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The Effect of Red Pepper (*Capsicum frutescens* L.) Powder Addition on the Antioxidant Activity and Total Phenolic Contents of Yogurt

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ABSTRACT

Background: The aim of this study is to increase the consumption acceptability of yogurt and to improve its functional properties by using red pepper (*Capsicum frutescens* L.) powder.

Methods: This study analyses yogurt with varying degrees red pepper powder addition (0, 0.2%, 0.5%, 1.5%, 2%). The mixtures were analyzed on days 1, 7 and 15.

Result: The antioxidant activity was found between 10.70 and 15.06 mM TE. Total phenolic compounds were between 250.19 and 577.50 mg GAE/kg. Important differences were revealed between the control and yogurts with red pepper powder with respect to total antioxidant activity and phenolic contents characteristics during storage ($p<0.05$). In conclusion, the obtained data can be used for the development of a functional product.

Key words: Antioxidant activity, Functional, Red pepper powder, Total phenolic contents, Yogurt.

INTRODUCTION

Growing in almost every region of the world, peppers are consumed and used both as natural food coloring and spices due to their remarkable colors, flavors and aromas (Ornelas-Paz *et al*., 2010). Phenolic compounds are natural antioxidants present in plant foods (Zeb, 2020). In addition, peppers are a type of plant that contains high levels of antioxidants. Peppers gain popularity so quickly, not only because of their taste, aroma or color, but also because of their health benefits. Since the color, shape, size and chemical structure of each pepper are different, the antioxidant properties, vitamins and phytochemicals of the peppers vary accordingly. In addition, peppers are known to contain high levels of polyphenols, flavonoids, quercetin and luteoline (Lee *et al*., 1995). Carotenoids, a substance that determines the color of fruits, are found in high amounts in pepper. Pepper-containing carotenoids are provitamin A (α and β-carotene and β-cryptoxanthine) and xanthophylls, which are oxygen-containing carotenoids (Howard, 2001). These compounds are fat-soluble and have an anti-ulcer effect, affect the immune system, protect against certain types of cancer and help prevent cardiovascular diseases and certain diseases, such as aging-related cataracts (Krinsky and Johnson, 2005). Capsaicinoids have some physiological and pharmacological effects. These effects are cancer prevention, antioxidantity, overweight prevention and anti-inflammation (Luo *et al*., 2011). Another effect of capsaicinoids is seen during the addition of capsaicinoids to vegetable oils. In this case, capsaicinoids may act as a lipid oxidation inhibitor (Si *et al*., 2012). In addition, ABTS and DPPH analyzes show that pepper pericarp and placenta have high chelation and scavenge properties against free radicals (Hyeon Sim and Young Sil, 2008). Many studies have been conducted to determine the antioxidancy of different pepper compounds and to determine the effect of pepper maturity on bioactive contents (Lee *et al*., 1995; Simmone *et al*., 1997; Howard *et al*., 2000; Marin *et al*., 2004). Red pepper is grown in almost every region of the world and is consumed in fresh or dried form (Rico *et al*., 2010). Red pepper is also preferred to give processed food products a bright red color and a sharp aroma (Lee *et al*., 2004; Rico *et al*., 2010). In another aspect, yogurt, which is an easy to digest nutrient, is often preferred because of its bioavailability and proteolytic properties (Shaboo and Ahmad, 2011). Yogurt, which has positive effects on the immune system, improves lactose intake and glucose control (Yadav *et al*., 2007). Yogurt also plays an important role in the relief of constipation and diarrhea problems and has significant effects on the removal of intestinal diseases (Adolfsson *et al*., 2004). Several studies have been conducted on the bioactive properties of yogurt. Studies of Min *et al*. (2012) and Damian (2013) on dietary fiber supplementation of yogurt, study of Lee and Paek (2003)

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on the cancer prevention property of yogurt, study of Sazawal *et al.* (2013) on the effect of yogurt on micronutrients, study of Shin *et al.* (2012) on the use of a mixture of yogurt with black garlic as an anti-inflammatory agent, study of McCowen *et al.* (2010) on omega-3 polyunsaturated fatty acids of yogurt, study of Shab-Bidar *et al.* (2011) on the vitamin D content of the yogurt, study of Cavallini *et al.* (2009) on isoflavones of yogurt and study of Jung and Park (2005) on green tea powder are examples, study of Šeregelj *et al.* (2019) on encapsulated natural bioactive compounds from red pepper waste are examples, study of Ogunyemi *et al.* (2021) on food spices are examples and study of Kiranawati *et al.* (2021) on the use of a mixture of yogurt with a large red chili puree as an antioxidant agent of studies done on the bioactive properties of yogurt. Yogurt is one of dairy products containing beneficial bacteria. Many researches have reported that yogurt has therapeutic and prophylaxis effects on some diseases such as cancers, infections, gastrointestinal disorders, asthma and antibioticassociated diarrhea (Beniwal *et al.*, 2003; Moghbel and Abbaspour, 2013; Karagül-Yüceer and Avsar, 2016; Fernandez and Marette, 2017). Functional yogurts enriched with micronutrients (iron, zinc, iodine and vitamin A) (Sazawal *et al.*, 2013), black garlic extract (Shin *et al.*, 2012), red ginseng extract (Kim *et al.*, 2008), spices oleoresins (Illupapalayam *et al.*, 2014), vitamin D (Shab-Bidar *et al.*, 2011), acacia fiber (Min *et al.*, 2012), sweet pumpkin (Jung *et al.*, 2011), MACA (*Lepidium meyenis*) hot water extract (Chung *et al.*, 2010), encapsulated natural bioactive compounds from red pepper waste (Šeregelj *et al.*, 2019), food spices (Ogunyemi *et al.*, 2021) and a large red chili puree (Kiranawati *et al.*, 2021) have been reported.

This study aims to reveal the antioxidancy and phenolic contents of yogurts added with red pepper powder. In this study, it is aimed to produce a healthy functional food which has high antioxidant level and contains red pepper powder.

MATERIALS AND METHODS

Material

Red pepper powder was obtained from a local store in Zonguldak. Sterilized full-fat cow's milk was used for yoghurt production. This cow milk was also obtained from a local store in Zonguldak. Skimmed milk powder (NFMP) was obtained from Pınar Dairy Products in İzmir. The frozen starter culture used for commercial purposes (YC-350, a blend of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrucckii* subsp. *bulgaricus*) was obtained from Chr. Hansen Co. (A/S, Horsholm, Denmark) and yogurt was produced. Preceding their usage, the strains were grown and kept up in reconstituted (11%, w/w) skimmed milk is exposed to pasteurization for 20 minutes at 85°C and incubated at 42±1°C. After the incubation, milk that will be used to produce yogurt is immunized with this culture at a ratio of 2.0% (1:1).

Yogurt production

The experiment was carried out in the research laboratories of the Department of Food Engineering at Zonguldak Bülent Ecevit University in Turkey during the 2019-20 and 2020-21 research periods. The technique used in the production of yogurt is described by Tamime and Robinson (1985). Cow milk with 3% fat, 3.6% protein, 12.1% solids level and acidity of 8 SH was used in yogurt production. Skimmed milk powder was added to the milk to increase the solids content to 15 grams per 100 grams. Red pepper was then added to the milk at concentrations of 0%, 0.2%, 0.5%, 1.5% and 2%. The milk was then homogenized (Homogenizer T65, Germany). The milk was heated at 85°C for 30 minutes and pasteurized. After pasteurization, the milk was quickly cooled to 45 degrees. As a starter culture, *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* was added to the mixture at the rate of 2% (w/v). Subsequently, incubation was carried out at 42±1°C and pH 4.6-4.7 for 3.5 hours. The milk was then allowed to stand for 12 hours at 4±1°C. Yogurt subject to analysis was kept at 4 degrees for 15 days. The process for the production of yogurt was repeated twice. Yogurt measurements were made on the first, 7th and 15th days.

Determination of total phenolic content

Total phenolic content of yogurt was determined according to Folin-Ciocalteu method. Gallic acid was used as standard in this determination process (Singleton and Rossi, 1965). The absorbance level was determined by spectrophotometer at 760 nm (Shimadzu Scientific Instrument, Tokyo, Japan). Total phenolic content concentration in yogurt was determined as gallic acid equivalent (GAE). This equivalent is reached using an equation provided from the standard gallic acid curve. The results reached with the study are expressed as mg GAE/kg.

Determination of total antioxidant activity

2,2'-azinobis (3-ethlybenzthiazoline)-6-sulfonic acid (ABTS-TEAC) assay

2,2'-azinobis (3-ethlybenzthiazolin-6-sulfonic acid) diammonium salt (ABTS+) radical cation was prepared by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate. $ABTS^{+}$ radical cation was diluted with PBS, pH: 7.4, to an absorbance of 0.70 (±0.02) at 734 nm equilibrated at 30°C. ABTS+ inhibition against Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was spectrophotometrically measured. The absorbance was measured at 734 nm in a spectrophotometer (Shimadzu Scientific Instruments, Inc., Tokyo, Japan). TEAC values of samples were calculated from the Trolox standard curve and expressed as Trolox equivalents (in μmol ml^{-1} of sample) (Re *et al.*, 1999).

Statistical analysis

All experiments and analyses were replicated two times. Data were statistically analyzed repeated measure ANOVA using SPSS for Windows 16.0. Differences between data were analyzed by Duncan analysis (SPSS, 2017).

RESULTS AND DISCUSSION

Evaluation of total antioxidant activity and phenolic contents

Total antioxidant activity and phenolic substance of samples are given in Fig 1 and 2. Yogurt with 2% red pepper powder (E) had the highest phenolic content (577.50 mg GAE/kg) and antioxidant value (15.06 mM TE) for all samples on the 7th day of storage.

Important differences were detected between the yogurts with red pepper powder and control with respect to total antioxidant activity and phenolic contents during storage ($p<0.05$). The analyses on yogurt containing red or green pepper juice have reached similar results (Kang *et al.*, 2018), for yogurt prepared with fermented red pepper combinations (Yu *et al.*, 2014), for yogurt prepared with encapsulated natural bioactive compounds from red pepper waste combinations (Šeregelj *et al.*, 2019), for yogurt prepared with a large red chili puree combinations (Kiranawati *et al.*, 2021) and for yogurt prepared with a food spice combinations (Ogunyemi *et al.*, 2021). Phenolic compounds which are present in high amounts in some plants are water soluble and have antioxidant effect (Zeb, 2020). The high amounts of phenolic compounds in red pepper may be caused by the native phytochemical compounds of red pepper (Yu *et al.*, 2014). Generally, it was observed that red pepper powder added to yogurt increased antioxidancy (Fig 1). Accordingly, it is possible to say that red pepper polyphenols contribute to the antioxidancy of yogurt. Antioxidant activity in yogurt containing red pepper is probably due to the high antioxidant effect of red pepper (Kapadiya *et al.*, 2016). High degradation of antioxidant phenolic compounds (Yildiz and Eyduran, 2009) and increased interaction of polyphenol with milk protein (Yüksel *et al.*, 2010) is caused by a decrease in antioxidant level during cold storage of yogurt.

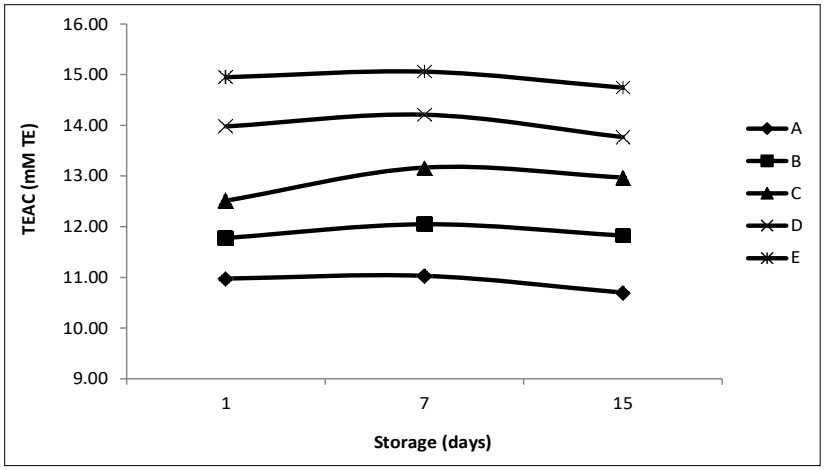

Fig 1: Total antioxidant activity of the experimental yogurts during storage at 4°C for 15 days:
A: 0% (Plain control sample), B: Yogurt with 0.2% red pepper powder, C: Yogurt with 0.5% red pepper powder, D: Yogurt with 1.5% red pepper powder, E: Yogurt with 2% red pepper powder.

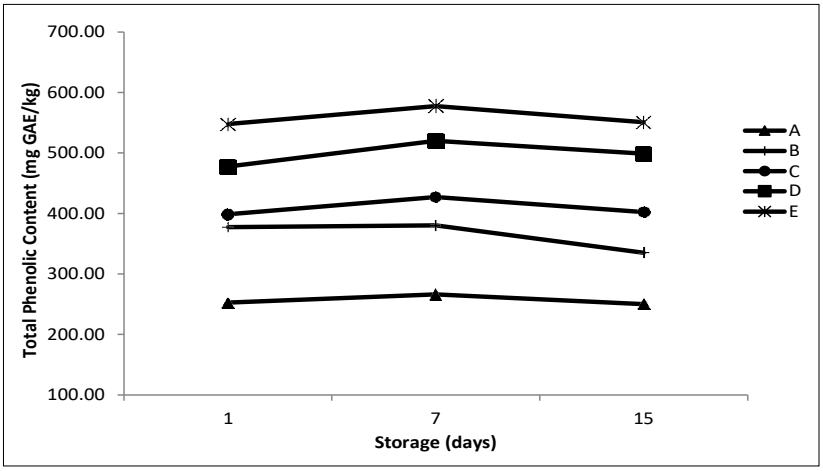

Fig 2: Total phenolic content of the experimental yogurts during storage at 4°C for 15 days:
A: 0% (Plain control sample), B: Yogurt with 0.2% red pepper powder, C: Yogurt with 0.5% red pepper powder, D: Yogurt with 1.5% red pepper powder, E: Yogurt with 2% red pepper powder.

CONCLUSION

This manuscript describes TEAC and Folin-Ciocalteu method for the antioxidant activity and total phenolic content analysis in yogurts with red pepper powder. The increase in the amount of red pepper powder added to the yogurt resulted in an increase in the phenolic content and antioxidancy of the yogurt. By providing high protection against pathologies related with free radicals, consumers will benefit greatly from these product forms. The yogurt containing red pepper powder might become a popular dairy product for consumers who prefer a unique taste. In conclusion, the results suggest that the red pepper powder can be used for manufacturing functionally fortified yogurt.

Conflict of interest: None.

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