

Development and Characterization of Fermented and Unfermented Whey Beverages Fortified with Vitamin E

Z. Sohrabi¹, M. H. Eftekhari^{2*}, M. H. Eskandari³, A. Rezaeianzadeh⁴, and M. M. Sagheb⁵

ABSTRACT

Regarding hemodialysis patients' limitations in consuming different foods, providing them with a product with health-promoting effects is important. The objective of this study was to develop a functional food from whey protein for this disease condition and assess its sensory, chemical, and nutritional characteristics. Fermented and unfermented beverages were prepared as a mixture of whey protein concentrate (8.5%), permeate (1.4% for fermented), mint flavor (0.01%), vitamin E (0.18%), and water. Ty17A starter was used for the fermented blend. After pasteurization, and homogenization, the blends were stored until the sensory, chemical, and nutritional evaluations were done. Data were analyzed by SPSS Software (version 16). The pH values of the fermented and unfermented beverages were 3.8 and 6.02, respectively. The medians of all sensory attributes were greater than 4 for the fermented and less than 3 for the unfermented beverages (except color). All sensory attributes other than the color were significantly different between beverages ($P < 0.05$). The overall acceptability of the unfermented beverage was less. No significant nutritional differences were seen between beverages. Some of the nutrients values in the beverages are desirable for hemodialysis patients (Protein: 7.9-8 g 100 g⁻¹, Fat: 0.4%, Trans fatty acids: 2%, Saturated fatty acids: 56.15% Phosphorus: 9.25-9.35 mg 100 g⁻¹, Potassium: 0.0295 %, Sodium: 62.5 mg 100 g⁻¹, and vitamin E: 400 mg 220 mL⁻¹). The microbial counts of both were safe. Vitamin E fortified fermented whey beverage might be a good recommendation for hemodialysis patients because of its protein quality, low fat, phosphorus, sodium, and potassium and high vitamin E contents.

Keywords: Fermentation, Functional food, Nutritional value, Sensory attributes, Whey protein concentrate.

INTRODUCTION

End Stage Renal Disease (ESRD), as a chronic condition, had a high prevalence in the range of 8-15% world-wide in 2013 (Jha *et al.*, 2013). According to previous reports,

from 120 countries, almost 1,900,000 people were afflicted with ESRD and received renal replacement therapy; of them, 68% were on HemoDialysis (HD) (Pakpour *et al.*, 2011). In ESRD patients, CardioVascular Disease (CVD) is the major cause of morbidity and mortality, especially for those on HD (Daud

¹Department of Community Nutrition, School of Nutrition and Food Sciences, Shiraz University of Medical Sciences, Shiraz, Islamic Republic of Iran.

² Department of Clinical Nutrition, School of Nutrition and Food Sciences, Shiraz University of Medical Sciences, Shiraz, Islamic Republic of Iran.

* Corresponding author, e-mail: h_eftrekhari@yahoo.com

³ Department of Food Science and Technology, School of Agriculture, Shiraz University, Shiraz, Islamic Republic of Iran.

⁴ Department of Epidemiology, School of Health, Shiraz University of Medical Sciences, Shiraz, Islamic Republic of Iran.

⁵ Department of Nephrology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Islamic Republic of Iran.



et al., 2012). Atherosclerosis as a life-threatening factor in HD patients is enhanced by oxidative stress, which contributes to the progress of CVD (Salehi *et al.*, 2013). Unfortunately, high oxidative stress is prevalent among HD patients (Putri *et al.*, 2014) and vitamin E deficiency, as a potent antioxidant, in these patients might aggravate the condition as well (Aguilera *et al.*, 2002). One of the most important CVD risk factors in HD patients is a syndrome called “Malnutrition-Inflammation Complex Syndrome” (MICS). This syndrome is an important cause of premature CVD in HD patients and is caused by a combination of non-nutritional and nutritional factors (Kalantar-Zadeh *et al.*, 2003), including dietary catabolism due to treatment regimens, nutrient losses in HD, metabolic acidosis, etc. To ameliorate this condition, at the first step, much attention was paid to supplementing HD patients with an anti-inflammatory protein source for improving nutritional status and clinical outcomes (Daud *et al.*, 2012).

Due to health-improving characteristics of whey protein including antihypertensive properties (Ballard *et al.*, 2013) and the high content of TGF- β (transforming growth factor-beta) in whey protein which prevents inflammatory status (Hering *et al.*, 2011), anti-oxidative properties(Xu *et al.*, 2011), and anabolic characteristics (Toedebusch *et al.*, 2012), application of a whey protein as a supplementary protein has achieved great attention. Whey Protein Concentrate (WPC) that is produced commercially can differ from 29-89% total protein volume (Toedebusch *et al.*, 2012) . Because of the importance of protein amount and quality in HD patients and their limitations in ingesting high amounts of phosphorus and some other nutrients, different studies have assessed the efficacy of various protein or amino-acid containing formulations. The formulations such as “amino acid functional cluster” (aminotrophic) (Sukar *et al.*, 2012) , oral amino acid supplement(Sundell *et al.*, 2009) , soy protein with isoflavone (Siefker and Disilvestro, 2006), and many other

supplements were analyzed in HD patients. On the other hand, blends of dairy and soy protein were used for manufacturing sport nutrition products in some studies (Butteiger *et al.*, 2013) , while whey protein supplement effects on weight gain in chronic obstructive pulmonary disease patients was also evaluated (Sugawara *et al.*, 2012). Additionally, due to high levels of oxidative stress in HD patients, providing them with sufficient vitamin E was assessed as well (Ahmadi *et al.*, 2013).

Therefore, the objective of this study was to produce a beverage from WPC and vitamin E and also to evaluate its nutritional, sensory, and physico-chemical properties. The beverage can provide patients’ nutritional needs and improve their well-being, and is considered to be produced for the first time in the world for use in this disease condition.

MATERIALS AND METHODS

This was an experimental study done in 2014 in Shiraz City (capital of Fars Province in Iran). A beverage specific to hemodialysis patients was manufactured using Whey Protein Concentrate (WPC) and vitamin E. The composition of the beverage was based on the patients’ requirements according to the disease condition, while no study was done using such components for preparing similar formulation. Two forms of beverages, fermented and unfermented types, were formulated. The beverage was fermented in order to improve the shelf life, taste, and acceptability of the product with comparison to the unfermented form.

The WPC powder (80% WPC: Supplied by Spauto cheese, USA Inc) was used for the manufacturing process. The WPC contained 5.96% fat, 80.97% protein and 2.83% ash; and the beverages were prepared in 230 mL bottles for each person. Almost 20% of the DRI (Dietary Reference Intake) value for protein was provided by the premix. In order to exclude the effect of shelf life on the nutritional and

physicochemical properties of the beverages, the beverages were produced every other day (in 2 days intervals).

Preparation of the Beverages:

The preparation methods are presented in Figure 1. The preparation of the fermented and unfermented beverages was done at semi-industrial scale at a dairy company. First, 8.5% WPC and 90.1% distilled water (60°C) were mixed completely and stirred gently. Then, it was kept at 50°C for 10 minutes for complete hydration of the mixture. For the fermented beverage, 1.4% permeate powder was also added to improve the fermentation process. Then, the blend was homogenized at 80°C under 200 bar in a double-stage homogenizer (double-stage homogenizer, GEA company, Italy). After this step, the homogenized blend was pasteurized at 90°C for 10 minutes. Gentle

Agitation of the mixture in all of the steps was necessary for inhibiting the high viscosity or protein denaturation of the beverage.

For the fermented beverage, the blend was cooled to approximately 45°C in the following step to be ready for the fermentation process. Yogurt starter culture (DELVO®-YOG TY-17A, DSM Food-Specialties, the Netherlands) including *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp *bulgaricus* was implemented for the fermentation process to give a pH of 3.8 after incubation at 45°C for 17 hours. Starter culture was initially prepared as a dilution in water before inoculation for better dissolving. To assess the fermentation kinetics of the whey beverage, pH measurements were done at different intervals during the fermentation process till achieving the targeted pH.

For both fermented and unfermented drinks, vitamin E (0.18% in the form of

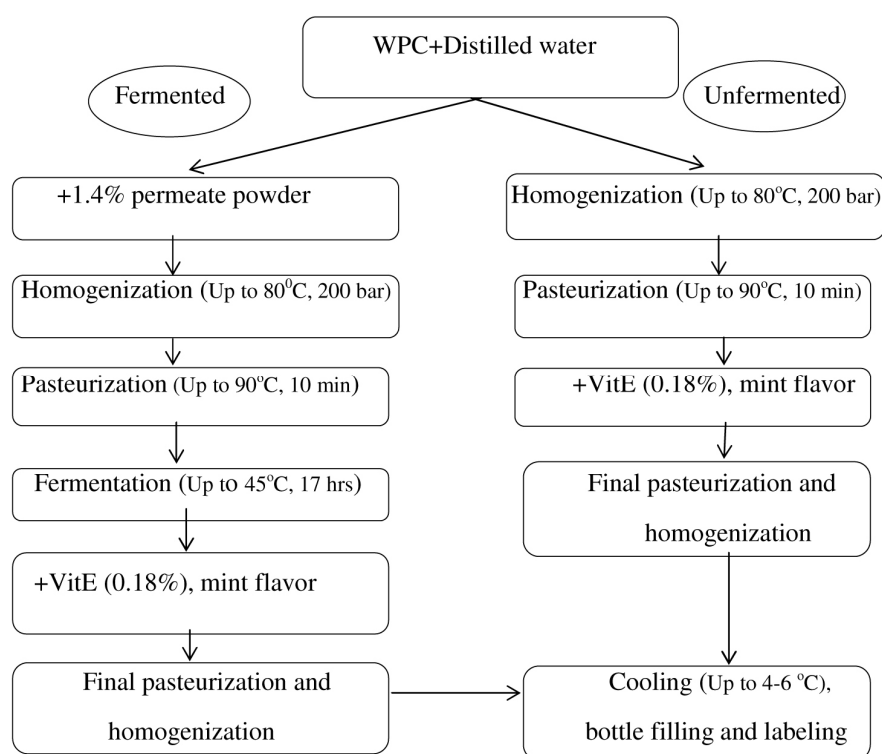


Figure 1. Preparation procedures of the fermented and unfermented beverages from whey protein.

(WPC: Whey Protein Concentrate, VitE: Vitamin E).



100% dl- α -tocopherol oil) was added to the mixture. Then, the blend was heated at 60°C and kept at this temperature for 10 minutes. Subsequently, thermal treatment for final pasteurization was performed by heating up to 75°C for 10 minutes followed by gentle stirring of the blend. The homogenization process for the second time was done using a double-stage homogenizer under 200 bar at 85°C. Eventually, 0.01% sterile mint flavor at a low level was added to the blend to improve the taste and acceptability of the final product. At the end, the blends were cooled in a cold water container to 4-6°C and then packaged in plastic bottles. Subsequently, the sealed bottles were labeled using specific labels designed for the beverages. At the end, each sample was stored at 4°C until the final evaluation of the nutritional, chemical, and sensory properties of the products after 2 weeks of storage.

Chemical and Sensory Analysis

The pH values of each homogenized blend were determined using a pH meter ((Metrohm 820 lab pH meter). A number of 20 panelists, some of the university students, staff, and some patients at hemodialysis centers of Shahid Faghihi and Namazi Hospitals (Hemodialysis Centers of Shiraz University of Medical Sciences (SUMS)), participated in the sensory evaluation of the product. Samples were served in similar 200 mL bottles encoded with numbers. A five-point scaled questionnaire designated for sensory evaluation based on the Iranian National Standards (ISIRI, 695) was used (AOAC, 2002). Different responses, including “very poor”, “poor”, “moderate”, “very good” and “excellent” were indicated by scores 1 to 5, respectively. Descriptive sensory analysis was done to assess texture, color, flavor, odor, and mouth feel. With the summation of the scores in each section, the total score of the questionnaires were calculated for assessing the overall acceptability.

Nutritional Analysis

Nutritional values of the beverages including the fat, saturated fatty acid, trans-fatty acid, carbohydrate, simple sugars, protein, fiber, calcium, potassium, sodium, phosphorus, and total calorie contents were assessed using specific chemical methods. Protein content was determined by Kjeldahl method (Peco Company: Iran) (ISIR 985.35)(AOAC985, 1999) and the gas chromatographic methods (Agilent Company: United States) were implemented for total fat and saturated fat contents determination, respectively (House, 1997). The High Performance Liquid Chromatography (HPLC) (Agilent Company: United States) was used for carbohydrate determination (Vidal-Valverde *et al.*, 1984). Calcium, sodium and potassium determination was done using flame emission spectrometric method (Fater Electronic Company: Iran) (ISIR 990.23) (AOAC695, 1999) and the spectrophotometric method (Hack Dr Company: Germany) (ISIR 990.23)(AOAC990, 1999) was used to measure phosphorus. Measurements of other nutrients were also performed according to the standard AOAC (Association of Official Analytical Chemists) procedures and calorie was determined by calculation methods. Duplicate analysis was performed for each test.

Microbial Analysis

Microbial counts of each beverage were done using the VRB Agar (Violet Red Bile Agar: Merck company, Darmstadt: Germany) for coliform count by pour plate method and incubated for 24 hours. For the unfermented preparation, the BP culture (Baired-Parker: Merck company, Darmstadt: Germany) was implemented for staff count with the surface method followed by incubation for 24 hours, while for the fermented preparation, yeast-mold count

with the surface method in the YGC culture (Yeast Extract Glucose Chloramphenicol: Merck company, Darmstadt: Germany) was done. For the yeast-mold count, the plates were incubated for 72 hours.

Statistical Analysis

SPSS version 16 statistical software package was used for all statistical analyses (SPSS Inc., Chicago, IL, USA). Man-Whitney U-test was implemented for comparing independent samples between the two beverages. P values less than 0.05 were considered significant.

RESULTS

Microbial Analysis

This analysis was done to ensure the product safety and prevent any contamination in the mixtures resulting from the procedures. According to the microbial analysis, the coliform, staff, yeast and mold counts for both the fermented and unfermented beverages were acceptable

The coliform counts for both the fermented and unfermented beverages were acceptable (< 10 CFU (Colony Forming Unit)). The staff count was also low for the unfermented mixture (< 10 CFU), while no yeast or mold was detected in the YG culture.

Chemical and Sensory Analysis

The pH values of the fermented and unfermented beverages were 3.8 ± 0.2 and 6.02 ± 0.1 , respectively. For the fermented beverage, pH measurements showed the fermentation kinetics of the beverage (pH: 6.02, 4.6, 4.3, 4.2, 3.95, 3.85, and 3.8 at 0, 5, 8, 10, 14, 16, and 17 hours during fermentation process, respectively). The fermented beverage had a brownish white color with a taste and odor similar to yogurt drink, while the unfermented one, almost with the same color (a bit lighter), had a low acceptability due to the taste and astringency in spite of the addition of flavor. The low pH of the fermented drink affected the taste and the acceptability of the blend by decreasing the astringency and adding an intense yogurt aroma to the mixture. The results of sensory evaluation of the samples are presented in Table 1. There were statistically significant differences ($P < 0.05$) in all attributes of sensory evaluation (texture, flavor, odor, and mouth-feel), except for color, between the two samples (Table 1). The medians of all sensory attributes of the fermented beverage were greater than 4, while for the unfermented one, for all of the attributes other than color, the medians were less than 3 (i.e. the threshold of acceptability). Hundred percent of all of the sensory attributes (texture, flavor, odor, color, and mouth-feel) reported by panelists for the fermented beverage was higher than 3 (3: threshold of acceptability), while for the

Table 1. Sensory evaluation of the fermented and unfermented beverages from whey protein.

Beverages	Mean±SD (Mean rank)					
	Texture ^a	Color	Flavor ^a	Odor ^a	Mouth feel ^a	Overall acceptability ^a
Fermented	4.2±0.77 (29.1)	4.2±0.72 (21.87)	4.4±0.68 (30.50)	4.75±0.44 (30.50)	4.1±0.72 (30.50)	21.7±1.3 (30.50)
Unfermented	2.4±0.82 (11.9)	4.1±0.64 (19.22)	1.4±0.51 (10.50)	1.95±0.6 (10.50)	1.2±0.41 (10.50)	11.1±1.77 (10.50)

^a Indicate a statistically significant difference ($P < 0.05$). Man-Whitney U-test was used for comparing independent variables.



unfermented beverage, most of the sensory attributes (flavor, odor, and mouth-feel), were scored 3 or less than 3 by the subjects. Indeed, all of panelists evaluated the fermented samples as acceptable, and unfermented ones as unacceptable. The overall acceptability of the unfermented sample, however, was less.

Nutritional Analysis

Nutritional values of the fermented and unfermented beverages are shown in Table 2. No significant differences were seen between the two samples in all of the nutrients. The values for most of the nutrients, especially for some of the important nutrients such as phosphorus, in both of the beverages were acceptable and desirable for the hemodialysis patients.

DISCUSSION

Results of the current study demonstrated that the fermentation process positively affected both the pH and acceptability of the whey beverage in comparison to the unfermented preparations. As a large

amount of protein in a solution causes astringency and can affect the taste and acceptability of the beverage, the fermentation process improved the acceptability of the beverage by reducing the astringency and, hence, the taste and acceptability of the beverage in the current study. It is noteworthy that using milk permeate, as a by-product of dairy production and a good source of lactose (Cote *et al.*, 2004), can help the fermentation process of whey protein concentrate in the processed beverage and the pH value of the fermented beverage decreased gradually during the fermentation process in 17 hours. All of the attributes of the sensory evaluation were better for the fermented sample than the unfermented one, except for the color which was almost the same for both of the preparations and was not significantly different. Heat treatment had very little effect on the fermented blend color as it was less white than the unfermented mixture. Concerning the results about flavor and mouth-feel, the present results were in accordance with those from Gallardo's study about a fermented whey beverage with added hydrocolloids (Gallardo-Escamilla *et al.*, 2007). However,

Table 2. Calorie and nutrients contents of the fermented and unfermented beverages from whey protein.

Nutrients	Beverages	
	Fermented drink	Unfermented drink
Energy (Kcal 100 g ⁻¹)	45.3±3.3	44.8±2.5
Protein (%)	8±0.10	7.9±0.09
CHO (%) ^a	2.43±0.25	2.40±0.20
Simple sugars (Mono-di) (%)	2.35±0.08	ND
Fat (%)	0.4±0.01	0.4±0.02
TFA (% of total fat) ^b	2±0.01	2±0.02
SFA (% of total fat) ^c	56.15±1.2	56.15±1.1
Fiber (%)	0	0
Na (mg 100 g ⁻¹) ^d	62.5±1.1	62.5±2
K (%) ^e	0.0295±0.001	0.0295±0.002
P (mg 100 g ⁻¹) ^f	9.35±0.2	9.25±0.3
Ca (%) ^g	0.0397±0.001	0.0395±0.002

^a Carbohydrate; Mono/di: Mono and disaccharides; ^b Trans Fatty Acids; ^c Saturated Fatty Acid; ^d Sodium; ^e Potassium; ^f Phosphorus; ^g Calcium, ND: Not diagnosed.

in another study, Pescuma *et al.* (2010) found that WPC fermentation by rationally selected lactic acid bacteria resulted in developing functional beverages with improved characteristics. Additionally, in another study by Evans *et al.* (2010), the same results were obtained according to the better sensory attributes of the beverages made from 80% WPC rather than those from SPC (Serum Protein Concentrate). However, at the same time, SPC beverage in their study had a higher pH value than that of the WPC beverage which might explain the better acceptability of the whey beverage to some extent, just the same as what was reported in the current study about the effects of higher pH value on the better acceptability of the fermented whey beverage. On the other hand, in Evan's study, the WPC beverage had an opaque appearance in comparison with the clear appearance of the SPC beverage (Evans *et al.*, 2010), but in the current study, the appearance of the fermented beverage from WPC was acceptable and the difference might be due to the fact that both of the fermented and unfermented beverages had almost the same color or appearance which was similar to the yogurt drink and there was no significant difference to be mentioned by the panelists.

As mentioned, one of the main reasons for the differences seen between the two experimental products according to the sensory attributes in the present study was the fermentation process, while in another study, a starter culture used for the fermentation process positively affected the sensory characteristics of the products, especially in those with whey milk as compared with skim milk. This demonstrated a desirable possibility of substituting milk with whey protein in producing acceptable fermented beverages, and this supported our hypothesis about using whey protein in production of an acceptable fermented beverage for specific groups. On the other hand, in the current study, one of the properties of the unfermented whey beverage was its

astringency which negatively affected the product acceptability. This problem was also found in a whey butter milk product in another study as compared with a product made up of sweet cream butter milk (without whey) (Jinjarak. *et al.*, 2006), and this was in accordance with the results of our study.

Based on the results, the protein content of the fermented mixture was more than milk (15 g vs. 7 g in 230 mL) (Mahan and Escott-Stump, 2008), while providing less phosphorus at the same time (22 vs. almost 232 mg) and less fat (1.8 vs. 3.45 g), and the trans fatty acid content was acceptable (Gallagher, 2008). It is noteworthy that a study by Forsum and Hambraeus (1977) was in the same line with the present study. They analyzed the nutritional properties of whey products and showed a good protein content with descent and significant nutritional value for these products for human diets. This showed that the fermented whey product with the better acceptability and nutritional value can be an appropriate recommendation, especially for hemodialysis patients who have limitations in ingesting protein sources due to the phosphorus content of these foods, and have some recommendations about reducing fat intake to prevent cardiovascular events as the leading cause of morbidity and mortality in these patients. And regarding the fact that casein, as the major milk protein component, is a phosphoprotein and contains a large amount of phosphorus (Kunz and Lonnerdal, 1990), implementing whey protein with low phosphorus content for developing a beverage can be a step forward in industry for the products specific to disease conditions such as hemodialysis and might be of great use. On the other hand, these patients also have deficiency of vitamin E, which is a powerful antioxidant, and they are exposed to different sources of oxidative stress regarding their treatment procedure (Aguilera *et al.*, 2002), while none of the protein sources can provide enough vitamin



E for these patients and providing them with a good amount of vitamin E (400 mg) plus protein can be an effective preventive modality that cannot be provided simultaneously by other protein food stuff. In line with the present study is a study conducted by Daud and his colleagues (Daud *et al.*, 2012) which focused on providing considerable amount of protein in the form of a beverage for hemodialysis patients with special limitations in consuming protein foods. But there are some differences with the present study including the drink content and supplementation with omega-3 instead of vitamin E. Additionally, there are some studies concentrating on vitamin E supplementation in hemodialysis patients (Ahmadi *et al.*, 2013; Daud *et al.*, 2013; Rusu *et al.*, 2013) which agree with the current study about the importance of supplementing these patients with vitamin E as a great antioxidant.

Another point to notice, regarding the nutrition analysis results, is the potassium and sodium contents of the mixtures, which are low and acceptable for hemodialysis patients who are recommended to consume less sodium and potassium due to their renal insufficiency and dependence on hemodialysis (sodium: 4.8% daily value, potassium: 2.26% DV) (Recommended intakes: Sodium, 2-3 g day⁻¹; Potassium, 2-3 mg 100 g⁻¹) (Willkens and Juneja, 2008), while other protein sources contain more sodium and potassium. In accordance with this fact, there are studies emphasizing the importance of potassium, sodium and phosphorus limitations in hemodialysis patients (Pollock and Jaffery, 2007; Savica *et al.*, 2008).

The only disadvantage related to whey protein ingestion according to the studies includes liver damage when consumed without exercising (Gürgen *et al.*, 2014). One of the limitations of the current study was lack of data concerning the effects of storage period on stability and properties of the beverages and another limitation is pertinent to the lack of vitamin E

measurements in the products that cannot ensure us about this vitamin stability in the beverages during the processing and storage period.

CONCLUSIONS

To sum up, it can be concluded that although the fermented whey beverage fortified with vitamin E, as a suitable anabolic supplement for hemodialysis patients, had almost the same nutritional value in comparison with the unfermented beverage, it had better acceptability and taste according to the sensory evaluation. Therefore, the fermented whey beverage fortified with vitamin E can be recommended as a supplementary or functional food for the HD patients, as it provides good amounts of protein, calorie, and some nutrients without containing high amounts of nutrients which should be consumed limitedly by them. On the other hand, providing this group with high protein, low phosphorus, and low fat foods fortified with an antioxidant can be a therapeutic remedy for malnutrition and other disorders in these complicated patients. This beverage can have unique characteristics due to anabolic, antioxidant, and anti-inflammatory properties of whey protein.

Further studies should be conducted for developing the best product with great nutritional value for these patients. Assessment of the product efficacy in patients nutritional well-being in future studies is of great importance as well.

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فرآوری و توصیف ویژگی های اختصاصی نوشیدنی های تخمیری و غیر تخمیری آب پنیر غنی شده با ویتامین E

ز. سهرابی، م. ح. افتخاری، م. ه. اسکندری، ع. رضاییان زاده، و م. م. ثاقب

چکیده

با توجه به محدودیت های بیماران همودیالیزی در مصرف انواع غذاها، فراهم کردن یک محصول با اثرات بهبود دهنده سلامتی برای آنها حائز اهمیت می باشد. هدف از این مطالعه تولید یک غذای فراویژه از پروتئین وی برای این شرایط بیماری و ارزیابی ویژگی های حسی، شیمیایی و تغذیه ای آن می باشد. نوشیدنی های تخمیری و غیر تخمیری به شکل مخلوطی از کنستانتره پروتئین وی (۸/۵٪)، پودر آب پنیر (۱/۴٪ برای تخمیری)، اسانس نعنای (۰/۰۱٪)، ویتامین E (۰/۱۸٪) و آب تهیه گردید. استارتر TY17A جهت مخلوط تخمیری به کار گرفته شد. پس از پاستوریزاسیون و هموژنیزاسیون، مخلوط ها تا زمان ارزیابی حسی، شیمیایی و تغذیه ای نگه داری شدند. اطلاعات با کمک نرم افزار SPSS (نسخه ۱۶) مورد ارزیابی قرار گرفتند. میزان pH نوشیدنی های تخمیری و غیر تخمیری به ترتیب عبارتند از ۳/۸ و ۶/۰۲. میانه تمامی ویژگی های حسی (به جز رنگ)، برای نوشیدنی تخمیری بیشتر از ۴ و برای نوشیدنی غیر تخمیری کمتر از ۳ بود. تمامی ویژگی های حسی به جز رنگ به طور معنی داری بین نوشیدنی ها متفاوت بود ($P < 0.05$). پذیرش کلی محصول غیر تخمیری پایین تر بود. هیچ گونه تفاوت تغذیه ای بین نوشیدنی ها وجود نداشت. بعضی از ارزش های مغذی در نوشیدنی ها برای بیماران همودیالیزی مطلوب می باشد (پروتئین: ۸-۷/۹٪، چربی: ۰/۴٪، اسید چرب ترانس: ۰/۲٪، اسید چرب اشباع: ۵۶/۱۶٪، فسفر: ۹/۳۵-۹/۲۵٪ میلی گرم/۱۰۰ گرم، پتاسیم: ۰/۰۲۹۵٪، سدیم: ۶/۲۵ میلی گرم/۱۰۰ گرم و ویتامین E: ۴۰۰ میلی گرم/۱۰۰ میلی لیتر). شمارش میکروبی هر دونمونه ایمن بود. نوشیدنی وی تخمیری غنی شده با ویتامین E به جهت کیفیت پروتئین، محتوای کم چربی، فسفر، سدیم و پتاسیم و محتوای بالای ویتامین E آن می تواند توصیه خوبی برای بیماران همودیالیزی باشد.