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Determination of Physicochemical, Rheological, Microbiological and Sensory Properties of Low Protein Yoghurt Substitutes Produced for PKU (Phenylketonuria) Patients

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ABSTRACT
Treatment of PKU (Phenylketonuria) is a lifelong special diet program starting from the newborn period. The aim of this study was to produce yoghurt substitute for PKU patients. A commercial low protein milk substitute, xanthan gum (1%), commercial yoghurt gelling agent (1.5%), starch (4%) and pectin (1.6%) were used to produce yoghurt substitute. Control yoghurt was produced from cow's milk. The fermentation of all samples was completed at the end of the 5th hour. The pH of the samples decreased during storage. The total solid matter of the corn starch and pectin added samples were higher than the those of others. Syneresis values of the samples with xanthan gum, pectin and commercial gelling agent were negligible. The shear stress values of xanthan gum, commercial gelling agent and starch added samples were found close to each other. The shear stress of the pectin added sample was the highest among the samples. L* values of the control and starch added samples, a* and b* values of pectin added sample were higher than those of other samples. The amount of protein and phenylalanine was higher in the pectin added sample than the other samples containing gelling. However, their values in all yoghurt substitute samples were found to be well below the upper limit value that can be consumed. Although the amount of phenylalanine tolerated in the body varies according to age, gender, weight and the degree of phenylketonuria, it is stated that up to 1000 mg per day. While numbers of *Lactobacillus delbrueckii* ssp. *bulgaricus* increased during storage, numbers of *Streptococcus thermophilus* increased by pectin addition and decreased in other samples. In terms of general acceptability in sensory analyses, the most preferred sample was the sample added containing commercial gelling agent. This sample was followed by the samples with corn starch and pectin. It was concluded that these yoghurt substitutes could support the missing alternative product range for the patients.

Keywords: Phenylketonuria, PKU patients, Hydrocolloid, Stabilizer, Milk substitute

1. Introduction

Phenylketonuria (PKU) is one of the most common congenital metabolic disorders which is a disease caused by mutations in the gene encoding the enzyme phenylalanine hydroxylase (Scriver and Kaufman, 2001). As a result of deficiency of this enzyme, the amino acid phenylalanine, which does not turn into tyrosine, and its metabolites (phenylpyruvic, phenyl lactic acid, phenyl acetic acid) formed as a result of its transamination, accumulate in the blood, urine and other body fluids of the patient, causing mental-motor retardation (Müslümanoğlu et al. 2014; Parlak 2018). When PKU is not treated, irreversible mental retardation, microcephaly, motor impairment, eczematous rash, autism, seizure development, developmental problems, abnormal behavior and psychiatric symptoms (Van Wegber et al. 2017; Erdal & Caferoğlu 2018), light hair, skin and eye color, widely spaced teeth, mold-like urine due to defect in melanin formation and sweat odor (Gaw et al. 1999; Davis et al. 2005) occurs.

The general principle in the treatment of PKU is special diet programs that should be started with the diagnosis in the neonatal period and their lifelong continuity. This diet aims to keep the blood Phe level within normal limits by minimizing the amount of Phe ingested with food (Lee & Newman 2003; Seçkin 2007; Özer et al. 2008). The diet program is different for each patient. A diet list is prepared according to the patient's height, age, body weight, type of phenylketonuria and blood Phe level (Anonymous 2017a). Products with high Phe content are removed from the diet lists of individuals with PKU. Milk and dairy products are among these products (Scriver & Kaufman 2001; Waisbren et al. 2007). This amino acid, which is found in 4% to 5% in almost all proteins, is 5.4 g / 100 g in cow's milk protein and forms the building block of many proteins, especially together with tyrosine (Kavas & Kınık 2005). The protein requirement necessary for the growth and development of the individual is met with special amino acid mixtures without Phe (Üstüner Top & Küçük Alemdar 2015).

The daily Phe amounts that patients with classical, moderate and mild phenylketonuria can tolerate are 20 mg/kg, 20-25 mg/kg and 25-50 mg/kg, respectively. (Thöny & Blau 2006; Akış 2012). According to recommendations for UK, Germany, the USA, France, the Netherlands, and the 2016 ESPKU (European Society for Phenylketonuria and Allied Disorders Treated as Phenylketonuria) guidelines, the allowed amount of Phe is 130-400 mg/day for 0-2 years old, 200-400 mg / day for 3-9 years old, 350-800 mg/day for 10-15 years old, 450-1000 mg/day for adolescent/adult and 120-400 mg/day for pregnancy. Tolerance for pregnancy will usually increase in later stages of pregnancy. Since Phe is an essential amino acid, excessive restriction is also harmful and, particularly in infancy, will result in impaired growth and cognitive development (Burgard et al. 2016).

There are home-made yoghurt substitutes recipes for PKU patients that are not industrially produced. Milk powder (20 g), yoghurt maker gel (3 g), lactose (1 g), and water (200 mL) are boiled. When the yogurt reaches the fermentation temperature, normal yoghurt (30 g) is added and a fermentation is carried out for 5 to 6 hours. In another way, 500 g of milk substitute (Taranis dalia), 40 g of starch and salt are boiled. Fifty grams of yogurt as a starter culture is added and left to ferment (Anonymous, 2017b). In another recipe, 400 mL of milk substitute and 40 g of corn starch are boiled for 5 minutes. Twenty g of yoghurt, 5 mL of lemon juice, 3 g of salt and 5 g of granulated sugar mixture is added to the starchy jelly that has come to the temperature of yoghurt fermentation. Fermentation takes 3 hours. In all recipes, yoghurts are stored at +4 ^{o}C after fermentation. In the last recipe, the protein, Phe and energy content of 1 serving of yoghurt is 0.22 g, 107.34 Kcal and 10-15 mg, respectively (Anonymous 2020).

It has been reported that the prevalence of PKU varies according to ethnic groups, being higher in white and native Americans, and lower in blacks, Asians and Spain (Walter et al. 2006). According to data released by Ministry of Health in 2006 revealed that prevalence decreased and rate was 1/4500. However, Turkey is still in the list of countries with the highest prevalence (TCSB 2006).

Lifelong diet is very important in PKU disease. Many foods are on the banned food list of PKU patients. There is a great need for alternative low-protein products for the limited diet lists of PKU patients. However, today there are not enough studies on the products that PKU patients can consume. Studies for low-protein milk and dairy products are very few. There is no industrially produced yoghurt substitute for PKU patients. These patients consume yoghurt substitutes produced in different formulations at home. Under these conditions, a standard product cannot be obtained. This situation can be dangerous for the health of very sensitive patients.

Scortegagna et al. (2021) observed in their study named “Evaluation and acceptability of alternative food recipes for patients with phenylketonuria” that there are few studies aiming to detail food products for the PKU population. They noted that as diet has a significant impact on the daily life of these patients, the number of studies to develop food products for patients with PKU needs to be increased.

In this study, it was aimed to produce yogurt substitutes that PKU patients can consume by using different stabilizers and to determine the properties of those yogurt substitutes.

2. Material and Methods

2.1. Materials

The research material consists of low protein milk substitute, yoghurt culture, low methoxylated pectin, xanthan gum, commercial gelling agent and corn starch. Since commercial gelling agent is an expensive product, in this study it was compared with other gelling agents that may be alternative in terms of their contribution to yoghurt substitution Because cow's milk contains high amounts of protein, low-protein milk substitute (The Taranis Dalia Low Protein Milk Substitute, Lactalis Nutrition Santé LNS, France) was used in yoghurt production. This milk substitute is made up of water, cream, lactose, curdled milk powder, maltodextrin, mono and diglycerides of fatty acids (palm and / or rapeseed oil). One hundred mL of low protein milk substitute has 200 mg of protein, 6.4 mg of phenylalanine, 6.7 mg of threonine, 6400 mg of carbohydrate, 2600 mg of fat and 17.5 mg of calcium. UHT milk (cow milk) supplied by Pınar Süt Mamülleri San. A.Ş. was used for the production of control sample. Low-protein milk substitute was not used in the control sample production. In the production of other samples, only low protein milk substitute was used as milk. As starter culture, a yoghurt culture (Doğadan Bizim Gıda ve Süt Ürünleri San. ve Tic. Ltd. Şti., Istanbul, Turkey) consisting of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* was used. Low methoxylated pectin (Arosel Gıda Katkı Maddeleri ve Mak. San. Dış Tic. Ltd. Şti., Istanbul, Turkey), xanthan gum (Çağdaş Kimya ve Gıda San Tic Ltd Şti., Istanbul, Turkey), commercial gelling agent and corn starch (Pak Gıda Üretim Pazarlama A. Ş., Istanbul, Turkey) were used as gelling agents. Low methoxylated pectin was chosen because it produces a stable gelation at low sugar concentrations, using very little calcium within wide pH limits (pH 2.5-6.5). Xanthan gum is preferred due to its high viscosity in small concentrations, its immediate dissolution in hot and cold water, its resistance to deterioration that may occur due to heat and physical applications, and its usability between pH 1-11. Commercial gelling agent yoghurt maker gel was obtained from Nestle Nutrition GmbH (Frankfurt, Germany). It is a vegetable based gelling agent which contains carob powder and calcium lactate. One hundred g of it contains 1.5 g carbohydrate and 5.3 g protein. This gel is preferred because it is a low

protein yoghurt ingredient. Corn starch was preferred due to its ability to make a gel in a wide pH range with a more fluid structure that is resistant to breakage.

2.2. Methods

2.2.1. Preparation and fermentation of milk substitute

For the low protein yoghurt substitute, before starting the study, yoghurt pre-tests were made using various proportions of gelling agents and it was decided to use 1% xanthan gum, 1.5% commercial gelling agent, 4% starch and 1.6% pectin. The lyophilized yoghurt culture was inoculated into some low protein milk heated to 42 ºC and activated for 1.2 hours in the incubator at 42 ºC. On the other hand, some low protein milk substitute was poured in equal amounts (1000 mL each) into four different containers. The consistency of the milks was increased by adding 1% xanthan gum, 1.5% commercial gelling agent, 4% starch and 1.6% pectin into each and heating milks. The low protein milk substitutes added xanthan gum, commercial gelling agent and starch turned into a gel after being heated at 82 ºC for 5 minutes, 88 ºC for 5 minutes and 94 ºC for 5 minutes respectively. Heat treatment was continued until gelation was achieved. The low protein milk substitute to which pectin was added did not turn into a gel when kept at 99 ºC for 2 minutes. It turned into a gel when cooled after inoculation. Then, the activated yoghurt culture was inoculated (2%) to the viscous milks brought to the inoculation temperature (42 ºC). After inoculation, the milks were separated into plastic containers (PP material) and left to incubate at 42 ºC. The same procedures were applied to the UHT milk for the control sample. pH values of the samples were followed during fermentation. After 5 hours of fermentation, yogurt sample produced from UHT milk and other yogurt substitute samples were stored at +4 ºC. The analyses of yoghurt and yoghurt substitutes were carried out on the 1st, 7^{th}, 14^{th} and 21^{st} days of storage.

The pH, syneresis, total solid matter, stable phase rheological properties and microbiological analyses were performed on the 1^{st}, 7^{th}, 14^{th} and 21^{st} days of storage. Sensory and color analyses of the samples were carried out on the 1^{st} day of storage, crude protein and Phe analyses were performed on the 7^{th} day of storage.

2.2.2. Physicochemical analyses

pH measurements were carried out by means of a digital pH meter named 300/310 branded WaterproofHand heldpH / mV / TemperatureMeter (Kosikowski 1982). Total solid amount of samples were carried out with using AOAC method (1990). Crude protein analysis was performed using the Kjeldahl method (IDF 1986). Phe content was calculated using HPLC system (Waters, USA) (Goldar et al. 2016). For the determination of syneresis values, 5 g of sample was weighed and centrifuged at 4500 rpm and 10 °C for 30 minutes and then serum amount calculated as% by modifying the method stated by Verbeken et al. (2006). Konica-Minolta Chroma MeterCR-5 device was used for color determination. The samples were homogenized and placed in the measuring cup. The measurement was recorded 5 times. L* (brightness), a* (+ red, - green) and b* (+ yellow, - blue) values of yoghurt samples were determined (Cueva & Aryana 2008).

2.2.3. Rheological analyses

Steady phase analysis was performed using a temperature controlled (peltier system) rheometer (TA Instruments DHR2, USA) in a specified parallel plate configuration (cone diameter 40 mm), 1-100 s^{-1} shear rate and 5 °C degrees (Barnes et al. 1993).

2.2.4. Microbiological analyses

Microbiological analyses were carried out by applying the method of spreading on the surface (TGK 2003). The anaerobic environment required for microbiological analyses was provided by Microbiology Anaerocult A kits obtained from Merck Germany. 35 mL of sterile water was transferred to the kits and the kits were put into anaerobic jars. De Man-Rogosa Agar (MRS, Oxoid CM 361) was used for *Lactobacillus delbrueckii* ssp. *bulgaricus* growth. The pH of the medium was lowered to 5.2 by using HCl. Petri plates were left to incubate for 72 hours at 37 ºC under anaerobic conditions. Growing colonies (30-300) were counted (Dave & Shah 1996). M17-Agar (Merck, Germany) was used for the growth of *S. thermophilus*. Petri plates were left to incubate at 37 ºC for 3 days under aerobic conditions. Round colonies (30-300) formed after incubation were counted (TGK 2003).

2.2.5. Sensory analyses

The samples were evaluated for color, appearance, taste, odor, texture, mouth consistency, spoon consistency and general acceptability. In sensory evaluation, the ratings of very unsatisfactory, unsatisfactory, average, good and very good were given 1, 2, 3, 4 and 5 points, respectively. This sensory evaluation was carried out by 6 panelists (Meilgaard et al. 2006).

2.2.6. Statistical analyses

In the statistical evaluation of sensory and color analyses results, the difference between samples was determined using one-way ANOVA analysis. During the evaluation of phsicochemical and microbiological analyses results, the difference between the groups was determined using the univariate general linear model procedure of the SPSS statistical software programme (SPSS Statistics 21.0 Inc., Chicago, IL, USA). Duncan's multiple comparison test was used to determine significant differences among the means at P<0.05 (Düzgüneş et al. 1978). All trials and measurements were repeated in triplicates.

3. Results and Discussion

3.1 Physicochemical properties

The fermentation was carried out in a controlled manner by observing the pH values of the yoghurt samples during the fermentation. Table 1 shows the pH values observed during fermentation.

Table 1- pH values of yoghurt and low protein yoghurt substitute samples during fermentation

Fermentation time (h)	*K*	*A1*	*A2*	*A3*	*A4*
0	6.42±0.03 ^{Aa}	6.31±0.03 ^{Ab}	6.04±0.02 ^{Bc}	6.34±0.01 ^{Ab}	5.83±0.02 ^{Ad}
1	6.21±0.02 ^{Ba}	6.00±0.00 ^{Bc}	6.11±0.01 ^{Ab}	6.03±0.05 ^{Bc}	5.76±0.04 ^{Bd}
2	5.95±0.03 ^{Ca}	5.66±0.02 ^{Cc}	5.66±0.03 ^{Cc}	5.87±0.01 ^{Cb}	5.59±0.05 ^{Cd}
3	5.16±0.03 ^{Dc}	5.21±0.01 ^{Dc}	5.37±0.02 ^{Da}	5.31±0.03 ^{Db}	5.16±0.04 ^{Dc}
4	4.84±0.02 ^{Ec}	4.63±0.02 ^{Ee}	4.78±0.03 ^{Ed}	4.89±0.01 ^{Eb}	5.05±0.02 ^{Ea}
5	4.50±0.04 ^{Fc}	4.51±0.02 ^{Fc}	4.52±0.03 ^{Fc}	4.60±0.03 ^{Fb}	4.80±0.04 ^{Fa}

K (the control group, the difference from other samples is that stabilizer was not used); A1 (1% xantham gum); A2 (1.5% commercial gelling agent); A3 (4% corn starch), A4 (1.6% pectin).

Lower-case letters present the differences between the different samples in the same fermentation time and upper-case letters show differences between the fermentation times of each samples (P<0.01).

At the end of the 5th hour, the fermentation was terminated. At the end of fermentation, the control sample had the lowest pH value. The sample with the closest pH value was the one xanthan gum. At the end of the 5th hour, the pH of the sample to which pectin was added was at the highest level.

pH values of yoghurt samples decreased during storage (Table 2). Generally, the highest pH was detected in the pectin added sample and the lowest pH in the control sample during storage. The difference between yoghurt types and difference between storage days in terms of pH value was found to be significant at the P<0.05 level. Commercial gelling agent, starch and pectin added yoghurts were found to be statistically similar. While it was determined that the control yoghurt was different from these, xanthan gum added yoghurt was found to be similar to both groups.

The commercial gelling agent contains Ca-lactate. Pathomrungsiyounggul et al. (2010) reported that Ca-lactate has a pH-lowering effect. The protective activity of lactates has been explained by various researchers as the addition of lactate reduces the water activity (aw) of the product to prevent microbial growth and provides microbial inhibition by lowering the intracellular pH (de Wit & Rombouts 1990). Lactates are both Gram (+) and Gram (-) a bacteriostatic agent that can inhibit bacteria is an agent. In the studies, it was determined that the effect of lactates on the product increased with the increase of its concentration, depending on the properties of the product (Bingöl & Bostan 2012). Although the commercial gelling agent contains calcium lactate, these effects were not observed in the sample containing the commercial gelling agent. The pH values and microorganism counts of that sample were not significantly lower from the other samples (Table 2). This may be due to the low calcium lactate ratio.

Goldar et al. (2016) produced a special yoghurt for PKU patients. Each of the yoghurt milks contained equal amounts of milk (5%), starch (2%), inulin (2%), butter (3.5%) and transglutaminase (0.1%). While two of the samples contained 4% permeate (composed of water, sugar, some minerals and non-protein nitrogen compounds), 1.5% and 2% non-dairy creamer, the other two samples contained 5% permeate 1.5% and 2% non-dairy creamer. There was water in the ratio of 80.4-81.9% in yoghurt milk. *L. delbrueckii ssp. bulgaricus* and *S. thermophilus* were used as starter. After incubated for 4 h at 42 °C, they were stored at 4 ºC for 2 weeks. The highest pH reduction (0.15 unit) was seen in the sample containing 4% permeate and 2% non-dairy creamer during storage. The reduction in pH can be attributed to activity of bacteria in yoghurt and production of lactic acid from lactose in permeate. The pH values of the samples in that study were considerably lower than those in this study. Cogan (1996) stated that yoghurt bacteria are also active at refrigerator temperature and can cause a significant decrease in pH.

Dry matter values of the samples are shown in Table 2. Among the samples to which gelatin was added on the 1st day of storage, the dry matter of the starch added sample was the highest. This was followed by the pectin added sample. The xanthan gummy sample had the lowest dry matter. It is an expected result that the solid matter values of the samples differ due to the differences in the water binding capacity of the substances used as thickener. When hydrocolloids are used as thickeners in foods, water is physically bound and the structure of the food material changes (Çakmakçı 2012). In a study conducted by Goldar et al. (2016) solid matter ratios of the samples varied between 14.80-17.00%. Total solid matter (%) of samples containing corn starch and pectin in this study were similar to those of that study. The solid matter of other samples in this study was significantly lower than those in that study. Difference between solid matter ratios in both studies may be due to the different materials used.

Table 2- Some physicochemical and microbiological properties of yoghurt substitute samples during the storage

Treatments	*Storage Periods (days)*	*pH*	*Total Solid Matter (%)*	*Syneresis (%)*	*L. delbrueckii ssp. bulgaricus (log cfu/g)*	*S. thermophilus (log cfu/g)*
Control	1	4.40 ± 0.20^{Ab}	10.80 ± 0.26^{Ca}	2.07 ± 0.02^{Eb}	4.98 ± 0.05^{Ca}	6.18 ± 0.05^{Bb}
	7	4.29 ± 0.02^{Aab}	12.00 ± 0.20^{Cb}	2.81 ± 0.03^{Ea}	5.52 ± 0.03^{Cc}	6.30 ± 0.10^{Bb}
	14	4.17 ± 0.01^{Aab}	11.30 ± 0.10^{Cb}	2.43 ± 0.02^{Ea}	5.30 ± 0.02^{Cb}	6.48 ± 0.20^{Bb}
	21	4.18 ± 0.73^{Aa}	12.21 ± 0.01^{Cb}	2.44 ± 0.02^{Ea}	5.00 ± 0.02^{Ca}	5.30 ± 0.02^{Bb}
Xantan gum	1	4.35 ± 0.01^{ABb}	11.10 ± 0.10^{Aa}	0.00 ± 0.00^{Db}	4.74 ± 0.05^{Aa}	6.84 ± 0.02^{Aa}
	7	4.33 ± 0.02^{ABab}	10.90 ± 0.21^{Ab}	0.00 ± 0.00^{Da}	5.04 ± 0.01^{Ac}	5.60 ± 0.05^{Aa}
	14	4.18 ± 0.01^{ABab}	10.80 ± 0.00^{Ab}	0.01 ± 0.00^{Da}	4.65 ± 0.02^{Ab}	5.60 ± 0.01^{Aa}
	21	4.24 ± 0.02^{ABa}	10.53 ± 0.09^{Ab}	0.02 ± 0.00^{Da}	4.40 ± 0.05^{Aa}	4.90 ± 0.03^{Aa}
Commercial gelling agent	1	4.45 ± 0.02^{Bb}	11.38 ± 0.01^{Ba}	0.03 ± 0.02^{Cb}	4.87 ± 0.06^{Ba}	7.30 ± 0.30^{Da}
	7	4.38 ± 0.02^{Bab}	11.40 ± 0.00^{Bb}	0.04 ± 0.01^{Ca}	5.11 ± 0.01^{Bc}	5.70 ± 0.01^{Da}
	14	4.41 ± 0.02^{Bab}	11.30 ± 0.03^{Bb}	0.12 ± 0.02^{Ca}	4.93 ± 0.05^{Bb}	5.41 ± 0.01^{Da}
	21	4.36 ± 0.00^{Ba}	11.18 ± 0.00^{Bb}	0.01 ± 0.00^{Ca}	4.82 ± 0.02^{Ba}	5.26 ± 0.03^{Da}
Starch	1	4.44 ± 0.02^{Bb}	16.70 ± 0.10^{Ea}	1.43 ± 0.01^{Bb}	4.60 ± 0.02^{Aa}	7.00 ± 0.03^{Ca}
	7	4.36 ± 0.01^{Bab}	16.58 ± 0.02^{Eb}	2.19 ± 0.01^{Ba}	4.70 ± 0.04^{Ac}	5.48 ± 0.02^{Ca}
	14	4.34 ± 0.01^{Bab}	16.52 ± 0.08^{Eb}	1.53 ± 0.01^{Ba}	4.74 ± 0.01^{Ab}	5.30 ± 0.05^{Ca}
	21	4.30 ± 0.01^{Ba}	16.98 ± 0.19^{Eb}	1.66 ± 0.03^{a}	4.62 ± 0.03^{Aa}	4.48 ± 0.02^{Ca}
Pectin	1	4.58 ± 0.03^{Bb}	14.20 ± 0.20^{Da}	0.03 ± 0.01^{Ab}	4.70 ± 0.02^{Ba}	4.30 ± 0.06^{Aa}
	7	4.52 ± 0.02^{Bab}	14.30 ± 0.30^{Db}	0.00 ± 0.00^{Aa}	5.48 ± 0.02^{Bc}	5.70 ± 0.03^{Aa}
	14	4.38 ± 0.02^{Bab}	14.19 ± 0.09^{Db}	0.01 ± 0.00^{Aa}	4.38 ± 0.02^{Bb}	5.48 ± 0.06^{Aa}
	21	4.54 ± 0.03^{Ba}	13.87 ± 0.10^{Db}	0.01 ± 0.00^{Aa}	4.20 ± 0.05^{Ba}	5.11 ± 0.03^{Aa}

Upper-case letters present the differences between the different samples in the same storage time and lower-case letters show differences between the storage times of each samples ($P<0.01$).

The syneresis is a structural defect and can be defined as the separation of the liquid phase held in the protein network spontaneously from the gel structure without any external effects (Lucey 2002). The syneresis is an undesirable feature that affects consumer choice of the product (Nunes et al. 2006).

The syneresis values of yoghurt samples during storage are shown in Table 2.

The syneresis values were higher in control yoghurt and starchy yoghurt than in the others. The effect of days and yoghurt varieties on the syneresis was found to be significant at the $P<0.01$ level. Syneresis of the samples at 7, 14 and 21 days were the same, while the 1st day was different from the others.

The product obtained in this study is a yoghurt substitute product and hydrocolloids were used to obtain a yoghurt-like structure. Hydrocolloids are compounds that can improve the texture of yoghurt. These compounds include long and branched molecules, which are able to establish links with each other or with other molecules present in the environment in the form of an emulsion. Additions of hydrocolloids to yoghurt are effective in absorbing water, increasing viscosity and strengthening and improving the texture of yoghurt (Mortazavian & Sohrabvandi 2006; Bahrami et al. 2013).

In the study conducted by Goldar et al. (2016) it has been observed that the rate of syneresis in yoghurt samples produced for patients with PKU decreased during the 14-day storage period. The reduction in serum separation may be due to the corn starch and inulin used in the formulation. In this study, irregularities were observed in serum separation between the storage days of the samples. In some samples no serum separation was observed on some days of storage. The reason for this is that the use of milk substitutes, not milk, in the production of yoghurt substitutes, differences in product composition or the level of stabilizers may have been high.

Crude protein, nitrogen and phenylalanine analyses were performed on yoghurt samples on the 7th day of storage. The results obtained are shown in Table 3.

Table 3- Crude protein, nitrogen and phenylalanine values of yoghurt substitute samples

Samples	*Nitrogen (%)*	*Crude Protein (%)*	*Phenylalanine (mg/L)*
K	0.50±0.10 [b]	3.19±0.02 [c]	60.03±2.47 [d]
A1	0.07±0.03 [a]	0.44±0.04 [a]	21.90±1.03 [b]
A2	0.07±0.02 [a]	0.44±0.01 [a]	15.59±0.92 [a]
A3	0.08±0.01 [a]	0.51±0.03 [a]	23.00±1.81 [b]
A4	0.12±0.04 [a]	0.76±0.02 [b]	28.78±1.01 [c]

K (the control group, without stabilizer); A1 (1% xantham gum); A2 (1.5% commercial gelling agent), A3 (4% corn starch); A4 (1.6% Pectin). ($P<0.01$)

In this study, the Phe amounts of yoghurt substitute samples are well below the recommended amounts. Considering that 1 serving (a bowl) of yoghurt is 180 grams, it has been determined that PKU patients can consume all yoghurt substitutes with stabilizer produced in this study when the values of Phe are considered.

Abdel-Salam & Effat (2010) prepared milk-based drink for PKU patients. Ultrafiltration was applied to a mixture of buffalo and cow milk (1:1) at 50-55 ºC. Glycomacropeptides (a protein source) (2.5%) and corn germ oil (3%) were added to ultra-filtered (UF) milk permeate (consisting of water, sugar, some minerals and non-protein nitrogen compounds) and emulsification was applied. The final product was then heated to 85 °C for 15 minutes, then rapidly cooled and stored at 4 °C. The protein value of the final product was 2.5%, and the amount of Phe 12 mg/100 mL. The protein and Phe values detected in that study are well above those in this study. This may be due to the use of buffalo and cow's milk in that study.

In the study conducted by Pimentel et al. (2014), low-protein foods were prepared according to recipe books specifically designed for phenilketonuria patients. Later, these foods were analyzed. Low protein homemade yoghurt (100 g) (daily basic food) contains corn starch (5.4 g), egg substitute (1.2 g), low protein milk substitute (84.4 g), natural yoghurt (9.0 g). Pimentel et al. (2014) reported that low-protein homemade yoghurt contained 0.9% protein and 21.3 Phe mg/g protein. In study conducted by Goldar et al. (2016), the protein amounts of the samples varied between 2.87-2.99%, while the Phe amounts varied between 24.09-27.64 mg/100g. Protein and Phe amounts in this study were also considerably lower than in that study.

Low-protein milk substitute differs from cow's milk in color. Therefore, the color of the low-protein yogurt substitute will be different from the color of yogurt produced from cow's milk. In addition, color analyses were carried out to determine whether the gelling agents used had an effect on the color. Color analyses were performed on yoghurt samples on the first day of storage. The highest L * values were seen in control yoghurt, the highest a * and b * values were seen in low protein yoghurt with pectin. The lowest L * value was observed in pectin added low protein yoghurt, the lowest a * value in control yoghurt and the lowest b * value in starch added low protein yoghurt. Results of color analyses are shown in the Table 4.

Table 4- Color properties of yoghurt and yoghurt substitute samples

Samples	*L**	*a**	*b**
K	91.11±0.00[e]	-1.93±0.01[a]	10.14±0.01[a]
A1	83.95±0.01[c]	0.09±0.01[c]	15.89±0.00[d]
A2	82.31±0.02[b]	0.09±0.01[c]	15.21±0.01[c]
A3	85.85±0.04[d]	-0.02±0.00[b]	13.75±0.02[b]
A4	81.98±0.01[a]	1.22±0.00[d]	17.04±0.01[e]

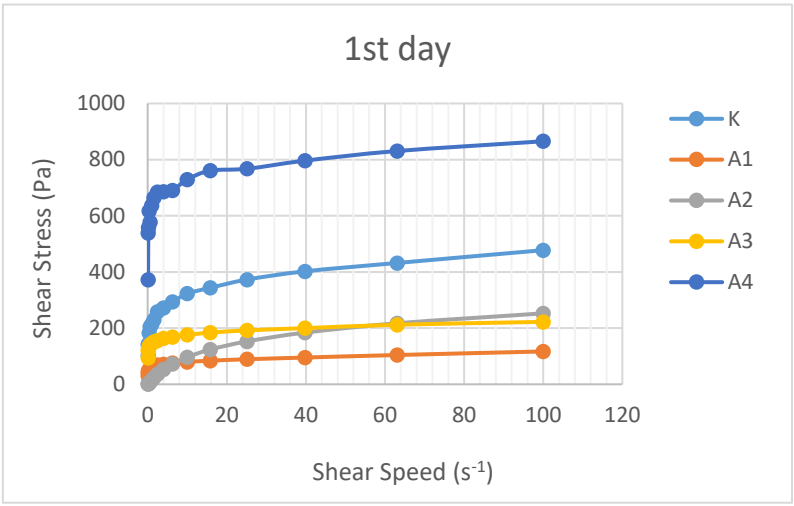
K (the control group, without stabilizer); A1 (1% xantham gum); A2 (1.5% commercial gelling agent); A3 (4% corn starch); A4 (1.6% pectin).

The difference between yoghurt types in terms of color was found statistically significant at $P\leq0.01$ level. Different stabilizers used may cause color change. In terms of a* values, only low protein yoghurt samples containing xanthan gum and commercial gelling agent were similar. In study conducted by Macit & Bakirci (2017), the L* value of the yoghurt sample containing xanthan gum was lower than that of the yoghurt sample containing starch. In that study, the b* value of the yoghurt sample containing xanthan gum was higher than that of the yoghurt sample containing starch. The results of their study were similar to the results of this study. It can be said that these two studies are similar in terms of the effect of xanthan gum and starch on values.

3.2. Rheology steady shear properties

The steady phase analysis in yoghurts was examined at 5 °C, between 1-100 s^{-1} shear rate. The shear stress graphs of yoghurt substitutes on the 1st and 21st days of storage at 1-100 (1/s) are shown in Figure 1.

a



b

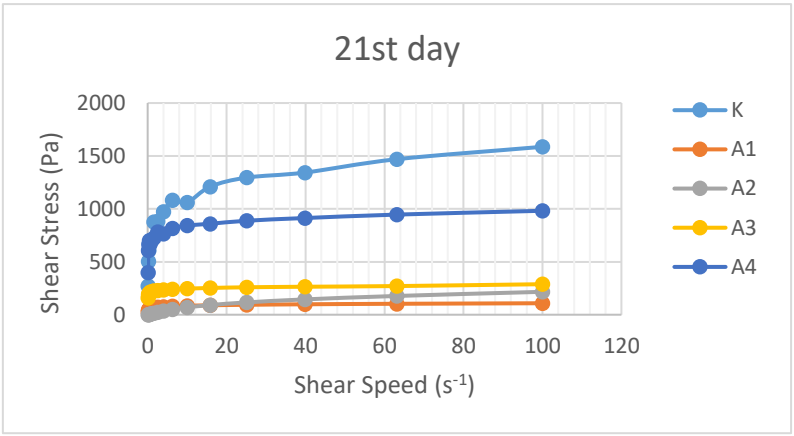


Figure 1- Shear rate-shear stress graph of yoghurts with different stabilizer added during the storage a: 1st day, b: 21st day K (the control group, without stabilizer), A1 (1% xantham gum), A2 (1.5% commercial gelling agent), A3 (4% corn starch), A4 (1.6% pectin).

When the shear stresses at shear rates on the 1st and 7th days of storage were examined, it was observed that the highest shear stress was in the pectin added low protein yoghurt sample. It was determined that the lowest shear stress was in the starch added low protein yoghurt sample. When the shear stresses at all shear rates were examined on the 14th and 21st days of storage, it was seen that the highest shear stress was in the control yoghurt. It was followed by a sample of pectin-added yoghurt. The fact that the shear stress of pectin added low protein yoghurt was high on the 1st and 7th days of storage, because the consistency was very dense. The decrease in its shear stress on the 14th and 21st days of storage can be explained by the deterioration of its structure during storage.

3.2. Microbiological properties

L. delbrueckii ssp. *bulgaricus* numbers in yoghurt samples generally increased until the 7th day of storage and then decreased. *L. delbrueckii* ssp. *bulgaricus* count was found in the control sample on all days of storage. This was followed by the yoghurt sample with commercial gelling agent. The difference between samples and days was found to be significant at the $P<0.01$ level. Statistically, while xanthan gum and starch added low protein yoghurt samples were similar, commercial gelling agent and pectin added low protein yoghurt samples were similar to each other. Control yoghurt was different from all of these samples.

On the first day of storage, the sample with commercial gelling agent had the highest number of *S. thermophilus*, while the sample with the lowest number of *S. thermophilus* had pectin added sample. In the control sample and the pectin supplemented sample, an increase, then a decrease was observed during storage. The *S. thermophilus* number of other samples decreased during storage. While the number of *S. thermophilus* is expected to increase, the reason for the decrease may be that the free water available by the bacteria in the environment is bound by thickeners.

In this study, the *S. thermophilus* counts changed more than the *L. delbrueckii* ssp. *bulgaricus* numbers. Similar results were obtained in the study of Macit & Bakirci (2017). They produced set type yoghurt using seven different stabilizers (sodium caseinate, gelatin, carrageenan, xanthan gum, guar gum, locust bean gum (LBG), native corn starch). Reducing water activity of stabilizers affected negatively the development of *S. thermophilus*. *S. thermophilus* needs higher water activity than *L. delbrueckii* ssp. *bulgaricus* (Macit & Bakirci 2017). The difference between the cultivars and between days was found to be significant at the P<0.01 level. Similarity was observed in xanthan gum and pectin added low protein yoghurt samples.

3.7. Sensory properties

Sensory properties of yoghurt and low protein yoghurt substitute samples were determined on the first day of storage. The results obtained by applying the hedonic test in terms of color, appearance, taste, odor, texture, consistency in mouth, consistency on spoon and general acceptability are shown in Figure 2.

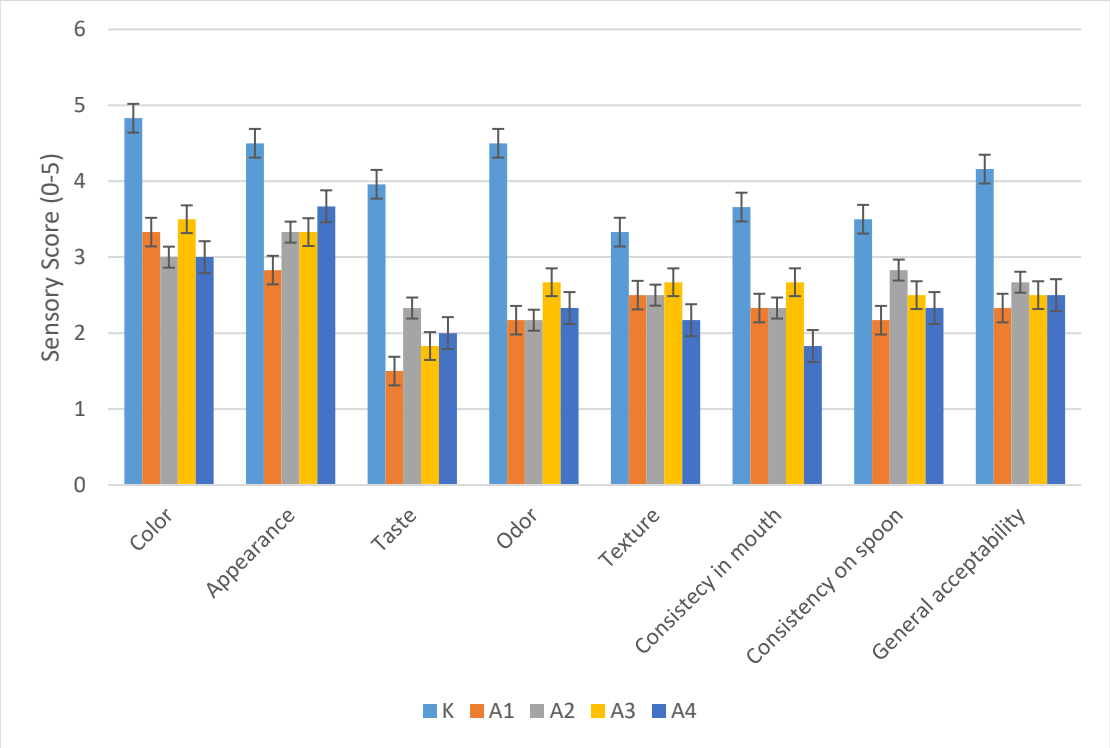


Figure 2- Sensory properties of yoghurt and low protein yoghurt substitute samples on the first day of storage
K (the control group, without stabilizer); A1 (1% xantham gum); A2 (1.5% commercial gelling agent); A3 (4% corn starch), A4 (1.6% pectin).

Control yoghurt became the most admired yoghurt in terms of all properties. It was thought that yoghurts produced using milk substitute are not as appreciated as the control yoghurt, since the sensory analyses of yoghurt substitute samples were performed by people who could consume yoghurt and did not accustomed to consuming milk substitute. In terms of taste, the commercial gelling agent supplemented sample followed the control sample with the highest score. It was followed by the example with pectin added. In terms of odor, texture and consistency in mouth, the starch-added sample was the most popular example of stabilizer-added yoghurt substitutes.

As a result of the sensory analyses, it was concluded that while xanthan gum, commercial gelling agent and starch were used at appropriate rates in the production of yoghurt substitutes, it was concluded that pectin was used at a high rate. The consistency of the pectin added low protein yoghurt sample was found to be harder and more brittle than desired, and it was proposed to reduce the amount used for possible subsequent applications.

As a result of the variance analysis performed, it was determined that the difference between samples was statistically significant at the P≤0.01 level in terms of color, taste and odor, while the difference between samples was statistically significant at the P≤0.05 level in terms of appearance and general acceptability. It was determined that the difference between the samples in terms of other criteria was not significant.

Macit & Bakirci (2017) examined the odor, appearance, consistency, taste and general acceptability properties of the set type yoghurts with stabilizer added. As in this study, the control example in that study was the most liked in terms of all features. In terms of all properties, the scores of the samples with starch added were higher than the scores of the samples with xsantan gum.

Those results are similar to those determined in this study. Xanthan gum particles are larger than casein fractions. When they enter the network of casein micelles, they cause a heterogeneous structure. Therefore, microstructural features are negatively affected. This may negatively affect the sensory properties of yoghurt with xanthan gum added (Macit & Bakirci 2017).

According to the sensory evaluations, in terms of all criteria, the samples with the average score from the highest to the lowest were the control samples, starch, commercial gelling agent, pectin and xanthan gum added low protein yoghurt substitute samples, respectively. Panelists have suggested that sweeteners can be added to low-protein yoghurt substitute samples in order to improve the taste-aroma. Evans et al. (2018) determined the food habits of children with PKU and their parents. In that study, it was found that children liked sweet foods more. Sweetened low-protein yogurt substitutes can be a particularly viable option for children. It was thought that it would be appropriate to use any sweetener that does not contain Phe or lactose as sweetener.

4. Conclusions

PKU patients consume yogurt substitutes prepared under home conditions. Industrial production must be done to obtain a standard product. The protein and phenylalanine amounts of the low-protein yogurt substitute samples produced in this study are at a level that PKU patients can consume. It was concluded that if sweetener is added, yogurt substitutes would be more appreciated in terms of taste. In future studies, the production of yogurt substitutes using probiotic bacteria can be tried. Since there are very few studies on this subject, it is necessary to increase the studies on the subject in order to standard production.

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