Drosophila as an emerging model organism for studies of food-derived antioxidants

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Abbreviations: AChE, acetylcholinesterase; AD, Alzheimer’s disease; ARE, antioxidant response element; CAT, catalase; Cnc, cap’n’collar; CncC, cap’n’collar isoform-C; CR, calorie restriction; dSir2, dSir2, Drosophila silent information regulator 2; EGFR, epidermal growth-factor receptor; GCLC, glutamate-cysteine ligase catalytic subunit; GPx, glutathione peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione-S-transferase; HO, heme oxygenase; HP, hydroperoxide; JNK, c-Jun N-terminal kinase; Keap1, Kelch-like ECH-associated protein 1; LPO, lipid peroxide; Maf, musculoaponeurotic fibrosarcoma; MAPK, mitogen-activation protein kinase; MDA, malondialdehyde; Mn, manganese; MMS, methyl methanesulphonate; Mth, methuselah; NF-\kappaB, nuclear factor-\kappaB; NQO1, NADPH:quinone oxidoreductase 1; NO, nitric oxide; Nrf2, nuclear factor erythroid 2-related factor 2; PC, protein carbonyl; PD, Parkinson’s disease; ROS, reactive oxygen species; RNS, reactive nitrogen species; RS, reactive species; RSS, reactive sulfur species; SOD, superoxide dismutase; T-AOC, total antioxidant capacity; TCA, tricarboxylic acid; TRR, thioredoxin reductase; TSH, total thiols.
Abstract: Dietary supplementation with antioxidants provides health benefits by preventing diseases caused by oxidative stress and damage. Consequently, there has been growing interest in the study of antioxidative foods and their active ingredients. Oxidative stress and antioxidative responses are mechanistically conserved from *Drosophila* to mammals. Therefore, as a well-established model organism with a short life cycle and advantages of genetic manipulation, the fruit fly has been increasingly employed to assess functions of antioxidants *in vivo*. In this review, the antioxidative defense mechanisms, methods used and assays developed in *Drosophila* to evaluate antioxidant supplementation, are highlighted. The main manifestations of antioxidation include reduction of reactive species, up-regulation of endogenous antioxidants, inhibition on oxidative damage to biomacromolecules, enhanced resistance against oxidative stress and extension of lifespan, which are related to the activations of nuclear factor erythroid 2-related factor 2-antioxidant response element pathway and other adaptive responses. Moreover, the key considerations and future perspectives for the application of *Drosophila* models in the studies of food-derived antioxidants are discussed.

**Keywords:** Fruit fly; Antioxidant; *In vivo* evaluation; Oxidative stress; Dietary supplementation

1. **Introduction**

Redox homeostasis, a delicate balance between production and removal of free radicals, plays critical roles in human health and is constantly regulated by oxidative stress and antioxidative defense systems (Carocho, Morales, & Ferreira, 2018). It is well established that free radicals generated in living organisms serve as essential signaling molecules delivering messages responsible for metabolic health (Ristow & Schmeisser, 2011). However, overproduction of free radicals, known as oxidative stress, can disturb redox homeostasis leading to severe diseases such as
cancer, atherosclerosis and neurological disorders (Carocho et al., 2018; Lobo, Patil, Phatak, & Chandra, 2010). This is frequently caused by presence of pro-oxidant compounds and various risk factors such as smoking, extreme exercise and electromagnetic radiation. Although living organisms possess endogenous systems to prevent radical-induced oxidative damage, they are sometimes insufficient to counteract extensive damage or not functional due to lack of antioxidants (Bayliak, Abrat, Storey, Storey, & Lushchak, 2019; Carocho et al., 2018; Tang et al., 2019). In these situations, dietary supplementation of antioxidants is helpful and effective (Y. Chen et al., 2018). For example, orange, melon, grape, peach, plum, apple and kiwi juices can effectively suppress the generation of free radicals in human plasma within 30 minutes (Ko et al., 2005).

Antioxidants such as tocopherol and ascorbic acid were initially used to protect against food deterioration by inhibiting oxidation processes (Cömert & Gökmen, 2018). Later on, they were found to be beneficial to human health by exerting protective effects against aging, inflammation, infection and many diseases including cancer, cataract, diabetes and neurodegenerative disorders (Cömert & Gökmen, 2018; Neha, Haider, Pathak, & Yar, 2019). Therefore, to improve the quality of life and reduce the cost of health care, considerable efforts have been made to identify dietary antioxidants and characterize their physiological functions. A number of in vitro analytical methods have been developed and widely used to evaluate the antioxidative properties of food-derived antioxidants (Alam, Bristi, & Rafiquzzaman, 2013; Cömert & Gökmen, 2018). Although these methods are relatively easy, simple and cost-effective, they fail to consider the complex biological events in vivo during consumption of antioxidants, including digestion, absorption, distribution and metabolism (Apak, Özyürek, Güçlü, & Çapanoğlu, 2016; Cömert & Gökmen, 2018). Therefore, to make accurate evaluations of food-derived antioxidants, employment of living model organisms is indispensable.

Most of in vivo antioxidant studies have been done using rodent models (Alam et al., 2013), which are costly, time-consuming and limited by ethical issues and availability of tools for genetic
manipulations (Panchal & Tiwari, 2017; Yadav, Srikrishna, & Gupta, 2016). However, these issues are almost negligible for the fruit fly *Drosophila melanogaster*, a well-established genetic model organism which has been widely used to study almost all biological processes (Piper & Partridge, 2018). It has been frequently used for modeling human diseases and as an *in vivo* tool for high-throughput screening of potential drugs and development of disease therapeutics (Staats, Lüersen, Wagner, & Rimbach, 2018; T. T. Su, 2019). The genome sequencing has revealed that 75% of the genes causing diseases in humans have homologs in *Drosophila*. Moreover, many human organs have functional analogous in *Drosophila*, with numerous similarities in digestion, absorption and metabolism (Bayliak et al., 2019; Piper & Partridge, 2018; Staats et al., 2018). Nevertheless, the employments of *Drosophila* in antioxidative investigations, compared to those using mouse models, are much less reported (Fig. 1). To promote the discovery and development of food-derived antioxidants, here, we compare understanding of oxidative stress and antioxidative mechanisms in *Drosophila* with mammals, discuss the emerging employment of *Drosophila* in antioxidant research, and its advantages as well as limitations to consider in practice.

2. Oxidative stress and endogenous antioxidant defense in mammals and *Drosophila*

2.1 Reactive species and oxidative stress

Overproduction of reactive species (RS), mainly reactive oxygen species (ROS) and reactive nitrogen species (RNS), is a sign of oxidative stress (Apak et al., 2016; Del Bo’, Martini, Porrini, Klimis-Zacas, & Riso, 2015). ROS are oxygen radicals, including superoxide anion (O$_2^-$), hydroxyl radical (HO$^-$), lipid radicals (ROO$^-$) and alkoxy radical (RO$^-$), and certain nonradicals with oxidizing and/or radical-convertible ability, such as hydrogen peroxide (H$_2$O$_2$), hypochlorous acid (HOCl) and atomic oxygen (O$^-$). RNS refer to a collection of nitric oxide (NO) and its derivatives (Apak et al., 2016). Various pathways of RS formation and transformation as well as their damage to biological systems have been extensively reviewed elsewhere (Bayliak et al., 2019; Luo, Mills, le
Similar to mammals, oxidative stress in *Drosophila* results from an imbalance between the RS production and the impaired ability to detoxify them or repair the damage that they made. This stress can be induced by exposure to oxidants or radiation (Nagpal & Abraham, 2017b; Tang et al., 2019; Hao Wang et al., 2019), consumption of high-calorie diets (Aksu et al., 2014; Colpo et al., 2018; H.-l. Wang et al., 2017), and ingestion of specific chemicals (Abolaji, Babalola, Adegoke, & Farombi, 2017; M.-D. Jiang, Zheng, Wang, & Wang, 2017; Khanam et al., 2017; Manjula, Subashini, Punitha, & Subramanian, 2017; Mohandas, Rao, Muralidhara, & Rajini, 2017; Nagpal & Abraham, 2019; Pb et al., 2020; J. Su, Jiang, Wu, Liu, & Wu, 2018). Moreover, stress-induced inflammation and immune responses frequently promote the RS production (Bayliak et al., 2019). As results of RS-induced oxidation reactions, elevated oxidation of biomolecules such as fatty acids, proteins and nucleic acids have also been used as indicators for oxidative stress (Samet & Wages, 2018).

### 2.2 Antioxidative defense

Like mammals, *Drosophila* possesses an endogenous system to prevent RS-induced oxidative damage. It consists of three defensive lines (Bayliak et al., 2019; Carocho et al., 2018). The first line is composed of antioxidative enzymes, mainly superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST), which scavenge RS by enzymatic reactions (Luo et al., 2020). Two forms of SOD are found in eukaryotic cells: one in the cytosol contains copper and zinc in the active site (copper and zinc-SOD, CuZn-SOD) and the other in the mitochondria contains manganese (manganese-SOD, Mn-SOD). They both catalyze the dismutation of superoxide radicals (O$_2^-$) into molecular oxygen (O$_2$) and H$_2$O$_2$. H$_2$O$_2$ is further decomposed to H$_2$O and O$_2$ by CAT, a family of heme-containing enzymes. In contrast, GPx is a selenium-containing enzyme which utilize reduced glutathione (GSH) as the substrate to convert...
peroxides and hydroperoxide into alcohols, water and oxidized glutathione (GSSG). In addition to GPx, GST, frequently involved in the metabolism of xenobiotics and carcinogens, can bind and conjugate electrophiles to GSH for neutralization (Carocho et al., 2018; B. Chen & Xu, 2019; Del Bo’ et al., 2015). The second defensive line is formed by non-enzymatic antioxidants, such as GSH, ubiquinone and uric acid, which rapidly stop the radical oxidation reactions by donating electrons (Luo et al., 2020; Poprac et al., 2017). The oxidized forms of ubiquinone and GSH can then be reduced by endogenous enzymes, such as NADPH:quinone oxidoreductase 1 (NQO1) and thioredoxin reductase (TRR) (Holmgren & Lu, 2010; Ross & Siegel, 2018). The third defensive line is managed by enzymatic antioxidants that repair damage of biomacromolecules caused by oxidative stress or remove the damaged biomacromolecules (Bayliak et al., 2019; Carocho et al., 2018). Examples for these include DNA repairing enzymes (polymerases, glycosylases and nucleases), proteolytic enzymes (proteinases, proteases and peptidases), protein disulfide oxidoreductases, and methionine sulfoxide reductase (Çakatay, 2010; Ighodaro & Akinloye, 2018).

2.3 Signaling pathways related to antioxidative defense

Antioxidative enzymes such as SOD, CAT, GPx, NQO1 and enzymes in the GSH synthesis system are transcriptionally regulated by the nuclear factor E2-related factor 2/antioxidant response element (Nrf2/ARE) pathway (Buendia et al., 2016). Nrf2, a member of the cap’n’collar (Cnc) family, has recognized as the major transcription factor in antioxidative defense and redox homeostasis (Pitoniak & Bohmann, 2015). Under normal physiological conditions, Nrf2 is sequestered in the cytosol by a Keap1 (Kelch-like ECH-associated protein1) homodimer, which facilitates its ubiquitination and proteasomal degradation. In response to oxidative stress, the Nrf2-Keap1 complex dissociates and allows the nuclear translocation of Nrf2. These Nrf2 proteins form heterodimers with small musculoaponeurotic fibrosarcoma (Maf) proteins in the nucleus and bind to AREs to activate expression of antioxidant and detoxification genes (Espinosa-Diez et al.,
In *Drosophila*, Cap’n’collar isoform-C (CncC), the sole *Drosophila* homolog of Nrf2, interacts with small Maf and Keap1 proteins, similar to their mammalian counterparts, to activate ARE-dependent gene expression (Pitoniak & Bohmann, 2015).

Nrf2 also interacts with other signaling pathway components such as the nuclear factor-κB (NF-κB), mitogen-activation protein kinases (MAPKs), p53, and homeodomain transcription factors, which are all highly conserved between *Drosophila* and mammals (Galenza & Foley, 2019; Ingaramo, Sánchez, & Dekanty, 2018). These molecules are believed to modulate Nrf2 activation steps, such as nuclear translocation and transcription activation, in a cell type- and gene-specific manner, but do not replace the Keap1-dependent ubiquitination and degradation of Nrf2 (Buendía et al., 2016; Ma & He, 2012). Especially, NF-κB, MAPKs and p53 all have bidirectional roles on the expression and activity of Nrf2, due to complex mechanisms of homeostasis regulation (Bellezza, Giambanco, Minelli, & Donato, 2018; Buendía et al., 2016; W. Chen et al., 2012; Lingappan, 2018). Lushchak (2014) indicated that antioxidative mechanisms in animals are closely related to the hierarchy of oxidative stress responses. Briefly, low-intensity stress up-regulates genes encoding antioxidant enzymes *via* the Nrf2-Keap1 pathway, intermediate-intensity stress up-regulates antioxidant enzymes and induces inflammation proteins mainly *via* the NF-κB and MAPKs pathways, whereas high-intensity stress leads to necrosis and/or apoptosis. Overall, the roles of NF-κB, MAPKs and p53 in Nrf2 pathway have yet to be fully investigated.
3. Effects of food-derived antioxidants in *Drosophila*

In addition to endogenous antioxidant defense, many food-derived antioxidants (Table 1), such as phenolic acids, flavonoids, and polysaccharides, also contribute to antioxidative responses in *Drosophila*. Commonly, they function in three aspects against oxidative stress *in vivo* (Leopoldini, Russo, & Toscano, 2011; H Wang et al., 2013). First of all, they can inactivate free radicals directly by the mechanisms of hydrogen atom transfer and single electron transfer. Secondly, they can protect organisms from oxidative damage by chelating and inactivating transition metals to prevent them from catalyzing certain oxidation reactions, resulting in reduction of free radicals indirectly. Thirdly, they can upregulate expression or activity of antioxidative enzymes, such as SOD, CAT, GST, GPx, TRR and GR.

Among these, the regulation of antioxidative enzymes by antioxidant supplementation has attracted most research in *Drosophila*. It has been reported that food ingredients, such as catechin, apple polyphenols and blueberry extract, exert antioxidative effects in wild type flies depending on SOD and CAT because these effects are significantly weakened in SOD or Cat mutants (Li, Chan, Huang, & Chen, 2007; Peng, Chan, Huang, Yu, & Chen, 2011; Peng et al., 2012). Moreover, the antioxidative benefit of curcumin in fruit flies was eliminated by co-supplementing disulfiram, a specific inhibitor of SOD (Suckow & Suckow, 2006). Further studies suggest that these regulations are primarily implemented through the CncC/ARE pathway. Food-derived antioxidants like curcumin and phlorizin are capable of inducing ARE-dependent gene expression by binding to or reacting with the cysteine thiol of Keap1 and CncC (Ma & He, 2012; Hao Wang et al., 2019).

Similarly, the antioxidative activity of apple phlorizin in *Drosophila* relies on the increased mRNA expressions of Cnc, Keap1, SOD, CAT, *Drosophila* silent information regulator 2 (dSir2) and glutamate-cysteine ligase catalytic subunit (GCLC), the first enzyme in the synthesis cascade of glutathione (Hao Wang et al., 2019). Another example comes from *Sargassum fusiforme*, a seaweed...
known as longevity-promoting vegetable in Northeast Asia. Its active ingredient the fucoidan SP2 showed antioxidative activity in old flies via activation of the CncC/ARE pathway (Y. Zhang et al., 2019). The SP2-dependent upregulation of CAT, CncC, GCLC and HO were significantly inhibited by co-supplementation of luteolin or all-trans-retinoic-acid, chemical inhibitors of the CncC/ARE pathway.

In addition to the CncC/ARE pathway, other signaling pathways may also participate in the regulation of the oxidative status by food-derived antioxidants in Drosophila. For example, the antioxidative royal-jelly proteins secreted by honey bees not only induce SOD, but also activate the epidermal growth-factor receptor (EGFR)-MAPK signaling pathway (Xin et al., 2016), which has been shown to promote epithelial regeneration to maintain tissue homeostasis (H. Jiang, Grenley, Bravo, Blumhagen, & Edgar, 2011). Notably, MAPK functions at the center of a signal transduction network which coordinates the induction of protective genes in response to oxidative stress (H. Jiang et al., 2011; Vrailas-Mortimer et al., 2011; M. C. Wang, Bohmann, & Jasper, 2003). JNK is another example of MAPK that can be activated by food-derived antioxidants in Drosophila. Apple phlorizin induces dSir2 leading to activation of the p53 pathway by acetylation, which in turn activates JNK (Ingaramo et al., 2018; Liang, Kume, & Koya, 2009; Hao Wang et al., 2019). Furthermore, the methuselah (mth) gene, which encodes a G protein–coupled receptor (GPCR) involved in stress response and aging (Lin, Seroude, & Benzer, 1998), has been implicated in mediating the antioxidative effects of various fruits and vegetables. Extracts from apples, berries and ginger down-regulate expression of mth to enhance oxidation resistance and promote lifespan in Drosophila (K.-S. Lee et al., 2010; Lin et al., 1998; Peng et al., 2011; Peng et al., 2012; Hao Wang et al., 2019; Zhou, Xue, Gao, Qin, & Du, 2018). Therefore, similar to mammals, food-derived antioxidants contribute to antioxidative defense in Drosophila through regulation of the CncC/ARE pathway and its related stress response signaling (Fig. 2).
4. Evaluation of food-derived antioxidants in Drosophila

Exogenous antioxidants, including vitamins, carotenoids, flavonoids, phenolic acids, polysaccharides, peptides and proteins, can be obtained from various foods (Cömert & Gökmen, 2018; B. Chen & Xu, 2019). Their antioxidative effects in vivo are mostly assessed in rodent models by analyzing the markers associated with oxidative damage or antioxidative defense in blood and tissues (Apak et al., 2016; Ghezzi, 2020). These markers are mainly biochemical parameters, such as RS levels, oxidative damage to biomacromolecules, and enzymic/non-enzymic antioxidants (Alam et al., 2013; Apak et al., 2016). As in mammals, these parameters can be measured in Drosophila. However, by taking advantage of short life cycle, high fecundity and genetic amenability, the Drosophila models also frequently use physiological indicators such as lifespan and viability together with genetic manipulations to evaluate the effect of antioxidative supplements (Table 1).

4.1. RS levels

Endogenous RS, including both ROS and RNS, can be directly measured in Drosophila to evaluate the antioxidative effects of food supplements. For example, supplementation with specific nutrients such as caffeic acid, curcumin, hesperidin and creatine, or certain food extracts, significantly reduced the ROS level (Abolaji et al., 2017; Casani, Gómez-Pastor, Matallana, & Paricio, 2013; Hosamani, Ramesh, & Muralidhara, 2010; S. R. Jahromi, Haddadi, Shivanandappa, & Ramesh, 2015; Jo & Imm, 2017; Krishna & Muralidhara, 2016; Prasad & Muralidhara, 2014; Wu et al., 2018). In these studies, 2’,7’-dichlorofluorescein diacetate (H2DCFDA), a highly fluorescent dye upon oxidation, was used to measure ROS levels in homogenized tissues. Also, H2DCFDA and other fluorescent probes such as dihydroethidium and N-borylbenzyloxy carbonyl-3,7-dihydroxyphenoxazine can be applied to ROS measurement in intact tissues (Chu et al., 2018; Fogarty et al., 2016). In contrast to ROS, the RNS levels were
determined by measuring NO. Creatine supplementation reduced the NO level in whole-body mitochondrial fractions of fruit flies (Hosamani et al., 2010). Notably, RS levels within the physiological range is critical for many redox-dependent signaling processes. Too high or too low RS levels can lead to adverse health effects (Luo et al., 2020). Therefore, the validity of RS reduction related to antioxidant supplementation should refer to their normal level in *Drosophila*.

### 4.2. Oxidative damage to biomacromolecules

Oxidative stress can damage biomacromolecules such as lipids, proteins, and DNA. Antioxidants can protect these molecules from oxidation-mediated changes (Apak et al., 2016). Notably, the oxidative damage to DNA occurs much less frequently than the damage to proteins and lipids (Dabrowska & Wiczkowski, 2017). Therefore, evaluation of oxidative damage on biomacromolecules in *Drosophila* mainly focus on oxidation of lipids and proteins.

#### 4.2.1. Lipid oxidation

Lipids are susceptible to oxidation due to the reactive unsaturated bonds in their molecular structures. Their initial and secondary oxidation products in *Drosophila* are commonly represented by hydroperoxide (HP) and malondialdehyde (MDA), respectively (Table 1). Usually, HP is quantified by iodometry or ferric thiocyanate test, and MDA is measured by thiobarbituric acid reactive substances assay (Apak et al., 2016). The diet containing tomato seed extract (Krishna & Muralidhara, 2016), geraniol or curcumin (Prasad & Muralidhara, 2014) inhibits HP generation in male flies with or without toxicant-induced stress. Interestingly, the MDA reduction by antioxidants depends on the specific conditions of the flies tested. These include gender (Shen et al., 2013), feeding duration (Qiu et al., 2020; Y. Zhang et al., 2019; Z. Zhang, Han, Wang, & Wang, 2014) and physiological disorders (Ali et al., 2019; Khanam et al., 2017; Siddique et al., 2016). For example, *Sargassum fusiforme* fucoidan (Y. Zhang et al., 2019) and royal jelly-collagen peptides (Qiu et al., 2020) inhibit MDA production in old flies (30~50 days), but not in young flies (7~10 days).
contrast, reduction of MDA by lutein, which was supplemented from the second day after eclosion, was found in 20-day old flies, but not in 35-day old flies (Z. Zhang et al., 2014). Moreover, the fly models of Parkinson’s disease (PD) or Alzheimer’s disease (AD), compared to healthy flies, were more sensitive to downregulation of MDA induced by antioxidants (Ali et al., 2019; Khanam et al., 2017; Siddique et al., 2016).

4.2.2. Protein oxidation

Oxidative effects on proteins include side-chain group oxidation, backbone cleavage, crosslinking, unfolding, and changes in hydrophobicity and conformation (Hawkins, Morgan, & Davies, 2009). Protein carbonyl (PC) and total thiols (TSH) are commonly used makers of protein oxidation in *Drosophila* (Table 1). PC can result from oxidative backbone cleavage and direct oxidation of amino acids like lysine, arginine, histidine, and proline. Oxidation of the thiol groups gives rise to production of the thiol radicals which are readily dimerized to sulfides (Dabrowska & Wiczkowski, 2017). The levels of PC and TSH can be determined by spectrophotometric methods using 2,4-dinitrophenyl hydrazine and di-thiobis-nitrobenzoic acid, respectively (Ali et al., 2019; Colpo et al., 2018; Krishna & Muralidhara, 2016; Mohandas et al., 2017; Prasad & Muralidhara, 2014; Siddique et al., 2016). It should be noted that glycation and binding with aldehydes resulting from lipid peroxidation also provide carbonyls for proteins (Apak et al., 2016). These may cause an overestimated level of protein oxidation if only the PC level is measured.

It has been reported that the levels of PC increase in flies during aging, in response to oxidative stress or in neurodegenerative conditions (Ali et al., 2019; Colpo et al., 2018; Krishna & Muralidhara, 2016; Prasad & Muralidhara, 2014; Siddique et al., 2016). These high levels of PC can be reduced by diets enriched with antioxidants. Also, the relatively low levels of TSH in flies exposed to acrylamide or manganese can be restored to normal by supplementing geraniol, curcumin or whey proteins (Mohandas et al., 2017; Prasad & Muralidhara, 2014). Moreover,
advanced oxidation protein products, a group of oxidized, dityrosine-containing proteins, may form insoluble aggregates with high molecular weights (Çakatay, 2010). It was found that caffeic acid supplementation reduced protein aggregation in flies with neuronal defects (Wu et al., 2018).

4.3. Endogenous antioxidants

Enzymes in the first line of antioxidative defense have been widely accepted as the biomarkers for in vivo antioxidant studies. As seen in Table 1, food-derived antioxidants can enhance expression or activity of antioxidative enzymes, mainly SOD, CAT, GPx and GST, in Drosophila with or without oxidative stress. Exceptionally, antioxidative enzymes in fruit flies with Parkinson’s disease (PD) or Alzheimer’s disease (AD) may be bi-directionally regulated by antioxidants, which can significantly lower the ROS level and the oxidative damage of lipids and proteins. For example, the activities of antioxidative enzymes (especially GST) were positively elevated by ascorbic acid, α-tocopherol (Casani et al., 2013), caffeic acid (Wu et al., 2018), curcumin (Prasad & Muralidhara, 2014) and Decalepis hamiltonii extract (S. R. Jahromi et al., 2015), but were negatively reduced by geraniol (Siddique et al., 2016), luteolin (Ali et al., 2019) and whey protein isolate (Mohandas et al., 2017). In addition, capsaicin supplementation decreased the levels of LPO and PC in the third instar larvae with carcinogenic exposure, but weakened the activities of GST and CAT (Khanam et al., 2017). It is implied that the effects of dietary antioxidants on antioxidative enzymes may be affected by the disordered homeostasis. Moreover, enzymes participated in the other two lines of antioxidative defense, such as TRR, NQO1 and glutathione reductase (GR), can be also up-regulated by supplementing antioxidants (Mohandas et al., 2017; Prasad & Muralidhara, 2014; Wu et al., 2018).

Unlike antioxidative enzymes, non-enzymatic antioxidants except GSH are less studied in Drosophila (Table 1). GSH-mediated metabolism plays a key role to protect cells from the oxidative stress (Prasad & Muralidhara, 2014). GSH and its oxidized form (GSSG) are the predominant redox
pair in cells (Samet & Wages, 2018). Decreases of the GSH level and the GSH:GSSG ratio, the
typical features of oxidative stress (Dabrowska & Wiczkowski, 2017), are able to be restored by
various antioxidants, such as curcumin (Prasad & Muralidhara, 2014), geraniol (Siddique et al.,
2016) and *Sargassum fusiforme* fucoidan (Y. Zhang et al., 2019). Furthermore, the total antioxidant
capacity (T-AOC) of tissue homogenate was employed to identify the effects of *Sipunculus nudus*
polysaccharides (J. Su et al., 2018) and edible bird’s nests (Q. Hu et al., 2016) on the non-enzymatic
antioxidants in *Drosophila*.

4.4. Resistance to oxidative stress

The resistance to oxidative stress in *Drosophila* is usually described by the survival curve during
exposure to H$_2$O$_2$ or paraquat (K.-S. Lee et al., 2010; Li, Chan, Huang, & Chen, 2008; Peng et al.,
2011; Peng et al., 2012; Tang et al., 2019; Hao Wang et al., 2019), or the mortality rate after a given
exposure time (Hosamani et al., 2010; S. R. Jahromi et al., 2015). Such experiments are generally
performed with 2-h-starved flies in vials containing a filter paper saturated with 1 mL of 20 mmol/L
paraquat or 30% H$_2$O$_2$ in a 6% glucose solution. Pre-consumption of food-derived antioxidants,
such as apple polyphenols, apple phlorizin, broccoli juice, blueberry extract, green tea catechins and
*Lyceum barbarum* polysaccharides, for more than 20 days significantly increased the average
survival time of wild-type flies that exposed to H$_2$O$_2$ or paraquat (K.-S. Lee et al., 2010; Li et al.,
2007, 2008; Peng et al., 2011; Peng et al., 2012; Tang et al., 2019; Hao Wang et al., 2019). This
effect was not observed in *SOD*$_{108}$ or *Cat*$_{1}$ mutant flies (Li et al., 2007; Peng et al., 2011; Peng et
al., 2012), indicating that both SOD and CAT play important roles mediating effects of these
antioxidants. Moreover, pre-consumption of α-tocopherol or cocoa significantly strengthened the
oxidative resistance of fruit flies, resulting in an extension of their average lifespan under hyperoxia
(Bahadorani, Bahadorani, Phillips, & Hilliker, 2008; Bahadorani & Hilliker, 2008).
4.5. Lifespan and healthspan

Free radical-caused oxidative damage is considered as a major determinant of lifespan. A considerable number of studies in various organisms indicate that the alleviation of oxidative stress by scavenging superfluous free radicals contributes to increase of life expectancy (Mockett, Orr, Rahmandar, Sohal, & Sohal, 2001; Ristow & Schmeisser, 2011). Extension of the *Drosophila* lifespan is usually judged by the significant increase of mean, median and/or maximum lifespans. Maximum lifespan, which is commonly calculated as the average survival time of the last ~5% of surviving flies, is proposed to reduce the sensitivity to sample size (W. Hu, Dai, & Li, 2013; Peng et al., 2012; J. Su et al., 2018). The mean lifespan is sample size independent, but does not convey information about age-specific patterns of mortality. By comparison, the median lifespan is the time when 50% of the population has died. It therefore reflects age-specificity (Jafari, 2010). To exclude the intervention of aging-unrelated factors such as genotype and stress condition (Bahadorani et al., 2008), the extension beyond normal lifespans should be primarily concerned for antioxidant evaluation (Mockett et al., 2001). Previous studies confirmed that the diets mixed with extracts from aronia, apple or blueberry prolonged the normal lifespan of fruit flies through upregulation of antioxidative functions (Jo & Imm, 2017; Peng et al., 2011; Peng et al., 2012; Hao Wang et al., 2019). Notably, the shorted lifespan of flies due to genetic manipulations or oxidative stress can be also extended by antioxidant supplementation (Ali et al., 2019; H.-I. Wang et al., 2017; Wu et al., 2018).

For aged humans and animals, lifespan extension is not always correlated with improved health conditions (Nguyen et al., 2016). More than 50% of the population aged over 65 suffer from one or more diseases for the rest of their lives (Niccoli & Partridge, 2012). The *Drosophila* healthspan has been defined as the period when fruit flies maintain greater than 50% of the maximum functional capacity of the wild-type control (Nguyen et al., 2016). Therefore, it might be more valuable to evaluate the health effects of antioxidant supplementation. As an important
indicator associated with the general health status of fruit flies, climbing ability in response to antioxidant consumption shows a positive correlation with lifespan (Chandrashekara & Shakarad, 2011; Jo & Imm, 2017; Peng et al., 2011; Peng et al., 2012; H.-l. Wang et al., 2017; Hao Wang et al., 2019; Wu et al., 2018). For example, extracts from apple, blueberry and rosemary elevate both lifespan and the climbing ability, *i.e.*, healthspan of fruit flies (Peng et al., 2011; Peng et al., 2012; H.-l. Wang et al., 2017).

### 4.6. Other measurable effects of antioxidants

Several studies have reported that antioxidants provided neuroprotective effects in flies against neurotoxicity or neurodegeneration by inhibiting activity of the acetylcholinesterase (AChE), an enzyme known to breakdown the neurotransmitter acetylcholine which leads to neurodegenerative disorders, and modulating endogenous antioxidative defenses (Ali et al., 2019; Samaneh Reiszadeh Jahromi, Haddadi, Shivanandappa, & Ramesh, 2013; Khanam et al., 2017; Prasad & Muralidhara, 2014). Moreover, loss of the dopaminergic neurons due to oxidative stress, known as one of the main symptomatic features of neurodegeneration, can be restored by antioxidant supplementation, thus contributing to behavioral improvements (Casani et al., 2013; Hosamani et al., 2010; Krishna & Muralidhara, 2016; Siddique et al., 2016). In addition, antioxidative benefits in *Drosophila* may also involve rhythmic regulation (Manjula et al., 2017; Subramanian, Kaliyamoorthy, Jayapalan, Abdul-Rahman, & Haji Hashim, 2017), antigenotoxicity (Fernandez-Bedmar, Anter, & Moraga, 2018), and immunomodulation (J. Su et al., 2018).

### 5. *Drosophila* models developed for antioxidant studies

As seen in Table 1, antioxidant studies used *Drosophila* with either normal oxidation status (*i.e.*, healthy wild-type *Drosophila*), or oxidative stress. The oxidative stress can be induced by feeding larvae or flies with chemicals or high-calorie diets (Table 2). The advantages of *Drosophila* also allow activation of the oxidative stress *via* genetic manipulations, *e.g.*, creating the antioxidative
deficiencies or mutants. Interestingly, the wild-type and stressed flies may respond to dietary antioxidants differently (Ali et al., 2019; Khanam et al., 2017; Mohandas et al., 2017; Siddique et al., 2016). For instance, luteolin (Ali et al., 2019), geraniol (Siddique et al., 2016) and capsaicin (Khanam et al., 2017) showed antioxidative effects in flies with AD or PD, but not in healthy flies. It has been reported that healthy wild-type flies supplemented with excessive antioxidants had a reduced level of ROS, thus weakened the CncC/ARE pathway-dependent transcription of antioxidative enzymes (Huangfu et al., 2013). Therefore, evaluation of the antioxidant effects under both normal and oxidative stress conditions can be very informative.

5.1. Chemical-induced oxidative stress in Drosophila

Various chemicals have been used to induce chronic or acute oxidative stress in Drosophila. These mainly include free radical generators, transition metals, and toxicants (Table 2). Dietary antioxidants can be supplemented before, during or after the use of chemicals, i.e., pre-, co-, or post-supplementation, to evaluate their effects.

5.1.1. Free radical generators

Paraquat and H$_2$O$_2$ have been widely used to induce oxidative stress as they are generators of superoxide anion radical and hydroxyl radical, respectively (Tang et al., 2019; Hao Wang et al., 2019). Generally, dietary antioxidants are pre-supplemented to increase the activity or expression of endogenous antioxidants and decrease the level of ROS and PC, thus enhance the resistance against the acute stress (Duavy et al., 2019; Park, Jung, Ahn, & Kwon, 2012; Qiu et al., 2020). The H$_2$O$_2$-induced oxidative stress in 5-day old flies was obviously alleviated after 3 days of supplementation with quercetin (Subramanian et al., 2017). Notably, both paraquat and H$_2$O$_2$ at a low concentration can elevate the activity and expression of the endogenous antioxidative enzymes by activating the CncC/Nrf2 pathway (Duavy et al., 2019; Pant, Dave, & Tiwari, 2013). However, when they are present at a high concentration, they can cause inflammatory response, growth arrest
and cell death (Sies, 2017). Therefore, the concentration of paraquat or H₂O₂ is a key factor to consider when using Drosophila to assess food-derived antioxidants.

5.1.2. Transition metals

Most transition metals induce oxidative stress by depleting GSH and protein-bound sulfhydryl groups (Stohs & Bagchi, 1995), and/or catalyzing the oxidation of low-molecular weight reductants, such as glucose, ascorbate and polyunsaturated fatty acids (Wolff, 1993). Ferrous iron (Fe²⁺) also promotes production of the hydroxyl radicals from H₂O₂ and the redox reactions between oxygen and biomacromolecules (Stohs & Bagchi, 1995). The Fe²⁺-induced oxidative stress in fruit flies can be alleviated by polyphenols such as gallic acid and epigallocatechin gallate (Jimenez-Del-Rio, Guzman-Martinez, & Velez-Pardo, 2010). Moreover, a 5-day consumption of diets containing 15 mmol/L manganese chloride gave rise to oxidative stress in 8~10-day old flies, which might be caused by the damage to antioxidative defense and mitochondrial function (Mohandas et al., 2017). Similarly, newly eclosed flies showed features of oxidative stress after a 10-day supplementation with diets containing 1.0 μg/mL Cd (VI), probably due to its suppression on the immune- and antiaging-related signaling pathways (J. Su et al., 2018). Both Mn- and Cd-induced overoxidation in Drosophila can be attenuated by co-supplementing antioxidative macromolecules, such as whey protein isolate (Mohandas et al., 2017) and Sipunculus nudus polysaccharides (J. Su et al., 2018).

5.1.3. Toxicants and drugs

Various toxicants, such as rotenone (Arumugam, Jayapalan, Abdul-Rahman, Hashim, & Subramanian, 2018), trichloroethylene (Abolaji et al., 2017), urethane (Nagpal & Abraham, 2017a), toluene (Pb et al., 2020) and methyl methanesulphonate (MMS) (Khanam et al., 2017), are capable of triggering oxidative stress in Drosophila. Typically, rotenone can penetrate cellular membranes independently of any transporters and cause mitochondrial dysfunction by binding with mitochondrial complex-I, thus promote ROS production (Arumugam et al., 2018). Several studies
confirmed that flies suffered oxidative stress after feeding on diets containing 500 μmol/L rotenone for 7~14 days. It can be attenuated by co-supplementing hesperidin (Arumugam et al., 2018; Manjula et al., 2017), creatine (Hosamani et al., 2010) or tomato seed extract (Krishna & Muralidhara, 2016). Similarly, trichloroethylene was used in flies to induce oxidative stress for evaluation of the antioxidative effects of *Citrus aurantium* hesperidin (Abolaji et al., 2017).

In addition to adult flies, *Drosophila* larvae with toxicant-induced oxidative stress can also be used for antioxidant studies. The urethane-induced oxidative stress in third instar larvae was alleviated by co-supplementation with gallic acid, quercetin or limonene (Nagpal & Abraham, 2017a). Likewise, the MMS-induced oxidative damage in third instar larvae was suppressed by dietary capsaicin. However, regardless of capsaicin supplementation, MMS increased the activities of GST and CAT probably by stimulating adaptive responses (Khanam et al., 2017). Interestingly, the newly eclosed flies from larvae growing in the media containing 200 mmol/L toluene, exhibited loss of the antioxidative defense due to toluene-induced reproductive and developmental toxicity. The loss was repaired by the co-supplementation of antioxidative *Boerhavia diffusa* L. extract (Pb et al., 2020). Apart from the toxic chemicals introduced above, some drugs, such as cyclophosphamide (Nagpal & Abraham, 2019), levodopa and chlorpromazine (M.-D. Jiang et al., 2017), may also be effective to trigger oxidative stress therefore can be used to study the effectiveness of antioxidants.

### 5.2. High-calorie diet-induced oxidative stress in *Drosophila*

An excessive high-calorie diet supplies energy substrate to the metabolic pathways in adipose and non-adipose tissues. This consequently accelerates oxidation of fatty acids and monosaccharides and stimulates the tricarboxylic acid (TCA) cycle (Bayliak et al., 2019; Paula et al., 2016). The elevated TCA cycle tends to overload the mitochondrial electron transport chain. As a result, mitochondrial dysfunction occurs which contributes to ROS production (Bayliak et al., 2019). For high-carbohydrate diets, both non-enzymatic glycosylation and autoxidation of monosaccharides
can lead to RS production (Bayliak et al., 2019). Therefore, the chronically excessive intake of carbohydrates and/or fats causes metabolic complications and RS overproduction, thus resulting in depletion of the antioxidative defenses and aggravation of biomolecular oxidation. For example, flies supplemented with diets rich in lard (10%–15%) or cholesterol, compared to the control with basal diet, showed significant changes on expression and activity of antioxidative enzymes and increased levels of the oxidized proteins and lipids (Colpo et al., 2018; H.-l. Wang et al., 2017). Consistently, dietary antioxidants, such as rosemary extracts (H.-l. Wang et al., 2017) and tea extracts (Kayashima et al., 2015), can attenuate the fat-induced stress.

The maintaining on 10%-carbohydrate (glucose or fructose) diets caused a higher LPO level and a weaker CAT activity in aged flies (50-day old), compared to the control of 2%-carbohydrate diet (O. V. Lushchak, Gospodaryov, Yurkevych, & Storey, 2016). The features of oxidative stress between 10%-fructose and 10%-glucose groups showed no significant difference, though fructose can produce more autoxidation products than glucose (Semchyshyn, Lozinska, Miedzobrodzki, & Lushchak, 2011). Carbohydrates may cause different effects of oxidative stress, partly due to their different pathways of utilization (O. V. Lushchak, Rovenko, Gospodaryov, & Lushchak, 2011). The replacement of sucrose with D-galactose in basal medium could cause oxidative stress-related aging in fruit flies, which was restorable by antioxidant supplementation (Aksu et al., 2014). Excessive monosaccharide consumption during the larval period promotes changes in the redox homeostasis of adults in carbohydrate- and sex-dependent manners. Gender difference in fly metabolism may also form background for the effects of carbohydrate type on antioxidant system, and produce different markers of oxidative stress in males and females (O. V. Lushchak et al., 2011).

5.3. Genetic modifications for antioxidant studies in Drosophila

The advanced genetic tools available in Drosophila allow both loss-of-function and gene overexpression studies on effects of antioxidants in vivo. Mutants of the antioxidative enzymes such
as SOD and CAT have been developed and widely used to determine whether the endogenous antioxidative mechanisms are activated by the antioxidative supplements. For example, as described above, the resistance of wild-type flies against oxidative stress was significantly enhanced by pre-supplementing apple polyphenols, blueberry extract or green tea catechins. Such effect was not observed in SOD\textsuperscript{n108} or Cat\textsuperscript{n1} mutant flies, indicating that both SOD and CAT play important roles mediating functions of these antioxidants (Li et al., 2007; Peng et al., 2011; Peng et al., 2012). The lifespan-related influences of cocoa on CuZn-SOD-deficient flies and Mn-SOD-deficient flies were opposites, probably due to the antioxidative activity in cytoplasm and the pro-oxidant activity toward mitochondria (Bahadorani & Hilliker, 2008).

Another example of the proteins contributing to ROS removal is DJ-1, a ubiquitously expressed redox-responsive protein acts as a transcriptional or translational regulator promoting expression of the genes involved in the antioxidative defense, as well as a free radical scavenger (Casani et al., 2013). DJ-1 orthologous genes in Drosophila, DJ-1\textalpha{} and DJ-1\textbeta{}, are both implicated in the protection against oxidative stress (Lavara-Culebras & Paricio, 2007). For example, compared to wild-type flies, DJ-1\textbeta{} mutants exhibited a higher level of oxidative biomarkers (such as ROS levels, PC and LPO), as well as a greater sensitivity to oxidative stress. Early supplementation with either α-tocopherol or ascorbic acid could suppress phenotypes of oxidative stress in the mutants (Casani et al., 2013).

The excessive generation of free radicals and the occurrence of oxidative stress have been known as a common component of many neurodegenerative disorders. Therefore, some transgenic Drosophila lines expressing neurodegeneration-related genes may be reliable for antioxidant studies (Kim, Jung, Ahn, Restifo, & Kwon, 2011). Examples for these include overexpression of either mutated (A30P and A53T) or wild-type human α-synuclein gene in the PD model (S. R. Jahromi et al., 2015; Siddique et al., 2016), accumulation of amyloid beta 40 (Aβ40) peptides in the AD model (Ali et al., 2019) and polyglutamine (MJDtr-Q78) expansion within ataxin-3 proteins in the
Machado-Joseph disease model (Wu et al., 2018). To generate these transgenic models, the GAL4-UAS system is commonly employed for activating gene expression, in which the UAS is an enhancer specifically targeted by the GAL4 protein. By crossing females carrying the driver elav-Gal4 to males of USA-A30P, USA-A53T (S. R. Jahromi et al., 2015; Siddique et al., 2016), USA-AB42 (Ali et al., 2019) or UAS-MJDtr-Q78 strain (Wu et al., 2018), the gene activation in offspring causes oxidative stress. Interestingly, this stress in transgenic flies can be also ameliorated by dietary antioxidants.

6. Key aspects to consider when using Drosophila models to study food-derived antioxidants

6.1. Choosing appropriate study conditions

Drosophila has a short life cycle of 10 days at 25°C. It consists of four stages: embryo (~1 day), larva (~4 days), pupa (~5 days) and adults. The larval stage can be further divided into three molting stages: 1st (~1 day), 2nd (~1 day) and 3rd (~2 days) instar. Dietary antioxidants are frequently supplemented in larval or adult stage. The juvenile larvae undergo rapid growth and cell proliferation until the 3rd instar, when most cells start to differentiate. Notably, development after the mid-3rd instar is independent of nutrient availability (Tennessen & Thummel, 2011). It is therefore suggested that antioxidants should be supplemented after the mid-3rd instar, i.e., ~3 days after egg-laying at 25°C. This is to avoid the potential developmental effects on the endogenous antioxidative defense. However, using 3rd instar larvae subjects to a limited period (about 2 days) of antioxidant supplementation. In contrast, Drosophila adults allow a long-term antioxidant supplementation, simulating the dietary intervention studies in mammalian models and humans. As shown in Table 1, flies eclosed within 3 days are most frequently used for antioxidant tests. Interestingly, age, genotype and gender of the flies tested are all closely related to the antioxidative capability and the oxidation levels, therefore can affect the protective effects of
antioxidants (Chu et al., 2018; Khavinson, Myl'nikov, Oparina, & Arutyunyan, 2001; Menshchikova, Zenkov, Weisman, Kandalintseva, & Prosenko, 2010; Mylnikov et al., 2005; Paithankar, Raghu, & Patil, 2018). To minimize gender effects in such studies, male is preferentially chosen to avoid the antioxidative interference from female estrogens (Aksu et al., 2014). Moreover, the antioxidative capability also partly depends on the circadian rhythm in Drosophila. Previous studies have reported that the antioxidative defense in wild-type flies has an acrophase at around 4:00 pm in a 12/12 light/dark daily cycle (Arumugam et al., 2018; Subramanian, Prasanna, Jayapalan, Abdul Rahman, & Hashim, 2014). The disrupted circadian rhythm can disturb the effects of antioxidants (Arumugam et al., 2018). Therefore, the circadian rhythm of fruit flies during the experimental process needs to be closely monitored. The antioxidative effects should be measured at a specific time or in a specific period without rhythmic interference.

6.2. Preparation of Drosophila diets

For antioxidant studies in Drosophila, feeding is a common method used for sample delivery (S.-H. Lee & Min, 2019). One of the most important factors to consider in the preparation of antioxidant diet is the sample concentration. Taking into account the average daily food intake (1~2 μL/day or about 1 mg/day) and the average body weight (about 1 mg) of Drosophila, the concentration can be reasonably calculated according to the recommended daily intake for humans (Fernandez-Bedmar et al., 2018; Toledano Medina et al., 2019). For example, the dosage of lyophilized tomato samples in fly diets was estimated by referring to the daily consumption of tomato in human diet, i.e. ~10% of the total vegetable intake (Fernandez-Bedmar et al., 2018). The dietary supplementation of 1~10 mg/mL green tea catechins for flies corresponds to the catechin concentration in regular tea infusions and beverages (Li et al., 2007). It should be noted that male and female differ in both average body weight and average daily food intake. Previous work indicated that the average body weight of male flies (approximately 700 μg) is significantly lower than that of female flies.
(1000~1200 μg) (Staats et al., 2018), and the mass of food intake in females is about three times larger than in males (Xin et al., 2016).

The advent of instant medium formulation simplifies the preparation of antioxidative diets by directly mixing the medium with water containing antioxidants at a required solid-liquid ratio. Samples should be dispersed evenly in the medium and consumed equivalently by individuals. For water-insoluble samples, sometimes, specific solvents such as dimethyl sulfoxide and ethanol within the tolerance dose may be needed (Richardson, Willoughby, & Humbert, 2015). However, their potential influences on the redox homeostasis should be considered. Moreover, some food-derived antioxidants are unstable and, therefore, their stable durations and protective measures need to be taken into account. For example, to investigate the antioxidative effects of tea catechin, acetic acid (0.5%) was added into the diet for a low-pH environment (pH 4~5) to maintain stable catechins (Li et al., 2007). Another example comes from tea polyphenols. Their stable time in the standard diet is 3 days. Accordingly, the polyphenols-supplemented diet was prepared freshly when needed and was renewed with a maximal interval of 3 days (Kayashima et al., 2015).

6.3. Monitoring feeding behaviour

As the main mode of antioxidant delivery, free feeding may cause false-positive results of antioxidative evaluation (S.-H. Lee & Min, 2019). Typically, secondary plant compounds used as dietary antioxidants may affect the diet taste and lead to the reduction of food intake, probably generating the effects of calorie restriction (CR). It was reported that CR could induce defense mechanisms for ROS detoxification and scavenging (Ristow & Schmeisser, 2011). Therefore, the change of food intake after adding antioxidant should be investigated to determine if CR occurs (Staats et al., 2018). The quantity of food intake in Drosophila can be indirectly calculated by measuring the co-ingestion of non-absorbable food dyes, e.g. the Blue No. 1 dye, the fluorescein dye and the sulforhodamine B sodium salt, or radioisotopes mixed in the diet (Jo & Imm, 2017;
Peng et al., 2011; Shaposhnikov et al., 2014; Staats et al., 2018; Tang et al., 2019). The food intake can also be determined by monitoring the extension of proboscis or using the assay of capillary feeding (Staats et al., 2018). Usually, the food intake can be quantified by scoring the intensity of body coloring, following visual observation, with photometric or fluorometric measurements at dye-specific wavelengths (Staats et al., 2018). Notably, when using the dye-based methods, the fly heads should be discarded before the treatment of fly samples, to avoid the interference of eye pigments on the measurement of food dyes (Samaneh Reiszadeh Jahromi et al., 2013; S. R. Jahromi et al., 2015). Moreover, water and food intake in flies can also be assessed by measuring the change of bodyweight following a standardized approach (Q. Hu et al., 2016). Interestingly, both the additions of *Lycium barbarum* polysaccharides (Tang et al., 2019) and royal jelly-collagen protein/peptide (Qiu et al., 2020; Xin et al., 2016) significantly increased the food intake of flies. However, its potential effect on antioxidant evaluation was unclear.

7. Summary and future perspectives

*Drosophila* has emerged as a model organism to study food-derived antioxidants *in vivo* following a standard approach (Fig. 3). Firstly, either 3rd instar larvae or 1~3-day-old male adult flies are recommended to use. Secondly, both wild type and flies under oxidative stress or with antioxidative defects can be chosen appropriately for the studies. Thirdly, the feeding assays need to consider concentration, dispensability and stability of the test samples in the basal diet, the influence of sample addition on feeding behaviors, the effects of circadian rhythm and feeding duration on antioxidative parameters, and the strategy of antioxidant intervention (*i.e.*, pre-, co-, or post-supplementation). Fourthly, the antioxidative activity can be evaluated by analyzing RS levels, endogenous antioxidants, oxidative damage of biomacromolecules, resistance against oxidative stress, and other benefits related to antioxidative improvements. Finally, the antioxidative mechanisms can be further explored by analyzing inactivation of free radicals and activation of...
specific signaling pathways such as CncC/ARE, MAPK, REL and p53.

The sophisticated genetic tools available in Drosophila allow temporally and spatially controlled loss-of-function and gain-of-function analyses of the genes of interest. Drosophila therefore holds a great potential as an excellent model organism for investigating the effects of nutrients and diet compositions on health and lifespan (Panchal & Tiwari, 2017). Although a broad range of transgenic and mutant flies have been generated and publicly available, only a small portion of them have been applied in exploring the antioxidative activity and mechanisms of foods and their extracts. Furthermore, increased Drosophila strains with oxidative phenotypes, which can be easily scored and amendable by antioxidant supplementation, are expected to be developed for the purpose of antioxidant screening. For example, the gstD-GFP reporter fly lines, with the monitorable GFP fluorescence positively related to oxidative stress, may be a valuable tool for the live monitoring of antioxidant responses (Sykiotis & Bohmann, 2008).

To understand the mechanism of feeding-delivered antioxidants in Drosophila, one of the main challenges is the mystery of their bioavailability (Jafari, 2010). Drosophila has striking similarities to mammals in both the digestive system and the intestinal bacterial community (S.-H. Lee & Min, 2019). It has been proposed that the host microbiome can influence the efficacy of antioxidant supplementation via three mechanisms: 1) the microbial metabolism results in activation, inactivation or derivative production of the antioxidants; 2) the microbial products act as competing ligands for the targeted receptor or enzyme of the antioxidants; and 3) the antioxidant-induced microbiome changes in composition or activity cause the off-target effects (Douglas, 2018). Therefore, understanding the in vivo fate of antioxidants, which is mainly affected by the digestive tract and intestinal microorganisms, is necessary for deciphering the antioxidant functions in vivo and their downstream consequences on redox homeostasis. Undoubtedly, the differences in pharmacokinetics and pharmacodynamics between Drosophila and mammals, which may produce false positives or false negatives for antioxidant evaluation (Gladstone & Su, 2011),
are also worthy of investigation.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**References**


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Figure captions:

Figure 1. The number of publications related to antioxidant studies using Drosophila or Mouse from Jan. 1990 to Dec. 2020. The data were obtained from the database of National Center for Biotechnology Information, U.S. National Library of Medicine (https://www.ncbi.nlm.nih.gov/pubmed/) by searching the title/abstract containing “Drosophila and antioxidant (or anti-oxidant)” or “mouse (or mice) and antioxidant (or anti-oxidant)”.  

Figure 2. The antioxidative mechanisms of food-derived antioxidants in Drosophila. Antioxidants can 1) inhibit the production of ROS/RNS through hydrogen atom transfer, single electron transfer and/or transition metal chelating; 2) promote the expression and synthesis of endogenous antioxidants via the CncC/ARE pathway; and 3) induce other adaptive responses to oxidative stress via the signaling pathways involving NF-kB (REL), MAPK, JNK and p53, which may or may not interact with the CncC/ARE pathway. Abbreviations used in this diagram: ARE, antioxidant response element; CAT, catalase; CncC, cap’n’collar isoform-C; GPx, glutathione peroxidase; HO1, heme oxygenase 1; JNK, c-Jun N-terminal kinase; Keap1, Kelch-like ECH-associated protein1; Maf, musculoaponeurotic fibrosarcoma protein; MAPK, mitogen-activation protein kinase; NQO1, NADPH:quinone oxidoreductase 1; REL, Relish; ROS, reactive oxygen species; RNS, reactive nitrogen species; SOD, superoxide dismutase.  

Figure 3. A proposed scheme to investigate antioxidative activities of foods and their extracts using Drosophila models. Abbreviations: ARE, antioxidant response element; CncC, cap’n’collar isoform-C; GPx, glutathione peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione S-transferase; HP, hydroperoxide; JNK, c-Jun N-terminal kinase; Keap1, Kelch-like ECH-associated protein1; LPO, lipid peroxide; MAPK, mitogen-activation protein kinase, MDA, malondialdehyde; NO, nitric oxide; PC, protein carbonyls; REL, Relish; ROS, reactive oxygen species; SOD, superoxide dismutase; T-AOC, total antioxidation capacity; TRR, thioredoxin reductase; TSH, total thiols.
### Table 1 Antioxidative effects of foods and their extracts in *Drosophila* models.

<table>
<thead>
<tr>
<th>Foods and their extracts</th>
<th>Dose</th>
<th>Subjects</th>
<th>Duration</th>
<th>Model descriptions</th>
<th>Antioxidative effects</th>
<th>Other benefits</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aloe vera juice</td>
<td>5 mL/L</td>
<td>Eggs</td>
<td>Larval period</td>
<td>Wild type</td>
<td>↑: activity of SOD and CAT</td>
<td>↑: egg-to-adult viability; lifespan; climbing ability</td>
<td>(Chandrashekara &amp; Shakarad, 2011)</td>
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<tr>
<td>Aronia melanocarpa extract</td>
<td>2.5 mg/mL</td>
<td>1–3-day old males</td>
<td>10 or 40 days</td>
<td>Wild type (Canton S)</td>
<td>↑: activity and expression of SOD, CAT and GPx</td>
<td>↑: lifespan; climbing ability; expression of longevity genes</td>
<td>(Jo &amp; Imm, 2017)</td>
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<tr>
<td>Ascorbic acid or α-tocopherol</td>
<td>0.25 mg/mL or 1 mmol/L</td>
<td>1–2-day old flies</td>
<td>12 days</td>
<td>DJ-1β mutant model</td>
<td>↑: CAT activity and Mn-SOD expression; ↓: level of ROS and MDA</td>
<td>↑: lifespan</td>
<td>(Casani et al., 2013; Lavara-Culebras, Muñoz-Soriano, Gómez-Pastor, Matallana, &amp; Paricio, 2010)</td>
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<tr>
<td>Apple phlorizin</td>
<td>0.5, 1.0 and 2.0 mg/mL</td>
<td>2-day old males</td>
<td>25 or 45 days</td>
<td>Wild type (Oregon K)</td>
<td>↑: activity and expression of CuZn-SOD, Mn-SOD and CAT; resistance to oxidative stress; ↓: MDA level</td>
<td>↑: lifespan; climbing ability; expression of cnc, Keap1, GCLC and dSir2; ↓: mth expression</td>
<td>(Hao Wang et al., 2019)</td>
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<tr>
<td>Apple polyphenols</td>
<td>10 mg/mL</td>
<td>Newly eclosed males</td>
<td>15–55 days</td>
<td>Wild type (Oregon-R-C); SOD mutant model; CAT mutant model</td>
<td>↑: activity and expression of CuZn-SOD, Mn-SOD and CAT; resistance to oxidative stress</td>
<td>↑: climbing ability; lifespan; expression of mth</td>
<td>(Peng et al., 2011)</td>
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<tr>
<td>Broccoli juice powder</td>
<td>50 mg/mL</td>
<td>2-day old males</td>
<td>20 days</td>
<td>Wild type (Oregon-R-C)</td>
<td>↑: resistance to oxidative stress; activity and expression of CuZn-SOD, Mn-SOD and CAT; ↓: HP level</td>
<td>↑: lifespan; climbing ability; expression of Rpn11</td>
<td>(Li et al., 2008)</td>
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<td>Blueberry extract powder</td>
<td>5 mg/mL</td>
<td>Newly eclosed males</td>
<td>10–55 days</td>
<td>Wild type (Oregon-R-C); paraquat-induced stress models; SOD mutant model; CAT mutant model</td>
<td>↑: resistance to oxidative stress; activity and expression of CuZn-SOD, Mn-SOD and CAT</td>
<td>↑: lifespan; climbing ability; expression of mth</td>
<td>(Peng et al., 2012)</td>
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<td>Supplement</td>
<td>Concentration</td>
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<td>Model</td>
<td>Effects</td>
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<td>Caffeic acid</td>
<td>0.5 and 1.0 mmol/L</td>
<td>Newly eclosed females</td>
<td>19 days ELAV-SCA3tr-Q78 transgenic model</td>
<td>†: expression of HO1, NQO1, GR, CAT, GPx, CuZn-SOD and Mn-SOD; ↓: ROS level, protein aggregation (Wu et al., 2018)</td>
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<td>Capsaicin</td>
<td>0.5 μg/mL</td>
<td>Third instar larvae</td>
<td>48 h  MMS-induced damage model [transgenic model (hsp70-lacZ)Bg']</td>
<td>↑: GSH level; ↓: level of MDA and PC; activity of GST and CAT (Khanam et al., 2017)</td>
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<td>Citrus aurantium</td>
<td>40 mg/g</td>
<td>1–3-day old flies</td>
<td>5 days Wild type (Harwich)</td>
<td>↑: activity of CAT and GST; TSH level; ↓: ROS level (Abolajii et al., 2017)</td>
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<td>Chlorella pyrenoidosa polysaccharides</td>
<td>0.25%, 0.5%, 1.0% (w/v)</td>
<td>Newly eclosed flies</td>
<td>5 days Wild type</td>
<td>↑: activity of SOD, GPx and CAT; ↓: AChE activity, β-galactosidase activity and expression; tissue damage; apoptotic index and DNA damage of midgut cells (Y. Chen et al., 2018)</td>
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<tr>
<td>Cocoa</td>
<td>50 and 100 mg/mL</td>
<td>Newly eclosed males</td>
<td>Until death Wild type (rosy'); CuZn-SOD or Mn-SOD gene-silenced model; hyperoxia, copper (II) or iron (III)-induced stress models</td>
<td>↑: resistance to hyperoxia stress; ↓: lifespan; egg-to-adult viability with copper or iron exposure (Bahadorani &amp; Hilliker, 2008); ↓: climbing ability</td>
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<tr>
<td>Coffee</td>
<td>1.5% (w/w)</td>
<td>Third instar larvae</td>
<td>1 day Wild type (Oregon-K); cyclophosphamide-induced stress model</td>
<td>↑: GSH level; activity of GST, CAT and SOD; ↓: MDA level; ↓: cyclophosphamide-induced lethal mutation (Nagpal &amp; Abraham, 2019)</td>
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<tr>
<td>Creatine</td>
<td>5 and 10 mmol/L</td>
<td>8–10-day old males</td>
<td>7 days Wild type (Oregon K); rotenone- or paraquat-induced stress model</td>
<td>↑: GSH level; resistance to oxidative stress; ↓: level of MDA, HP, NO and ROS; ↓: dopamine level; mitochondrial activities (Hosamani et al., 2010)</td>
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<tr>
<td>Curcumin</td>
<td>250 μmol/L</td>
<td>1–2-day old flies</td>
<td>14 days Wild type (Canton-S and Ives)</td>
<td>↑: resistance to oxidative stress; ↑: climbing ability; spontaneous locomotion; ↓: expression of longevity assurance genes (K.-S. Lee et al., 2010)</td>
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<tr>
<td>Substance</td>
<td>Concentration</td>
<td>Treatment</td>
<td>Age</td>
<td>Effect(s)</td>
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<tr>
<td><strong>Curcumin</strong></td>
<td>0.5 and 1.0 mg/g</td>
<td>Newly emerged flies</td>
<td>7 or 21 days</td>
<td>Wild type (Oregon R) †: activity and expression of SOD †: lifespan ↓: MDA level ↓: expression of aging-related genes (Shen et al., 2013)</td>
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<tr>
<td><strong>Curcumin</strong></td>
<td>5 and 10 μmol/L</td>
<td>8–10-day old males</td>
<td>7 days</td>
<td>Wild type (Oregon K); acrylamide-induced stress model †: level of GSH and TSH; activity of TRR, GST, SOD and CAT ↑: climbing ability; activity of SDH and CS; dopamine level ↓: level of ROS, HP and PC ↓: mortality; AChE activity (Prasad &amp; Muralidhara, 2014)</td>
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<td><strong>Curcuma longa</strong> rhizome powder</td>
<td>0.25–0.70 g/100 mL</td>
<td>Newly emerged flies</td>
<td>-</td>
<td>Wild type †: activity of SOD and CAT †: lifespan (Rawal, Singh, Gupta, &amp; Mohanty, 2014)</td>
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<tr>
<td><strong>Decalepis hamiltonii extract</strong></td>
<td>0.1% and 0.5% (w/v)</td>
<td>2-day old males</td>
<td>21 days</td>
<td>PD models with missense mutations (A30P and A53T) of α-synuclein gene †: activity of SOD and CAT; resistance to oxidative stress ↓: level of MDA and ROS ↑: climbing ability; circadian rhythm of locomotor activity (S. R. Jahromi et al., 2015)</td>
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<tr>
<td><strong>Decalepis hamiltonii extract</strong></td>
<td>0.1% and 0.5% (w/v)</td>
<td>2-day old males</td>
<td>14 days</td>
<td>Wild type (Oregon K) †: activity of SOD and CAT; resistance to oxidative stress; GSH level ↓: AChE activity ↓: MDA level (Samaneh Reiszadeh Jahromi et al., 2013)</td>
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<tr>
<td><strong>Decalepis hamiltonii extract</strong></td>
<td>0.1% (w/v)</td>
<td>First instar larvae</td>
<td>Up to 55th day of adult stage</td>
<td>Wild type (Oregon K) †: activity of SOD and CAT ↑: cognitive ability (Haddadi, Jahromi, Shivanandappa, &amp; Ramesh, 2013)</td>
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<tr>
<td><strong>Edible bird's nests</strong></td>
<td>1, 3 and 9 g/kg</td>
<td>Flies eclosed within 8 h</td>
<td>29 days</td>
<td>Wild type †: CAT activity; T-AOC ↑: lifespan; resistance to heat-stress; fecundity (Q. Hu et al., 2016)</td>
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<tr>
<td><strong>Emblica officinalis fruit juice</strong></td>
<td>20 mL/100 mL</td>
<td>Newly emerged flies</td>
<td>-</td>
<td>Wild type †: activity of SOD and CAT ↑: lifespan (Rawal et al., 2014)</td>
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<tr>
<td><strong>Geraniol</strong></td>
<td>10, 20 and 40 μmol/L</td>
<td>Flies</td>
<td>24 days</td>
<td>PD models with missense mutations (A30P and A53T) of α-synuclein gene †: GSH level; ↓: level of MDA and PC; GST activity ↑: climbing ability; dopaminergic level (Siddique et al., 2016)</td>
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<tr>
<td><strong>Ginger extract</strong></td>
<td>1 and 2 mg/mL</td>
<td>3-day old males</td>
<td>30 days</td>
<td>Wild type (w1118) †: expression of CAT and Mn-SOD ↑: lifespan; metabolic function ↓: MTH expression (Zhou et al., 2018)</td>
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<tr>
<td><strong>Green tea catechin</strong></td>
<td>10 mg/mL</td>
<td>2-day old males</td>
<td>20 days</td>
<td>Wild type (Oregon-R-C); †: activity and expression of CAT, GuZn-SOD ↑: lifespan (Li et al., 2007)</td>
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<td>Extract/Polysaccharides</td>
<td>Concentration/Dilution</td>
<td>Species/Model</td>
<td>Age</td>
<td>Activity</td>
<td>Protection</td>
<td>Ref.</td>
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<tr>
<td>Hesperidin</td>
<td>0.1%</td>
<td>Flies</td>
<td>14 days</td>
<td>Wild type; clock mutant Cry2; rotenone-induced oxidative stress model</td>
<td>↓: MDA level; ↑: activity of SOD, CAT and GST; GSH level</td>
<td>(Arumugam et al., 2018; Manjula et al., 2017)</td>
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<tr>
<td>Ilex paraguariensis</td>
<td>1mL/30mL</td>
<td>Diet</td>
<td>10 days</td>
<td>Cholesterol-induced oxidative stress model</td>
<td>↑: GST activity; ↓: MDA and PC levels</td>
<td>(Colpo et al., 2018)</td>
<td></td>
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<tr>
<td>Lycium barbarum and Lentinus edodes polysaccharides</td>
<td>0.2–2 mg/g</td>
<td>Newly emerged flies</td>
<td>7 or 21 days</td>
<td>Wild type</td>
<td>↑: activity of T-SOD, CuZn-SOD and CAT; resistance to oxidative stress</td>
<td>(Tang et al., 2019)</td>
<td></td>
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<tr>
<td>Lycopene</td>
<td>2.5, 7.5 and 22.5 mg/kg</td>
<td>Newly emerged flies</td>
<td>15 or 30 days</td>
<td>Wild type (Oregon-K)</td>
<td>↑: SOD activity; ↓: MDA level; ↑: lifespan; sexual potency; fertility</td>
<td>(W. Hu et al., 2013)</td>
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<tr>
<td>Lutein</td>
<td>0.03 and 0.1 mg/mL</td>
<td>2-day-old male flies</td>
<td>20, 30 or 35 days</td>
<td>Wild type (Oregon-R-C)</td>
<td>↑: activity and expression of CuZn-SOD, Mn-SOD and CAT; resistance to oxidative stress</td>
<td>(Z. Zhang et al., 2014)</td>
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<tr>
<td>Luteolin</td>
<td>5–20 μmol/L</td>
<td>Newly eclosed male flies</td>
<td>30 days</td>
<td>Human Aβ42 transgenic model</td>
<td>↑: GSH level; ↓: level of MDA and PC; activity of SOD, GPx and GST</td>
<td>(Ali et al., 2019)</td>
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<tr>
<td>Rosemary</td>
<td>0.5 and 1.5 mg/mL</td>
<td>2-day-old male flies</td>
<td>45 days</td>
<td>Lard-induced oxidative stress model (Oregon-R-C)</td>
<td>↑: activity and expression of CuZn-SOD, Mn-SOD and CAT; ↑: lifespan; climbing ability; expression of Mth and HRF2</td>
<td>(H.-l. Wang et al., 2017)</td>
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<tr>
<td>Royal jelly-collagen peptide powder</td>
<td>1–5 mg/mL</td>
<td>Newly unmated males</td>
<td>7, 21 or 42 days</td>
<td>Wild type (Canton-S)</td>
<td>↑: activity of T-SOD, CAT and GPx; resistance to oxidative stress</td>
<td>(Qiu et al., 2020)</td>
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<tr>
<td>Royal jelly</td>
<td>1.25%, 2.50%</td>
<td>Newly</td>
<td>7 or 21 days</td>
<td>Wild type (Canton-S)</td>
<td>↑: activity of T-SOD and CuZn-SOD; ↓: lifespan; fecundity, expression of S6K,</td>
<td>(Xin et al., 2016)</td>
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<tr>
<td>Natural Products</td>
<td>Concentration</td>
<td>Life Stage</td>
<td>Duration</td>
<td>Model</td>
<td>Protecive Effect</td>
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<tr>
<td><strong>Proteins</strong></td>
<td>5.0% (w/w)</td>
<td>Emerged flies</td>
<td>CuZn-SOD expression ↓: MDA level</td>
<td>MAPK and Egfr ↓: level of HP and ketodienes</td>
<td>(Mylnikov et al., 2005)</td>
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<tr>
<td><strong>Rubus fruit juices</strong></td>
<td>~2.3%</td>
<td>Second instar larvae</td>
<td>Low-activity model</td>
<td>MDA level</td>
<td>(Mylnikov et al., 2005)</td>
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<tr>
<td><strong>Sargassum fusiforme</strong></td>
<td>0.8 and 1.6 g/L</td>
<td>Virgin flies</td>
<td>Wild type</td>
<td>↑: activity of SOD, GPx and CAT; GSH/ GSSG ratio ↓: level of MDA and GSSG</td>
<td>(Y. Zhang et al., 2019)</td>
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<tr>
<td><strong>Rubus fruit juices</strong></td>
<td>0.125–0.5 mg/mL</td>
<td>Flies closed</td>
<td>Cd-induced immune damage</td>
<td>↑: activity of SOD, GPx and T-AOC ↓: MDA and GSSG</td>
<td>(J. Su et al., 2018)</td>
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<tr>
<td><strong>Tea polyphenols</strong></td>
<td>0.25%, 0.5% and 1%</td>
<td>Third instar larvae</td>
<td>γ-radiation induced oxidative stress model (Oregon-K)</td>
<td>↑: activity of SOD, GST and CAT; GSH level ↓: LPO level</td>
<td>(Nagpal &amp; Abraham, 2017b)</td>
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<tr>
<td><strong>Whey protein isolate</strong></td>
<td>0.25% and 0.5%</td>
<td>8–10-day old males</td>
<td>Mn-induced stress model (Oregon-K)</td>
<td>↑: TRR activity; level of GSH and TSH ↓: Manganese chloride-lethality</td>
<td>(Mohandas et al., 2017)</td>
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</table>

Abbreviations: AChE, acetylcholine esterase; Cnc, cap’n’collar; CncC, cap’n’collar isoform-C; CS, citrate synthase; CuZn-SOD, Copper and zinc superoxide dismutase; dSir2, Drosophila silent information regulator 2; EgFr, epidermal growth-factor receptor; GCLC, glutamate-cysteine ligase catalytic subunit; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione S-transferase; HO, heme oxygenase; HP, hydroperoxide; Hsp27, heat shock protein 27; Keap1, Kelch-like ECH-associated protein1; LPO, lipid peroxide; MAPK, mitogen-activation protein kinase; MDA, malondialdehyde; MMS, methyl methanesulphonate; Mn-SOD, manganese superoxide dismutase; ND, no detection; NO, nitric oxide; Nrf2, nuclear factor erythroid 2-related factor 2; PC, protein carbonyls; PD, Parkinson's disease; ROS, reactive oxygen species; SDH, succinate dehydrogenase; SLRL, sex-linked recessive lethal; SOD, superoxide dismutase; T-AOC, total antioxidation capacity; TRR, thioredoxin reductase; TSH, total thiols.
<table>
<thead>
<tr>
<th>Stress inducers</th>
<th>Inducer concentration</th>
<th>Subjects</th>
<th>Inductive duration</th>
<th>Oxidative markers</th>
<th>Other effects</th>
<th>References</th>
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<tbody>
<tr>
<td><strong>Models induced by free radical generators</strong></td>
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<tr>
<td>H$_2$O$_2$</td>
<td>Filter paper moistened with 100 µL of 88 µmol/L H$_2$O$_2$ in a 1% sucrose solution</td>
<td>5-day old flies (Canton-S)</td>
<td>4 h</td>
<td>↑: PC level</td>
<td>↑: expression of heat shock protein-70; IL-6 homolog and nitric oxide synthase</td>
<td>(Subramanian et al., 2017)</td>
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<tr>
<td>Paraquat</td>
<td>Filter paper saturated with 20 µmol/L paraquat in a 5% sucrose solution</td>
<td>2–3-day old male flies (Oregon R)</td>
<td>24 h</td>
<td>↑: ROS level; ↓: expression and activity of CuZn-SOD, Mn-SOD and CAT</td>
<td>↑: mortality; AChE activity</td>
<td>(Park et al., 2012)</td>
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<tr>
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<td>Filter paper saturated with 10 µmol/L paraquat in a 4% sucrose solution</td>
<td>2-day-old flies (Canton-S)</td>
<td>60 h</td>
<td>↑: level of ROS and MDA; activity of CAT and GST; expression of CAT and SOD</td>
<td>↑: mortality; ↓: climbing ability; expression of gstD1 and mth</td>
<td>(Duavy et al., 2019)</td>
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<td></td>
<td>Filter paper saturated with 0.44 mg/g diet</td>
<td>1–5-day old flies (Harwhich)</td>
<td>7 days</td>
<td>↑: MDA level</td>
<td>↑: mortality; ↓: cell viability</td>
<td>(dos Santos Nunes et al., 2019)</td>
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<tr>
<td><strong>Models induced by toxicants or drugs</strong></td>
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<tr>
<td>Acrylamide</td>
<td>5 mmol/L diet</td>
<td>8–10-day old males (Oregon K)</td>
<td>7 days</td>
<td>↑: level of ROS and HP; GST activity; ↓: activity of TRR, SOD and CAT; level of GSH and TSH</td>
<td>↑: mortality; AChE activity; ↓: climbing ability; dopamine level; citrate synthase activity</td>
<td>(Prasad &amp; Muralidhara, 2014)</td>
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<tr>
<td>Cyclophosphamide</td>
<td>2.3 µmol/g diet</td>
<td>Third instar larvae (Oregon K)</td>
<td>1 day</td>
<td>↑: LPO level; ↓: activities of GST, CAT and SOD; GSH level</td>
<td>↑: lethal mutation</td>
<td>(Nagpal &amp; Abraham, 2019)</td>
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<tr>
<td>Methyl methanesulphonate</td>
<td>0.5 µg/mL diet</td>
<td>Third instar larvae [transgenic (hsp70-lacZ)Bg9]</td>
<td>48 h</td>
<td>↑: level of LPO and PC; activity of GST and CAT; ↓: GSH level</td>
<td>↑: β-galactosidase activity and expression; intestinal damage; ↓: AChE activity</td>
<td>(Khanam et al., 2017)</td>
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<tr>
<td>Rotenone</td>
<td>500 µmol/L diet</td>
<td>8–10-day old males (Oregon K)</td>
<td>7 or 14 days</td>
<td>↑: level of ROS, NO, HP, PC and MDA; ↓: activity of SOD, Gpx and T-AOC; GSH level</td>
<td>↑: mortality; AChE activity; ↓: climbing ability; dopamine level, mitochondrial activities</td>
<td>(Hosamani et al., 2010; Krishna &amp; Muralidhara, 2016); (Manjula et al., 2017)</td>
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<tr>
<td>Toluene</td>
<td>200 mmol/L diet</td>
<td>Third instar larvae (Oregon wild-type)</td>
<td>Until eclosion</td>
<td>↑: level of CAT, GST and SOD</td>
<td>↓: fecundity; fertility; lifespan; developmental time</td>
<td>(Pb et al., 2020)</td>
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<tr>
<td>Trichloroethylene</td>
<td>1 µmol/g diet</td>
<td>1–3-day old flies</td>
<td>5 days</td>
<td>↑: ROS level</td>
<td>↑: AChE activity</td>
<td>(Abolaji et al., 2017)</td>
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<tr>
<td>Models induced by transition metals and radiation</td>
<td>(Harwich)</td>
<td>↓: activity of CAT and GST; TSH content</td>
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<tr>
<td>Cadmium</td>
<td>1.0 μg/mL diet</td>
<td>Flies eclosed within 8 h 10 days</td>
<td>↑: MDA level ↓: activity of SOD, GPx and T-AOC ↑: MDA level ↓: activity of SOD, GPx and T-AOC ↓: NO level; activation of immune- and antiaging-related pathways</td>
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<tr>
<td>Manganese chloride</td>
<td>15 mmol/L diet</td>
<td>8–10-day old male flies 5 days</td>
<td>↑: level of MDA and PC; GST activity ↓: TRR activity; level of GSH and TSH ↓: NO level; activation of immune- and antiaging-related pathways ↓: NO level; activation of immune- and antiaging-related pathways ↓: NO level; activation of immune- and antiaging-related pathways</td>
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<tr>
<td>γ-radiation</td>
<td>10 Gy at a dose rate of 1.8 Gy/min</td>
<td>Third instar larva (Oregon-K) -</td>
<td>↑: LPO level ↓: GSH level; activity of GST, CAT and SOD ↓: GSH level; activity of GST, CAT and SOD ↓: GSH level; activity of GST, CAT and SOD ↓: GSH level; activity of GST, CAT and SOD ↓: GSH level; activity of GST, CAT and SOD</td>
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<tr>
<td>Models induced by fats or carbohydrate</td>
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<tr>
<td>Lard</td>
<td>10% of diet</td>
<td>2-day old male flies (Oregon-R-C) 45 days</td>
<td>↓: activity and expression of CuZn-SOD, Mn-SOD and CAT ↓: NO level; activation of immune- and antiaging-related pathways ↓: NO level; activation of immune- and antiaging-related pathways ↓: NO level; activation of immune- and antiaging-related pathways ↓: NO level; activation of immune- and antiaging-related pathways ↓: NO level; activation of immune- and antiaging-related pathways</td>
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<tr>
<td>Cholesterol</td>
<td>0.5 μmol/g diet</td>
<td>2–3-day-old male flies (Harwich) 10 days</td>
<td>↑: MDA and PC levels ↓: expression of CuZn-SOD, Mn-SOD and CAT ↓: survival against heat, cold or starvation ↓: survival against heat, cold or starvation ↓: survival against heat, cold or starvation ↓: survival against heat, cold or starvation</td>
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<tr>
<td>D-galactose</td>
<td>6% (w/w) of diet (instead of sucrose in the basal diet)</td>
<td>Oregon-R flies 4 weeks</td>
<td>↑: level of MDA and AOPP ↓: CuZn-SOD activity ↓: CuZn-SOD activity ↓: CuZn-SOD activity ↓: CuZn-SOD activity ↓: CuZn-SOD activity</td>
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Abbreviations: AChE, acetylcholine esterase; AOPP, advanced oxidative protein product; CuZn-SOD, Copper and zinc superoxide dismutase; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione S-transferase; HP, hydroperoxide; LPO, lipid peroxide; MDA, malondialdehyde; Mn-SOD, manganese superoxide dismutase; NO, nitric oxide; PC, protein carbonyls; ROS, reactive oxygen species; SOD, superoxide dismutase; T-AOC, total antioxidation capacity; TRR, thioredoxin reductase; TSH, total thiols.
Figure 1

The graph shows the number of Fruit fly- and Mouse-related publications from Jan. 1990 to Dec. 2020. The x-axis represents the publication date, while the y-axis represents the number of publications. The data is depicted with two trends, one for Fruit fly-related publications and another for Mouse-related publications.
Figure 2

Oxidative stress

- Free radical generators
- Drugs
- Toxicants
- Radiation
- Transition metals
- High-calorie diets

Antioxidants

- MAPK
- p53

RNS

- ROS

Keap1

Antioxidant enzymes: SOD, CAT, GPx, HO1, NQO1, etc.

Transcription
**Figure 3**

<table>
<thead>
<tr>
<th>Selection of models</th>
<th>Drosophila subjects</th>
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<tbody>
<tr>
<td>1) Wild type with normal oxidative status</td>
<td>1) Larva (mostly third instar larvae)</td>
</tr>
<tr>
<td>2) Models with antioxidative defects (such as SOD, Cat and DJ-1β mutants)</td>
<td>2) Adult (mostly 1~3-day old males)</td>
</tr>
<tr>
<td>3) Models with oxidative stress (induced by chemicals or high-calorie diets)</td>
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</table>

<table>
<thead>
<tr>
<th>Feeding</th>
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<tbody>
<tr>
<td>1) Preparation of the testing diet (considering concentration, dispersibility and stability of the samples)</td>
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<tr>
<td>2) Feeding assay (without calorie restriction)</td>
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<tr>
<td>3) Administration (with regular circadian rhythm and multiple feeding durations)</td>
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<tr>
<td>4) Induction of oxidative stress (before, during or after antioxidant supplementation)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Antioxidant evaluation</th>
</tr>
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<tbody>
<tr>
<td>1) Measurement of reactive species (ROS, H₂O₂, NO, etc.)</td>
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<tr>
<td>2) Assay detecting endogenous antioxidants (SOD, CAT, GST, GPx, TRR, GSH/GSSG, T-AOC, etc.)</td>
</tr>
<tr>
<td>3) Assessment of oxidative damage to biomacromolecules (LPO, HP, MDA, PC, TSH, etc.)</td>
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<td>4) Evaluation of resistance against oxidative stress (e.g., caused by paraquat or H₂O₂)</td>
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<td>5) Analyses on physiological benefits related to antioxidative activities (lifespan, climbing ability, neuroprotection, immunomodulation, etc.)</td>
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</tbody>
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<th>Antioxidant mechanisms</th>
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<td>1) Chemical mechanisms (i.e., free radical scavenging, reducing capacity and metal chelating)</td>
</tr>
<tr>
<td>2) Effects on the CncC/ARE pathway</td>
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<td>3) Other adaptive responses (MAPKs, REL, p53, JNK, etc.)</td>
</tr>
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</table>