Food Bioscience 49 (2022) 101828

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Lactose hydrolyzing activity of the lactase immobilized polycaprolactone and silk fibroin-based nanofiber and nitrocellulose membrane

Sümeyye Yılmaz-Karaoğlu [a], Begüm Gürel-Gökmen [b], Tuğba Tunali-Akbay [a,*]

[a] Vocational School of Health Services, Fenerbahce University, Istanbul, Turkey
[b] Department of Basic Medical Sciences, Dentistry Faculty, Marmara University, İstanbul, Turkey

ARTICLE INFO

Keywords:
Lactose reduced milk
Immobilization
Bioactive surface
Electrospinning
Lactose intolerance

ABSTRACT

This study aimed to investigate the activity of lactase immobilized polycaprolactone and silk fibroin (PCL/SF)-based nanofiber and nitrocellulose membrane for the preparation of lactose-reduced milk. PCL/SF -based nanofiber was prepared by using the electrospinning method. The lactase enzyme was immobilized using the physical adsorption method on both surfaces, and then the optimum operating temperature and pH of the immobilized enzymes were determined. The efficiency of the immobilized lactase enzyme was determined in both goat and cow milk. The nutrient content of milk was also analyzed before and after the incubation of nanofiber and membrane with milk. The lactose hydrolysis efficiency of the lactase immobilized nitrocellulose membrane was found to be higher than the lactase immobilized PCL/SF -based nanofiber. Lactose was hydrolyzed 59% in cow milk and 87% in goat milk by using lactase immobilized nitrocellulose membrane. 42% of lactose was also hydrolyzed in cow milk and 21% was hydrolyzed in goat milk by using lactase immobilized PCL/SF-based nanofiber. However, the use of these two bioactive surfaces did not change the fat and protein composition of both cow and goat milk. In conclusion, lactase immobilized nitrocellulose membrane was found to be more advantageous in the production of lactose-reduced milk than the lactase immobilized polycaprolactone/silk fibroin nanofiber.

1. Introduction

Lactase is an enzyme that hydrolyzes lactose to glucose and galactose in the body (Schaafsma, 2008). It is also utilized to produce lactose-free or lactose-reduced dairy products in both free and immobilized forms (Ladero et al., 2003). Lactase has been immobilized on many support materials such as alginate, agarose, DEAE cellulose, K-carrageenan, zeolite, Sephadex, porous glass, nylon, chitosan, polyvinyl alcohol polymers, Eupergit C (epoxy-activated acrylic beads), and polyurethane foams (Peppler & Reed, 1987; Souza et al., 2018). The type of support materials used in immobilization processes plays an important role in terms of the functions of the produced bioactive catalyst surfaces (Jin et al., 2015; Zdarta et al., 2018). The support material should be easily accessible, non-toxic, and have high biological compatibility with the enzyme. Large-surface-area supports are always helpful in achieving optimum immobilization efficiency. Natural polymer materials such as cellulose, chitin, chitosan, starch, and other natural polymer materials with a wide range of sources have been used in many studies due to their ease of modification, nontoxicity, and pollution-free properties, as well as a variety of functional groups and good biocompatible properties (Zhang et al., 2013). Menaa et al. (2008) showed that when the carrier surface was modified with hydrophobic groups, a high loading capacity of protein could be produced. The hydrophobicity/hydrophilicity of the supports may have an effect not only on the immobilized ratio but also on the immobilized enzyme activity. The crosslinker and porogen utilized in the preparation of the polymer beads or nanofibers might alter the porous structure of the polymer, hence affecting enzyme immobilization. Furthermore, some desired functional groups can be added by polymerization with other monomers or modification with different groups (Zhang et al., 2012).

In this study, the lactase enzyme was immobilized either onto the polycaprolactone (PCL) and silk fibroin (SF) -based nanofiber or nitrocellulose membrane (NC) to hydrolyze milk lactose. By blending PCL and SF, it is possible to form a nanofiber with different properties, and the newly formed nanofiber will be suitable for food contact (Nazeer et al., 2019). The NC membrane is readily accessible support that may be used to bind enzymes to perform repeating reactions in a variety of organic and aqueous solvents (Kumar & Kanwar, 2012). Therefore, this study aimed to investigate a suitable support material for the

* Corresponding author. Marmara University, Dentistry Faculty, Department of Basic Medical Sciences, Basibuyuk, Maltepe, İstanbul, Turkey.
E-mail address: ttunali@marmara.edu.tr (T. Tunali-Akbay).

https://doi.org/10.1016/j.fbio.2022.101828
Received 7 March 2022; Received in revised form 2 June 2022; Accepted 3 June 2022
Available online 23 June 2022

Abbreviations

PCL/SF	Polycaprolactone and Silk Fibroin-based Nanofiber
NC	Nitrocellulose Membrane
ONPG	ortho-nitrophenyl-β-galactopyranoside
DMF	dimethylformamide
DMC	dichloromethane
OGD	The Mean Gray Value

immobilization of lactase enzyme, which can be used to alleviate lactose intolerance and hydrolyze lactose in milk under appropriate conditions. One of the most common food intolerances today is lactose intolerance due to lactase deficiency. The primary treatment strategy for this condition is to stop lactose intake, replace lactose with alternative foods, use enzymatic substitutes and lactase supplements, and maintain intake of minerals and vitamins such as calcium and vitamin D (Fraissl et al., 2011; Ugidos-Rodríguez et al., 2018). However, in some cases, the cause of lactose intolerance is not lactase deficiency but hypersensitivity to the sugars present in milk. In these cases, the best strategy is to avoid lactose-containing products, use lactose-reduced dairy products, or focus on reducing the lactose in the milk used (Bouchoucha et al., 2021). Therefore, in this study, the lactose hydrolysis activity of two different bioactive surfaces was investigated to obtain lactose-reduced milk.

2. Material and methods

2.1. Materials

The chemicals used in this study were polycaprolactone (PCL) (Corbion Purac, Netherlands), silk fibroin (SF) (China), lactose (Sigma Aldrich, USA), bovine serum albumin (Sigma Aldrich, USA), ortho-nitrophenyl-β-galactopyranoside (ONPG) (Sigma Aldrich, USA), lactase enzyme (Mayasan, Turkey), nitrocellulose filter paper (Sartorius, Germany).

Analytical grade compounds used in this study were obtained from Sigma Aldrich (USA). Cow and goat milk samples were obtained from local dairies.

2.2. Preparation of PCL/SF-based nanofiber

An electrospun PCL/SF-based nanofiber was prepared using an electrospinning machine (Inovenso NE200, Turkey), 2 g of PCL and 2 g of SF were mixed with 20 mL of a mixture of dimethylformamide (DMF) and dichloromethane (DCM) (1:4) at room temperature for 24 h to prepare a PCL (10%, w/v)/SF (10%, w/v) mixture (Gurel-Gokmen et al., 2021). The electrospinning conditions were listed in Table 1.

2.3. Characterization of the PCL/SF-based nanofiber and nitrocellulose membrane

The PCL/SF -based nanofiber and nitrocellulose membrane was characterized by using the scanning electron microscope (SEMoscope Tabletop Compact SEM model, Inovenso, Turkey) and the Fourier transform infrared spectroscopy (FT-IR) (Shimadzu, Japan). FTIR was performed over the range of 600–4000 wavelength (cm^{-1}).

Table 1
The conditions of the electrospinning.

Voltage	26 kV
Distance	15,2 cm
Flow rate	1 mL/h
Temperature	37 °C

2.4. Immobilization of lactase enzyme on PCL/SF -based nanofiber and NC membrane

The PCL/SF -based nanofiber and nitrocellulose membrane was cut into 3 × 3 cm pieces and washed with phosphate buffer (0.02 M, pH 6.5). Then, 2 mL of 5000 NLU (neutral lactose unit) lactase enzyme was added to each piece. The PCL/SF -based nanofibers were incubated at 37 °C and the NC membrane at room temperature for 2 h. After the incubation, the PCL/SF -based nanofiber and NC membrane were washed three times with phosphate buffer (0.02 M, pH 6.5).

2.5. The free and immobilized lactase enzyme activity

In the lactase activity determination o-nitrophenyl-beta-D-galactopyranoside (ONPG) is used. ONPG is structurally similar to lactose with the exception that glucose is substituted with an o-nitrophenyl group. When ONPG, a colorless compound, is cleaved, *o*-nitrophenol (ONP), a yellow compound, is released. The intensity of this yellow color can be detected by measuring the absorbance at 420 nm.

2.7 mL phosphate buffer (0.02 M, pH 6.5), 0.2 mL free lactase enzyme (5000 NLU/g), and 0.3 mL ONPG (5 mM) were incubated at 37 °C for 10 min to determine free lactase activity. To measure immobilized lactase enzyme activity, PCL/SF -based nanofiber or NC membrane immobilized with lactase were cut into 1 × 1 cm squares and incubated with phosphate buffer and ONPG solution. After the incubation period, 2 mL of 1 M Na_2CO_3 (stop solution) was added to the mixture. At 420 nm, the change in absorbance was measured (Ansari & Husain, 2012).

2.6. Coomassie Brilliant Blue staining of lactase immobilized PCL/SF -based nanofiber and NC membrane

The PCL/SF-based nanofiber (3 × 3 cm) and NC membrane (3 × 3 cm) were stained with 1% (w/v) Coomassie Brilliant Blue solution for 30 min after lactase was immobilized on the PCL/SF -based nanofiber and NC membrane. After the staining step, the PCL/SF -based nanofiber and nitrocellulose membrane was washed with distilled water and kept in a 10% (v/v) acetic acid solution to remove the excess dye. Then, the nanofiber and membrane were photographed using a Canon EOS 700D camera with an 18–55 lens. and high-resolution jpeg files of the membranes were obtained. Quantitative measurements of the color intensity of the membranes in the jpeg files were calculated by using ImageJ software, and the mean gray values of the membrane photographs were calculated (Schindelin et al., 2015). The mean gray value (OGD is the average of the gray values in the image. This value is obtained by dividing the sum of the gray values of all pixels by the number of pixels. The values obtained with OGD were used to determine the color intensity between images. A high OGD value indicates a low color intensity of the image file.

2.7. Determination of the optimal working temperature of the immobilized lactase enzyme

0.5 g/mL and 1 g/mL free lactase, lactase immobilized PCL/SF-based nanofiber and lactase immobilized NC membrane was dipped separately into the 20 mL milk sample and incubated at 25 °C, 37 °C, and 50 °C for 24 h to determine the effect of temperature on the activity of immobilized lactase enzyme. The principle of the procedure consists of two steps: (a) hydrolysis of milk lactose by the free and immobilized lactase and (b) the enzymatic determination of glucose in the hydrolyzed milk sample by the glucose oxidase method (Lott & Turner, 1975). The activity of the lactase enzyme was monitored by measuring the milk glucose level released by the hydrolysis of lactose. The glucose level was determined by using a commercial kit (Glucose GOD-PAP, Biolabo, Germany). In this method, the enzyme glucose oxidase catalyzes the conversion of *β*-D-glucose to D-glucono-*δ*-lactone, releasing hydrogen

peroxide in the process. Milk samples were diluted 50-fold before the glucose measurement.

2.8. Determination of the optimal working pH of the immobilized lactase enzyme

Phosphate buffer solution (0.02M) was prepared at different pH values with 0.5 intervals between pH 5.0–8.0. The activity of the free and immobilized enzyme was determined using the prepared buffer solutions. The pH value at which the lactase enzyme showed the highest activity was determined.

2.9. Incubation of the prepared nanofiber and membrane with goat's and cow's milk

Because the manufacturer of the free lactase enzyme recommended a 24-h incubation at 4 °C for the hydrolysis of lactose in milk, the lactase-immobilized PCL/SF nanofiber and NC membrane were incubated for 24 h at 4 °C in goat and cow milk samples.

2.10. Calculation of the percentage of lactose hydrolysis in milk

The percentage of lactose hydrolyzed in the milk samples was calculated using the following formula. The percentage of lactose hydrolysis of free lactase was assumed to be 100%.

$$Lactose\ hydrolysis\ (percentage) = A\ x\ 100\ B$$

A: Glucose concentration after the incubation of milk samples with lactase immobilized
PCL/SF -based nanofiber or NC membrane
B: Glucose concentration after the incubation of milk samples with free lactase enzyme

2.11. Protein determination in milk

The milk protein level was determined before and after the incubation of milk samples with lactase-immobilized PCL/SF -based nanofiber or lactase-immobilized NC membrane according to the method of Bradford (Bradford, 1976).

2.12. Fat determination in milk

The milk fat level was determined before and after the incubation of milk with lactase-immobilized PCL/SF -based or lactase-immobilized NC membrane according to the method of Lucas et al. (1978).

2.13. Statistical analysis

Data analysis was performed using the GraphPad Prism 6.0 package program (GraphPad Software, San Diego, CA, USA). Analysis of variance (Tsekovska et al., 2012) followed by Tukey's multiple comparison tests was used to compare the results of the groups. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Scanning electron microscopy (SEM) and FTIR analysis: characterization of the PCL/SF -based nanofiber and nitrocellulose membrane

In the SEM image of the PCL/SF-based nanofiber, a homogeneously distributed, bead-free morphology was examined (Fig. 1). In the SEM analysis in which the diameter of the thinnest fiber was determined approximately 54.7 nm and the diameter of the thickest fiber was determined approximately 379.9 nm (Fig. 1F). The mean fiber diameter

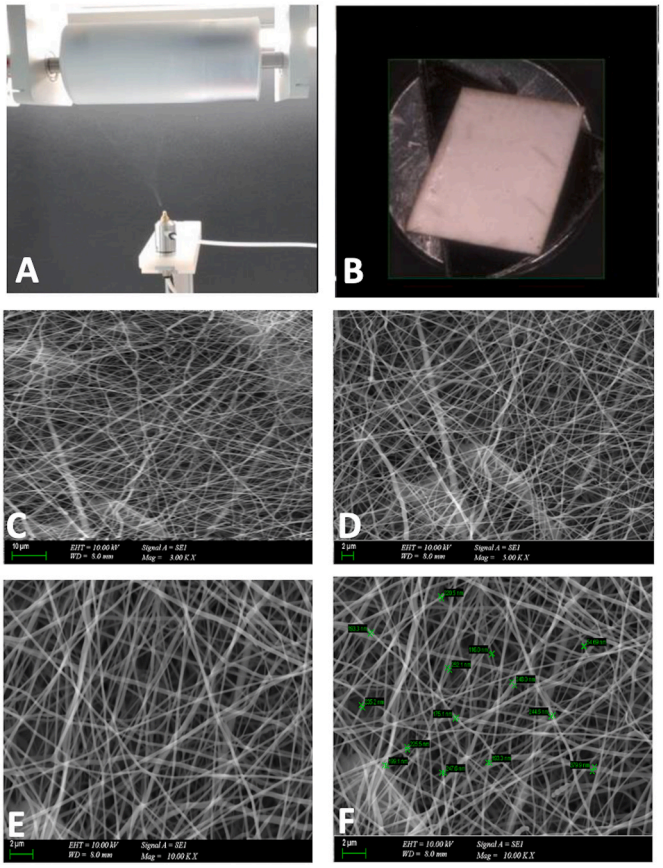


Fig. 1. Characterization of PCL/SF -based nanofiber by SEM. **A:** Electrospinning of PCL/SF-based nanofiber, **B:** PCL/SF-based nanofiber, **C:** SEM image of PCL/SF nanofiber (X3000), **D:** SEM image of PCL/SF nanofiber (X5000), **E:** SEM image of PCL/SF nanofiber (X10000) **F:** PCL/SF based nanofiber diameters **PCL/SF:** Polycaprolactone/silk fibroin.

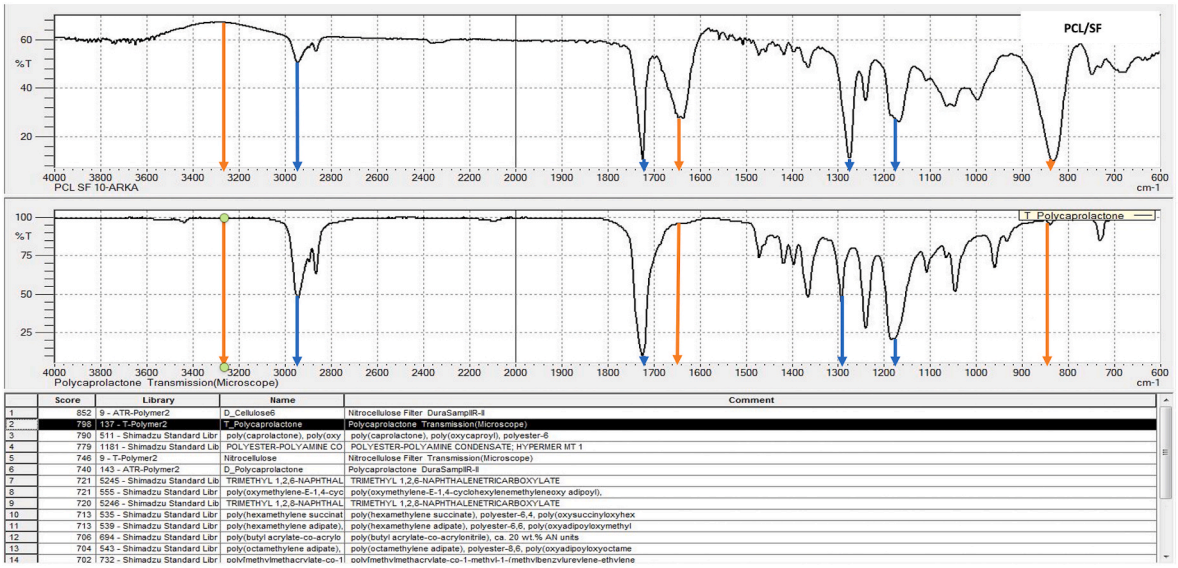
of the PCL/SF-based nanofiber was determined as approximately 220 nm by calculating the average of the measured diameters of fibers from Fig. 1F. In the FTIR spectrum of PCL/SF nanofiber, CH planar vibration band at 2946 cm^{-1}, and CO tension at 1720 cm^{-1}, 1290 cm^{-1} and 1190 cm^{-1} were detected. These four peaks demonstrated the presence of PCL in the nanofiber's structure. In addition, there was NH stretch at 3250 cm^{-1}, amide I (CO stretch) at 1650 cm^{-1}, and amide II at 850 cm^{-1} absorption bands. These three peaks represent the presence of SF in the nanofiber we produced (Fig. 2).

In Fig. 3, 2000 and 5000 times magnified SEM images of the NC membrane were also presented. NC membrane (Sartorious, 11406-47-ACN Membrane Filter Disc) has a 0.45 μm pore size, and 47 mm diameter. It is a regenerated cellulose membrane filter.

When the FTIR spectrum of nitrocellulose was examined, C═O stress at 1740 cm^{-1}, C–H planar vibration band at 1380 cm^{-1}, NO_2 stress at 1247 cm^{-1} and N–O group vibrations at 827 cm^{-1} were detected (Fig. 4). When compared to the cellulose IR results, the characteristic peak changes were specific to the structure of nitrocellulose.

3.2. The efficiency of lactase immobilization on PCL/SF-based nanofiber and NC membrane

The stained blank PCL/SF based nanofiber and lactase immobilized PCL/SF-based nanofiber were presented in Fig. 5A and B. When the OGD values of the blank PCL/SF-based nanofiber and lactase enzyme immobilized PCL/SF-based nanofiber were compared, it was detected that the color intensity was 5.5% higher in the lactase enzyme immobilized PCL/SF-based nanofiber.



	Score	Library	Name	Comment
1	852	9 - ATR-Polymer2	D_Cellulose6	Nitrocellulose Filter DuraSamplIR-II
2	788	137 - T-Polymer2	T_Polycaprolactone	Polycaprolactone Transmission(Microscope)
3	790	511 - Shimadzu Standard Libr	poly(caprolactone), poly(oxy	poly(caprolactone), poly(oxycaproyl), polyester-6
4	779	1181 - Shimadzu Standard Lib	POLYESTER-POLYAMINE CO	POLYESTER-POLYAMINE CONDENSATE; HYPERMER MT 1
5	746	9 - T-Polymer2	Nitrocellulose	Nitrocellulose Filter Transmission(Microscope)
6	740	143 - ATR-Polymer2	D_Polycaprolactone	Polycaprolactone DuraSamplIR-II
7	721	5245 - Shimadzu Standard Lib	TRIMETHYL 1,2,6-NAPHTHAL	TRIMETHYL 1,2,6-NAPHTHALENETRICARBOXYLATE
8	721	555 - Shimadzu Standard Libr	poly(oxymethylene-E-1,4-cyc	poly(oxymethylene-E-1,4-cyclohexylenemethyleneoxy adipoyl),
9	720	5246 - Shimadzu Standard Lib	TRIMETHYL 1,2,8-NAPHTHAL	TRIMETHYL 1,2,8-NAPHTHALENETRICARBOXYLATE
10	713	535 - Shimadzu Standard Libr	poly(hexamethylene succinat	poly(hexamethylene succinate), polyester-6,4, poly(oxysuccinyloxyhex
11	713	539 - Shimadzu Standard Libr	poly(hexamethylene adipate),	poly(hexamethylene adipate), polyester-6,6, poly(oxyadipoyloxymethyl
12	706	694 - Shimadzu Standard Libr	poly(butyl acrylate-co-acrylo	poly(butyl acrylate-co-acrylonitrile), ca. 20 wt.% AN units
13	704	543 - Shimadzu Standard Libr	poly(octamethylene adipate),	poly(octamethylene adipate), polyester-8,6, poly(oxyadipoyloxyoctame
14	702	732 - Shimadzu Standard Libr	poly(methylmethacrylate-co-1	poly(methylmethacrylate-co-1-methyl-1-(methylbenzylureylene-ethylene

Fig. 2. FTIR spectrum of PCL/SF-based nanofiber **PCL/SF:** Polycaprolactone/silk fibroin.

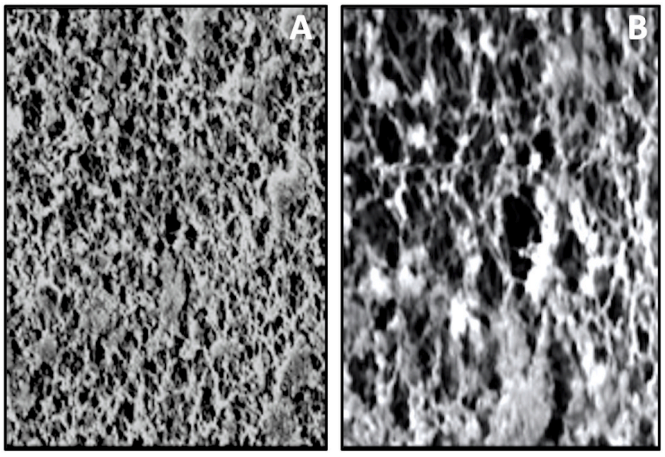


Fig. 3. Characterization of NC membrane by SEM. **A:** Nitrocellulose membrane (X2000), **B:** Nitrocellulose membrane (X2000) **NC:** Nitrocellulose.

The stained blank NC membrane and lactase immobilized NC membrane was presented in Fig. 5C and D. When the OGD values of the blank NC membrane and the lactase immobilized NC membrane were compared, the color intensity was 8.4% higher in the lactase immobilized NC membrane.

3.3. The activity of the free and immobilized lactase enzyme activity

There was no significant difference between the free lactase activity and lactase immobilized NC membrane activity. The activity of the lactase immobilized PCL/SF-based nanofiber was found to be significantly lower than the free lactase and lactase immobilized NC membrane activities (Table 2).

3.4. The effect of pH and temperature on the activity of free and immobilized lactase enzyme

The activities of free lactase enzyme, lactase enzyme immobilized to PCL/SF-based nanofiber, and lactase enzyme immobilized to nitrocellulose membrane at different temperatures and pH ranges were presented in Fig. 6A, B, and 6C. The optimum working temperature of free lactase and lactase enzyme immobilized on the NC membrane was determined as 25 °C, and the optimum working temperature of lactase enzyme immobilized on PCL/SF- based nanofiber was.

The optimum working pH value of free lactase enzyme, lactase enzyme immobilized to the NC membrane, and lactase enzyme immobilized to PCL/SF-based nanofiber was determined as 6.5 (Fig. 6A, B, and 6C).

3.5. Lactose hydrolysis percentages of immobilized lactase enzyme in cow's milk and goat milk

When the percentage of lactose hydrolysis in cow and goat milk was evaluated, the percentage of lactose hydrolysis by using lactase immobilized NC membrane was significantly higher than the lactase immobilized PCL/SF-based nanofiber (Table 3). When the lactose hydrolysis percentages of lactase immobilized PCL/SF-based nanofiber and NC membrane in cow and goat milk were compared, the highest percentage of lactose hydrolysis was detected with the lactase immobilized NC membrane in goat milk (Table 3).

3.6. Evaluation of the nutritional content of cow and goat milk before and after the treatment with lactase enzyme immobilized on PCL/SF- based nanofiber and NC membrane

The protein level of goat and cow milk treated with lactase immobilized PCL/SF-based nanofiber was significantly higher than that of untreated milk and goat and cow milk after treatment with lactase immobilized NC membrane (Table 4 and Table 5).

There is no statistically significant difference between the fat levels of untreated goat and cow milk and the fat levels of goat and cow milk after treatment with lactase immobilized PCL/SF-based nanofiber and NC membrane (Tables 4 and 5).

4. Discussion

The optimal operating conditions are different for free and immobilized enzymes. The substrate-binding properties of the enzyme change depending on the properties of the surface on which it is immobilized (Mohamad et al., 2015). In this study, to investigate the lactose hydrolysis activity of the free and immobilized lactase enzyme in cow and goat milk samples, the lactase enzyme was immobilized on the PCL/SF-based nanofiber and on the NC membrane. Depending upon the support,

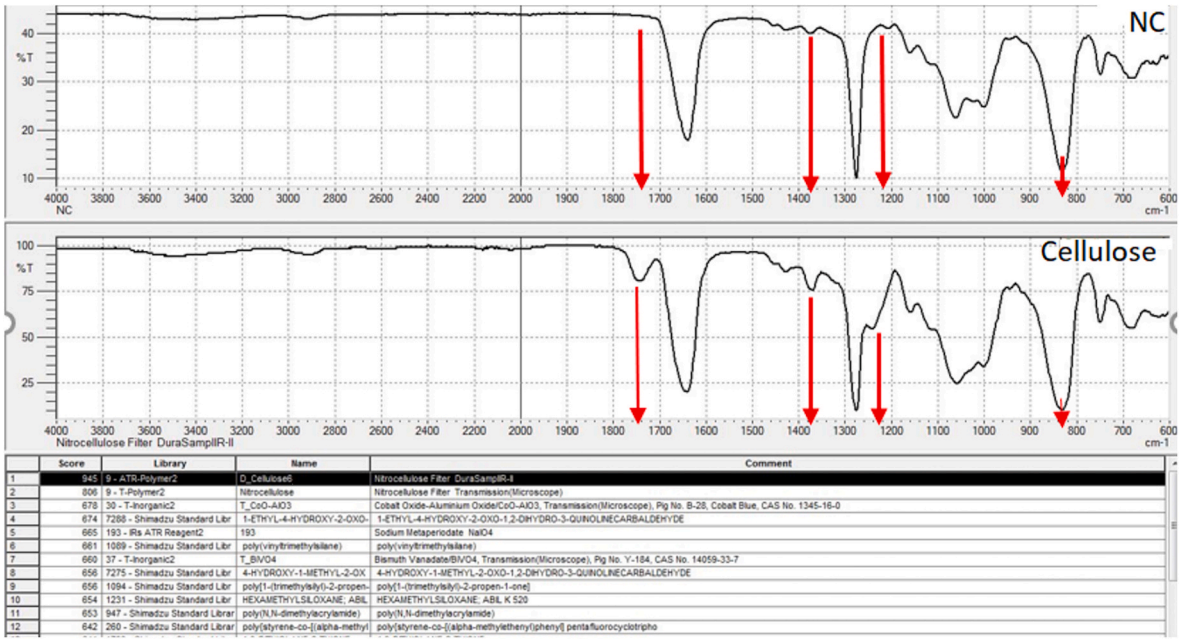


Fig. 4. FTIR spectrum of NC membrane **NC:** Nitrocellulose.

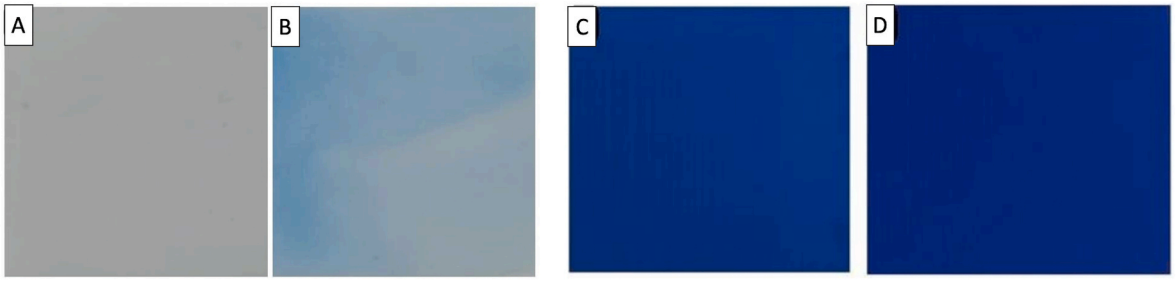


Fig. 5. Stained Lactase Immobilized PCL/SF-based nanofiber and NC membrane. **A:** Blank PCL/SF-based nanofiber after staining with Coomassie Brilliant Blue, **B:** Lactase immobilized PCL/SF-based nanofiber after staining with Coomassie
Brilliant Blue, **C:** Blank NC membrane after staining with Coomassie Brilliant Blue, **D:** Lactase immobilized NC membrane after staining with Coomassie Brilliant Blue.

Table 2
Activities of free and immobilized lactase enzyme.

	Free Lactase		NC		PCL/SF	
	Mean	SD	Mean	SD	Mean	SD
Activity (μmol/min)	0.004	0.0001	0.004	0.0001	$0.002^{\Delta *}$	0.0001

SD: Standard deviation, **PCL/SF:** Lactase immobilized polycaprolactone and silk fibroin-based nanofiber, **NC:** Lactase immobilized nitrocellulose membrane **(Δ):** $p < 0.05$ statistically significant compared to free lactase activity, (*): $p < 0.05$ statistically significant compared to lactase immobilized nitrocellulose membrane.

mechanism of activation, and method of immobilization, optimal conditions for optimum enzyme activity vary for free and immobilized enzymes (Tümtürk et al., 2007). SEM analysis of PCL/SF-based nanofiber showed nanofibrous scaffold development without bead formation and successful blending confirmed by FTIR analysis. The fibers made by combining PCL and SF combine the mechanical advantages of PCL with the biological benefits of SF (Singh et al., 2020). The availability of functional groups that can react with lactase was the primary rationale for choosing this blend, as PCL has hydrophobic and SF has hydrophilic properties. The nitrocellulose membrane was also chosen because of the possible hydrophobic and dipolar interactions between the enzyme and the nitrocellulose membrane, enabling the enzyme to maintain its orientation.

In this study, the activity of both free and immobilized lactase enzymes was influenced by pH and temperature variations. The decreased lactase activity at extreme pH and temperatures was likely to be related to changes in enzyme structure that are important for enzymatic activity. When the optimal working pH and temperature values of free and immobilized lactase were evaluated, the optimum working temperature for lactase immobilized PCL/SF based nanofiber differed from the free lactase and lactase immobilized nitrocellulose membrane and the optimum working pH of the immobilized lactase did not change compared to free lactase. The activity of lactase immobilized NC was shown to be less impacted by temperature changes than the free lactase and lactase immobilized PCL/SF-based nanofiber.

There was no significant activity difference between the free enzyme and the lactase immobilized NC membrane, the activity of the enzyme immobilized PCL/SF-based nanofiber was lower than that of the others. The Coomassie Brillant Blue staining was used to determine the immobilization efficiency of lactase onto the PCL/SF-based nanofiber and nitrocellulose membrane. As a negative control, PCL/SF-based nanofiber and nitrocellulose membrane were prepared without the addition of lactase. This method revealed the presence of lactase on the surface because the intensity of the blue color was directly proportional to the

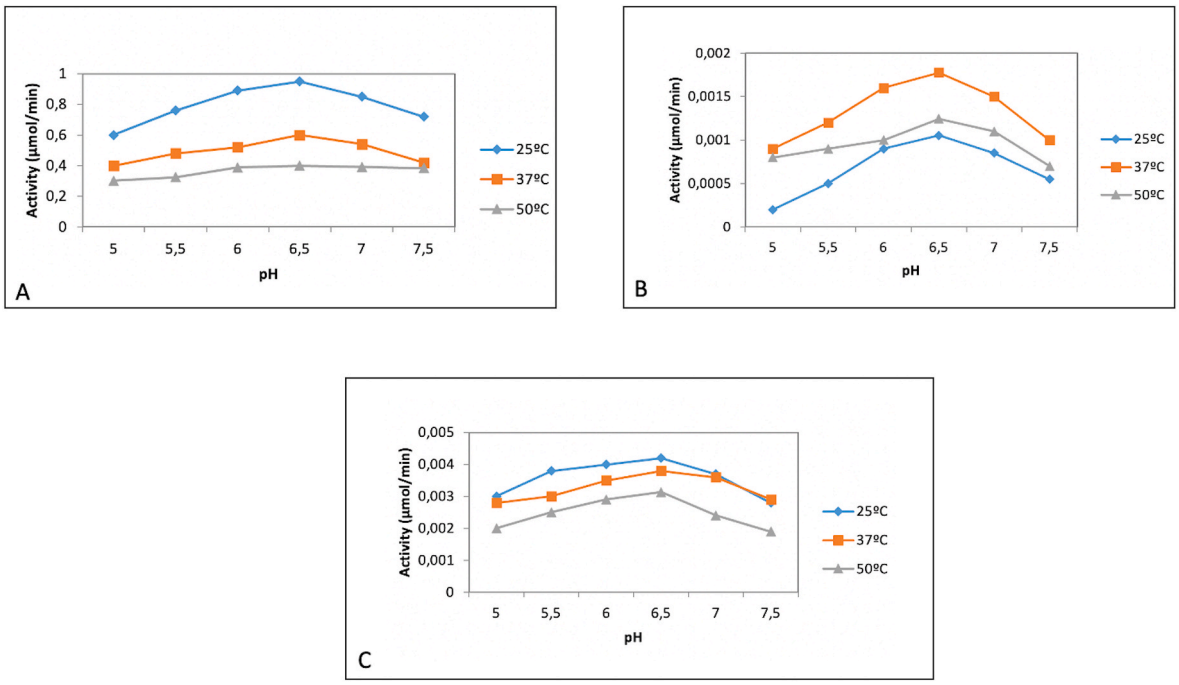


Fig. 6. Determination of the optimum working temperature and pH of the free and immobilized lactase enzyme. **A:** Free Lactase enzyme, **B:** Lactase immobilized to PCL/SF-based nanofiber, **C:** Lactase immobilized to NC membrane.

Table 3
Percentage of lactose hydrolysis in cow and goat milk samples.

	Lactase immobilized NC membrane		Lactase immobilized PCL/SF nanofiber	
	Mean	SD	Mean	SD
Lactose Hydrolysis in cow milk (%)	59.2	1.5	41.8*	0.9
Lactose Hydrolysis in goat milk (%)	87	1.1	21.0*	1.6

SD: Standard deviation, n = 20.
*: $p < 0.05$, compared to lactase enzyme immobilized NC membrane.
PCL/SF: Polycaprolactone/silk fibroin **NC:** Nitrocellulose.

Table 4
Nutritional content of untreated cow milk and cow milk treated with immobilized lactase.

COW MILK	Untreated milk		Milk treated with lactase immobilized NC membrane		Milk treated with lactase immobilized PCL/SF nanofiber	
	Mean	SD	Mean	SD	Mean	SD
Protein (% g)	2.07	0.06	2.07	0.05	2.16 Δ *	0.12
Fat (% g)	3.51	0.07	3.51	0.07	3.51	0.07

SD: Standard Deviation, n = 16, **PCL/SF**: Polycaprolactone and silk fibroin.
NC: Nitrocellulose *: $p < 0.05$ compared to the milk treated with lactase immobilized NC membrane, **Δ:** $p < 0.05$ compared to the untreated milk.

presence of lactase. Lactase immobilization efficiency on PCL/SF-based nanofiber was not as effective as lactase immobilization efficiency on nitrocellulose membrane. This can be attributed to the absence of the lactase enzyme on the surface of PCL/SF-based nanofiber. As a result, the lactose hydrolyzing activity of the lactase-immobilized PCL/SF nanofiber was lower than that of the nitrocellulose performance. This was most likely due to the lower immobilization of lactase on PCL/SF nanofiber compared to nitrocellulose. The observed variability between PCL/SF-based nanofiber and nitrocellulose was most likely due to differences in support efficiency, but modifying the immobilization

Table 5
Nutritional content of untreated goat milk and goat milk treated with immobilized lactase.

GOAT MILK	Untreated milk		Treatment with lactase immobilized NC membrane		Treatment with lactase immobilized PCL/SF nanofiber	
	Mean	SD	Mean	SD	Mean	SD
Protein (% g)	2.86	0.09	2.93	0.13	3.05 Δ *	0.13
Fat (% g)	3.50	0.05	3.50	0.05	3.50	0.05

SD: Standard Deviation, n = 16, **PCL/SF**: Polycaprolactone and silk fibroin.
NC: Nitrocellulose *: $p < 0.05$ compared to the milk treated with lactase immobilized NC membrane, **Δ:** $p < 0.05$ compared to the untreated milk.

procedure on PCL/SF nanofiber using cross-linking techniques can be helpful to increase the lactase activity and provide more effective lactase immobilization to the PCL/SF membrane. Although the low activity of the PCL/SF-based nanofiber can be partially attributed to the use of the physical adsorption method for enzyme immobilization, the lactase enzyme was immobilized on the PCL/SF-based nanofiber in a pre-planned manner without the use of crosslinking agents because cross-linking agents were considered unsuitable for food contact. These findings demonstrated that the NC membrane is an appropriate support material for the immobilization of lactase enzymes without the need for any cross-linking agents.

Lactase immobilization on PCL/SF nanofibers can limit the freedom of the immobilized lactase enzyme, making it difficult for the enzyme to adapt to a catalytic conformation. Non-biospecific interactions can also occur between the lactase enzyme and the PCL/SF nanofiber, resulting in undesirable changes in enzyme conformation and microenvironment variation. The presence of fewer lactase enzymes on the PCL/SF nanofiber surface compared to NC resulted in decreased lactase activity.

When the protein and fat content of cow and goat milk were compared before and after treatment with immobilized lactase, it was found that the content of milk protein increased after treatment with the PCL/SF-based nanofiber immobilized with lactase. The lactase enzyme, which cannot be physically adsorbed onto the PCL/SF-based nanofiber,

likely passes into the milk and causes an increase in the amount of protein measured. In milk samples treated with lactase immobilized NC membrane, the protein content did not change. No significant change was observed in the amount of fat in the milk after treatment with immobilized enzymes. In this study, NC membrane containing immobilized lactase was found to have the same activity as free lactase. The lactase immobilized NC membrane can also prevent the formation of hydroxymethylfurfural (HMF), which occurs during the production of lactose-free milk. HMF is formed as a result of the Maillard reaction (Francisquini et al., 2019). Since the lactase enzyme used in the production of lactose-free milk and dairy products hydrolyzes the lactose in milk and converts it into glucose and galactose, protein-bound lysine and other –NH ends show higher reactivity and create a more suitable environment for the Maillard reaction. Moreover, the added lactase enzyme has been reported to interfere with the reaction by causing an increase in numerous free amino acids with undesirable end activities (Jansson et al., 2014; Messia et al., 2007). Therefore, when lactase immobilized membranes are used, no free lactase remains in the milk after heat treatment of milk and does not cause undesirable reactions.

5. Conclusion

The use of lactase immobilized NC membrane may be a suitable option to obtain lactose-reduced milk without changing the nutritional content of milk during lactose hydrolysis. The lactose hydrolysis efficiency of the immobilized lactase was higher in goat milk compared to cow's milk. This higher hydrolysis can be due to goat milk's high blocking effect on nanofiber or membrane compared to cow's milk, which facilitates the interaction of lactose with the lactase enzyme.

Author statement

Sümeyye Yılmaz Karaoğlu: Investigation, Validation, Formal analysis, Writing-Original Draft, Visualization.

Begüm Gürel-Gökmen: Investigation, Formal analysis.

Tuğba Tunalı-Akbay: Conceptualization, Methodology, Supervision, Writing –Review & Editing, Project Administration.

Declaration of competing interest

The authors declare that they have no conflicts of interest that are relevant to the content of this article.

Acknowledgements

This study was funded by a grant from Marmara University Scientific Research Project (TYL-2021-10180) units.

References

Ansari, S. A., & Husain, Q. (2012). Lactose hydrolysis from milk/whey in batch and continuous processes by concanavalin A-Celite 545 immobilized Aspergillus oryzae β galactosidase. *Food and Bioproducts Processing, 90*(2), 351–359.

Bouchoucha, M., Fysekidis, M., Rompteaux, P., Raynaud, J.-J., Sabate, J.-M., & Benamouzig, R. (2021). Lactose sensitivity and lactose malabsorption: The 2 faces of lactose intolerance. *Journal of Neurogastroenterology and Motility, 27*(2), 257.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248–254.

Fraissl, L., Leitner, R., & Missbichler, A. (2011). Novel formulation of neutral lactase improves digestion of dairy products in case of lactose intolerance. *Clinical and Translational Allergy, 1*(1), 104.

Francisquini, J. D., Rocha, J., Martins, E., Stephani, R., Henrique Fonseca da Silva, P., Toledo Renhe, I. R., et al. (2019). 5-Hydroxymethylfurfural formation and color change in lactose-hydrolyzed Dulce de leche. *Journal of Dairy Research, 86*(4), 477–482.

Gurel-Gokmen, B., Taslak, H. D., Ozcan, O., Ipar, N., & Tunali-Akbay, T. (2021). Polycaprolactone/silk fibroin electrospun nanofibers-based lateral flow test strip for quick and facile determination of bisphenol A in breast milk. *Journal of Biomedical Materials Research Part B, 109*(10), 1455–1464.

Jansson, T., Clausen, M. R., Sundekilde, U. K., Eggers, N., Nyegaard, S., Larsen, L. B., et al. (2014). Lactose-hydrolyzed milk is more prone to chemical changes during storage than conventional ultra-high-temperature (UHT) milk. *Journal of Agricultural And Food Chemistry, 62*(31), 7886–7896.

Jin, L., Li, Y., Ren, X.-H., & Lee, J.-H. (2015). Immobilization of lactase onto various polymer nanofibers for enzyme stabilization and recycling. *Journal of Microbiology and Biotechnology, 25*(8), 1291–1298.

Kumar, A., & Kanwar, S. S. (2012). Catalytic potential of a nitrocellulose membrane-immobilized lipase in aqueous and organic media. *Journal of Applied Polymer Science, 124*(S1), E37–E44.

Ladero, M., Perez, M., Santos, A., & Garcia-Ochoa, F. (2003). Hydrolysis of lactose by free and immobilized β-galactosidase from Thermus sp. strain T2. *Biotechnology and Bioengineering, 81*(2), 241–252.

Lott, J. A., & Turner, K. (1975). Evaluation of Trinder's glucose oxidase method for measuring glucose in serum and urine. *Clinical Chemistry, 21*(12), 1754–1760.

Lucas, A., Gibbs, J., Lyster, R., & Baum, J. (1978). Creamatocrit: Simple clinical technique for estimating fat concentration and energy value of human milk. *British Medical Journal, 1*(6119), 1018–1020.

Messia, M. C., Candigliota, T., & Marconi, E. (2007). Assessment of quality and technological characterization of lactose-hydrolyzed milk. *Food Chemistry, 104*(3), 910–917.

Mohamad, N. R., Marzuki, N. H. C., Buang, N. A., Huyop, F., & Wahab, R. A. (2015). An overview of technologies for immobilization of enzymes and surface analysis techniques for immobilized enzymes. *Biotechnology & Biotechnological Equipment, 29*(2), 205–220.

Nazeer, M. A., Yilgor, E., & Yilgor, I. (2019). Electrospun polycaprolactone/silk fibroin nanofibrous bioactive scaffolds for tissue engineering applications. *Polymer, 168*, 86–94.

Peppler, H., & Reed, G. (1987). Enzymes in food and feed processing. *Biotechnology, 7a*, 578–580.

Schaafsma, G. (2008). Lactose and lactose derivatives as bioactive ingredients in human nutrition. *International Dairy Journal, 18*(5), 458–465.

Schindelin, J., Rueden, C. T., Hiner, M. C., & Eliceiri, K. W. (2015). The ImageJ ecosystem: An open platform for biomedical image analysis. *Molecular Reproduction and Development, 82*(7-8), 518–529.

Singh, R., Eitler, D., Morelle, R., Friedrich, R. P., Dietel, B., Alexiou, C., et al. (2020). Optimization of cell seeding on electrospun PCL-silk fibroin scaffolds. *European Polymer Journal, 134*, Article 109838.

Souza, C. J. F., Garcia-Rojas, E. E., & Favaro-Trindade, C. S. (2018). Lactase (β-galactosidase) immobilization by complex formation: Impact of biopolymers on enzyme activity. *Food Hydrocolloids, 83*, 88–96.

Tsekovska, R., Boyanova, M., Mironova, R., & Ivanov, I. (2012). Impact of glycation inhibitors on the biologic activity of recombinant human interferon-gamma. *Biotechnology & Biotechnological Equipment, 26*(sup1), 170–174.

Tümtürk, H., Karaca, N., Demirel, G., & Şahin, F. (2007). Preparation and application of poly (N, N-dimethyl acrylamide-co-acrylamide) and poly (N-isopropyl acrylamide-co-acrylamide)/κ-Carrageenan hydrogels for immobilization of lipase. *International Journal of Biological Macromolecules, 40*(3), 281–285.

Ugidos-Rodríguez, S., Matallana-González, M. C., & Sánchez-Mata, M. C. (2018). Lactose malabsorption and intolerance: A review. *Food & Function, 9*(8), 4056–4068.

Zdarta, J., Meyer, A. S., Jesionowski, T., & Pinelo, M. (2018). A general overview of support materials for enzyme immobilization: Characteristics, properties, practical utility. *Catalysts, 8*(2), 92.

Zhang, D.-H., Yuwen, L.-X., Xie, Y.-L., Li, W., & Li, X.-B. (2012). Improving immobilization of lipase onto magnetic microspheres with moderate hydrophobicity/hydrophilicity. *Colloids and Surfaces B: Biointerfaces, 89*, 73–78.

Zhang, Dong-Hao, Yuwen, Li-Xia, & Peng, Li-Juan (2013). Parameters affecting the performance of immobilized enzyme. *Journal of Chemistry, 2013*, 1–7. https://doi.org/10.1155/2013/946248, 946248. In this issue.