Approach to Determine Agricultural Products' Redox Bioactivity

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Food products contain hundreds of chemical constituents in varying concentrations and the comprehensive analysis of their bioactivity can reveal unique distinctive patterns. The screening of the biological properties of a wide variety of both lipophilic and hydrophilic antioxidant substances using a rapid and low-cost methodology is a critical step toward establishing the classification of the endogenous bioactivity of foodstuffs.

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1. Introduction

The term “stress” was firstly introduced in biological sciences as a non-specific response of an organism against several exogenous factors. In 1956, Hans Selye was the first scientist to refer to this term in the context of human physiology, describing generic signs and symptoms that are responsible for several pathologies and illnesses. It was only in 1970 when the term “oxidative stress” was mentioned by Paniker [1], indicating the detrimental effects of oxidizing agents, such as hydrogen peroxide (H_2O_2). Over the years, this concept has been redefined, and nowadays, oxidative stress is described as “an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage” [2]. The inability of an organism to regulate free radicals is a critical step toward the induction of oxidative stress, which is counterbalanced by the activities of endogenous and exogenous antioxidants. Therefore, the protective role of antioxidants against oxidative stress has been widely investigated. Furthermore, antioxidants have been proposed as additives in the food industry to delay, retard or prevent the development of rancidity or other flavor deterioration in food products due to oxidation. Antioxidants exert their protective effects by scavenging free radicals, chelating metal ions, converting hydroperoxides to non-radical species, absorbing UV radiation or deactivating singlet oxygen [3]. Their mechanism of action depends mainly on the chemical structure of the antioxidant.

Regardless of the mechanism of action, it was supposed that antioxidants “cure” oxidative stress, caused by the overproduction of free radicals or the inability of the endogenous antioxidant mechanisms to effectively neutralize them. Nevertheless, the use of antioxidants in large-scale clinical trials have shown no significant beneficial results. Therefore, “cure” was not suitable to describe their role in oxidative stress, which eventually occurs due to an alteration of the thiol redox state, leading to a disruption of cellular signaling and of physiological function.

Based on the alterations of thiol redox circuits and the up- or down-regulation of reactive oxygen species (ROS), the scientific literature has distinguished oxidative stress in “oxidative eustress” and “oxidative distress” [4][5]. The first is responsible for providing beneficial signal transduction and regulating several biochemical reactions due to low ROS levels, whereas the latter is responsible for disrupting redox signaling and causing molecular damage due to excessive ROS levels. It has to be noted that low oxidative events do not always participate in physiologically positive actions, while high oxidative events do not always exert negative actions, indicating that the complex and fine regulation of ROS generation is not easily discrete. An example of the difficulty to define high and low amounts of oxidants and their relative functions is preconditioning, a mechanism by which small amounts of an oxidant provided to cells at different times and in different amounts result in a resistance to the damage caused by high amounts of oxidants. All of these occurrences, described as stress, could be topographically distinct, since the regulation or damage could be restricted to a specific site, while also occurring at the same time in different sites.

2. The Importance of Quality Assessment in Food Industry

The determination of food authenticity and quality includes the identification of mislabeled products that do not meet the requirements to be characterized as bio-functional [6]. Due to the high production costs in the food industry, in many cases, high-quality foods/ingredients are replaced by similar less expensive kinds, even those of dubious quality [7]. The adoption of undeclared procedures, the food adulteration, and the incorrect declaration of the origin of raw materials or of the production method are some of the common practices for downgrading production costs, also leading to lower food quality [7].

Nowadays, the food quality assessment attracts considerable interest as consumers come into contact with a wide variety of food products on a daily basis, and among the main selection criteria is food certification. Since globalization has permitted the unlimited facile trade of an increasing number of food products, their traceability and certification of quality has become one of the cornerstones of the European Union (EU)’s food safety policy [8]. Therefore, there is a strong trend for the development of tools that will enable the food industry to satisfy the underlining consumer need to be ensured that their food products are of high quality and that they exert beneficial effects to human health based on their bioactivity [7]. Many thinkers of the 21st century consider that consumers can be the critical “revolutionary mass” that will attempt the next historical socio-economic revolution, changing the current structure of the food production model, but mainly restructuring the existing model of food labeling and the health-translational potency of these labels.

For food products protected by geographical indications and traditional specialties and, to be more specific, the Protected Geographical Indications (PGI), Protected Designation of Origin (PDO), and Traditional Specialties Guarantee (TSG), the EU Regulations EC N. 510/2006 [9] and 1151/2012 [10] require several protection measures against mislabeling. Thereupon, Regulation N. 668/2014 implements specific rules for the application of Regulation N. 1151/2012 of the European Parliament and of the Council on quality schemes for agricultural products and foodstuffs [11]. Except for the geographical indications, the EU Commission has established rules, principles, and requirements that farmers need to comply with in order to be certified for organic farming [12]. Depending on each separate Member State, the authorities in charge of the control system for organic production may confer their

control competences to one or more public control authorities or delegate control tasks to one or more private control bodies. The Commission must be aware of the list of designated control authorities and approved control bodies in each Member State [13]. Furthermore, all food producers, processors or traders who wish to market their food as organic need to be aware of the registration process with the control agency or body. Yearly inspections and the checking of complying with the rules of organic production are well operated by the control agencies or bodies of each Member State. Although in most cases, the incorporated traceability systems guarantee the geographical origin of food products [9], and also, conforming with the organic farming rules is a well-described requirement by the EU Commission, concerning their retail market, the food industry urgently needs screening methods for the unlabeled foods to provide proofs of their quality in order to educate and inform consumers whether a food product is “good” or “bad” for their health. This “gap” must be filled using certain protocol schemes that shall examine endogenous bioactive properties of the final food products with a homogenized system.

Geographical, climatic, pedological, geological, botanical, and agricultural parameters affect the ratios and patterns of bio-elements in nature, and these variations are incorporated into the plant or animal tissues throughout the food chain and through direct contact with the natural environment. Food products contain hundreds of chemical constituents in varying concentrations and the comprehensive analysis of their bioactivity can reveal unique distinctive patterns. The screening of the biological properties of a wide variety of both lipophilic and hydrophilic antioxidant substances using a rapid and low-cost methodology is a critical step toward establishing the classification of the endogenous bioactivity of foodstuffs.

3. Characterization of the Food Product Quality on the Basis of the Antioxidant Profile

Dietary antioxidants, present in several food products, possess the ability to neutralize the excess of free radicals, which are produced as by-products of normal cell metabolism [14]. Therefore, the consumption of food products that are rich in bioactive compounds with antioxidant properties might protect against the onset and the progression of pathological conditions associated with disturbances of redox homeostasis [14]. In addition to the benefits for human health, the antioxidant profile affects the shelf life and the flavor stability of a food product and protects its ingredients from oxidations, which could lead to quality degradation.

An important issue in the global food industry is the inability of the consumers to recognize food products that are not only a source of primary nourishment, but are also capable of exerting beneficial health effects [15]. Therefore, it is critical to develop a specific scheme of laboratory analyses that could examine the food product quality based on their antioxidant properties and categorize them according to their bioactivity [16].

Up to the present time, several antioxidant assays have been introduced to investigate the antioxidant properties of conventional antioxidants, foodstuffs, dietary supplements, and biological samples (**Table 1**). In the table below, natural products, such as wines [17], berries [18], honey [19], herbs [20], grape seed extracts [21], and plant extracts [22], have been examined using experimental protocols that investigate their endogenous biological properties, such as antiradical potency, as well as their reducing capacity and DNA protective activity in relation with their

antioxidant power. In order to characterize the bioactivity of a food product, in terms of its antioxidant potency, the first and crucial step is the adoption of a battery of reliable and valid antioxidant markers.

Table 1. Summary table demonstrating the antioxidant properties of various natural products evaluated using in vitro cell-free screening techniques. All results in [17][18][21][22] are expressed as mean ± standard deviation (SD). All results in [19][20] are expressed as mean ± standard error of the mean (SEM). IC_{50} (Half maximal inhibitory concentration): The concentration of the sample required for the inhibition of the 50% of the corresponding free radicals. $AU_{0.5}$ (Absorbance unit 0.5): The concentration of the tested sample required for the achievement of an absorbance value of 0.5. * Refers to the concentration of each sample that has the ability to scavenge 20% of the free radical (IC_{20}).

Samples	Antioxidant Assays						References
	$ABTS^{\bullet+}$ Scavenging Assay	**$DPPH^{\bullet}$ Scavenging Assay**	**$O_2^{\bullet-}$ Scavenging Assay**	**$OH^{\bullet}$ Scavenging Assay**	**Reducing Power Assay**	**Plasmid DNA Relaxation Assay**	
Wine extracts	IC_{50} (μg/mL)	IC_{50} (μg/mL)	IC_{50} (μg/mL)	IC_{50} (μg/mL)	$AU_{0.5}$ (μg/mL)	IC_{50} or IC_{20} * (μg/mL)	
Xinomavro	7.3 ± 0.19	13.4 ± 0.42	34.5 ± 3.17	304.8 ± 29.57	4.9 ± 0.07	260.5 ± 27.4	[17]
Agiorgitiko	8.2 ± 0.04	14.5 ± 0.62	32.0 ± 0.37	491.2 ± 30	8.3 ± 0.59	116.1 ± 19.4	
Assyrtiko	18.4 ± 1.05	28.4 ± 2.27	73.9 ± 0.75	165.7 ± 13.03	13.0 ± 0.21	220.3 ± 14.1 *	
Malagouzia	43.5 ± 1.33	89.4 ± 4.14	268.5 ± 33.62	409.1 ± 19.03	48.1 ± 0.66	150.1 ± 15.0 *	
Honey	IC_{50} (mg/mL)	IC_{50} (mg/mL)	IC_{50} (mg/mL)	IC_{50} (mg/mL)	$AU_{0.5}$ (mg/mL)	IC_{50} (mg/mL)	
Oak	2.96 ± 0.81	7.14 ± 0.02	1.98 ± 0.04	1.22 ± 0.04	1.87 ± 0.19	2.98 ± 0.11	[19]
Eryngium creticum	4.03 ± 0.08	9.95 ± 0.025	7.48 ± 0.37	1.04 ± 0.06	3.60 ± 0.3	6.04 ± 0.19	
Fir and vanilla	1.03 ± 0.01	6.51 ± 0.32	1.01 ± 0.01	1.05 ± 0.06	2.41 ± 0.01	1.60 ± 0.17	
Forest with oak honeydew	0.90 ± 0.01	4.61 ± 0.29	1.24 ± 0.01	1.24 ± 0.02	1.79 ± 0.06	1.55 ± 0.15	

Samples	Antioxidant Assays						References
	$ABTS^{\bullet+}$ Scavenging Assay	**$DPPH^{\bullet}$ Scavenging Assay**	**$O_2^{\bullet-}$ Scavenging Assay**	**$OH^{\bullet}$ Scavenging Assay**	**Reducing Power Assay**	**Plasmid DNA Relaxation Assay**	
Flower (1)	1.99 ± 0.1	15.04 ± 0.3	4.32 ± 0.14	0.68 ± 0.01	3.71 ± 0.25	9.02 ± 0.41	
Flower (2)	1.45 ± 0.02	8.47 ± 0.69	2.63 ± 0.02	0.66 ± 0.01	2.28 ± 0.01	6.86 ± 0.68	
Herb extracts	IC_{50} (µg/mL)	IC_{50} (µg/mL)	IC_{50} (µg/mL)	IC_{50} (µg/mL)	$AU_{0.5}$ (µg/mL)	IC_{50} (µg/mL)	
Origanum vulgare	7.85 ± 0.56	6.60 ± 1.50	12 ± 1.07	-	7.5 ± 0.41	35 ± 3.06	
Salvia officinalis	19.07 ± 0.09	26.68 ± 1.22	6.5 ± 0.25	-	8 ± 0.35	54 ± 4.51	[20]
Aloysia citrodora	8.29 ± 1.13	10.25 ± 0.25	49 ± 2.39	-	3.5 ± 0.13	26 ± 2.14	
Rosmarinus officinalis	12.27 ± 0.38	11.63 ± 4.32	14.5 ± 1.09	-	7.5 ± 0.48	25 ± 1.27	
Fruit extracts	IC_{50} (mg/mL)	IC_{50} (mg/mL)	IC_{50} (mg/mL)	IC_{50} (mg/mL)	$AU_{0.5}$ (mg/mL)	IC_{50} (mg/mL)	
Lycium barbarum	0.67 ± 0.01	2.33 ± 0.03	-	-	-	1.80 ± 0.05	[18]
Grape seed extracts	IC_{50} (µg/mL)	IC_{50} (µg/mL)	IC_{50} (µg/mL)	IC_{50} (µg/mL)	$AU_{0.5}$ (µg/mL)	IC_{50} (µg/mL)	
Mavrotragano	5.0 ± 0.4	3.5 ± 0.3	-	400 ± 55	-	0.65 ± 0.07	
Voidomato	7 ± 0.9	3.5 ± 0.5	-	200 ± 24	-	1 ± 0.08	[21]
Moshato	8 ± 0.7	3.5 ± 0.5	-	400 ± 39	-	1 ± 0.1	
Vinsanto	10 ± 1.1	4 ± 0.2	-	300 ± 38	-	0.95 ± 0.08	
Athiri	15.0 ± 1.6	5.0 ± 0.4	-	310 ± 25	-	1.05 ± 0.07	
Mandilaria	9.0 ± 1.0	9.0 ± 0.9	-	390 ± 35	-	1.05± 0.12	

Samples	Antioxidant Assays						References
	$ABTS^{\bullet+}$ Scavenging Assay	$DPPH^{\bullet}$ Scavenging Assay	$O_2^{\bullet-}$ Scavenging Assay	$OH^{\bullet}$ Scavenging Assay	Reducing Power Assay	Plasmid DNA Relaxation Assay	
Plant extracts	IC_{50} (µg/mL)	IC_{50} (µg/mL)	IC_{50} (µg/mL)	IC_{50} (µg/mL)	$AU_{0.5}$ (µg/mL)	IC_{50} (µg/mL)	
Mentha microphylla	29 ± 1.2	15 ± 0.6	-	240 ± 43	-	0.5 ± 0.09	[22]
Mentha longifolia	28 ± 1.0	36 ± 1.2	-	325 ± 24	-	1.55 ± 0.18	
Sideritis raeseri ssp. raeseri	31 ± 0.6	38 ± 1.5	-	>800	-	2.20 ± 0.06	
Salvia pomiferassp. calycina	19 ± 1.0	19 ± 0.6	-	170 ± 13	-	1.25 ± 0.10	
Salvia fruticosa	16 ± 0.5	29 ± 0.6	-	350 ± 14	-	0.95 ± 0.08	
Salvia sclarea	25 ± 0.5	20 ± 1.2	-	210 ± 4	-	1.10 ± 0.18	
Salvia officinallis	21 ± 1.0	17 ± 0.6	-	300 ± 14	-	0.90 ± 0.07	

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