

Investigating the Texture and Antioxidant Capacity of Papain and Trans-glutaminase Enzyme-treated Yogurt with Different Carbohydrates – Glucose, Sucrose and Maltodextrin

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Abstract

Yogurt is considered as a functional food, which is a complex mixture of different biological components. Functional foods are designed to have physio-logical benefits and reduce the risk of chronic diseases beyond basic nutritional functions, and may be similar in appearance of conventional food. Therefore, the consumption of dairy products is highly recommended. In this study, the average composition milk was purchased in a local supermarket. Subsequently, ultrafiltration of milk was carried out with a tubular membrane, placed in a laboratory-developed cross-flow membrane module. Pore size and active surface area of tubular membrane were 5 nm and 0.005 m², respectively. A static turbulence promoter was placed inside of membrane tube. Retentate of ultrafiltration membrane was treated with different concentrations of papain at temperature 50 °C for 10 minutes and subsequently, deactivation of enzymatic activity was performed at temperature 70 °C for 20 minutes. After deactivation of catalytic activity of papain, milks were fermented with yogurt starter culture (Thermophilic YoFlex® culture) at temperature 45 °C for 6 hours. During fermentation, transglutaminase and different types of carbohydrates, such as glucose, maltodextrin and sucrose were introduced with the aid of changing texture and antioxidant capacity of yogurt. Antioxidant capacity and hardness of yogurt, prepared with cow's milk were 0.44 mmol eqv. ascorbic acid/L and 0.58 Newton, respectively. It was found that application of enzyme (both papain and transglutaminase) and maltodextrin increased the antioxidant capacity of yogurt. Furthermore, it was found that hardness of yogurt was increased by addition of carbohydrate.

Keywords

yogurt, papain, transglutaminase, antioxidant capacity, hardness

1 Introduction

Yogurt is a well-known fermented milk product, which is a heterogeneous system of carbohydrate, protein and fat. It is produced by the anaerobic fermentation of milk with starter cultures, mostly *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. When an adequate amount of lactic acid is produced, the milk proteins coagulate. This coagulated milk is known as yogurt. There are two types of yogurt i.e. set yogurt and stirred yogurt. The functional food sector has great possibility in the field of product development [1]. Milk proteins are good sources of bioactive peptides. Enzymatic hydrolysis of proteins is frequently used for the generation of bioactive peptides from protein molecules. Bioactive peptides have positive impact on wide range of health benefits. They can control and modulate important physiological functions through their countless activities, such as antimicrobial, antihypertensive, antithrombotic, immunomodulatory and

antioxidant activities [2]. Cow's milk allergenicity is a very complex disorder with different immune reactions. Immune-mediated reactions may be IgE-mediated or non-Ig-mediated, whereas food intolerance may be different in every geographical regions. Most of the IgE-mediated allergy occurs in young children in the first 6 months. Milk allergy depends on the characteristics of the population, e.g. geographical origin, atopic status and age. The Cow's milk is a member of the "Big-8" food allergens. Cow's milk contains 30–35 g/L of protein. After the acidification, two fractions are generated i.e. lactoserum (whey) and coagulum. The major milk allergens are α s1-casein, α -Lactalbumin and β -Lactalbumin [3, 4]. Milk contains numerous amounts of equol, which is a metabolite of daidzein. The equol offers antioxidant capacity in milk. Furthermore, milk proteins (α -, β - and κ - caseins) offer antioxidant capacity in milk [5, 6].

Acid whey formation during the yogurt production creates main problem for the development of texture of yogurt. The acceptance of yogurt is better when the acid whey is removed [7]. Centrifugation as well as several filtration methods, such as Ultrafiltration (UF) and Reverse Osmosis (RO) are used to concentrate proteins and subsequently yogurt preparation. Application of concentrated milk proteins during yogurt preparation increases quality product with high protein concentration and enhance the gel-strength of yogurt [8]. The problems associated with size exclusion-based membrane filtration process are generation of concentration polarization on membrane surface as well as membrane fouling. Generation of concentration polarization on membrane surface is the cause of severe flux declination, which affect the quality of product [9, 10]. To reduce the chance of membrane fouling during filtration process, it is important to increase the turbulence intensity on vicinity of membrane surface with the use of static turbulence promoter [11]. The enzymatic modification of proteins by use of microbial transglutaminase and papain offers unique physical and chemical properties of food products. Recently, it has been reported that microbial transglutaminase improves the yogurt texture, i.e. it increases hardness and gel-strength of yogurt. Furthermore, application of transglutaminase increases the viscosity and preventing of whey separation from yogurt [12]. The transglutaminase is a transferase enzyme, which promotes cross-linking of amino acid residues (like lysine and glutamine) of protein chain [12, 13]. The papain is an endopeptidase enzyme, extracted from the fruit of *Carica papaya*. Papain belongs to the cysteine protease family, which cannot generate bitter taste of food products [14]. This enzyme is relatively heat stable. The molecular weight of papain enzyme is 23,406 Da with 8.75 isoelectric point. Optimum catalytic activity is at pH 5–8. In food industry, papain is used for to produce protein hydrolysate. In juice and brewing industries, papain is used for clarification of juice and fermentation broth [15].

Object of this study was investigating the texture and antioxidant capacity of papain and trans-glutaminase enzyme-treated yogurt with different carbohydrates, such as glucose, sucrose and maltodextrin.

2 Material and methods

2.1 Material

Extended self-life type of cow's milk was purchased in a local supermarket (G-Roby, Hungary). A freeze-dried yogurt culture (FD-DVS YF-L812 Yo-Flex®) was procured from Chr. Hansen, Denmark. This yogurt culture

contains *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* strains. The culture can produce set and drinking-type of yogurt with very mild flavor and extra high viscosity. Microbial transglutaminase (activity 100 U/g) from Barentz Ltd., Hungary, papain (nominal activity: 30000 U/mg) from HiMedia Laboratories, India were purchased. Ascorbic acid and 2,4,6 Tri(2-pyridyl)-triazine from HiMedia Laboratories, India, sodium acetate, iron (III) chloride, citric acid and acetic acid from Merck, Germany were procured. Ultrasil P3-11 was purchased from Ecolab-Hygiene Kft, Hungary.

2.2 Methods

The detailed process flow diagram of the yogurt preparation is shown in Fig. 1. In first step, milk was concentrated with 5 nm-pore-sized membrane and subsequently, retentate of filtration process was collected at aseptic condition. Concentrated milk was treated with papain and after deactivation of catalytic activity of papain, it was fermented with yogurt starter culture. Effects of transglutaminase and different types of carbohydrates, such as glucose, maltodextrin and sucrose during fermentation were studied.

2.2.1 Membrane filtration

In this study, the first step was the pre-concentration of milk using 5 nm-pore-sized ceramic membrane. The tubular membrane was placed inside the laboratory-developed membrane house, designed by the Department of Food Engineering, Szent István University, Hungary. Detailed description of membrane module is mentioned elsewhere [16]. Inside the membrane tube, static turbulence promoter was placed. Concentrate milk was prepared by batch-mode filtration process until Volumetric Concentration Ratio (VCR) was achieved 2. The Volumetric Concentration Ratio was determined according to Eq. (1):

$$\text{VCR} = \frac{\text{Initial feed volume}}{\text{Concentrate volume}} \quad (1)$$

The permeate flux was calculated according to Eq. (2) [17]:

$$J = \frac{V}{A \times t}, \quad (2)$$

where J is the permeate flux [L/(m²×h)], V is volume [L], A is active membrane surface [m²], t is time [s]. The effective area of the membrane was 0.005 m². The 3 L of milk sample was poured in feed tank of membrane module and filtration experiment was performed until 1.5 L permeate was achieved. Filtration process was performed

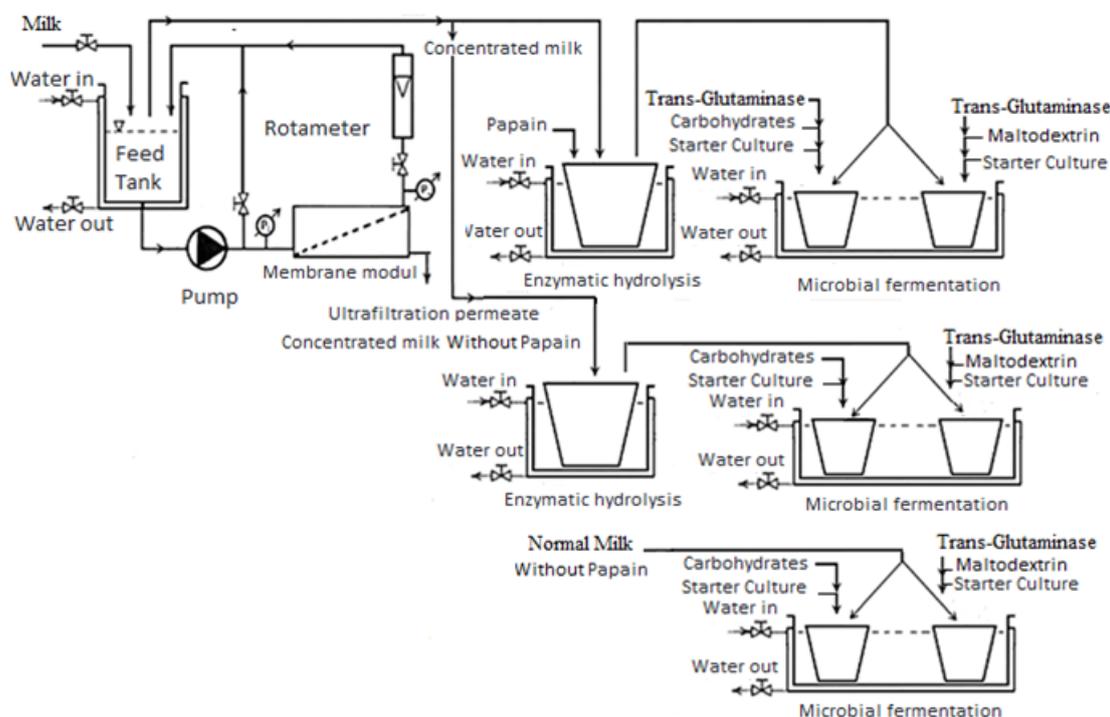


Fig. 1 Process flowsheet for yogurt preparation

with constant temperature 25 ± 1 °C. Temperature of milk in feed tank was maintained by a thermostat. During the filtration process the transmembrane pressure and the recirculation flow rate were 3 bar and 100 L/h, respectively.

Before membrane filtration of milk, membrane compaction was performed with transmembrane pressure 4 bar and recirculation flow rate 200 L/h, until the water flux became steady. After filtration of milk, membrane was cleaned with ultrasil and citric acid in a subsequent way. During cleaning with ultrasil and citric acid, transmembrane pressure 0.8 bar and recirculation flow rate 200 L/h were employed. Finally, membrane was cleaned with de-ionized water. During cleaning with water, transmembrane pressure 5 bar and recirculation flow rate 200 L/h were employed [18].

2.2.2 Enzymatic hydrolysis and fermentation

In present study, 3 L of milk was concentrated with membrane and ~1.5 L of concentrated milk from retentate channel was collected after the end of filtration experiment. Concentrated milk was treated with different concentration of papain, such as 0.0008 % (w/v), 0.0016 % (w/v), 0.0032 % (w/v) in separate Erlenmeyer flasks. In each Erlenmeyer flask, sample (concentrated milk) volume was 300 mL. They are designated as N1, N2 and N3, respectively. Enzymatic hydrolysis of milk protein with papain,

was performed at temperature 50 °C for 10 minutes. Subsequently, deactivation of enzyme was performed at temperature 70 °C for 20 minutes. After deactivation of catalytic activity of papain, the samples were cooled to temperature 45 °C and subsequently, 45 mL of papain-treated milk from each category was distributed in 50 mL of falcon tube. In some cases, 3 % (w/v) of different types of carbohydrates, such as glucose, sucrose and maltodextrin were added in the respective tubes. Furthermore, in some tubes, 0.002 % (w/v) transglutaminase, and maltodextrin with transglutaminase were added. Besides that, 45 mL of membrane retentate concentrated milk was poured into some tubes and similar like before, 3 % (w/v) of individual carbohydrate was added into them. Normal milk was poured into different falcon tubes with similar fashion. Total 29 samples were generated. Sample codes and their detailed descriptions are mentioned in Table 1. Suspension of yogurt culture was prepared by dissolving 0.18 of lyophilized starter culture with 30 mL of concentrated milk and subsequently 1 mL of suspension was inoculated to 45 mL of milk in each sample tube. All tubes were placed in a water bath at temperature 45 °C for 6 hours. Subsequently, temperature of all samples was reduced to room temperature (~ 25 °C) and they were removed from the top of yogurt. Finally, all samples were placed to a refrigerator at 5 °C for one day for aging.

Table 1 Codes and descriptions of yogurt samples

Sl. no	Sample codes	Description
1	N1	Concentrated milk treated with 0.0008 % (w/v) of papain
2	N1+G	Concentrated milk treated with 0.0008 % (w/v) of papain + Glucose
3	N1+S	Concentrated milk treated with 0.0008 % (w/v) of papain + Sucrose
4	N1+M	Concentrated milk treated with 0.0008 % (w/v) of papain + Maltodextrin
5	N2	Concentrated milk treated with 0.0016 % (w/v) of papain
6	N2+G	Concentrated milk treated with 0.0016 % (w/v) of papain + Glucose
7	N2+S	Concentrated milk treated with 0.0016 % (w/v) of papain + Sucrose
8	N2+M	Concentrated milk treated with 0.0016 % (w/v) of papain + Maltodextrin
9	N3	Concentrated milk treated with 0.0032 % (w/v) of papain
10	N3+G	Concentrated milk treated with 0.0032 % (w/v) of papain + Glucose
11	N3+S	Concentrated milk treated with 0.0032 % (w/v) of papain + Sucrose
12	N3+M	Concentrated milk treated with 0.0032 % (w/v) of papain + Maltodextrin
13	NM	Normal milk
14	NM+G	Normal milk + Glucose
15	NM+S	Normal milk + Sucrose
16	NM+M	Normal milk + Maltodextrin
17	N1T	Concentrated milk treated with 0.0008 % (w/v) of papain + Transglutaminase
18	N1T+M	Concentrated milk treated with 0.0008 % (w/v) of papain + Transglutaminase + Maltodextrin
19	N2T	Concentrated milk treated with 0.0016 % (w/v) of papain + Transglutaminase
20	N2T+M	Concentrated milk treated with 0.0016 % (w/v) of papain + Transglutaminase + Maltodextrin
21	N3T	Concentrated milk treated with 0.0032 % (w/v) of papain + Transglutaminase
22	N3T+M	Concentrated milk treated with 0.0032 % (w/v) of papain + Transglutaminase + Maltodextrin
23	NMT	Normal milk + Transglutaminase
24	NMT+M	Normal milk + Transglutaminase + Maltodextrin
25	UFM	Ultrafiltered milk
26	UFM+G	Ultrafiltered milk + Glucose
27	UFM+S	Ultrafiltered milk + Sucrose
28	UFM+M	Ultrafiltered milk + Maltodextrin
29	UFM+T+M	Ultrafiltered milk + Transglutaminase + Maltodextrin

2.2.3 Monitoring texture

Among the texture characteristics, hardness is one of the most important parameters, which is often used to determine the freshness of food. In this study, the hardness is measured by the maximum force (in Newton), which is needed to break the surface of yogurt. The hardness of yogurt was determined by texture analyzer (TA.XT plus, Texture Analyzer, Stable Micro Systems Ltd., England). The yogurt samples were placed in a thermostat, where temperature was maintained 20 °C. The experiment was carried out with a 25 mm cylinder probe (Patch number 2161). The penetration depth was 5 mm in every case. The movement of the probe was 1 mm/s, when it was in air. When the probe touched the superficial surface of yogurt, its speed turned to 0.1 mm/s (test speed). Penetration depth of cylindrical probe within yogurt was 5 mm with 0.1 mm/s. Software Exponent (Stable Micro Systems, 2006, version 5.0) was used to calculate force as well as hardness. Around 45 mL of yogurt sample in each tube was used to analyze hardness of yogurt. The peak force during the first compression cycle is defined as hardness or firmness. Peak force during the 5 mm compression is represented with Newton unit.

2.2.4 Antioxidant capacity

Antioxidant capacity of freshly prepared yogurt samples was measured by the Ferric Reducing Antioxidant Power (FRAP) Assay according to the procedure described by Benzie and Strain with some modifications [19]. From each falcon tube, 10 g of yogurt were collected and centrifugation was performed with a laboratory centrifuge (Z 206 A Model, Hermle Labortechnik GmbH, Germany). During centrifugation of samples, rotor speed 5600 rpm and temperature ~25 °C were used for 20 minutes. Subsequently, 1 mL of supernatant was collected for assay. In assay, 300 mM of sodium acetate buffer (pH 3.6), 20 mM of ferric chloride and 2,4,6-Tripyridyl-S-TriazineAscorbic acid solution (10 mM 2,4,6-Tripyridyl-S-Triazine with 40 mM of hydrochloric acid) were mixed with the ratio 10:1:1. 9 mL of freshly prepared reagent was mixed with 1 mL of appropriate diluted sample and all test tubes were incubated for 30 min at temperature 37 °C. Ascorbic acid was used as reference. After incubation, the absorbance of the samples was measured with a spectrophotometer (DR/2400 Portable Spectrophotometer, Hach, USA). Spectrophotometric measurement was performed with 593 nm of wavelength.

3 Results and discussion

3.1 Membrane filtration

The composition of cow's milk is represented in Table 2. In first step, milk was concentrated by ultrafiltration membrane. It was observed that permeate flux was declined with increase of the VCR (Fig. 2). With increase of VCR, permeate flux was reduced due to formation of concentration polarization on membrane surface. As membrane separation process was performed with 5 nm membrane and batch-mode process, protein concentration was increased in retentate side along with filtration time. Concentrated proteins were deposited on membrane surface and gel layer resistance was increased with time progress. In Fig. 2, it is shown that the initial value of permeate flux was 37 L/(m²×h) which was declined to 24 L/(m²×h) when VCR reach to 2. In present investigation, static turbulence promoter was applied inside of membrane tube to reduce the formation of concentration polarization on membrane surface. It created turbulence on membrane surface, which offered tangential force on membrane surface. On the other hand, trans-membrane pressure offered driving force on membrane surface. Combination of these two forces, formation of concentration polarization and gel-layer resistance were reduced. In the present investigation, membrane with pore size 5 nm was used. It increased the concentration of proteins in retentate end of membrane module.

Table 2 Average composition of the Cow's milk, according to Naszálytej Tejfeldolgozó és Kereskedelmi Zrt, Hungary

Component	Concentration in % (w/v)
Fat	1.5
Protein	2.9
Lactose	4.6
Minerals	0.13

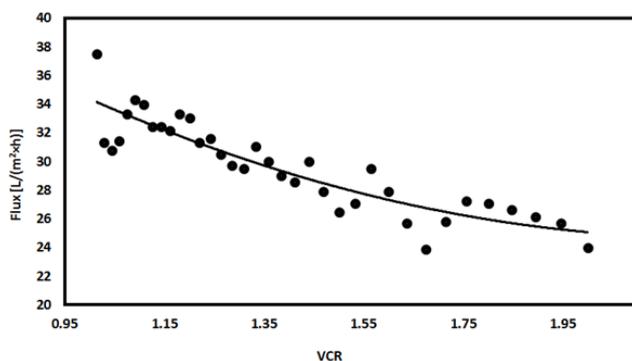


Fig. 2 Permeate flux during membrane filtration of milk

3.2 Texture analysis

Results of harness of yogurt samples are represented in Table 3. Hardness of yogurt, prepared by normal milk was 0.58 Newton and it was increased when yogurt was prepared with concentrated milk. This is justified by the fact that high solid (milk protein) concentration in ultra-filtered-milk increased the viscosity as well as hardness of yogurt samples [20, 21]. Hardness of yogurt, prepared with carbohydrate addition was increased compare to yogurt, prepared without carbohydrate. This can be justified by the fact that yogurt starter cultures, namely *Lactobacillus bulgaricus* and *Streptococcus thermophilus* produced exo-polysaccharides from carbohydrate, which have potential contribution to impart smooth texture, higher viscosity and lower syneresis [22, 23]. It has

Table 3 Hardness of different types of yogurts

Sl. no	Sample codes	Hardness (Newton)
1	N1	1.58
2	N1+G	1.53
3	N1+S	1.07
4	N1+M	1.5
5	N2	1.94
6	N2+G	2.08
7	N2+S	1.74
8	N2+M	1.8
9	N3	1.43
10	N3+G	2.03
11	N3+S	1.73
12	N3+M	1.75
13	NM	0.58
14	NM+G	0.59
15	NM+S	0.59
16	NM+M	0.65
17	NIT	1.8
18	NIT+M	1.9
19	N2T	2
20	N2T+M	1.9
21	N3T	2.6
22	N3T+M	2.7
23	NMT	0.73
24	NMT+M	0.7
25	UFM	1.5
26	UFM+G	1.9
27	UFM+S	1.65
28	UFM+M	1.6
29	UFM+T+M	1.8

been reported that exo-polysaccharide yield from the species of *Streptococcus thermophilus* was ~1,029 mg/L in milk medium in presence of 0.5 % whey protein concentrate [24]. Furthermore, intrinsic viscosity, ranging from 1.5–4.7 dL/g had been reported for exo-polysaccharides, produced by starter culture *Streptococcus thermophilus* and *Lactobacillus bulgaricus* [25]. Transglutaminase also had a positive effect on the hardness of yogurt. This can be justified by the fact that transglutaminase promoted cross-linking of amino acids lysine and glutamine in protein chain, which reduced the loss of whey proteins, increased the solid content (protein) in yogurt and developed tight protein network. As a result, hardness of yogurt was increased [12, 20].

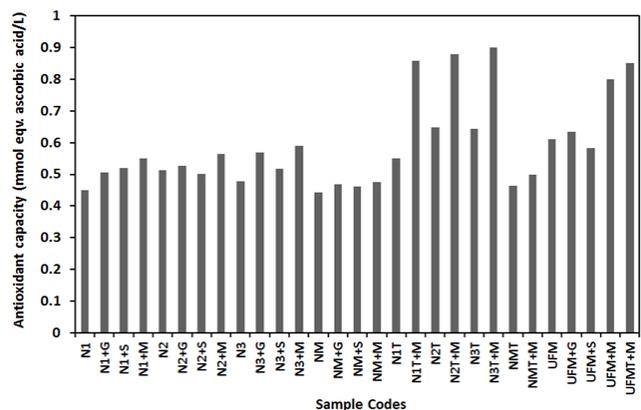
3.3 Antioxidant capacity

The aim of this study was to examine the antioxidant capacity of different types of yogurt samples. Antioxidant capacity of different types of yogurts is represented in Fig. 3. Antioxidant capacity in yogurt, prepared by conventional method was 0.44 mmol eqv. ascorbic acid/L. It was increased in yogurt, prepared with papain compare to normal cow's milk yogurt. Papain prefers to cut at (hydrophobic amino acid)-(Arg or Lys or Glu or His or Gly or Tyr) in milk protein [26, 27]. With increase of the concentration of papain, more amounts of peptide bonds in milk proteins were cleaved and hydrophobic amino acids were exposed. Hydrophobic amino acids in peptide chain offer antioxidant capacity [28].

Antioxidant capacity in yogurt samples, prepared with transglutaminase was increased compare to yogurt sample, prepared with normal milk. This can be justified by the fact that cross-linking of amino acids lysine and glutamine in protein chain was done in presence of transglutaminase. It reduced the loss of whey proteins, where hydrophobic amino acids were present at terminal end. Furthermore, addition of carbohydrate increased the antioxidation capacity of yogurt samples because during microbial fermentation, exo-polysaccharides were produced from carbohydrate by starter cultures, which increased the water holding capacity in yogurt [29].

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