 Texture profile analysis

Yogurt is a colloidal dispersion of casein aggregates created by the disulfide bonds between k-caseins and denatured whey proteins when the pH is reduced. Casein micelles come together to form a three-dimensional structure that gives the yogurt a semi-solid texture. Initially the firmness index of Y_1 was higher (1.25±0.053 N) than Y_2, Y_3 and Y_4. Until day 21 of storage, all yogurt samples exhibited an increase in the amount of force required to break the yogurt gel as modified dietary fiber might act as stabilizers and showed an increase in the viscoelasticity of the gel in yogurts [12, 28]. At day 28 of storage, all yogurt samples exhibited a minor drop in breaking point (Table 5.5). Increased firmness in sample Y_2, Y_3 and Y_4 which had maximum concentration of modified fiber may be owing to an increase in total solids, reinforcing, and improving the interaction between casein aggregates and the fiber network, resulting in a firmer yogurt gel after storage at 4°C. Moreover, presence of modified dietary fiber lead to more moisture absorption and a more robust protein network, could increase the firmness [3, 12]. It might be linked to a further reduction in pH, which causes the gel structure to shrink, and increases gel strength. The increased gel strength may reveal dynamic network features that make structural rearrangements more likely [9]. A similar kind of result was reported with the incorporation of fruit by-products like passion fruit peel powder and carrot cell wall particles into yogurt [8, 23].

The set-type yogurts should be spoonable, hard, and free of slimy texture. Cohesiveness is an important parameter for set-type yogurts because it measures how much force is needed to remove yogurt from a spoon or lips while eating [24]. Except for the control sample and Y_1, cohesiveness increased during storage. Springiness is the capacity of the yogurt gel to restore its structure (in mm) following the penetration test and demonstrates textural integrity [21]. When compared to control yogurt with no fiber on day one of storage, the yogurt sample with fiber had higher springiness values. Springiness values were shown to rise in all yogurt samples during the storage. The fall in pH and metabolic activity of starter cultures, which finally leads to structural shrinkage and rearrangement, might explain the rise in springiness and cohesiveness of fiber-supplemented yogurt after storage [24]. Similar to our findings, other researchers have shown that yogurt's textural properties improve with storage time [24, 26].

Table 5.5 Texture profile analysis of plain and fiber enriched yogurts over 28 days of storage at 4°C

Parameter	Sample	Storage period (Days)				
		1	**7**	**14**	**21**	**28**
Firmness (N)	C	1.80±0.03^{e}	1.68±0.01^{d}	1.70±0.01 ^{d}	1.94±0.01^{e}	1.89±0.02^{c}
	C_{UM}	1.07±0.01^{b}	1.23±0.01^{b}	1.29±0.02 ^{b}	1.35±0.02^{c}	1.21±0.01^{a}
	Y_1	1.25±0.05^{d}	1.40±0.04^{c}	1.47±0.02 ^{c}	1.61±0.05^{d}	1.59±0.01^{b}
	Y_2	1.15±0.04^{c}	1.85±0.06^{e}	1.90±0.03^{f}	1.86±0.05^{e}	2.06±0.07^{d}
	Y_3	0.09±0.001^{a}	0.67±0.04^{a}	1.74±0.02 ^{e}	0.46±0.03^{a}	2.74±0.08^{e}
	Y_4	0.05±0.001^{a}	0.76±0.05^{a}	0.82±0.01 ^{a}	0.96±0.08^{b}	1.64±0.01^{b}
Cohesiveness (N)	C	0.45±0.06^{a}	0.74±0.09^{a}	0.76±0.01 ^{a}	0.67±0.07^{a}	0.81±0.08^{a}
	C_{UM}	2.06±0.05^{c}	2.32±0.05^{c}	2.38±0.05 ^{d}	2.42±0.07^{c}	2.48±0.07^{c}
	Y_1	1.15±0.08^{b}	1.49±0.07^{b}	1.53±0.04 ^{b}	1.65±0.08^{b}	0.84±0.01^{a}
	Y_2	1.37±0.09^{b}	1.67±0.08^{b}	1.74±0.08 ^{c}	1.81±0.09^{b}	2.01±0.09^{b}
	Y_3	2.04±0.11^{c}	2.67±0.10^{c}	2.81±0.09 ^{e}	2.90±0.09^{d}	3.01±0.12^{d}
	Y_4	2.82±0.12^{d}	3.01±0.16^{d}	3.32±0.15f	3.59±0.17^{e}	3.98±0.17^{e}
Springiness (mm)	C	0.53±0.003^{a}	0.54±0.004^{b}	0.54±0.005 ^{a}	0.55±0.004^{a}	0.56±0.005^{a}
	C_{UM}	0.53±0.001^{a}	0.54±0.001^{b}	0.56±0.001 ^{b}	0.58±0.001^{b}	0.61±0.001^{b}
	Y_1	0.52±0.003^{a}	0.53±0.004^{a}	0.61±0.005 ^{d}	0.69±0.005^{d}	0.85±0.006^{d}
	Y_2	0.55±0.004^{b}	0.52±0.003^{a}	0.59±0.004 ^{c}	0.62±0.004^{c}	0.64±0.001^{b}
	Y_3	0.51±0.003^{a}	0.52±0.003^{a}	0.56±0.004 ^{b}	0.61±0.004^{c}	0.77±0.005^{c}
	Y_4	0.52±0.002^{a}	0.55±0.004^{b}	0.56±0.005 ^{b}	0.58±0.004^{b}	0.78±0.006^{c}

C: lactose free yogurt without added fiber; C_{UM}: lactose free yogurt with 1.0% (w/v) unmodified fiber; Y_1: lactose free yogurt with 1% (w/v) modified fiber; Y_2: lactose free yogurt with 1.5% (w/v) modified fiber; Y_3: lactose free yogurt with 2% (w/v) modified fiber, Y_4: lactose free yogurt with 2.5% (w/v) modified fiber.

5.3.5 Flow behavior

The flow behavior of the yogurts was determined with the help of a power law model. The shear stress (τ) versus shear rate (γ) data of the yogurts (Fig 5.1) were fitted with power law model, and the data were summarized in Table 5.6. The flow behaviour index was less than 1, which implies that the yogurt behaves like shear thinning, where the viscosity decreases under shear strain. The structural network in the yogurt partially breaks down and rearranges under shared stress conditions, causing the viscosity of the sample to decrease. The flow behavior index of the control sample was 0.257. However, with the incorporation of fiber the flow behavior index of the yogurt varied markedly for Y_1 and Y_2, the flow behviour index increases may be due to the formation of the strong network at low fiber concentration whereas, the flow behavior index of yogurt decreases with an increase in fiber concentration from 2 to 2.5%. With the increase in fiber concentration in the yogurt, the supramolecular interaction in the yogurt became weak, making it easy to break the structure at shear strain, thereby decreasing the flow behavior index [19].

5.3.6 Dynamic rheology

The oscillatory rheological behviour of the yogurts were studied in term of storage (G') and loss (G") module (Fig 5.2). The rheological behaviour of yogurt is a result of the formation of a three-dimensional network composed of casein and denatured whey protein. Mild acidification leads to the formation of aggregates by way of hydrophobic and electrostatic bonds, resulting in the gel structure. The firmness of a yogurt gel is related to the total solids content and type of protein. The addition of pea peel dietary fiber influenced the rheological behaviour of the yogurt ($p < 0.05$). The G' and G" curves of the yogurt without fiber showed greater values than the yogurts added with fiber. However, the variation in dietary fiber concentration ranged from 1% to 2.5% and had no significant influence. This behaviour suggests that the addition of dietary fiber interferes with the formation of the protein network, decreasing the gel strength. Dietary fiber or structure breaker prevents the formation of a protein network by forming the complexes with protein aggregates of yogurt by a hydrogen bond [11]. The G' value was higher than G", implying that yogurt had a rigid solid-like structure The Y_2 sample showed the highest rigidity and the control sample showed the lowest rigidity.

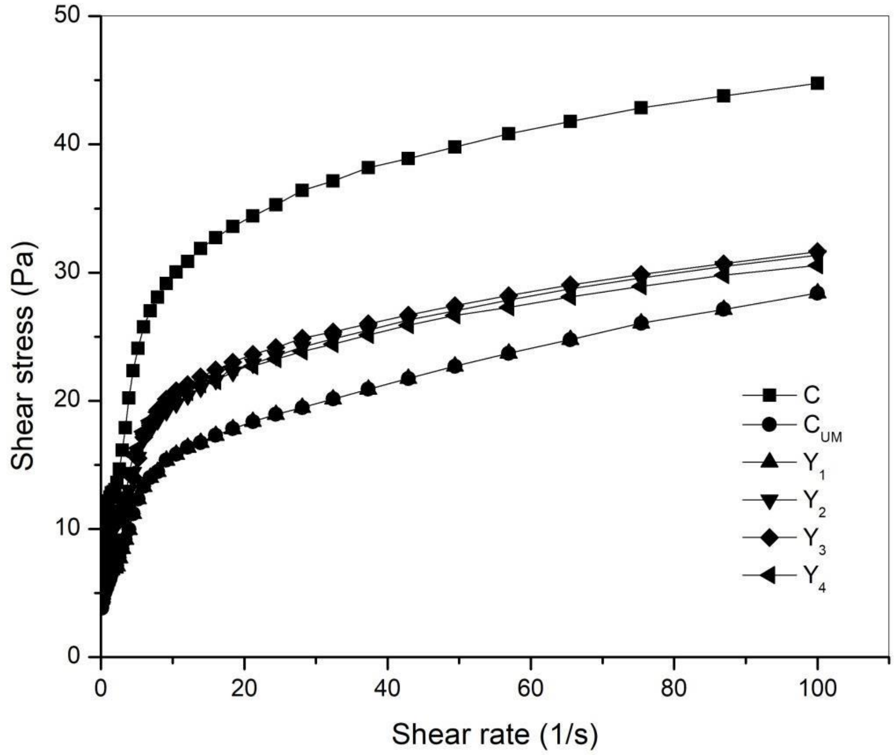


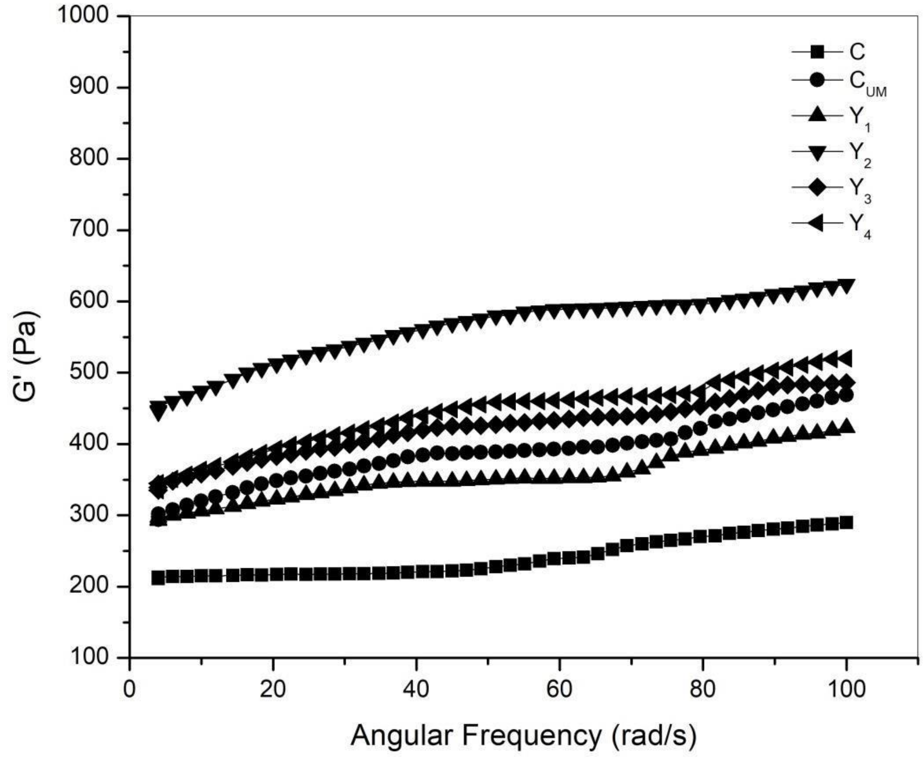
Fig 5.1 Flow curves (shear stress vs. shear rate) of lactose free set type yogurt enriched with pea peel modified and unmodified dietary fiber

C: lactose free yogurt without added fiber; C_{UM}: lactose free yogurt with 1.0% (w/v) unmodified fiber; Y_1: lactose free yogurt with 1% (w/v) modified fiber; Y_2: lactose free yogurt with 1.5% (w/v) modified fiber; Y_3: lactose free yogurt with 2% (w/v) modified fiber, Y_4: lactose free yogurt with 2.5% (w/v) modified fiber.

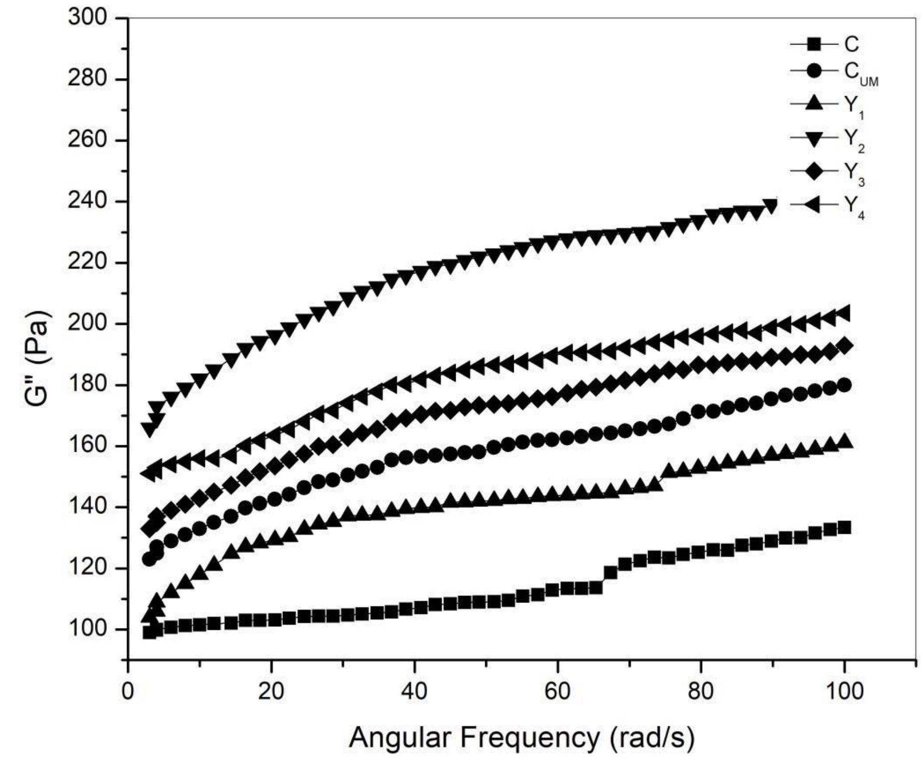
Table 5.6 Flow behaviour of the yogurts was determined using power law model

Sample	n	K (Pa s^n)	R^2	χ^2	RMSE
C	0.257	14.685	0.960	5.565	2.359
C_{UM}	0.316	6.755	0.985	0.816	0.903
Y_1	0.314	6.785	0.984	0.849	0.921
Y_2	0.262	9.773	0.979	1.353	1.163
Y_3	0.238	10.857	0.968	1.900	1.378
Y_4	0.221	11.233	0.969	1.583	1.258

C: lactose free yogurt without added fiber; C_{UM}: lactose free yogurt with 1.0% (w/v) unmodified fiber; Y_1: lactose free yogurt with 1% (w/v) modified fiber; Y_2: lactose free yogurt with 1.5% (w/v) modified fiber; Y_3: lactose free yogurt with 2% (w/v) modified fiber, Y_4: lactose free yogurt with 2.5% (w/v) modified fiber.



(a)



(b)

Fig 5.2 Frequency sweep rheograms by changes of G' and G'' of lactose free set type yogurt (a) storage modulus and (b) loss modulus

5.3.7 Microstructure

To better comprehend the structure of the casein network, SEM micrographs of each yogurt samples were taken. Between control and fiber-enriched yogurts, SEM analyses showed subtle differences in the compactness of gel structure, pore diameters, and the three-dimensional network of casein micelles seen in Fig 5.3. The typical microstructure of the casein network in plain yogurt could be displayed by globular shapes of casein micelle aggregation are interspersed by void zones filled with whey in a three-dimensional network [19, 16]. Control yogurt showed a high number of crosslinks between casein micelles and pores marking the initially whey-filled cavities. Yogurt was significantly affected by fiber addition with regard to structure formation and gel characteristics depending on fiber size and concentration [24]. The fiber particles might also serve as anchor points to help casein aggregate and form networks at low fiber concentrations. SEM micrographs of yogurts with added fiber Y_1 show that casein micelles adhere to fiber particles, which showed that at low fiber concentrations, casein network stabilisation occurs which hindered to a certain extent due to steric effects caused by the existent fiber network. As a result more compact casein clusters are formed by pea peel fiber; Y_1, Y_2, Y_3 and Y_4 as shown in Fig 5.3. Similar effects were observed by Espírito-Santo et al. [11] and Kieserling, et al. [16] in yogurts supplemented with passion fruit fiber and pectin rich orange fiber. The casein network was interspaced by void zones filled with whey and attached with starter cultures in a control yogurt micrograph. The reduced hardness and increased syneresis found in plain yogurt during storage might be explained by the poor cross-linking observed between casein micelles and the existence of larger pores [24, 19]. Yogurt containing dietary fiber appeared to have more organized and dense molecular packing. Furthermore, when the concentration of fibers increased, the size of network holes decreased. The starter cultures seemed to be entrenched in the enriched yogurt's fiber network.

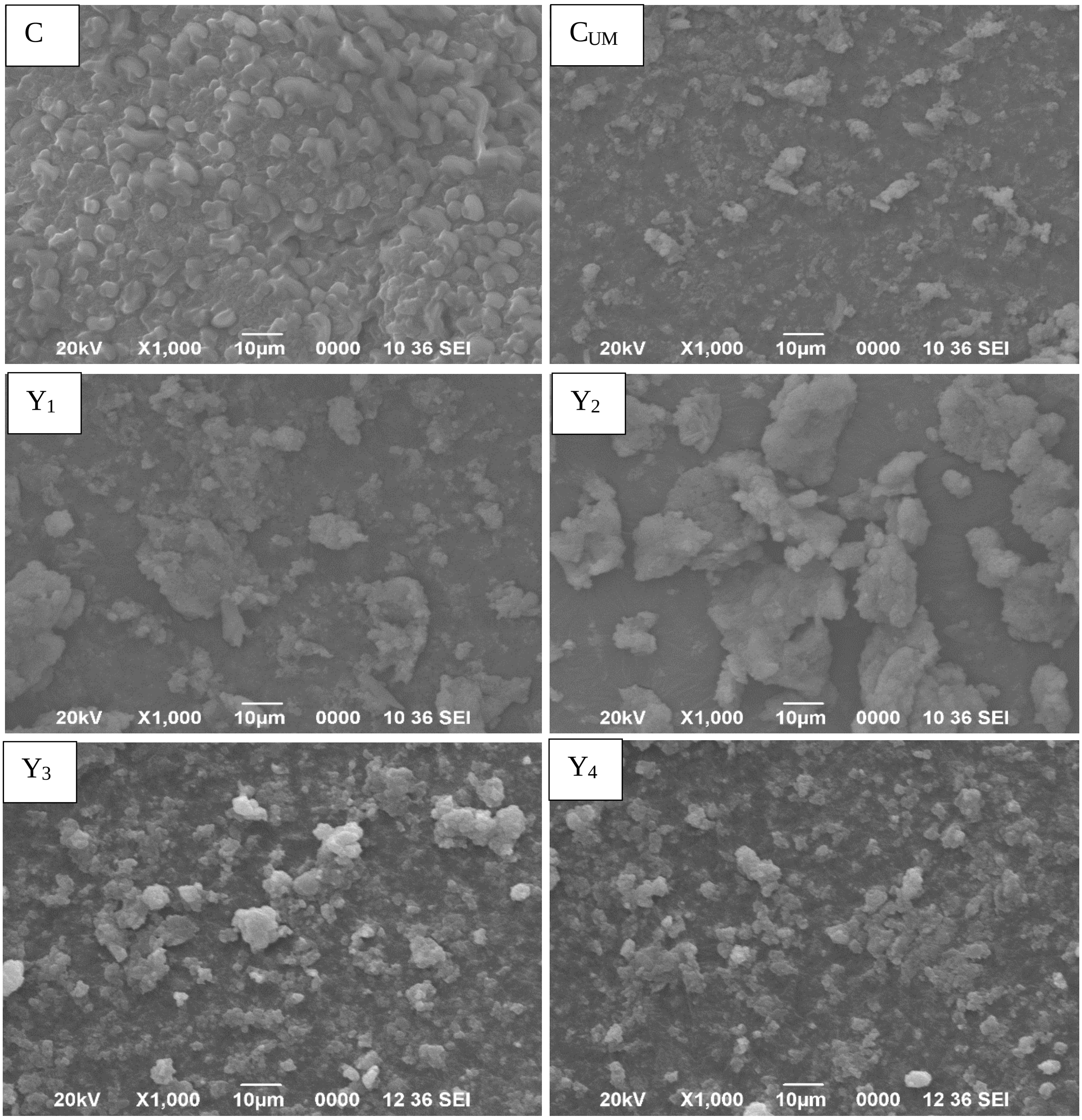


Fig 5.3 SEM of lactose free set type yogurt enriched with modified and unmodified dietary fiber from pea peel

C: lactose free yogurt without added fiber; C_{UM}: lactose free yogurt with 1.0% (w/v) unmodified fiber; Y_1: lactose free yogurt with 1% (w/v) modified fiber; Y_2: lactose free yogurt with 1.5% (w/v) modified fiber; Y_3: lactose free yogurt with 2% (w/v) modified fiber, Y_4: lactose free yogurt with 2.5% (w/v) modified fiber.

5.3.8 Sensory properties

Table 5.7 shows the sensory qualities of yogurts enriched with pea peel dietary fiber after 24 h of storage. The Y_1 showed no effect on the appearance of the probiotic yogurt (p >

0.05). The control yogurt sample had the highest acidity compared to all fiber-supplemented yogurts. The appearance of all the samples showed no significant difference, which could be due to the proper blending of fiber and the presence of lactose-free milk which made the yogurt slightly yellowish in color. The control yogurt had the lowest taste, texture, and overall acceptability scores, indicating that the experimental yogurts had superior organoleptic qualities because of the pea peel dietary fiber addition. There was no significant difference in perceived taste, texture, or overall acceptability between the yogurts supplemented with 1.0% w/v or 1.5% w/v pea peel dietary fiber ($p > 0.05$), but in terms of overall acceptability, Y_2 stands out as the most acceptable. These results are similar to those reported by Feng et al. [14] for goat milk yogurt enhanced with jujube pulp where 3% jujube pulp imparted a greater perceived overall acceptability than 1% or 9% jujube pulp, particularly in terms of eliminating the goat flavour [7, 29].

Table 5.7 Organoleptic attributes of lactose free set type yogurts supplemented with different concentrations of modified and unmodified pea peel fiber

Sample	Appearance	Acidity	Texture	Odour	Consistency	Overall acceptability
C	8.00 ± 0.50^d	8.06 ± 0.55^d	6.64 ± 0.54^a	7.50 ± 0.53^c	6.36 ± 0.25^a	7.50 ± 0.51^b
C_{UM}	7.42 ± 0.35^b	6.82 ± 0.31^a	7.45 ± 0.51^c	5.32 ± 0.30^a	7.35 ± 0.42^b	6.45 ± 0.32^a
Y_1	7.97 ± 0.56^c	7.53 ± 0.59^c	7.98 ± 0.49^d	8.51 ± 0.62^d	7.12 ± 0.32^b	8.10 ± 0.52^d
Y_2	7.86 ± 0.51^c	7.32 ± 0.67^b	8.56 ± 0.68^e	8.95 ± 0.65^d	7.89 ± 0.41^c	8.35 ± 0.33^d
Y_3	7.12 ± 0.39^a	7.31 ± 0.61^b	7.34 ± 0.61^b	7.15 ± 0.63^c	7.45 ± 0.40^c	7.85 ± 0.42^c
Y_4	7.32 ± 0.25^a	7.28 ± 0.48^b	7.02 ± 0.60^b	6.06 ± 0.31^b	7.00 ± 0.25^b	7.54 ± 0.25^b

C: lactose free yogurt without added fiber; C_{UM}: lactose free yogurt with 1.0% (w/v) unmodified fiber; Y_1: lactose free yogurt with 1% (w/v) modified fiber; Y_2: lactose free yogurt with 1.5% (w/v) modified fiber; Y_3: lactose free yogurt with 2% (w/v) modified fiber, Y_4: lactose free yogurt with 2.5% (w/v) modified fiber.

5.4 Conclusion

In the present study lactose free set type yogurt was developed with enzymatically modified and unmodified pea peel dietary fiber. Modified fiber addition was observed to interfere with the protein network and its interaction during storage, it also helped to reduce syneresis and improve the yogurt's textural qualities. The sensory quality of lactose free yogurt fiber gave slightly yellowish color to the entire sample and up to 1.5%

modified fiber concentration was found to be the best sample in overall attributes. Therefore, it can be concluded that lactose free yogurt with fiber can be successfully utilized and gave huge scope for lactose intolerant people with improving gut problem by modulating the intestinal bacteria by incorporating prebiotic and probiotic supplements.

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