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Fruit juice drink production containing hydrolyzed collagen

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ABSTRACT

Fruit juice drinks containing hydrolyzed collagen were formulated and produced as a new functional drink from orange, apple and white grape juice blends containing ingredients such as hydrolyzed fish collagen, citric acid, ascorbic acid, and natural mint flavour. Difference and preference sensory tests were conducted to select the most preferred formulation containing an addition of 1–3% hydrolyzed collagen. The results indicate that the formulation with the addition of 2.5% hydrolyzed collagen was significantly preferred. Drinks at pH 3.96–4.04 were pasteurized at 95 °C until reaching P value necessary for 3D decimal reduction of *Alicyclobacillus acidoterrestris*. Addition of hydrolyzed collagen increased the protein content of the drinks from 0.56 to 2.22–2.48 g/100 mL. The *in vitro* bioavailability results indicated that the orange (95.37%) and apple (90.71%) drinks showed a higher bioavailability (5–14%) than the white grape juice blends. The ascorbic acid content (81.39–113.5 mg/100 mL), total phenolic content (86.93–117.43 mg GAE/100 mL), and antioxidant capacity ABTS assay results (104–127 μmol TEAC/100 mL) varied widely in the drinks.

1. Introduction

Functional food sales in the world's key markets are in the areas of health such as: cardiovascular, gut and bone diseases as well as anti-ageing. The global functional food market holds a strong position worldwide to quote some examples, 38.4% of the Japanese, 31.1% of the USA, and 28.9% of the European markets in 2010. The functional beverages sector accounts for approximately 12.5% of the world market (Anon, 2011). Fruit juice products are suitable for functional food production due to their nutritive and technological properties. Hence, new ingredients having functional properties are of interest for the development of novel functional beverages. One of the remarkable ingredients of the food industry is hydrolyzed collagen which contains 8 out of 9 essential amino-acids and glycine, and the proline concentration is nearly 20 times higher than other protein-rich foodstuffs (Anon, 2009). In humans and animals, 25% of their body protein is in the form of collagen. Due to the decrease of collagen synthesis in the body, its demand for the skin, hair, and bone tissues increases with ageing (Iwai et al., 2005). Some clinical studies reported that 10 g daily oral intake of hydrolyzed collagen decreased joint pain (Moskowitz, 2000; Ruiz-Benito et al., 2009), reduced the skin wrinkles (Tanaka, Koyama, & Nomura, 2009), and improved skin properties (Matsumoto, Ohara, Ito, Nakamura, & Takahashi, 2006). Other studies have suggested that a hydrolyzed collagen enriched diet can improve bone collagen metabolism (Guillerminet et al., 2010; Koyama et al., 2001), reduce pain in patients with osteoarthritis of knee or hip when the blood concentration of hydroxyproline increases (Moskowitz, 2000), and also prevent osteoporosis (Bonjour, 2005). Oesser, Adam, Babel, and Seifert (1999) determined the high bioavailability of collagen where the distribution of labelled

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amino acids in skin was confirmed, and 58% of the peak value of the labelled amino acids remained 192 h after oral administration in mice.

Hydrolyzed collagen is obtained by hydrolyzing gelatin with an enzyme or an acid and is commercially available with molecular weights from 500 up to 20,000 Da. Understanding the properties of hydrolyzed collagen and the concomitant food type is very important for innovated products. As a food ingredient, usage of low molecular weight (2000–5000 Da) hydrolyzed collagen is preferred as it prevents precipitation and turbidity problem in food drinks (Moskowitz, 2000). Collagen can react with acidic polysaccharides and tannins, which decrease sensory quality of the products, and therefore, high polyphenolic content foods are not suitable for production of functional food with hydrolyzed collagen. In food legislations, there is no restriction for the amount of collagen usage in foods. However, 2 to 30% hydrolyzed collagen addition to liquid foods is proposed in view of its positive effects. In addition, a hydrolyzed collagen concentration higher than 30% can cause a viscosity increase in beverages which in turn can have an important effect on the processing line and product quality (Takemori, Yasuda, Mitsui, & Shimizu, 2007). Based on all the properties discussed above, beverages can be satisfactorily formulated with hydrolyzed collagen to improve their functional and nutritional properties.

Functional drinks, in liquid form, are mostly produced by the cosmetic industry for the improvement of moisture-retaining properties of the skin and wrinkles prevention by boosting collagen levels. Collagen drinks, originating from Asian countries such as Japan and China as well as from the USA (Lotte collagen, Colla-plus, Meiji and Shiseido, among others) are sold as a liquid beauty elixir. In addition to these liquid products, a DXN Cobeauté Collagen Powder Drink (DXN Marketing Sdn. Bhd., Selangor, Malaysia) is also available in a premixed powder form, which is a unique product that is formulated from hydrolyzed collagen and natural orange juice but also sold as a supplementary beauty drink. However, although the absorption of specific amino acids from hydrolyzed collagen has been studied clinically in different matrices (Moskowitz, 2000; Ohara, Ito, Iida, & Matsumoto, 2009; Tanaka et al., 2009; Walrand, Chiotelli, Noirt, Mwewa, & Lassel, 2008), fruit juice drink product of hydrolyzed collagen was not studied or published in the food literature. Therefore, hydrolyzed collagen addition to the natural fruit juice without sugar and preservatives can be a new innovative approach for the production of healthy and functional new drink where perceived natural character will be an important factor (Siegrist, 2008). In the nutritionally adequate and well-balanced diets, collagen need is mostly provided as a result of eating fish, meat and offal. However, when normal diet do not provide sufficient collagen to meet the nutritional requirements of an individual, functional foods with hydrolyzed collagen can be consumed as part of the normal diet to satisfy the need. Our aim was to develop a food product which contains hydrolyzed collagen and sustain the daily necessary intake of collagen within the functional fruit juice drinks which can be easily and freshly consumed. Our formulation is based on 100% fruit juices and the production was done according to the fruit juice industry process. Therefore, this will be a unique market sold as a fruit juice drink but with potential health-promoting properties.

The aim of this study was to investigate the development of natural fruit juice-based drinks containing hydrolyzed collagen namely collagen juice drink (CJD); added citric acid, ascorbic acid and natural mint were added for improved flavour. The other objectives are to determine the temperature-time data for pasteurization of the functional drink as well as to examine the *in vitro* bioavailability of hydrolyzed collagen in CJD.

2. Materials and methods

2.1. Materials

The hydrolyzed collagen (Peptan™, F/2000 Da) enzymatically derived from fish was provided by Rousselot (2011) SAS, a Vion Company (Puteaux, France). The following were purchased from local markets: ascorbic acid (99% purity, Applichem), the natural mint flavour (Aromsa Besin Aroma ve Katkı Maddeleri San. ve Tic. A.Ş., Gebze-Kocaeli, Turkey), the citric acid (Merck KGaA, Darmstadt, Germany), orange and apple juice (Dimes Gıda San. ve Tic. A.Ş., Tokat, Turkey), and white grape juice from Kavaklıdere Şarapları A.Ş. Ankara, Turkey. Juices were filled into glass jars having 190 mL volume with a 90.7 mm height.

2.2. Methods

The formulation, production, and pasteurization of the CJD were carried out at the Ege University, Food Engineering Department Pilot Plant.

2.2.1. Formulation and Production

Four different CJDs were formulated as orange, orange-white grape, apple and apple-white grape drinks from 100% fruit juices. The final °Brix values of the CJD were adjusted to 10.4–13.5 by addition of ingredients according to the water to fruit juice proportions considering the limits in the regulation of Turkish Food Codex for fruit drinks without alcohol (NO: 2007/26) which is in accordance with Directive 2012/12/EU of The European Parliament and of The Council. Citric acid (0.1–0.2%) was added to the drinks for acidification and taste improvement. Due to its antioxidant properties, 0.1% of ascorbic acid was added into the CJD where the limit for addition is considered as a GRAS in the Turkish Food Codex regulation on food additives which is drafted in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

Natural mint flavour (0.02–0.03%) was added to the CJD to mask collagen taste and odour due to its fish origin as suggested by the flavour producing company "Aromsa Besin Aroma & Katkı Maddeleri San. & Tic. A.Ş., Gebze-Kocaeli, Turkey".

In pre-trials, five different concentrations of hydrolyzed collagen were added (1, 1.5, 2, 2.5 and 3%) to the CJD due to the amount that allows the physiological and/or pharmacological effects to be exerted. In case of a liquid food such as beverages, the intake level preferably ranges from 1 to 10% by weight (Matsumoto, Ohara, Nakajima, Sugihara, & Takasaki, 2010). Sensory difference and preference tests were conducted to select the most preferred formulations and percent of hydrolyzed collagen for each product series.

Table 1 – Selected T_{ref}, D and z values of *A. acidoterrestris* for fruit juice drink containing hydrolyzed collagen.

CJD	T_{ref} (°C)	D value (min)	z value (°C)	Reference
Orange	80	54.3	9.5	Komitopoulou et al. (1999)
Orange-white grape	80	54.3	9.5	Komitopoulou et al. (1999)
Apple	80	41.15	12.2	Komitopoulou et al. (1999)
Apple-white grape	90	11.1	8.5	Bahçeci & Acar (2007)

Selected formulations were produced by mixing and homogenizing the ingredients using an Ultra-turrax at 1800 rpm for 10 min. The homogenized CJD were then preheated to 60 °C, filled into the 190 mL glass jars and pasteurized at 95 °C in a steam jacketed open vessel until reaching the calculated target pasteurization (P) value and then cooled.

2.2.2. *Pasteurization criterion*

As suggested by Silva and Gibbs (2001) the criterion for high acid fruit products is 2D or 3D (decimal reduction time in spore population for a given T_{ref}) with *Alicyclobacillus acidoterrestris* as a reference microorganism. In this study, the target P value was determined for a 3D of a given reference microorganism. The target P values were calculated according to the thermal resistance values of the reference microorganism according to literature data for different juice media and are given in Table 1.

During pasteurization the slowest heating point was determined experimentally by measuring the temperature from 3 different points using 5; 5.5; and 6 cm (Cu-CuNi) thermocouples. The 5.5 cm thermocouple was found suitable for the 90.7 mm height jars to measure the cold point temperature of the liquid drinks. Cold point and medium temperatures were recorded in 1 min intervals with an Ellab CTF 84 Model Digital Thermometer (Ellab A/S, Hilleroed, Denmark) and the experimental P values calculated by using Eq. (1) and Eq. (2).

$$P = \sum\left(L(T) \times \frac{\Delta time}{timeref}\right) \quad (1)$$

where,

$$L(T) = 10^{\frac{T-Tref}{z}} \quad (2)$$

$\Delta time$ = *time between samples in minutes*
$timeref = 1 minute PU = Pateurization Unit$
L = *Lethality*

There were 26 glass jars pasteurized in one production cycle for each product according to crate capacity of a steam jacketed vessel with 70 cm height and 50 cm width (Milwall, John Fraser and Son Ltd., London, UK). The cold point and the medium temperatures were measured twice for two replicate productions. When the target P value was reached, cooling of the jars was maintained until the cold point temperature decreased below 40 °C.

2.2.3. *Compositional analyses*

The ascorbic acid content was determined according to the AOAC (2006) Method (967.21). The total phenolic content was measured with Folin and Ciocalteu reagent, using the method described by Singleton and Rossi (1965) and expressed as mg gallic acid equivalents (GAE)/100 mL juice. The linearity range for this assay was determined as 1.0–4.0 mg/L GAE ($R^2 = 0.996$), with an absorbance range of 0.10–0.37 AU at 760 nm. Antioxidant capacity was established with the 2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging assay (trolox equivalent antioxidant capacity (TEAC)), the procedure followed the method of Arnao, Cano, and Acosta (2001) and Rice-Evans, Miller, and Paganga (1996). A sugar analysis was carried out using the Lane–Eynon method and °Brix, and the pH was determined according to Cemeroğlu (1992). Protein analysis was accomplished according to the Bradford (1976) method.

2.2.4. *Collagen analysis*

Collagen content of the samples was determined according to the AOAC (2006) Method (990.26).

2.2.4.1. *Standard curve preparation.* A standard curve was prepared with a trans-hydroxyproline standard stock solution using a 60 μg/mL initial concentration. A 10, 20, 30, and 40 mL filtrate of the standard stock solution of hydroxyproline was taken and volume was adjusted to 100 mL which contains 0.6, 1.2, 1.8, 2.4 μg/mL hydroxyproline. Then, 2 mL of these solutions were transferred to the reaction tubes. After the addition of 1 mL of the oxidant solution (0.006 M chloramine T in 0.8 M citrate buffer, pH 6.0), the volume was adjusted to 100 mL, the reaction tubes were shaken at room temperature for 30 min. Afterwards, 2 mL of reactive colour reagent (10 g dimethylamine benzaldehyde was dissolved in 35 mL of 65% perchloric acid for daily usage) were added and the mixture was shaken at 60 °C for 15 min. After cooling the tubes under tap water, the absorbance was read at 558 nm using a VIS 6000 spectrophotometer (Pop Optizen, Mecasys Co., Ltd., Daejeon, Korea) and the calibration curve was created. All standard solutions were prepared immediately before use.

2.2.4.2. *Sample preparation.* A 4 g CJD sample was weighed. After the addition of 30 mL of 3.5M sulphuric acid (H_2SO_4), the sample was placed in an oven (Memmert GmbH, Heilbronn, Germany) at 105 °C for 12 h. The sample volume was adjusted to 500 mL with water; the solution was then filtered through a Whatman No.1 (110 mm) filter paper and then 2 mL of the filtrate of the sample was transferred to the reaction tubes and then a similar procedure was applied to the samples as given in the standard curve preparation.

The collagen content was calculated according to Eq. (3) and Eq. (4).

$$Hydroxyproline\,(\%) = \frac{Y \times 2.5}{W \times V} \quad (3)$$

$$Collagen\ content\left(\frac{mg}{100mL}\right) = Hydroxyproline\,(\%) \times 8^{*} \quad (4)$$

Y: Hydroxyproline concentration from calibration curve (μg/2 mL)
W: Amount of sample (g)
V: Volume (mL) completed to 100 mL
*Conversion factor of hydroxyproline to collagen

2.2.4.3. *In vitro bioavailability analysis*. The procedure was applied according to Miller, Schricker, Rasmussen, and Van Campen (1981). A pancreatin–bile acid solution was prepared by weighing 4 g pancreatin and 25 g bile acid and then dissolving them in 0.1 M $NaHCO_3$ solution and the volume was completed to 250 mL by the addition of 0.1 M $NaHCO_3$.

2.2.4.4. *Preparation of dialysis tube*. The dialysis tube dimensions were 25 mm wide, with a 16 mm inside diameter, and a 12,000 Da pore size.

Before use, tubes were first left in tap water for 3–4 h then 0.3% sodium sulphite solution at 80 °C for 1 min, followed by rinsing in pure water (60 °C – 2 min). Rinsed tubes were left in 0.2% sulphuric acid at 60 °C for 1 min. For removing acid solution, they were washed with hot pure water again. From one side tubes were tied up and used in experiment. The dialyses tubes can be used several times; before the next time usage, the dialysis tubes were washed with deionized water, dried, and were left in hot pure water.

2.2.4.5. *In vitro bioavailability analysis of collagen*. Drink samples of 50 g were homogenized with a 15,750 unit of pepsin. The solution was adjusted to pH 2.0 with 1 M HCl and incubated at 37 °C for 2 h in a water bath. Then, 20 mL of this solution were mixed with a 5 mL pancreatin–bile acid solution, and then titrated with 0.5 M $NaHCO_3$ until pH 7.5 was reached. Assays were carried out in triplicate and the mean value was used. Later, 25 mL of pure water, 0.5 M $NaHCO_3$, and the necessary amount of 0.5 M $NaHCO_3$ that was found after titration were added to the inside of the dialysis tubes and the other side was tied up. Dialysis tube was then settled into a 250 mL erlenmeyer. Then the 20 mL homogenized sample with pepsin was transferred to the erlenmeyer containing the dialysis tube, adjusted to pH 5.0 with the addition of a pancreatin–bile acid solution and incubated at 37 °C for 2 h in a shaking water bath. After the incubation, the dialysis tube was washed with deionized water and dialysate was used for collagen analysis (Gil-Izquierdo, Terreres, & Barberan, 2001).

Bioavailability of collagen in the samples was calculated using Eq. (5).

$$Bioavailability\,(\%) = \frac{Amount\ of\ collagen\ in\ dialysate}{Amount\ of\ total\ collagen\ in\ sample} \times 100 \quad (5)$$

2.2.5. *Microbiological analysis*

According to the Microbiological criterion of the Turkish Food Codex (2011/03) for fruit drinks, the glass jars were incubated at 30 °C for 10 days for physical and pH control. The total viable count (TVC), yeast and mold count (YMC), and *Escherichia coli* O157:H7 counts of the CJD were determined before and after pasteurization by plate counting method (Karapınar, 1995).

2.2.6. *Sensory evaluation*

Difference and preference tests were conducted to select the most preferred formulations. A dosing characteristics test of the samples was performed by a trained panel. Each sample was served at 5 °C. The evaluation was made on a descending scale of 1 to 4 (very good, good, bad, and very bad) according the effect of fish collagen taste (Altuğ & Elmacı, 2011). A sensory test of 4 trials was performed with 20 panellists.

2.2.7. *Statistical analysis*

The experimental data were subjected to an analysis of variance (ANOVA) and the Duncan test was used to see the differences among the samples using the SPSS 16 software package (Chicago, IL, USA) and $P < 0.05$ was considered (Cochran & Cox, 1992). All measurements were carried out in triplicate while production of drinks was in duplicate. Data were considered statistically significant at $P < 0.05$ and denoted with different superscripts.

3. Results and discussion

3.1. Formulation

Drink formulations which were decided as a result of the difference and preference tests are given in Table 2. Commercial

Table 2 – Formulation of fruit juice drinks containing hydrolyzed collagen.

CJD	Ingredients	Ratio
Orange	Orange juice	50
	Water	50
	Hydrolyzed collagen	2.5[a]
	Citric acid	0.2[a]
	Ascorbic acid	0.1[a]
Orange-white grape	Orange juice	50
	White grape juice	30
	Water	20
	Hydrolyzed collagen	2.5[a]
	Citric acid	0.2[a]
	Ascorbic acid	0.1[a]
Apple	Apple juice	70
	Water	30
	Hydrolyzed collagen	2.5[a]
	Citric acid	0.1[a]
	Ascorbic acid	0.1[a]
	Natural mint flavour	0.03[a]
Apple-white grape	Apple juice	50
	White grape juice	26
	Water	24
	Hydrolyzed collagen	2.5[a]
	Citric acid	0.1[a]
	Ascorbic acid	0.1[a]
	Natural mint flavour	0.02[a]

[a] % concentrations of ingredients were calculated according to percent of (fruit juice + water = 100 mL) volume.

products of 100% fruit juices such as orange (10.1% Brix), white grape (12.5% Brix), and apple (11.7% Brix) were chosen for the production of CJD due to their high antioxidant activity. In addition, 0.1% ascorbic acid, which has an antioxidant activity, was added to the formulations.

Considering the sensory analysis results of each product, 2.5% concentration of hydrolyzed collagen was preferred ($P < 0.05$) and added to the CJD. The natural mint aroma (0.02–0.03%) was added to apple drinks to mask the collagen slight taste and odour due to its fish origin, which was not sensed in the orange drinks. Acidification of the CJD with 0.1–0.2% citric acid according to juice type maintained the taste of the CJD besides decreasing the pH value.

Sensory analysis of the given formulations had the highest overall acceptance; therefore, CJD can be successfully fortified with hydrolyzed collagen and produced commercially.

3.2. *Pasteurization of the CJD*

As a pasteurization criterion, *A. acidoterrestris* was chosen as the reference microorganism which causes quality deterioration in high acid foods. It is a thermoacidophilic, non-pathogenic, and spore forming bacterium that has been isolated and identified previously in several spoiled commercial pasteurized fruit juices such as orange and apple (Komitopoulou, Boziaris, Alison Davies, Broughton-Delves, & Adams, 1999; Maldonado, Belfiore, & Navarro, 2008; Pettipher, Osmundsen, & Murphy, 1997; Silva & Gibbs, 2001; Sukasih & Setyadjit, 2008). Silva, Gibbs, Vieira, and Silva (1999) suggested the use of *A. acidoterrestris* spores as the target of the pasteurization processes in high-acid fruit products because *A. acidoterrestris* spore germination and growth at 10^6 cfu/mL under acidic conditions was reported in orange juice stored at 44 °C for 24 h (Pettipher et al., 1997) and in apple, orange, and grapefruit juices stored at 30 °C (Komitopoulou et al., 1999).

The target P value was chosen according to a 3D decimal reduction of the target microorganism which is recommended in the commercial canning industry (Silva & Gibbs, 2010). The T_{ref} and z values of *A. acidoterrestris* were selected from literature data (Table 1) according to juice type. It can be observed from Table 3 that the target P value for apple-white grape juice is considerably smaller than other drinks due to the T_{ref} and z value of the target microorganism. Reference data selection for each CJD was done according to juice type. For the blends, the highest volume of the juice was taken into consideration as a reference. Therefore, for orange-white grape juice blends, Tref, D and z values were chosen according to orange juice. For apple-white grape juice blends, the parameters for apple juice are chosen but due to temperature increases within a shorter time than apple juice in the preliminary experiments, T_{ref} was chosen as 90 °C, otherwise 80 °C for other CJDs (Table 1). The pasteurization parameters for each CJD productions are given in Table 3.

The pasteurization processes were carried out at 95 °C. The initial cold point temperatures of the preheated drinks were changed to between 59.1 and 64.6 °C. The come up time was changed to between 9 and 13 min where the processing time was 18 to 32 min and cooling time was 14 to 17 min. When the target P value was reached, the cooling process was started. To ensure microbiological safety under commercial processing conditions, the experimental P values were kept higher than the target ones.

The initial microbial populations are shown in Table 4. The counts of the microbial populations for TVC and YMC were low initially and were approximately 10^1 CFU/g due to the use of commercial pasteurized fruit juices. *E. coli* O157:H7 was not determined before pasteurization.

The CJD were incubated at 30 °C for 10 days after pasteurization and no growth was determined in any of the juices. The pasteurization process was found adequate to inactivate the total microbial load in the CJD which resulted in an acceptable microbiological product quality.

Table 4 – Microbial populations of fruit juice drink containing hydrolyzed collagen before and after pasteurization.

CJD	TVCb (CFU/g)	YMC[c] (CFU/g)	*E. coli* O157:H7 (CFU/g)
Orange	4.8×10^b/nd[a]	4.0×10^b/nd	nd[d]/nd
Orange-white grape	8.0×10^b/nd	1.5×10^b/nd	nd/nd
Apple	2.5×10^b/nd	$<10^b$/nd	nd/nd
Apple-white grape	6.2×10^b/nd	$<10^b$/nd	nd/nd

[a] Results were given before/after pasteurization.
[b] TVC: mesophilic total viable count.
[c] YMC: yeast and mold count.
[d] nd: not determined.

Table 3 – Pasteurization parameters at 95 °C of the fruit juice drink containing hydrolyzed collagen.

CJD	$T_{initial}$ (°C)	Come up time (min)	Process time (min)	Cooling time (min)	Target PU value (3D)	Measured PU value
Orange	61.2 ± 1.6	13	21	16	162.9	239.99
	64.6 ± 1.4	12	22	18		252.67
Orange-white grape	62.0 ± 1.2	8	24	17	162.9	195.771
	61.5 ± 0.5	7	18	14		184.95
Apple	61.5 ± 0.7	9	23	16	123.45	125.05
	64.5 ± 0.5	9	20	16		140.32
Apple-white grape	59.1 ± 0.2	9	23	16	33.3	41.40
	61.5 ± 0.5	9	32	17		38.05

$T_{initial}$: Initial cold point temperature of the drinks in glass jars.

Table 5 – Chemical composition and antioxidant capacity of pasteurized fruit juice drink containing hydrolyzed collagen.

CJD	pH	°Brix	Protein (g/100 mL)	Reducing sugars (g/100 mL)	Total sugar (g/100 mL)	Sucrose (g/100 mL)	Ascorbic acid (mg/100 mL)	Total phenolic (mg GAE/ 100 mL)	ABTS (μmol TEAC/ 100 mL)
Orange	3.96[a]	10.4 ± 0.3[a]	2.42 ± 0.01[a]	3.86 ± 0.11[a]	6.15 ± 0.25[a]	2.18 ± 0.22[a]	104.5 ± 0.4[a]	86.03 ± 0.74[a]	115 ± 0.23[a]
Orange-white grape	4.02[a]	13.5 ± 0.5[b]	2.22 ± 0.06[b]	6.49 ± 0.13[b]	6.69 ± 0.06[a]	0.09 ± 0.08[b]	113.5 ± 4.7[a]	114.9 ± 0.54[b]	104 ± 0.15[b]
Apple	4.04[a]	10.9 ± 0.4[a]	2.48 ± 0.01[a]	6.38 ± 0.3[b]	6.97 ± 0.23[a]	0.55 ± 0.3[b]	81.39 ± 0.1[b]	87.56 ± 0.27[a]	127 ± 0.29[c]
Apple-white grape	3.97[a]	13.5 ± 0.1[b]	2.39 ± 0.04[a]	7.37 ± 0.28[c]	7.79 ± 0.05[b]	0.39 ± 0.32[b]	85.12 ± 0.5[b]	117.43 ± 1.07[b]	105 ± 0.13[d]

[a,b,c,d] Data are given as means ± SD. Means with different superscript letters in the same column are significantly different ($P < 0.05$).

3.3. *Compositional analyses of CJD*

The results of composition analyses of the CJD after pasteurization are given in Table 5. The samples were initially adjusted to pH 4.0–4.09 by the addition of 0.2% citric acid before pasteurization. The change in sample pH values before and after pasteurization was <0.5 which maintains the Turkish Food Codex regulation and the Microbiological Criteria 2011/03 requirement. Therefore, pasteurization process of the CJD is considered to be suitable according to the pH value requirement. The °Brix values of the CJD were adjusted to a range of 10.4 to 13.5 according to the juice–water proportions in the formulations.

As shown in Table 5, total sugar content varied between 6.15 and 7.79 g/100 mL. The orange drink had significantly higher sucrose content (2.16 g/100 mL) than the other CJDs (0.09–0.55 g/100 mL), the reason being that orange juice contains higher percentage of sucrose resulting from the citrus fruits compared to the apple and white grape juices. Glucose and fructose are the main sugars of the grapes whereas fructose is the dominant one in apples. Sucrose, glucose, and fructose represent about 80% of the total soluble solids of orange juice, and the ratios of sucrose:glucose:fructose are generally about 2:1:1 (Chaudhary, Yadav, & Grewal, 2014; Fuleki, Pelayo, & Palabay, 1994; Lee & Coates, 2000; Roberts & Gaddum, 1937).

Before the addition of ascorbic acid to the drinks, the ascorbic acid contents were measured as 19.25, 19.65, 0.79 and 0.39 mg/100 mL for orange, orange-grape, apple, and apple-grape drinks, respectively. Orange drinks possessed the highest ascorbic acid content ($P < 0.05$) due to the high ascorbic acid content of the raw material. For each 100 mL CJD, the ascorbic acid content increased to approximately 80 mg. The ascorbic acid content of the pasteurized CJD varied between 81.39 and 113.5 mg/100 mL after the addition of 0.1% ascorbic acid to the formulations. The ascorbic acid content of the drinks was decreased to 2–6% after the pasteurization process. Also with the addition of ascorbic acid to the drinks, inhibition of the oxidation of the blending components including the collagen was aimed.

The amount of total phenolics in the CJD varied widely between 86.93 and 117.43 mg GAE/100 mL. The highest level of phenolics in the drinks was found in the apple-grape, while the lowest was in the orange. The total phenolic content in the orange drink formulations of the present study was close to those described by Stella, Ferrarezi, Santos, and Monteiro (2011), for ready-to-drink orange juice and nectar, in which the total phenolic content varied from 18.7 to 54.2 mg of gallic acid equivalents/100 mL. As mentioned before, the addition of white grape juice, with its high phenolic content, increased the total phenolic content values of the drink blend. Usage of white grape juice in the formulations had shown important effect as a phenolic compound source. The radical scavenging capacities of the drinks were determined using the ABTS assay. In the ABTS assay, the values ranged from 104 to 127 μmol TEAC/100 mL. Similar results for orange juice were reported previously by Stella et al. (2011) where the total antioxidant activity varied from 57.88 to 315.42 μmol TEAC/100 mL in the ready-to-drink orange juice and from 87.59 to 349.32 μmol TEAC/100 mL in the nectar. Despite the total phenolic content of the drinks with white grape juice being higher, the ABTS assay results did not vary markedly.

The protein content of the drinks was measured around 0.56 ± 0.02 g/100 mL, initially. The addition of 2.5% Peptan™, F type hydrolyzed collagen increased the protein content of the CJD to 2.22–2.48 g/100 mL. The results of the collagen analysis conform to the amount of addition. The hydrolyzed collagen addition facilitates production of protein enhanced nutritional drinks.

Collagen and *in vitro* collagen contents of the drinks and bioavailability results are given in Table 6.

The present study demonstrates that the *in vitro* bioavailability of hydrolyzed collagen is very high. A 2.5 mg/100 mL hydrolyzed collagen addition to the drinks increased the collagen content to 2.05–2.27. It is also known from *in vivo* metabolism that more than 90% of the hydrolysates are digested and quickly absorbed after oral ingestion (Asghar & Henrickson, 1982; Oesser et al., 1999) where the *in vivo* bioavailability studies in humans are important to confirm the *in vitro* findings. In addition, chronic intake of hydrolyzed collagen affects platelet adhesion and aggregation which should be tested by clinical studies (Roberts, McNicol, & Bose, 2004).

Table 6 – The collagen *in vitro* bioavailability of the pasteurized fruit juice drinks containing hydrolyzed collagen.

CJD	Collagen (%)	*In vitro* (%)	Bioavailability (%)
Orange	2.27 ± 0.08[a]	2.08 ± 0.10[a]	95.37 ± 2.13[a]
Orange-white grape	2.07 ± 0.07[a]	1.86 ± 0.04[a]	83.38 ± 0.57[b]
Apple	2.05 ± 0.05[a]	1.86 ± 0.05[a]	90.71 ± 0.22[ac]
Apple-white grape	2.19 ± 0.14[a]	1.89 ± 0.10[a]	86.34 ± 1.05[bc]

[a,b,c] Data are given as means ± SD. Means with different superscript letters in the same column are significantly different ($P < 0.05$).

The bioavailability results were found higher for orange (95.37%) and apple (90.71%) drinks, where the grape juice addition decreased that value to 83.38 and 86.34 for orange-grape and apple-grape blends, respectively ($P < 0.05$). As Ferguson (2001) mentioned, the decrease in bioavailability could be due to the phenolic compounds in the white grape juice which is related to its perceived role as 'antinutrients', particularly due to its ability to reduce the digestibility of proteins, either by direct precipitation or by inhibition of enzyme activity. From a bioavailability standpoint, orange juice is the most appropriate for CJD production. However, all the CJD products could be viewed as good sources of health-promoting nutritional factors due to the high bioavailability of hydrolyzed collagen.

4. Conclusion

Health-conscious consumers are increasingly seeking functional foods in an effort to control their own health and well-being. Hydrolyzed collagen can be used as a main ingredient in the development of functional food products. According to clinical studies, a 5–10 g daily intake of hydrolyzed collagen for three to six months helps bone/joint and skin health. Hydrolyzed collagen can be added to beverages to improve their nutritional and functional properties without causing any technological problem in production due to its low viscosity and high solubility in water. Our results show that hydrolyzed collagen addition to drinks increases the protein content of the product markedly and the bioavailability results were satisfactorily high for all drinks. The finding of this research revealed that the fruit juice drinks produced with a substitution up to 2.5% hydrolyzed collagen will help in increasing the intake of protein and be successfully used as a commercial drink product. Formulations could affect bioavailability of the hydrolyzed collagen and consumer acceptability of the product where the orange juice formulation provided the highest *in vitro* bioavailability.

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