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A Lactose-Free Yogurt Using Membrane Systems and Modified Milk Protein Concentrate: Production and Characterization

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***Abstract*—**Using membrane technology and modification of milk protein structural properties, a lactose free yogurt was developed. The functional, textural and structural properties of the sample were evaluated and compared with the commercial ones. Results showed that the modification of protein in high fat set yogurt resulted in 11.55%, 18%, 20.21% and 7.08% higher hardness, consistency, water holding capacity, and shininess values compared with the control one. Furthermore, these indices of modified low fat set yogurt were 21.40%, 25.41%, 28.15% & 10.58% higher than the control one, which could be related to the gel network microstructural properties in yogurt formulated with modified protein. In this way, in comparison with the control one, the index of linkage strength (A), the number of linkages (z), and time scale of linkages (λ_{rel}) of the high fat modified yogurt were 22.10%, 50.68%, 21.82% higher than the control one; whereas, the average linear distance between two adjacent crosslinks (ξ), was 16.77% lower than the control one. For low fat modified yogurt, A, z, λ_{rel}, and ξ indices were 34.30%, 61.70% and 42.60% higher and 19.20% lower than the control one, respectively. The shelf life of modified yogurt was extended to 10 weeks in the refrigerator, while, the control set yogurt had a 3 weeks shelf life. The acidity of high fat and low fat modified yogurts increased from 76 to 84 and 72 to 80 Dornic degrees during 10 weeks of storage, respectively, whereas for control high fat and low fat yogurts they increased from 82 to 122 and 77 to 112 Dornic degrees, respectively. This behavior could be due to the elimination of microorganism's source of energy in modified yogurt. Furthermore, the calories of high fat and low fat lactose free yogurts were 25% and 40% lower than their control samples, respectively. Generally, results showed that the lactose free yogurt with modified protein, despite of 1% lower protein content than the control one, showed better functional properties, nutritional properties, network parameters, and shelf stability, which could be promising in the set yogurt industry.

***Keywords*—**Lactose free, low calorie, network properties, protein modification.

I. Introduction

THE consumption of dairy products such as yogurt is limited for people who are lactose intolerant. On the other hand, decreasing or avoiding the consumption of dairy products has nutritional consequences of reducing the protein, calcium, phosphorus, and vitamin D intake of people [1]. Considering the high advantages of dairy product consumption, the application of different techniques for reduction or elimination of lactose is a growing trend in dairy industry [2]. Syneresis and decrease in curd consistency are regarded as defects of set yogurt. These problems can be reduced or eliminated by the addition of stabilizers, an increase in the level of milk solids and the addition of exopolysaccharide (EPS)-producing starter cultures [3]. These actions can result in the greater elastic structure of the final product, an increase in mold population and flavor change during shelf life [4].

Herein, by using modified manufacturing conditions, incorporation of modified protein and optimization of lactose free yogurt formula, we attempted to gain enhanced functional and rheological properties, extended shelf life and a lower production cost.

II. Materials and Methods

To adjust the lactose, protein, fat, acid, dry matter and milk mineral, the ultrafiltration, nanofiltration, and reverse-osmosis membrane techniques were employed. Protein separation was performed using polysulfone (PSF) membranes with molecular weight cut-off (MWCO) of 10 kDa at 50 °C and 3 bar transmembrane pressure (TMP). For modification of protein, the chemical reactions of citrate buffer and sodium dihydrogen phosphate were used before ultrafiltration (UF) membrane process, while, sodium poly-phosphate chemical reaction and transglutaminases enzymatic reaction were employed after UF membrane process. Lactose separation was conducted using PSF amide with MWCO of 300 Da at 5 °C and 20 bar TMP. Milk mineral separation was performed using nonporous membrane at 5 °C and 30 bar TMP. The recombined milk was obtained by mixing determined ratios of milk fat, protein, milk mineral and water. Then, the recombined milk was heated at 70 °C for 5 min, homogenized at 200 bar, followed by pasteurization at 95 °C for 5 min. A commercial freeze-dried yogurt culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (FD-DVS YFL904) was provided by Chr. Hansen (Horsholm, Denmark). Fermentation was conducted at 42 °C for 6 hours.

For evaluation of the physicochemical properties of yogurt during shelf life, the protein content was determined using the official Association of Official Agricultural Chemists (AOAC) method (976.06) [5]. The total solids content was determined after drying samples at 105 °C using an air-dry oven (DHG-924OA, Shanghai Yiheng Technology Ltd, China). The pH was measured using a Jenway 3010 model pH meter (Essex, UK). Titratable acidity was determined as Dornic by titrating with 0.1N NaOH; using phenolphthalein as an indicator [6]. Texture

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Analyzer (CNS Farnell Com, UK) equipped with a 36 mm diameter cylinder probe with 50% strain, 100 mm/min rate, and 30 mm depth was employed in puncture test. Hardness (peak force (N)) and consistency (the positive area below the force-deformation curves) through the puncture were determined using the Brookfield TexturePro CT v1.0 software [7]. The rheological properties of yogurts were characterized by using a controlled stress/strain rheometer (Physica MCR 301 rheometer) equipped with a cone-plate geometry (40 mm of diameter, 4° cone angle, and 0.206 mm gap). The temperature was fixed at 25 °C using a Peltier device and then each sample was equilibrated at least for 10 min before the rheological tests. To minimize the loss of water, samples were coated around their periphery with light silicone oil. Strain sweep experiments were performed from 0.10 to 1000% strain amplitude using a controlled shear rate mode at 25 °C and a constant frequency of 1 Hz. Data were analyzed in the linear (LVE) and non-linear (n-LVE) viscoelastic regions, the strain limit (at which biopolymers enter from the linear viscoelastic to non-linear viscoelastic region, γ_L) and stress limit (τ_y), the extent of strain overshoot (G''_{max}/G''_{LVE}), and stress at flow point (τ_{FP}) were determined by this test. Also, dynamic measurements were carried out in the 0.10-100 rad/s frequency range within the LVE range (strain amplitude of 0.1%). Network parameters were obtained as [7]:

$$G^*(\omega) = A \times \omega^{\frac{1}{z}} \quad (1)$$

$$\xi = \left(\sqrt[3]{\frac{G'_p N_A}{RT}}\right)^{-1} \quad (2)$$

$$G' = \int_{-\infty}^{+\infty} \frac{H\lambda^2\omega^2}{1+\lambda^2\omega^2} d(ln(\lambda)) \quad (3)$$

$$G'' = \int_{-\infty}^{+\infty} \frac{H\lambda\omega}{1+\lambda^2\omega^2} d(ln(\lambda)) \quad (4)$$

here G'_p is plateau storage modulus (Pa), R is gas constant (J/mol.K), T is temperature (K), N_A is Avogadro number (atoms/mole), A (Pa) is the network strength, z is the network extension (-) which is related to the number of interacting rheological units within the network and H is the continuous relaxation spectrum [8]. To solve the 7 parameters transformation problem, a determinant of a matrix procedure was employed using MATLAB 2020b. For statistical analysis, a completely randomized factorial design was employed. All experiments were repeated three times. Any significant difference between means (from three replicates) was determined by Tukey's honest significant difference test at a significance level of $P < 0.05$ using MSTATC statistical software (version 1.42, MSTATC director, Michigan State University, USA). Data fitting was carried out by MATLAB 2020b, using the curve fitting toolbox and Levenberg–Marquardt algorithm.

III. Results and Discussion

Physicochemical and network properties of both high fat and low fat lactose free set yogurts and control samples were evaluated. The fat content of high and low fat yogurts were 3 and 1.5%, respectively. On the other hand, the protein contents of modified yogurt and control one were 3 and 4%, respectively. Results showed that in spite of 1% less protein content of modified lactose free yogurt, the functional properties of this sample were significantly higher than the control one ($P < 0.05$).

According to Table I, the modified high fat yogurt showed 11.55%, 18%, 20.21% and 7.08% higher hardness, consistency, WHC (water holding capacity) and shininess than the control one, respectively. Likewise, the modified low fat yogurt showed 21.40%, 25.41%, 28.15% and 10.58% higher hardness, consistency, WHC and shininess than the control one, respectively. These behaviors could be related to the microstructure of gel network within the modified yogurt.

As seen in Table II, the chemical-enzymatic modification of milk protein induced significant changes in network parameters of set yogurt. Accordingly, the indexes of linkage strength (A), density of linkages (z), and timescale of interactions (λ_{rel}) of high fat modified yogurt were 22.10%, 50.68% and 21.82%, higher than the control one, respectively, whereas the distance between linkages (ξ) was 16.77% lower. Similarly, low fat modified yogurt showed 34.30%, 61.70% and 42.60% higher A, z, and λ_{rel} values, respectively, and 19.20% lower ξ value than the control one. In other words, the modification of milk protein structure improved the gel network of the set yogurt. In this way, a gel network with greater strength, more extensive network and lower distance between linkages (which characterizes the effective space available for solute diffusion) was observed in treated yogurt in respect to the control one. Also, the higher relaxation time of the modified yogurt network suggested a higher mean lifetime of the junction zones and could be associated with the formation of intermolecular aggregates due to the fine-stranded conformation of the modified protein. Based on the aforementioned concepts, the network parameters supported the texture analyzer and WHC results [9]-[12]. Also, the modified protein resulted in the better functional properties of low fat yogurt compared with high fat yogurt ($P < 0.05$), which could be explained by a greater protein in dry matter of low fat sample (almost 50%) compared with high fat yogurt (37%).

Li et al. [13] investigated the effect of modified micellar casein proteins on set yogurts properties. They reported that the modified proteins improved the properties of casein network structure, syneresis and firmness of yogurts. It is worth mentioning that in their research the same percentage of protein in the control and treated samples was employed. Also, Lesme et al. [14] employed nanoparticulated whey protein aggregates to improve the textural properties of fat-free yogurts. Their results showed that with increasing protein concentration, the firmness and the cross-link density of the protein network decreased. In addition, Hossain et al. [15] used microparticulated whey protein to imitate the role of fat on the yogurt gel network properties. The results showed that with only more than 7% microparticulated protein usage, the reduced fat yogurt can be produced with a similar texture to full-fat

yogurts (3% fat).

According to Table III, the modified low fat yogurt showed a minimum value of strain limit (0.64 ± 0.10). This parameter depends on the deformability of the gel networks and the molecular architecture of the biopolymers [16]. The low γ_L value suggested a long timescale of interaction within gel networks, so indicating the required time for new entanglement to replace those disrupted by external small deformation in amplitude oscillatory test is long. τ_y is considered as the starting point of the weakening of the gel strength. The result indicated that the modified high fat yogurt showed a maximum value of the limiting stress (80.20 ± 6.16 Pa) which suggested the fragile hydrogel nature of this sample. The rise in strain higher than γ_L caused a drop in the magnitude of G'_{LVE} for all the yogurts over the entire strain. G''_{LVE} of control yogurts (low and high fat) showed a similar behavior with G'_{LVE}, whereas, the modified yogurts showed an overshoot. According to classification of Hyun et al. [17], the former group showed strain thinning behavior and the latter ones showed weak strain overshoot behavior, which suggested that the structure formed within the control yogurts was weaker than other samples. On the other hand, the modification of protein can form a structured gel network. These complex structures resist against deformation up to a certain amount of strain where G″ increases to the highest value (G''_{max}), which has been observed in soft glassy materials [18]. Sim et al. [19] also indicated that the overshoot may be related to the balance between the formation and degradation of the junction zones. Also, strain thinning behavior is observed when with an increase in strain polymer chains disentangle and align with the flow field [20]. The magnitude of τ_f (Pa) (where $G' = G''$) was the highest for high fat modified yogurt (183.45 ± 4.85 Pa), which reflected the highest dynamic yield stress characteristic among other yogurt. Beyond the flow point, materials change from viscoelastic to elastoviscous behavior where the materials demonstrate irrecoverable deformation [21], [22].

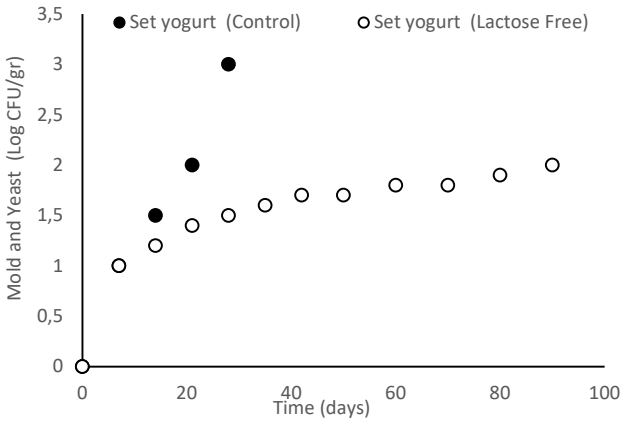

Fig. 1 Population dynamics of yeasts and mold in high fat lactose free set yogurt and control sample

TABLE I
PHYSICOCHEMICAL PROPERTIES OF HIGH FAT AND LOW FAT LACTOSE FREE SET YOGURT AND CONTROL SAMPLES

Sample		Hardness (gr)	Consistency (gr.s)	Water holding capacity (%)	Shininess (%)
Full fat	Control	129.52 ± 1.05[b]	2700.81 ± 20.10[b]	55.57 ± 0.81[b]	86.50 ± 1.05[b]
	Modified	145.70 ± 0.83[a]	3187.12 ± 14.53[a]	66.60 ± 0.41[a]	92.55 ± 0.81[a]
Low Fat	Control	115.36 ± 2.10[b]	2089.12 ± 10.21[b]	48.51 ± 1.05[b]	88.20 ± 1.05[b]
	Modified	139.60 ± 1.71[a]	2620.12 ± 5.91[q]	62.17 ± 0.59[a]	94.80 ± 1.05[a]

[a-b] Means of two replicates in the same column with same superscripts do not differ significantly (p > 0.05)

TABLE II
NETWORK PROPERTIES OF HIGH FAT AND LOW FAT LACTOSE FREE SET YOGURT AND CONTROL SAMPLES

Sample		A (Pa.s$^{1/z}$)	z (-)	λrel (s)	ξ (nm)
Full fat	Control	939.83 ± 19.05[b]	8.88 ± 0.38[b]	68.51 ± 5.10[b]	11.83 ± 0.22[a]
	Modified	1147.53±25.10[a]	13.38 ± 0.38[a]	83.45 ± 4.85[a]	9.13 ± 0.41[b]
Low Fat	Control	815.52 ± 1.05[b]	7.41 ± 0.22[b]	39.10 ± 1.75[b]	12.84 ± 0.36[a]
	Modified	1094.70 ± 21.30[a]	11.98 ± 0.28[a]	55.76 ± 2.55[a]	10.37 ± 0.45[b]

[a-b] Means of two replicates in the same column with same superscripts do not differ significantly (p > 0.05)

TABLE III
LINEAR AND NONLINEAR VISCOELASTIC PROPERTIES OF HIGH FAT AND LOW FAT LACTOSE FREE SET YOGURT AND CONTROL SAMPLES

Sample		γ_c(%)	τy(Pa)	τ_f(Pa)	$\frac{G''_{max}}{G''_{LVE}}(-)$
Full fat	Control	11.83 ± 2.10[a]	39.68 ± 3.83[b]	138.51 ± 5.52[b]	-
	Modified	1.53 ± 0.13[b]	80.20 ± 6.16[a]	183.45 ± 7.25[a]	1.93 ± 0.18
Low Fat	Control	5.52 ± 0.80[a]	27.37 ± 3.50[b]	98.62 ± 3.05[b]	-
	Modified	0.64 ± 0.10[b]	69.15 ± 4.28[a]	126.02 ± 4.55[a]	1.50 ± 0.10

[a-b] Means of two replicates in the same column with same superscripts do not differ significantly (p > 0.05)

To evaluate the stability of yogurts, the mold and yeast population and acidity of them were analyzed during two months (Fig. 1). Results indicated that modified yogurt could sustain for 10 weeks in the refrigerator, whereas the control set yogurt had a 3 weeks shelf life. The changes in acidity of high fat and low fat modified yogurt were from 76 to 84 and 72 to

80 Dornic degrees during 10 weeks storage, whereas they were 82 to 122 and 77 to 112 Dornic degrees for high fat and low fat control samples, respectively. This behavior could be due to the elimination of energy source for the microorganisms in modified yogurt. The lactose content of the modified yogurt was less than 0.01%, whereas the control samples had 3 to 4% residual lactose. It is worth mentioning that, the calorie of high fat lactose free yogurt was 48 kcal per 100 gr which was 25% lower than that of the control high fat yogurt with 64 kcal energy per 100 gram. Similarly, the low fat modified yogurt and control sample had 25.5 kcal/100 gr and 41.5 kcal/100 gr calories, respectively, with 40% less calories in comparison with the control one.

IV. Conclusion

In this study, a yogurt was developed by both modification of the manufacturing process and the milk protein as its ingredient. According to Hyun classification, the result indicated that modified yogurts (low and high fat) belonged to weak strain overshoot class while control yogurts (low and high fat) belonged to strain thinning class. Some of the most common problems in yogurt industry are syneresis, decrease in curd consistency and the post acidification of yogurt in shelf life. Herein, to decrease the lactose content and adjust the protein, fat, acid, dry matter and milk mineral of yogurt samples, a combination of UF, nano-filtration and reverse osmosis were employed. Furthermore, for modification of protein a number of chemical reactions with citrate buffer, sodium dihydrogen phosphate, sodium poly-phosphate and enzymatic reaction of Transglutaminase (TG) were employed. These modifications reinforced the functional properties of yogurt. The aforementioned protocol has additional advantages such as increase in curd consistency, decrease in syneresis even with 1% lower protein content compared with control one, decrease in lactose content to less than 0.01%, production of low calorie yogurt, prevention of Millard reaction with its following problems, manufacturing of valuable ingredients as by product such as lactose powder and milk mineral powder, increase in shelf life from 3 to 10 weeks in refrigerator and decrease in production cost.

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