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Determination of Color Parameters, Phenolic Status and Antioxidant Potential in Some Industrial and Traditional Bread Varieties

Bazı Endüstriyel ve Geleneksel Ekmek Çeşitlerinde Renk Parametreleri, Fenolik Durum ve Antioksidan Potansiyelinin Belirlenmesi

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Abstract

Bread and especially wheat bread as the basic food of society is one of indispensable food sources of human nutrition. The golden brown color caused by baking bread dough is the result of non-enzymatic chemical reactions and usually Maillard reaction (MR) and caramelization affect to this. Amino gropes and reducing sugars contained in bakery products can perform caramelization and MR simultaneously with effect of temperature. Processing conditions of bakery products also have a very important effect on the antioxidant activity. As bread types, francala bread, grissini, rusk and traditional oven-dry (Şebinkarahisar region) bread were examined. Antioxidant substances in bread crust have an important place in preventing colon cancer for terms of health. In this study, types of bread made from wheat flour; samples were taken from crust of francala bread, grissini, rusk and traditional (Şebinkarahisar region) breads, and reactions occurring with effect of heat were measured with tone angle Hue (°), Chroma (C) and Browning Index (BI). Amount of antioxidant substance formed was determined by 2,2-

Özet

Toplumun temel besinlerinden biri olan ekmek ve özellikle buğday ekmeği, insan beslenmesinin vazgeçilmez besin kaynaklarından biridir. Ekmek hamurunun pişirilmesi ile oluşan altın sarısı renk kahverengileşmesi, enzimatik olmayan kimyasal reaksiyonların sonucudur ve Maillard Reaksiyonu (MR) ve karamelizasyonun etkili olduğu bilinmektedir. Unlu mamüllerin içerdiği amino gropları ve indirgen şekerler sıcaklıklığın etkisi ile karamelizasyon ve MR'yi aynı anda gerçekleştirebilir. Unlu mamullerin işleme koşulları antioksidan aktivitesi üzerinde de oldukça önemli etkiye sahiptir. Ekmek kabuğunda meydana gelen antioksidan maddeler sağlık açısından kolon kanseri önlemede önemli bir yere sahiptir. Bu çalışmada, buğday unundan yapılan ekmek tipleri; francala ekmek, galeta, etimek ve geleneksel fırın kurusu (Şebinkarahisar yöresi) ekmeklerinin kabuk kısımlarından örnekler alınarak ısı etkisi ile oluşan reaksiyonlar sonucu meydana gelen ton açısı Hue (°), Chroma (C) ve Browning Indeksi (BI) değerleri ölçülmüştür. Oluşan

diphenylpicrilhydrazil (DPPH) and total phenolic content (TPC) methods. TPC value to bread crusts is 122,76±2,62 mg GAE/100g in francala bread, 91,30±21,83 mg GAE/100g in rusk, 54,49±16,76 mg GAE/100g in oven-dry bread and 85,60±16,39 mg GAE/100g in grissini bread. %DPPH values are 45,62±0,43 in francala bread, 21,57±14,84 in rusk, 17,30±0,53 in grissini bread and 12,59±8,14 in oven-dry bread, respectively. H, C, BI, DPPH and TPC results were analyzed by Pearson Correlation Test and one-way analysis of variance (ANOVA).

Keywords: Antioxidant, Bread types, DPPH, TPC, Traditional bread

antioksidan madde miktarı 2,2-difenilpikrilhidrazil (DPPH) ve toplam fenolik madde içeriği (TFM) yöntemleri ile belirlenmiştir. Ekmek kabuklarına TFM değeri francala ekmeğinde 122,76±2,62 mg GAE/100g, peksimette 91,30±21,83 mg GAE/100g, fırın kurusu ekmekte 54,49±16,76 mg GAE/100g ve grisini ekmeğinde 85,60±16,39 mg GAE/100g'dır. %DPPH değerleri sırasıyla francala ekmeğinde 45,62±0,43, peksimette 21,57±14,84, grisini ekmeğinde 17,30±0,53 ve fırın-kuru ekmekte 12,59±8,14'tür. H, C, BI, DPPH ve TFM sonuçları Pearson Korelasyon Testi ve tek yönlü varyans analizi (ANOVA) ile analiz edildi.

Anahtar kelimeler: Antioksidan, Ekmek çeşitleri, DPPH, TFM, Geleneksel ekmek

Abbreviations: TPC, Total phenolic content; GAE, gallic acid equivalent

1. INTRODUCTION

Cereal products, fruits and vegetables contain phytochemicals that have nutritional complementary effects. The phenolic compound classes in cereals are benzoic and cinnamic acid derivatives, anthocyanidins, quinones, flavonols, chalones, flavones and amino phenolic compounds (Liyana-Pathirana & Shahidi, 2005). Antioxidants have been focus of attention in recent years with their ability to scavenge / remove free radicals. There are many defense mechanisms to prevent the formation of reactive oxygen species and damage they cause. These mechanisms are known as antioxidant defense systems or simply antioxidants (Altınışık, 2000).

Bread and bread products have an important role in human nutrition. In general, wheat bread is considered a good source of energy and indispensable food for the human body (Różyło, 2014). While white bread is preferred by most consumers, bioactive compounds, especially antioxidants (phenolic compounds) found in cereal, are especially concentrated in bran and aleurone layer (Mateo Anson et al., 2011). In particular, whole grain bread is a rich source of fiber and bioactive compounds such as oligosaccharides, fatty acids, sulfur amino acids, minerals, B vitamins, phytosterols, and antioxidants (Gani et al., 2012). Chemical reactions occurring in bread production; enzymatic production of sugars (30-70°C), starch gelatinization

(50-65°C), caramelization (50-65°C), protein denaturation and coagulation (60-70°C), yeast inactivation (45-50°C) and enzymes (60-80°C), MR (230-250°C) play an important role especially in determining the quality characteristics of bread (Hui et al., 2008).

The yellow-gold color, which is browning, colored compounds occur during the baking of the dough, which is caused by non-enzymatic chemical reactions, especially MR and caramelization. MR products (melanoidins) appear in bread crusts where reducing sugars and amino acids, proteins and/or other nitrogen-containing compounds are heat treated together. Caramelization is a complex reaction due to direct heating of carbohydrates, especially sucrose and reducing sugars. (Fennema & Tannenbaum 1996). The suitable temperature for estimating crust browning in bread crust using dried and ground breadcrumbs is 250°C (Zanoni et al., 1995). In addition, browning development can be effectively evaluated by measuring color during baking and is exponentially related to baking time. (Ramírez-Jiménez et al., 2000). Recently, a strongly correlated brownness model has been developed between concentration of melanoidin resulting from MR and color development in bread with baking (Purlis, 2010). MR can be associated with the formation of toxic and mutagenic compounds, as well as the formation of antioxidative products (Martins et al., 2000). Caramelization and MR can occur simultaneously (Villota et al., 2019); Both reactions depend on temperature, water activity and pH (Zanoni et al., 1995). Therefore, bread baking is a process in which heat and mass transfer occur at the same time (Purlis & Salvadori, 2009a).

Product formulation and processing conditions of bakery products also affect hydroxymethyl furfural color properties and antioxidant activity (Ertop & Sarikaya, 2017).

Different biological activities of melanoidids formed in the last stages of MR are known, including prebiotic, metal chelating, antihypertensive and antioxidant capacity (Rufian-Henares & Morales, 2007; Patrignani et al., 2016). MR products protect biological tissues from oxidation and this is associated with cancer, cardiovascular and neurological diseases that adversely affect human health (Pérez-Burillo et al., 2018; Pastoriza & Rufián-Henares, 2014).

Effect of baking conditions and dough supplements on amount of antioxidant and Phase II-Enzyme modulated, protein-bound 2,4-dihydroxy-2,5-dimethyl-1-(5-acetamino-5-methoxycarbonyl-pentyl) in bakery products 3-oxo- 2H-pyrrole (pronyl-L-lysine) has been studied in quantitative studies. These studies revealed that pronyl-L-lysine antioxidant is high in bread crust, low in crumbs and absent in unprocessed flours. Pronyl-L-lysine amounts were found to be strongly influenced by intensity of heat treatment, increasing cooking time from 70 to 210 minutes or increasing the cooking temperature from 220 to 260°C resulted in a 3 to 5-

fold increase in these antioxidant concentrations in shell, respectively (Lindenmeier & Hofmann, 2004). Pronyl-L-lysine, an important antioxidant substance in bread crust, has been shown to have a beneficial effect against chemically induced colonic preneoplastic progression in rats (Selvam et al., 2008).

In this study, industrial bread varieties with baking temperatures between 230-250°C; oven bread (225-230°C) (Elgün, 1981); In order to evaluate the relationship between the color parameters of grisini bread (175-250°C) (Garipoğlu, 2019), rusks and traditional Giresun Şebinkarahisar oven dry bread (230-250°C) (Anonymous, 2015) with the total phenolic substance and antioxidant content of the same samples; Hue (°) angle (H), chroma (C) and Browning index (BI) values were measured. Oven-dried berad, which is a traditional food according to others, is kept in a quenched oven for 3 days at 40°C after cooking process is finished in a stone oven, and its shelf life is cool and dry for approximately 2 years due to its low water activity (Anonymus, 2018).

Samples were taken from outer crust parts of these breads with advanced browning reaction and color parameters formed here were analyzed according to Hunter color system. Using obtained *L**, *a**, *b** data, H, C and BI values were calculated. Amount of antioxidant substance formed was determined by 2,2-diphenylpicrilhydrazil (DPPH) and total phenolic substance determination (TPC) methods. The results obtained were evaluated by ANOVA and Pearson Correlation statistical tests.

2. MATERIALS and METHODS

2.1. Materials

In this study, a total of 4 types of bread were used. Breads purchased commercially from Giresun city; grissini bread, rusk, francala bread and Giresun Şebinkarahisar traditional oven-dry bread. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), gallic acid, Folin & Ciocalteu phenol reagent purchased from Sigma-Aldrich. Sodium carbonate and 98% ethyl alcohol were purchased from Merck Company.

2.2. Methods

2.2.1. Extraction of Antioxidant Compounds

In the extraction of antioxidant compounds, firstly, 10 mg bread crusts were crushed in a ceramic mortar and then 20 ml of 60% ethyl alcohol solution was added. It was shaken for 2

hours on a horizontal shaker and filtered through coarse filter paper and extract was made up to 50 ml with distilled water.

2.2.2. Determination of Total Phenolic Substance

300 µL sample extract was placed into test tubes and 400 µL of Folin & Ciocalteu's phenol and 1.5 ml sodium carbonate (20%) were added. Then, in same way, tubes containing 20, 40, 60 and 80 µg gallic acid were prepared, 400 µL of Folin & Ciocalteu's phenol and 1.5 ml of sodium carbonate (20%) were added and 2 minutes later, tubes were completed to 5 ml with distilled water.

The prepared mixtures were kept at room temperature for 45 minutes and measured at 765 nm with Shimadzu UV-1700 Double Beam Scanning UV-Vis brand Spectrophotometer. The total phenolic concentration was calculated from calibration graph created with gallic acid and results were expressed as gallic acid equivalent (GAE) (Bae & Suh, 2007).

2.2.3. DPPH Radical Scavenging Property

415 µL of sample extracts containing antioxidant substance were taken into a test tube and final volume was completed to 1 ml with ethanol. 0.4 ml of 2, 2-diphenylpicrylhydrazil (DPPH) solution at a concentration of 0.004% prepared daily in ethanol was added to samples and shaken in vortex. Then test tubes were incubated for 30 minutes in a dark environment and readings were made at 517 nm wavelength in the spectrophotometer. The radical scavenging property of each sample was calculated using following equation and the %inhibition (1) corresponding to sample amounts was determined (Brand-Williams et al., 1995).

% Inhibition = (A control-A example) / Acontrol (1)

A Control: Absorbance of DPPH solution and sample containing ethanol

A Example: Absorbance of DPPH solution and sample containing sample

2.2.4. Color Parameters

Color measurement of bread crusts were done with a Hunter colorimeter (3NH Technology Co. Ltd. NR10QC Colorimeter). L^* (brightness), a^* (greenness / redness), and b^* (blueness / yellowness) values were determined in each sample. From L^*, a^* and b^* values obtained at result of analysis, hue (H), chroma (C) and browning index (BI) values were calculated with following equations. Hue angle is defined as a color circle and red-purple colors take angle values of 0°-360°. The chroma value indicates saturation of color. Chroma values decrease in dull colors and chroma values increase in vivid colors. BI value increases depending on the

amount of caramelization and MR products. The equations (2), (3), (4) used in obtaining C, H and BI values are given below (Askari et al., 2008; Mutlu & Ergüneş, 2008).

$$Chroma = \sqrt{a^2 + b^2} \quad (2)$$

$$BI = \frac{100X[\frac{(a+1.75XL)}{(5.645XL+a-3.012Xb)} - 0.31]}{0.17} \quad (3)$$

$$H = \arctan^{b/a} \quad (4)$$

2.2.5. Statistical Analysis

Statistical analyses were performed with SPSS (version 25 for Windows, SPSS Inc.) software. Number, arithmetic mean and standard deviation were evaluated using one-way analysis of variance (ANOVA). Tukey test, one of the Post Hoc Tests, was used to determine between which groups the difference was and relationship between DPPH, TFM and Hue was compared with the Pearson Correlation test. The significance level of analyzes is 0.05 (p-value). All data were presented as mean ± standart deviation.

3. RESULTS and DISCUSSION

In the study, H, C and BI values were calculated by considering the *L**, *a**, *b** values of the crust samples taken from bread types also antioxidant amounts and TPC values were measured Average Hue value for oven-dry bread type bread calculated with *L**, *a** and *b** values in the samples taken from outer crust parts of bread types was 64.94±2.55; it is 57.69±1.53 for bakery bread and BI value was the lowest with 105.32±22.23 for oven-dry bread and 71.78±19.79 for grissini bread (Table 1). According to the Hue angle color scale, oven-dry bread, rusk and grissini bread are light yellow but francala bread reddening was observed (Figure 1). Francala bread crust %DPPH value is 45.62±0.43 and oven dry bread is 12.59±8.14, and chroma values are close to each other for all bread types.

According to ANOVA analysis, there was no statistically significant difference between BI and C, but there was a significant difference in Hue (°) due to 1>4 (1 different 4) and 3>4 (3 different 4) (Table 3). The comparison of the phenolic/antioxidant activity properties of bread types with the Pearson Correlation Test is given in Table 4. While there is a strong and significant positive correlation (r=0.895, p<0.01) between DPPH and TPC values, there is a negative strong and significant (r=-0.804, r=-0.875.p) relationship between Hue and DPPH and TPC values. <0.01) relationship was detected (Table 4).

Table 1. Color parameters in bread types.

Bread Types		*L**	*a**	*b**	Hue (°)	Chroma	Browning Index
	N	X̄±ss	X̄±ss	X̄±ss	X̄±ss	X̄±ss	X̄±ss
Oven-dry Bread	3	53,30±6,78	14,50±1,81	30,94±0,90	64,94±2,55	34,19±1,33	105,32±22,23
Rusk	3	60,76±2,66	16,06±0,66	28,82±1,01	60,87±0,17	33,00±1,21	92,25±5,87
Grissini Bread	3	66,91±6,94	14,43±2,00	28,34±2,85	63,07±0,89	31,81±3,44	71,78±19,80
Francala Bread	3	49,02±8,84	16,17±0,65	25,63±2,24	57,69±1,53	30,31±2,19	98,01±17,17
Total	12	57,51±9,16	15,29±1,49	28,44±2,57	61,64±3,11	32,33±2,42	89,34±20,22

X̄: mean, ss: standard deviation

Table 2. DPPH activity and total phenolic content in bread types.

Bread Types	N	% DPPH	Total Phenolic Content (mg GAE/100g)
Oven-dry Bread	3	12,59±8,14	54,49±16,76
Rusk	3	21,57±14,84	91,30±21,83
Grissini Bread	3	17,30±0,53	85,60±16,39
Francala Bread	3	45,62±0,43	122,76±2,62
Total	12	24,27±15,13	88,64±28,77

X̄: mean, ss: standard deviation

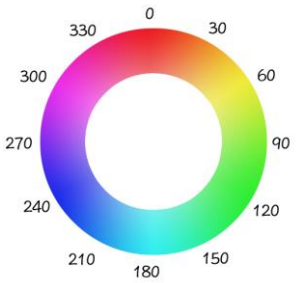

Figure 1. Hue (°) angle color scale (Anonymous. 2000)

Table 3. Hue (°), Chroma and BI mean values One Way ANOVA analyses.

	Groups	**N**	**X̄±ss**	**F**	**p**
H (°)	Oven-Dry Bread(1)	3	64,90±2,55[a]	12,020	**0,002**
	Rusk (2)	3	60,87±0,16[a]		
	Grissini Bread(3)	3	63,07±0,89[bc]		
	Francala Bread(4)	3	57,70±1,53[ac]		
	Total	12	61,64±3,11		
C	Oven-Dry Bread(1)	3	34,19±1,32[a]	1,655	0,253
	Rusk (2)	3	33,00±1,21[a]		
	Grissini Bread(3)	3	31,81±3,44[a]		
	Francala Bread(4)	3	30,31±2,19[a]		
	Total	12	32,33±2,42		
BI	Oven-Dry Bread(1)	3	105,32±22,23[a]	2,268	0,158
	Rusk (2)	3	82,25±5,87[a]		
	Grissini Bread(3)	3	71,78±19,79[a]		
	Francala Bread(4)	3	98,01±17,16[a]		
	Total	12	89,34±20,22		

N: Number of samples, X̄: mean, ss: standard deviation, [a, b, c,] Values with the same superscript letters in the same line are non-significant at $p < 0.05$.

Table 4. TPC and DPPH Pearson Correlation Test.

		% DPPH	TPC	Hue (°)
% DPPH	r	1	0,895**	-0,804**
	p		0,000	0,000
TPC	r		1	-0,875**
	p			0,000
H (°)	r			1
	p			

*p<0,01

4. CONCLUSION

The bread making process generally has a positive effect on phenolic profile and antioxidant activities of whole wheat products (Tian et al., 2021). In the study, in the samples taken from the crust parts of traditional and industrial bread varieties that were heat-treated at 175-250°C, BI value of oven-dry bread produced by traditional method was 105.32±22.23 and H was 64.94°±2.55 %DPPH radical scavenging value in bread made from wheat flour is 48.3±0.02 (Msaddak et al., 2017). The TPC value of loaf bread known as francala bread is 579.27° ± 3.93 (Tian et al., 2021). TPC values were 122.76±2.62 mgGAE/100g and antioxidant activity %DPPH 45.62±0.43 in francala bread and this shows that TPC value is lower in shell part and antioxidant activity is approximately same. In the oven-dry bread produced by traditional method TPC value is 54.49±16.76 and DPPH 12.59±8.14%. According to the results of the statistical analysis, there is a strong positive correlation between the phenolic/antioxidant activity values of the bread types examined and significant relationship between Hue value and DPPH and TPC values, there was a strong and significant negative relationship.

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