

## Bölüm 6

# HPLC İLE ANTIOKSİDAN AKTİVİTE TAYİN YÖNTEMLERİ

Nesibe ARSLAN BURNAZ<sup>1</sup>

### GİRİŞ

Serbest radikaller, dış orbitallerinde bir veya daha fazla eşlenmemiş elektrona sahip oldukları için kararsız atomlar veya moleküllerdir. Bu nedenle, kararlı hale gelmek için diğer moleküllerle reaksiyona girme eğilimindedirler. Serbest radikallerin büyük bir kısmı oksijen kaynaklıdır <sup>(1,2)</sup>. Aerobik organizmalar için hayati öneme sahip olan oksijen, kontrollü koşullarda genellikle metabolik bir amaca hizmet ederken, bazen normal şartlar altında elektron taşıma zincirinden sızar ve moleküler oksijen ile birleşerek reaktif oksijen türlerini (ROT) oluşturur <sup>(3-6)</sup>. ROT yüksek derecede reaktif moleküller olup lipitler, proteinler veya DNA gibi hücrel moleküller ile reaksiyona girebilir ve onlara zarar verebilir <sup>(7,8)</sup>. Biyolojik olarak önemli olan ROT'lar, süperoksit anyonu ( $O_2^{\cdot-}$ ), tekli oksijen ( $^1O_2$ ), hidrojen peroksit ( $H_2O_2$ ), hidroksil radikali ( $\cdot OH$ ), hipokloröz asit ( $HOCl$ ), peroksinitrit ( $ONOO^-$ ) radikallerini içerir <sup>(8-11)</sup>. ROT'lar, hücrede çeşitli bölgelerde gerçekleşen biyokimyasal reaksiyonları etkileyerek hücrel hasara neden olabilir <sup>(7,12)</sup>. Bununla birlikte, çeşitli hastalıklar, alınan ilaçlar, hava kirliliği, radyasyon ve yetersiz beslenme gibi sayısız faktörler ROS üretimini olumsuz yönde etkileyerek oksidatif strese yol açabilir <sup>(1,13-15)</sup>. Oksidatif stres “oksidan oluşumu ile antioksidan onarım kapasitesi arasındaki dengesizlik” olarak tanımlanabilir <sup>(5)</sup>. Oksidatif stres kanser, ateroskleroz, Alzheimer, Parkinson, astım, romatoid artrit, inflamasyon gibi çeşitli hastalıklara yol açan ciddi hücre hasarına neden olur <sup>(1,16-19)</sup>. Ayrıca, ROT nedeniyle oluşan hasar hücrede yaşlanma sürecini de hızlandırmaktadır. Bu süreci yavaşlatmak, oksidasyonu önlemek ve olumsuz etkilerinden korunmak için hücre antioksidan olarak adlandırılan molekülleri kullanır <sup>(20-21)</sup>. Doğal antioksidanların önemi temel olarak sağlığı geliştirici özellikleri ile ilgilidir. Antioksidanlar, serbest radikallerin saldırısına bağlı patolojilere karşı organizmanın savunma mekanizmalarından sorumludur. Bu nedenle, antioksidanların alımı, kanser, Parkinson, Alzheimer, ateroskleroz gibi oksidatif stresin neden olduğu dejeneratif hastalıkların önlenmesinde rol oynar <sup>(19,22-24)</sup>.

<sup>1</sup> Dr. Öğretim Üyesi, Gümüşhane Üniversitesi, nsbburnaz@gmail.com

## KAYNAKLAR

1. Phaniendra A, Jestadi DB & Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. *Indian Journal of Clinical Biochemistry*. 2015;30(1):11-26.
2. Shalaby EA & Shanab SMM. Antioxidant compounds, assays of determination and mode of action. *African Journal of Pharmacy and Pharmacology*. 2013;7(10):528-539.
3. Cadenas E & Davies KJA. Mitochondrial free radical generation, oxidative stress, and aging. *Free Radical Biology and Medicine*. 2000;29(3-4):222-230.
4. Buonocore G, Perrone S & Tataranno M. Oxygen toxicity: chemistry and biology of reactive oxygen species. *Seminars in Fetal and Neonatal Medicine*. 2010;15.(4):186-190.
5. Halliwell B & Gutteridge JM. (2015). *Free radicals in biology and medicine*, (Fifth edit.). USA: Oxford University Press.
6. Krumova K & Cosa G. Overview of reactive oxygen species. 2016;1:1-21.
7. Wu D & Cederbaum AI. Alcohol, oxidative stress, and free radical damage. *Alcohol Research & Health*. 2003;27(4):277-284.
8. Valko M, Leibfritz, D, Moncol, J, et al. Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology*. 2007;39(1):44-84.
9. Böhm F, Edge R, Burke M, et al. Dietary uptake of lycopene protects human cells from singlet oxygen and nitrogen dioxide-ROS components from cigarette smoke. *Journal of Photochemistry and Photobiology B: Biology*. 2001;64(2-3):176-178.
10. MacDonald-Wicks LK, Wood LG & Garg ML. Methodology for the determination of biological antioxidant capacity in vitro: a review. *Journal of the Science of Food and Agriculture*. 2006;86(13):2046-2056.
11. Niederländer HA, Van-Beek TA, Bartasiute A, et al. Antioxidant activity assays on-line with liquid chromatography. *Journal of Chromatography A*. 2008;1210(2):121-134.
12. Zorov DB, Juhaszova M, & Sollott SJ. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiological reviews*. 2014; 94(3):909-950.
13. Sies H. Oxidative stress: oxidants and antioxidants. *Experimental Physiology: Translation and Integration*. 1997;82(2):291-295.
14. Devasagayam TPA, Tilak JC, Bloor KK, et al. Free radicals and antioxidants in human health: current status and future prospects. *Japi*. 2004;52(4):794-804
15. Numan MS, Jacques PB & Laëtitia M. Impact of air pollutants on oxidative stress in common autophagy-mediated aging diseases. *International Journal of Environmental Research and Public Health*. 2015;12(2):2289-2305.
16. Pham-Huy LA, He H, & Pham-Huy, C. Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science: IJBS*. 2008;4(2):89-96.
17. Badarinath AV, Rao KM, Chetty CMS, et al. A review on in-vitro antioxidant methods: comparisons, correlations and considerations. *International Journal of PharmTech Research*. 2010;2(2):1276-1285.
18. Rahman T, Hosen I, Islam MT, et al. Oxidative stress and human health. 2012;3(7):997-1019.
19. Moharram HA & Youssef MM. Methods for determining the antioxidant activity: a review. *Alex. J. Fd. Sci. & Technol*. 2014;11(1):31-42.
20. Oliveira, Barbara F, José Augusto Nogueira-Machado, and Miriam M. Chaves. "The role of oxidative stress in the aging process." *The Scientific World Journal* 10 (2010): 1121-1128.
21. Lobo V, Patil A, Phatak A, et al. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy reviews*. 2010;4(8):118-126.

22. Langseth L. (1995). *Oxidants, antioxidants, and disease prevention*. Brussels: ILSI Europe.
23. Oberley TD. Oxidative damage and cancer. *The American Journal of Pathology*. 2002;160(2):403-408.
24. Doughari JH. (2012). *Phytochemicals: extraction methods, basic structures and mode of action as potential chemotherapeutic agents*. Rijeka, Croatia: INTECH Open Access Publisher.
25. Seifried HE, Anderson DE, Fisher EI, et al. A review of the interaction among dietary antioxidants and reactive oxygen species. *The Journal of Nutritional Biochemistry*. 2007;18(9):567-579.
26. Hamid AA, Aiyelaagbe OO, Usman LA, et al. Antioxidants: Its medicinal and pharmacological applications. *African Journal of Pure and Applied Chemistry*. 2010;4(8):142-151.
27. Rajagopal PL, Sreejith KR, & Premaletha K. Natural colorants as safe additives: A review. *Worldwide J. Multidisciplinary Res. Develop*. 2016;2(7):28-32.
28. Ghatak PD & Chandan KS. Antioxidant Additives in Food Preservation and Human Health. *Food Toxicology*. 2016: 377-390.
29. Goulas V, Gomez-Caravaca AM, Exarchou V, et al. Exploring the antioxidant potential of Teucrium polium extracts by HPLC-SPE-NMR and on-line radical-scavenging activity detection. *LWT-Food Science and Technology*. 2012;46(1):104-109.
30. Rehecho S, Hidalgo O, de-Cirano MGI, et al. Chemical composition, mineral content and antioxidant activity of Verbena officinalis L. *LWT-Food Science and Technology*. 2011;44(4):875-882.
31. Koleva II, Niederländer HAG & van-BEEK TA. An on-line HPLC method for detection of radical scavenging compounds in complex mixtures. *Analytical Chemistry*. 2000;72(10):2323-2328.
32. Koleva, II & Niederländer HAA. Application of ABTS radical cation for selective on-line detection of radical scavengers in HPLC eluates. *Analytical Chemistry*. 2001;73(14):3373-3381.
33. Dapkevicius A, van-BEEK TA & Niederländer HAG. Evaluation and comparison of two improved techniques for the on-line detection of antioxidants in liquid chromatography eluates. *Journal of Chromatography A*. 2001;912(1):73-82.
34. Shi SY, Zhang YP, Jiang XY, et al. Coupling HPLC to on-line, post-column (bio) chemical assays for high-resolution screening of bioactive compounds from complex mixtures. *TrAC Trends in Analytical Chemistry*. 2009;28(7):865-877.
35. Raudonis R, Raudone L, Jakstas V, et al. Comparative evaluation of post-column free radical scavenging and ferric reducing antioxidant power assays for screening of antioxidants in strawberries. *Journal of Chromatography A*. 2012;1233:8-15.
36. Koşar M, Dorman HD, Başer KHC, et al. Screening of free radical scavenging compounds in water extracts of Mentha samples using a postcolumn derivatization method. *Journal of Agricultural and Food Chemistry*. 2004;52(16):5004-5010.
37. Shui G & Leong LP. Screening and identification of antioxidants in biological samples using high-performance liquid chromatography– mass spectrometry and its application on *Salacca edulis* Reinw. *Journal of Agricultural and Food Chemistry*. 2005;53(4):880-886.
38. Raudonis R, Jakštas V, Burdulis D, et al. Investigation of contribution of individual constituents to antioxidant activity in herbal drugs using postcolumn HPLC method. *Medicina*. 2009;45(5):382-394.
39. Çelik SE, Özyürek M, Güçlü K, et al. Determination of antioxidants by a novel on-line HPLC-cupric reducing antioxidant capacity (CUPRAC) assay with post-column detection. *Analytica Chimica Acta*. 2010;674(1):79-88.
40. Brand-Williams W, Marie-Elisabeth C & Berset CLWT. Use of a free radical method to evaluate

- antioxidant activity. *LWT-Food Science and Technology*. 1995;28(1):25-30.
41. Anagnostopoulou MA, Kefalas P, Papageorgiou VP, et al. Radical scavenging activity of various extracts and fractions of sweet orange peel (*Citrus sinensis*). *Food Chemistry*. 2006;94(1):19-25.
  42. Kedare SB & Singh R.P. Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*. 2011;48(4):412-422.
  43. Bondet V, Brand-Williams W & Berset CLWT. Kinetics and mechanisms of antioxidant activity using the DPPH free radical method." *Food Science and Technology-Zurich*. 1997;30:609-615.
  44. Xie J & Schaich KM. Re-evaluation of the 2, 2-diphenyl-1-picrylhydrazyl free radical (DPPH) assay for antioxidant activity. *Journal of Agricultural and Food Chemistry*. 2014;62(19):4251-4260.
  45. Koşar M, Dorman D, Başer KHC, et al. An improved HPLC post-column methodology for the identification of free radical scavenging phytochemicals in complex mixtures. *Chromatographia*. 2004;60(11-12):635-638.
  46. Zhang Y, Li Q, Xing H, et al. Evaluation of antioxidant activity of ten compounds in different tea samples by means of an on-line HPLC-DPPH assay. *Food Research International*. 2013;53(2):847-856.
  47. Miller NJ, Rice-Evans C, Davies MJ, et al. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical Science*. 1993;84(4):407-412.
  48. Re R, Pellegrini N, Proteggente A, et al. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*. 1999;26(9-10):1231-1237.
  49. Prior RL & Cao G. In vivo total antioxidant capacity: comparison of different analytical methods1. *Free Radical Biology and Medicine*. 1999;27(11-12):1173-1181.
  50. Chanda S, & Dave R. In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. *African Journal of Microbiology Research*. 2009;3(13):981-996.
  51. Pellegrini N, Del-Rio D, Colombi B, et al. Application of the 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation assay to a flow injection system for the evaluation of antioxidant activity of some pure compounds and beverages. *Journal of Agricultural and Food Chemistry*. 2003;51(1):260-264.
  52. Raudonis R, Bumblauskiene L, Jakstas V, et al. Optimization and validation of post-column assay for screening of radical scavengers in herbal raw materials and herbal preparations. *Journal of Chromatography A*. 2010;1217(49):7690-7698.
  53. Karaçelik, AA, Küçük M., Iskefiyeli Z, et al. Antioxidant components of *Viburnum opulus L.* determined by on-line HPLC-UV-ABTS radical scavenging and LC-UV-ESI-MS methods. *Food Chemistry*. 2015;175:106-114.
  54. Benzie IFF & Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*. 1996;239(1):70-76.
  55. Albayrak S, Sağdıç O & Aksoy A. Bitkisel ürünlerin ve gıdaların antioksidan kapasitelerinin belirlenmesinde kullanılan yöntemler. *Erciyes Üniversitesi Fen Bilimleri Enstitüsü Fen Bilimleri Dergisi*. 2010;26(4): 401-409.
  56. Okan OT, Varlıbaş H, Öz M, et al. Antioksidan analiz yöntemleri ve Doğu Karadeniz Bölgesinde antioksidan kaynağı olarak kullanılabilir odun dışı bazı bitkisel ürünler. *Kastamonu Üniversitesi Orman Fakültesi Dergisi*. 2013;13(1):48-59.
  57. Huang D, Ou B & Prior RL. The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*. 2005;53(6):1841-1856.
  58. Burnaz NA, Küçük M & Akar Z. An on-line HPLC system for detection of antioxidant com-

pounds in some plant extracts by comparing three different methods.” *Journal of Chromatography B*. 2017;1052:66-72.

59. Shi S, Guo K, Tong R, et al. Online extraction–HPLC–FRAP system for direct identification of antioxidants from solid Du-zhong brick tea. *Food Chemistry*. 2019;288:215-220.
60. Apak R, Güçlü K, Özyürek M, et al. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *Journal of Agricultural and Food Chemistry*. 2004;52(26):7970-7981.
61. Apak R, Güçlü K, Özyürek M, et al. Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay. *Microchimica Acta*. 2008;160(4):413-419.
62. Özyürek M, Güçlü K & Apak R. The main and modified CUPRAC methods of antioxidant measurement. *TrAC Trends in Analytical Chemistry*. 2011;30(4):652-664.
63. Olgun FA, Ozturk BD & Apak R. Determination of Synthetic Food Colorants in Powder Beverage Samples by On-line HPLC–Cupric Reducing Antioxidant Capacity (CUPRAC) Assay with Post-Column Detection. *Chromatographia*. 2016;79(3-4):199-208.