



Original research

Functional protein beverage with milk and egg white: physical properties, formulation, protein contents and amino acids components

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ABSTRACT

Generally, between protein sources, whey proteins are often used in ready-to-drink protein beverages, but in the current study, because of the important nutritional role, egg white protein was investigated. Beverages were formulated with two different amounts of egg white powder (EWP) (14 and 16%), cocoa powder (1 and 2%) and sugar (2.8 and 3.8%), carrageenan stabilizer (0.05%) with two types of (pasteurization and sterilization) milk. The stability, viscosity, total solids and protein content of samples were measured at 1st, 5th, 7th, and 10th days of storage at 4°C. The results showed that Egg White Protein (EWP) could increase viscosity up to 1.9 to 3.5 times ($P < 0.05$). In spite of the direct relation between the increase of EWP amount and viscosity increase, this relation was totally reversed during storage time. There was also a positive relationship between temperature increase and viscosity. On the other hand, EWP acted as a stabilizing agent and significantly caused to decrease phase separation and sedimentation of the samples and it was direct relation between the amount of EWP and temperature with stability of the samples. Also, the total solids content of the treated samples became doubled compared to the control samples. This drink contained good amounts of amino acids such as glutamic acid, aspartic acid, leucine, lysine, valine and serine. Totally, the best formulated beverage was (14% egg white powder, 2% cocoa powder, 3.8% sugar and 0.05% stabilizer) with protein content of 14.6% and optimal viscosity and the best stability.

Keywords: Egg white, Chocolate milk, Protein beverage, Physical properties

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1. Introduction

From the past to present, dairy products have a significant share of nearly 43% of the market of functional foods and drinks. One of the new functional food products is ready-to-drink protein beverages (RTD), to build muscle and gain athletic power. In general, ready-to-drink beverages are manufactured using milk proteins, including whey protein isolate (WPI), whey protein concentrate (WPC), milk protein isolate (MPI), milk protein concentrate (MPC) and micellar casein concentrate (MCC) as the sources of protein (Dairy Export Council, 2017). Due to the high nutritional and functional benefits of milk, egg or soy proteins compared to other sources, these proteins are often used in supplemented and sport drinks (Perotti et al., 2019; Phillips et al., 2009). Of all of these proteins, whey protein is often selected as a source for ready-to-drink protein beverages because it is nutritious, and has good flavor, digestibility and functional properties. Also, milk is recognized as a functional drink such as whey-based drinks

(Romana et al., 2018; Chavan et al., 2015). Dairy-based whey drinks often are produced with fermentation of whey protein concentrate or a mix of milk with whey protein concentrate or whey protein isolate (Knijnenburg et al., 2019; Ozer & Avnikrmaç, 2010). Whey was first used in commercial beverages, in the Switzerland in 1952, by Rivella Red to manufacture carbonated, milk serum-based beverage with 35% whey by volume (Galaz, 2019). There are many different formulations for producing this beverage, such as hydrolyzed WPC, skim milk powder, cocoa, liquid glucose, sugar, and vegetable oil with the composition of 52% protein, 10% fat and 6.6% ash. (Sinha et al., 2007). In 1952, soy flour with 42% dissolved protein in milk was produced as the first commercial soy protein supplement (Shurtleff et al., 2010). Ready to drink soy-cow milk blend is one of another common protein drink. For example, 40-50% soymilk is blended with 50-60% of skim milk powder and has a good physicochemical and sensorial attribute similar to cow milk (Rehman et al., 2007). Besides these, egg protein is one of the important sources of protein that can provide efficiency and body power for daily activity

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(Applegate, 2000). Egg white contains many functionally important proteins such as ovalbumin, ovotransferrin, ovomucoid, ovomucin and lysozyme (Abeyrathne et al., 2014). Ovalbumin is the major egg white protein consisting of 54% of the total egg white proteins. It also has a unique amino acid con (leucine); therefore, egg consumption is necessary as a routine diet (Layman et al., 2009). When some of the other proteins are added to milk, they change physical and rheological properties of it. For example, in soy-cow milk blends, by increasing soy milk in the formulation, the amount of protein content, viscosity, and instability of samples were increased (Mazaheri-Tehrani & Yasamini-Farimani, 2011). Whey protein concentrate is used to modify the physical properties and enhance the nutritional value of fermented milk products (Amatayakul et al., 2006). Adding WPC up to 2% has a positive effect on physical properties similar to stabilizers, such as propylene glycol alginate and locust bean gum (Ozen & Kilic, 2009). In another hand, many RTD protein beverages usually have 7-8% protein on average which they are made with MPI or WPI. Rising of the protein content, technologically, is hard because the resulted beverage texture becomes thicken. One of the difficulties for production of protein beverage is viscosity reduction in products with a protein content of up to 14%, somehow that their texture must be similar to that of milk with the natural milky taste with no off-flavor (González-Tello et al., 2009; Halton et al., 2004). Regard to the above descriptions and difficulty of producing RTD protein beverages, the aim of this study was adding EWP to these beverages and measuring physical and rheological properties of the resulted product as a functional food.

2. Material and Methods

2.1. Materials and sample preparation

The influence of EWP addition on viscosity, total solids, sedimentation (%) and protein content of chocolate milk during shelf-life was investigated on samples produced by (80 %v/v) of milk (pasteurized or sterilized) and egg white powder (14 and 16 %v/v) (Narin, Hamedan, Iran), cocoa powder (1 and 2 %v/v) (Bendrop, France), sugar (2.8 and 3.8% v/v) (Hafttappah, Iran) and carrageenan gum (0.05 %v/v) (foodINc, China). in the other words, this study was done on four samples with EWP: pasteurized milk mixed with 2% cocoa powder + 3.8% sugar + 14% egg white powder (S1P), pasteurized milk mixed with 1% cocoa powder + 2.8% sugar + 16% egg white powder (S2P), sterilized milk with 2% cocoa powder + 3.8% sugar + 14% egg white powder (S1S), sterilized milk with 1% cocoa powder + 2.8% sugar + 16% egg white powder (S2S), and 2 control samples without EWP: pasteurized milk mixed with 1.3% cocoa powder + 3.3% sugar (control p), sterilized milk with 1.3 % cocoa powder + 3.3% sugar. Beverages were kept in PET bottles for one, five, seven, and ten days) at 4 °C. Also 0.05% carrageenan gum also was added to all of formulations (Lottfian et al., 2019).

2.2. Viscosity Measurement

The apparent viscosity of the samples was measured by a Brookfield viscometer (DVII-RV, Brookfield Engineering Laboratories, INC., USA) by spindle No.2 in six speeds ranging from 30 to 200 rpm at intervals of 5 rpm (Prakash et al., 2010; Hasani et al., 2017).

2.3. Sediment measurement or stability

The precipitate in chocolate milk is defined as gravity-based separation of cocoa particles. A 10 mL samples were centrifuged at 3000 rpm and 21 °C in a centrifuge (Sigma Instrument) for 5 min (Karami, 2018). After centrifugation, the separated phases were recorded, and the degree of precipitation in all samples was expressed as mL/100 mL of milk (Prakash et al., 2010).

2.4. Measurement of total solids

Before the test started, the temperature of the sample was reached to 20 ± 2 °C, carefully mixed, weighted and dried in the oven for 2 h at 102 ± 5 °C (Iranian national standard, 637) and percent of total solids was calculated.

2.5. Measurement of protein

Protein content was measured using the Kjeldahl method (AOAC, 1995, No. 999.06).

2.6. Measurement of Amino acids

Identification of the amino acid content was done using the method of British Pharmacopoeia (2011). The sample was hydrolyzed with hydrochloric acid at 110 °C for 24 h. Sodium citrate buffer solution was then added to the hydrolyzed sample and measured using a machine of amino acid analyzer (HP Hewlett 1100) in 3 repetitions.

2.7. Statistical analysis

The experimental design used was as follows: four substrates (S1P, S2P, S1S, and S2S) and 2 control (P, S) samples. Each combination repeated in triplicate. All of the obtained characteristics of the samples during 10 days of the shelf-life were studied in comparison with control samples using three-way analysis of variance by the general linear model. All of the results were statistically analyzed with SPSS statistical analysis software (SPSS 19, PASW) and Microsoft Excel to show possible differences between means.

3. Results and Discussion

3.1. The effect of formulation and thermal process on viscosity

Fig. 1 indicates the effect of different formulations and heating on the viscosity of chocolate milk samples. The results showed that adding EWP increased viscosity up to 1.9 to 3.5 times more than the control sample ($P < 0.05$). In the first day, the highest viscosity value was S2S sample (89.13 cp) and the lowest it was S1P (43.66 cp).

Despite the direct relationship between increasing of EWP percentage and reducing the amount of cocoa and sugar with viscosity increasing, the viscosity of the samples that were contained a higher percentage of EWP, decreased during storage time, while the samples with lower percentages of EWP and control samples showed an increasing trend.

It can be concluded that adding of EWP can lead to viscosity increase, but if its percentage go higher than 14%, it can lead to viscosity reduction during the storage period. Also, the viscosity of samples prepared with sterilized milk was higher than those prepared with pasteurized milk, which confirmed the positive effect of temperature on viscosity increasing. Either by raising the percentage of EWP, in the presence of carrageenan, the viscosity increased significantly. The reason for this can be related to the chemical structure and interaction of these agents. Egg white proteins make significant functional properties such as gel formation, foaming, and emulsifying characteristic. Many studies have shown that these functional properties are related to the presence of hydrophobic groups and sulfhydryl groups (SH) in the protein core (Van der Plancken et al., 2005). Same groups also exist in carrageenan. Therefore, sulfate groups are hydrophilic and well-bonded with water molecules, and on the other hand, its anionic structure forms a mixture of casein and gel (Spagnuolo et al., 2005). The other factor that affects the texture and viscosity of the milk is protein content (Katsiari et al., 2002). As shown in this study, the highest protein content had the highest viscosity. In similar results, gelatin addition increased viscosity in yogurt, and the more whey protein concentrate caused viscosity increase in the mixed drink of cow's and soy milk (Vahid Moghadam et al., 2013). In another study, the processing of chocolate flavored milk showed higher apparent viscosity resulting from the higher sugar contents (Prakash et al., 2010). Viscosity changes in dairy protein beverage formulations during thermal processing showed that a consistent and similar ingredient could be gained in a variety of formulations (Kelleher et al., 2018).

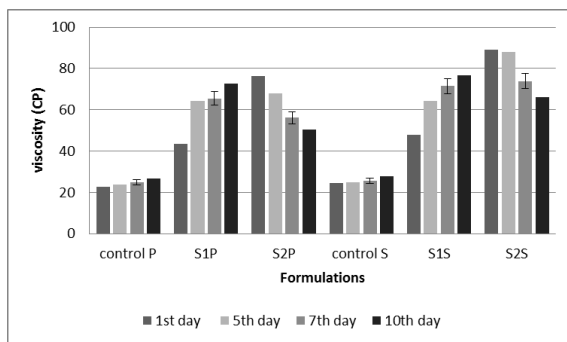


Fig. 1. Viscosity changes of the samples during the storage period (P: pasteurized, S: sterilized, 1: formulation 1 (14% egg white), 2: formulation 2 (16% egg white)).

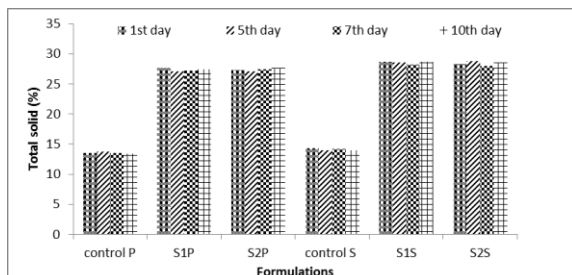


Fig. 2. The Total solid changes of the samples during storage time (%) (P: pasteurized, S: sterilized, 1: formulation 1 (14% egg white), 2: formulation 2 (16% egg white)).

According to the National Dysphagia Diet (NDD), viscosity values lower than 50 mPas or cp is called thin liquid (NDD 2002).

Thus, formulations with 14 % EWP content and control samples are considered thin beverages in the 1st day of production, but 16 % EWP content sample is classified in nectar-like liquids (> 50 mPas).

3.2. The effect of formulation type and thermal process on sedimentation amount and stability

Table 1 indicates the amount of samples sedimentation during the shelf life.

Results showed that EWP acted as a stabilizing agent and compared to the control sample, had a significant effect on reducing the amount of sediment and preventing from phase separation of the drinks. Also, it was a direct relationship between increasing of EWP percentage and the temperature with the stability of samples. According to Table 1, the highest and the lowest amount of sediment were obtained for the control p and the S2P sample, respectively. Also, all samples showed less stability and more sediment with time passing. Drinks with suspension stabilities higher than 70% can be packaged as stable products (Vasquez-Orejarena et al., 2018). In this study, the highest stability value of $88.9 \pm 0.2\%$ was achieved with the highest amount of EWP (16%). The precipitate in cocoa milk occurs due to the deposition of cocoa particles during storage, and to avoid it, stabilizers must be used to reduce phase separation. Also, the addition of protein compounds can be effective on the stability and avoidance of phase separation in chocolate drinks (Lotfian et al., 2018). In this study, the simultaneous effect of adding EWP and carrageenan (0.05%) on the amount of sedimentation during the shelf life was studied. Salimian et al. (2012) showed that adding carrageenan gum to chocolate milk made a high-viscose low-sediment drink. Also, too much sugar can increase the amount of sediment in chocolate milk (Prakash et al., 2010).

Proteins have many physicochemical functional properties that affect food characteristics during preparation, transformation and, storage. Many functional properties of them depend on hydrophobic groups in the molecular surface and the interrelation and reactions of them with other molecules. Egg white has some of the proteins such as ovomucin that contains sulfate esters, disulfide groups and large amounts of cysteine. These groups can react between negative ions such as sulfate esters and cysteine amino acids with positive ions of milk proteins. Also, other reactions occur between carbohydrate groups in glycoprotein of EWP and some of the amino acids in milk. In general, the stability factor for the stabilization of chocolate milk and the formation of a stable colloidal system is due to functional properties of hydrophilic groups (Lotfian et al., 2018). In another study, the effect of whey protein concentrate on dessert syneresis was investigated and, the results showed that WPC reduced syneresis amount (Celeguin et al., 2016). Also, the addition of isolated whey protein in orange-flavored soft drink had a significant effect on product stability (Prado et al., 2015), that it can be applied in chocolate drink formulation.

The main challenge of WPC based beverages is the sedimentation of insolubilized proteins because of low pHs leading protein sedimentation. Therefore, sweet whey is more useful rather than acidic whey (Britten and Giroux 2001). On the other hand, egg white is an alkaline solution and can raise the pH level of samples, therefore sedimentation of protein in our sample was normal (Zhao et al., 2014).

Table 1. The effect of formulation type and thermal process on viscosity (cp) on sedimentation amounts (%) during storage time at the refrigerated temperature.

Formulation	Thermal process	Viscosity(CP)				Sedimentation amount (%)			
		Storage time (day)				Storage time (day)			
		1	5	7	10	1	5	7	10
Control P	Pasteurization	22.8 ^f	23.96 ^q	25.03 ^{pq}	26.70 ^o	12.7 ^{efd}	13.16 ^{bcd}	13.73 ^{ab}	14.10 ^a
Control S	Sterilization	24.43 ^{pq}	24.75 ^{pq}	25.50 ^p	27.73 ⁿ	12.76 ^{ed}	13.06 ^{cd}	13.55 ^{abc}	14.03 ^a
S1P	Pasteurization	43.66 ^m	64.33 ⁱ	65.55 ^h	72.83 ^e	11.73 ^{ijkl}	12.06 ^{ghi}	12.33 ^{efg}	12.60 ^{efd}
S1S	Sterilization	48 ^l	64.20 ⁱ	71.46 ^f	76.73 ^c	11.45 ^{kl}	11.63 ^{jkl}	12.13 ^{ghi}	12.43 ^{efg}
S2P	Pasteurization	76.26 ^c	67.86 ^g	56.13 ^j	50.26 ^k	11.15 ^{ml}	11.37 ^k	11.77 ^{ijkl}	12.03 ^{hijk}
S2S	Sterilization	89.13 ^a	88.13 ^b	73.96 ^d	66.06 ^h	11.75 ^{ijkl}	11.30 ^{ml}	11.65 ^{jkl}	12.01 ^{hijk}

P: pasteurized, S: sterilized, 1: formulation 1 (14% egg white), 2: formulation 2 (16% egg white). The different letters in each column indicate statistically significant differences ($p < 0.05$).

3.3. The effect of formulation and thermal process on the total solid content

As shown in Fig. 2, the total solid content of the treated samples was twice as much as the control sample, therefore adding EWP had a significant effect on total solids amount, but it was not significantly different between the treated samples. On the other hand, there was not any change in total solids of samples during storage time. Also, the total solid of samples prepared with sterilized milk was higher than those prepared with pasteurized milk, which confirmed the effect of temperature on total solids. In a similar study, oat flour (1.50 to 2.30 % w/w) and MPI (2.50 to 4.00 % w/w) were used for the production of protein beverage with total protein content from 5.07% to 6.47%. Total solid of the resulted beverages was from 17.8-20.6% (Vasquez-Orejarena et al., 2018). The amount of total solid has a positive effect on viscosity and stability of beverages. The obtained results suggested that use of EWP in the level of 14% in a chocolate milk beverage with 22 % total solid yielded appropriate content of protein, viscosity, and stability (Mazaheri-Tehrani & Yasamini-Farimani, 2011).

3.4. The effect of formulation type and thermal process on protein content

The results showed that the addition of egg white had a significant effect on the increase of protein content in chocolate milk samples. According to Fig. 3, the protein content increased from 3.1% in control samples to 14.63% (4.7 times) in treatments. This protein content is an important factor for the production of protein beverages. Many RTD protein beverages usually have 7-8% protein on average, which they are made with milk protein isolate or whey protein isolate. These beverages do not have good texture and flavor. One of the problems in these drinks is that high protein content cannot be used in such a way that physical properties, such as viscosity and concentration, to be acceptable. For example, if the percentage of protein source increases, the concentration of beverages increases dramatically. One of the important issues to protein beverage production (with protein content up to 14%), is viscosity reduction of these products, such a way that they must be agreeable and have similar texture to that of milk with the natural milky taste with no off-flavor (González-Tello et al., 2009; Halton et al., 2004). The protein content of formulated beverages in the current study was up to 14% that means these beverages can be categorized as protein drinks.

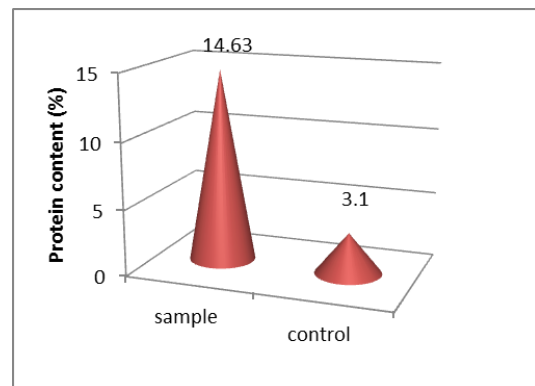


Fig. 3. Comparison of protein content in treated and control samples.

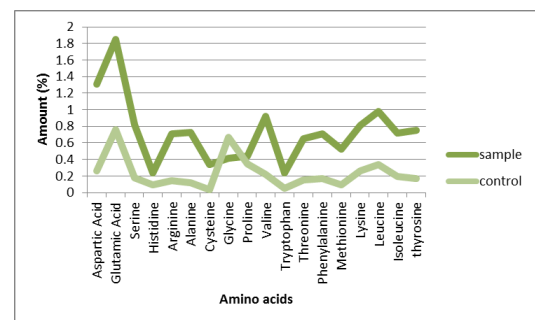


Fig. 4. The amino acids of the samples compared with control (%).

3.5. The effect of formulation on amino acids profiles

Ovalbumin and the rest of the egg white proteins are considered as a good source of amino acids. The amounts of amino acids present in treatment and control samples are presented in Fig. 4.

Proteins in egg whites contain eight essential amino acids (leucine, isoleucine, valine, lysine, arginine, etc.), which are essential for the athlete's diet to build and maintain muscle mass. It has the best values of BCAA (branched amino acids) that stimulates protein synthesis in the muscle and can equally reduce mental and mental fatigue during exercise. As shown in Fig. 4, this beverage contains high levels of glutamic acid of 1.85 ± 0.99 , followed by spastic acid of leucine, lysine, valine and serine, which indicates high amounts of essential amino acids.

In the same research, whey drink 35% was tested at and the highest amino acids were histidine 114 mg, glycine 62 mg and glutamine 53 mg/l (Pescuma et al., 2010). Also, the highest amount of amine acid in whey concentrate is related to glutamic acid and

leucine, which are essential amino acids that are found in sufficient amounts in whey protein drink (Sinha et al., 2007).

4. Conclusion

Abstract The protein content and quality in RTD protein drink are more important factors rather than other characteristics. While manufacturers try to produce beverages with higher protein content, with the addition of the percentage of protein, their consistency and the viscosity are greatly increased, that it is not so desirable for production of RTD protein drink. So, by choosing the right percentage of high-quality EWP, we could to produce a dairy drink with much higher protein content than commercial ones, with desire concentration and viscosity as well as the texture, similar to that of chocolate milk. On the consumer's point of view, not only the efficiency of RTD beverage is important, but also the appearance of products, amount of beverage sedimentation and phase separation are key factors for their overall acceptance. In this study, apart from the role of gum, it was possible to use EWP as a stabilizer that significantly decreased the amount of sedimentation and stability improved. Totally, on behalf of the key role of egg and milk for protein fulfillment of children and adolescents and development of physical and mental activities, this research introduced a commercial beverage with unique nutritional and technological aspects.

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