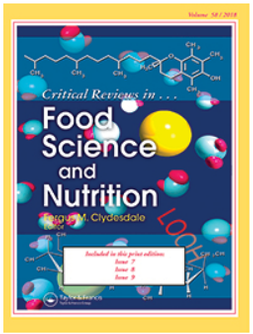

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Interactions between phytochemicals from fruits and vegetables: Effects on bioactivities and bioavailability

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ABSTRACT

The combinations of two or more phytochemicals bring about changes in the ultimate biological effects and/or the bioavailability of each component. A number of mixtures of pure bioactive compounds or phytochemical-containing plant extracts provide synergy with regard to antioxidant status, anti-inflammation, anti-cancer and chemoprevention of several oxidative stress and metabolic disorders *in vitro*. The biological activities of food phytochemicals depend upon their bioaccessibility and bioavailability which can be affected by the presence of other food components including other bioactive constituents. The interactions between phytochemicals during intestinal absorption could result in changes in the bioavailability of the compounds, which in turn affects the intensity of their bioactivities. This paper provides an overview of combined biological effects of phytochemical mixtures derived from fruits and vegetables with a focus on anti-oxidative, anti-inflammatory and anti-carcinogenic activities. The bioavailability impairment or enhancement caused by the co-consumption of dietary phytochemicals is also discussed. Finally, research gaps for future studies on phytochemical interactions are identified.

KEYWORDS

Bioactive compounds; phytochemical interaction; antioxidant synergy; bioavailability; antioxidant capacity; anti-inflammation; anti-carcinogen

Introduction

The risks of many chronic disorders (e.g. cancer, diabetes, or cardiovascular diseases) may be reduced by the regular consumption of fruits and vegetables or other plant-based foods (Graf et al., 2005; Wang, 2012a; Fang, 2014). A number of bioactive compounds naturally present in fruits and vegetables are effective agents for reducing the onset of oxidation and inflammation (Hollman et al., 1999; Galvanoa et al., 2004; Maiani et al., 2009; Metzler et al., 2013). The combinations of different pure bioactive compounds or their extracts from food sources can enhance the benefits conferred by individual bioactive compounds (Becker et al., 2007; Hidalgo et al., 2010; Wang et al., 2011; Jiang et al., 2015). For instance, α-tocopherol mixed with a flavonol (kaempferol or myricetin) is more effective in inhibiting lipid oxidation induced by free radicals than each component alone (Zhou et al., 2005a) or the mixture of resveratrol, chrysin, and curcumin synergistically suppressed the proliferation of colorectal cancer cells (Iwuchukwu et al., 2011). In some cases, however, the combination of phytochemicals may lower the biological effects if they are combined in inappropriate ratios (Hidalgo et al., 2010; Jiang et al., 2015), or if the participant compounds form hydrogen bonds at active hydroxyl groups that decrease their capability to scavenge free radicals (Hidalgo et al., 2010), or they do not have proper orientation / distribution in lipid/water phases to facilitate the interaction (Liang et al., 2009a).

Bioactive compounds may interfere with intestinal absorption of other compounds. There are multiple phytochemicals in a single fruit or vegetable. People usually consume many vegetables in meals or fruits in desserts or drinks. Thus, concurrently consumed bioactive compounds may affect the intestinal absorption of each other. The interactions of phytochemicals may enhance or reduce the bioavailability of a given compound, depending on the facilitation/competition for cellular uptake and transportation taking place between them (Reboul et al., 2007a; Claudie et al., 2013; Fale et al., 2013). For example, β-carotene increases the bioavailability of lycopene in human plasma (Böhm and Bitsch, 1999), and quercetin-3-glucoside reduces the absorption of anthocyanins in rat jejunum mounted in Ussing chambers (Walton et al., 2006).

The interactions of some major classes of food bioactive compounds such as carotenoids and flavonoids with other macromolecules (food matrices, blood proteins, digestive enzymes or intestinal transporters) have been reviewed (van den Berg, 1999; Parada and Aguilera, 2007; Yang et al., 2011; Gonzales et al., 2015). Nevertheless, there is no comprehensive review to date that provides overall understanding of bioactivity and bioavailability interactions among dietary bioactive compounds. Therefore, this paper presents for the first time interactive effects on bioactivities and bioavailability of dietary phytochemical combinations derived from fruits and vegetables.

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Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/bfsn.

Effects of phytochemical interactions on biological activities

Modes of phytochemical interaction

Bioactive compound mixtures may produce a biological effect higher or lower than the summative effects of each single component. The effects of phytochemical interactions can be classified as potentiation, addition, synergy, or antagonism. There is confusion in literature about the difference between potentiation and synergy (Efferth and Koch, 2011). If the phytochemical mixture containing two compounds, in which one is active and the other is inactive, produces a greater effect than that of its single active component, the effect is defined as potentiation: the presence of the inactive compound enhances the potency of the active one (Chou, 2006; Efferth and Koch, 2011). If each component of the mixture is active, their mixture can produce an additive, synergistic or antagonistic effect. In additive phytochemical interactions, the combined effect is equal to the sum potency of individual components of the mixture. In synergistic or antagonistic interactions, the combined effect is analyzed by isobologram or combination index that shows greater or less (respectively) than addition (Chou, 2006). These definitions of potentiation, addition, synergy and antagonism will be used in this review.

Methods to analyze phytochemical combination data

Because plant phytochemicals are natural drugs, one can analyze results of phytochemical combination studies by using methods used for drug combination analysis. These approaches have been described in detail elsewhere (Tallarida, 2001; Chou, 2006; Bulusu et al., 2016). The following methods have been commonly used to determine the types of interaction of binary mixtures of phytochemicals:

(i) Isobologram (Tallarida, 2001):illustrates an iso-dose effect of two phytochemicals (Efferth and Koch, 2011). A concave isobologram indicates synergy, and a convex one indicates antagonism (Figure 1).

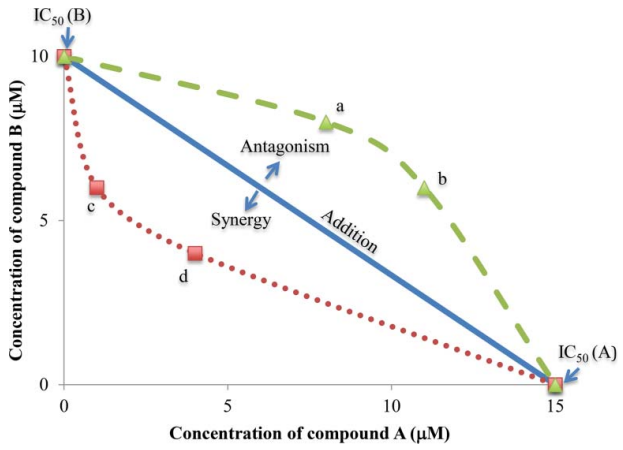

Figure 1. Isobologram of binary mixture at 50% activity (IC_{50}): Addition: a line formed from IC_{50} of compound A and IC_{50} of compound B: any combination points fall in this line indicate additive effect; Synergy: lower left area of addition line: any combination points that fall in this area (e.g., points c and d: form a concave curve) indicate synergistic effect; Antagonism: upper left area of addition line: any combination points that fall in this area (e.g., points a and b: form a convex curve) indicate antagonistic effect.

(ii) Combination Index (CI) (Chou, 2006): calculation of CI is shown in equation (1) and (2). CI < 1 indicates synergy; CI = 1 addition; CI > 1 antagonism.

For binary combination at 50% activity:

$$CI_{50} = \frac{C_A}{IC_{50}(A)} + \frac{C_B}{IC_{50}(B)} \quad (1)$$

where CI_{50} is Combination Index for the binary mixture at 50% activity; C_A and C_B is the proportional dose of compound A and compound B (respectively) in the mixture that shows 50% activity; $IC_{50}(A)$ and $IC_{50}(B)$ is the single dose of each compound A and B that provides 50% activity.

For *n*-phytochemical combination at x% inhibition:

$$(CI_x)_n = \sum_{j=1}^{n} \frac{(C)_j}{(IC_x)_j} \quad (2)$$

where $(CI_x)_n$ is Combination Index for *n*-compound mixture at x% activity; C_j is the proportional dose of each compound in *n*-phytochemical mixture that shows x% activity; $(IC_x)_j$ is the single dose of each compound that provides x% activity.

Each of these approaches relies on a dose–effect plot of each component, from which the IC_{50} value (defined as the concentration of a compound that gives 50% activity) is determined and used for determination of interaction mode. These methods are used for assays where measured activity is a value reversely related to the compound concentration: a lower IC_{50} indicates a higher activity. Software (CompuSyn, Combenefit, CalcuSyn, SynergyFinder™, Genedata Screener®, Chalice) may be employed for analysis of the performance of the mixture (Bulusu et al., 2016). In some studies, the dose–effect curve (IC_{50} value) of each phytochemical component is determined but neither method is used to analyze the interaction mode. The interaction mode in these studies is determined by comparing the experimental effect of the mixture with the sum effects of each component. The latter, in 1:1-ratio mixtures, is calculated by taking the average of the IC_{50} values of the two components. Synergy or antagonism may appear possible, but may not be validly claimed because this calculation is correct only when the components are combined at their equal-potency ratio (IC_{50}-of-compound-1: IC_{50}-of-compound-2) (Chou, 2006). The terms: potential synergy or potential antagonism will be used to describe such results in this review.

When the assessments of biological activities of phytochemicals do not rely on IC_{50} values, the interaction mode is not determined by either isobologram or combination index method, but by comparing the experimental effect of the mixture with the sum of effects of each phytochemical component. The two methods may not be appropriate to assess the contribution of individual phytochemicals to the interactive effects observed in complex mixtures containing multi phytochemical components (such as those occurring in food or herbal extracts) (Efferth and Koch, 2011). In practice, the interactive

effects seen in an extract mixture can be compared to that obtained from the mixtures of isolated principle active phytochemicals at concentrations equivalent to those in the extracts (Efferth and Koch, 2011). If these two effects are equal, the ultimate effects observed in the combined extracts are from the interaction between the major active phytochemicals. If they are unequal, there are compounds in the extracts other than the major active phytochemicals contributing to the effects (Efferth and Koch, 2011).

Interactive effects of bioactive compounds on antioxidant activities

Methods for studying antioxidant interactions

It is important to select an appropriate well-developed and validated antioxidant assay that can effectively measure the antioxidant power of bioactive compounds or food extracts. Antioxidant interactions can be studied by either chemical-based or biological-based assays.

Chemical assays

Chemical assays have been extensively used because they are simple, cost less, and samples are easy to handle (Prior et al., 2005; Schaich, 2005; Niki, 2010). Chemical assays can be classified into two groups according to their reaction mechanism: hydrogen atom transfer (HAT) or electron transfer (ET). In a HAT-based assay, there is competition between the antioxidant and the target molecular probe (substrate) for the reaction with peroxyl radicals generated by an azo compound (Huang et al., 2005; Özyürek et al., 2008). The peroxyl radicals preferentially abstract hydrogen atoms from the antioxidant resulting in the suppression of the reaction between the radicals and the probe. Oxygen radical absorbance capacity (ORAC), total radical trapping antioxidant parameter (TRAP), inhibition of induced low-density lipoprotein (LDL) autoxidation and crocin bleaching assays are based on the HAT mechanism (Huang et al., 2005). In an ET-based assay, the antioxidant capacity is evaluated from the changes in the color of a chromogenic oxidant when reduced by an antioxidant (Huang et al., 2005; Özyürek et al., 2008). The reaction is initiated by the transfer of electrons from the antioxidant to the oxidizing reagent (Huang et al., 2005). An array of ET-based assays has been developed: 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS)/Trolox equivalence antioxidant capacity (TEAC), Folin – Ciocalteu reagent (FCR), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay, ferric ion reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC) (Huang et al., 2005; Özyürek et al., 2008).

It is essential to consider the solubility of the antioxidants in the reaction media (Özyürek et al., 2008). In most chemical assays, the reaction medium is single-phase water-based or alcohol-water-based. The main assay reagents usually have high affinity towards aqueous solvents (Özyürek et al., 2008). Thus, the majority of these methods study the interactions between hydrophilic antioxidants in homogeneous systems. HAT- and ET-based assays are run in hydrophilic homogeneous media. Some assays, such as ABTS/TEAC and CUPRAC assays are applicable for both hydrophilic and lipophilic antioxidants (MacDonald−Wicks et al., 2006; Özyürek et al., 2008). These methods enable efficient measurement of the total antioxidant capacity of food samples containing both water- and oil-soluble antioxidants, and are often selected for studying the interactions between hydrophilic and/or lipophilic antioxidant mixtures. Like the ET-based assay they use a neutral or univalent-charged chromophore (Özyürek et al., 2008). Other chemical assays use heterogeneous media (multi-phase) media. These multi-phase systems allow the investigation of phytochemical interactions at a water–lipid interface and avoid the interferences of organic solvents with interactive effects and permit the assessment of the performance or interactions of antioxidants in physiological conditions (Roberts and Gordon, 2003). Some multi-phase assays investigate oxidative inhibition of emulsions (water-in-oil, oil-in-water) (Becker et al., 2007; Yin et al., 2012), or of artificial biological membranes (liposomes or micelles) (Stahl et al., 1998; Shi et al., 2004; Zhou et al., 2005a; Bermudez-Soto et al., 2007; Dai et al., 2008).

Biological assays

In vitro biological models: food products (bread, chicken breast, turkey meatball), cultivated living cells, human plasma, and human low-density lipoprotein, and *in vivo* biological models: animals and humans have been used to examine phytochemical interactions (Fuhrman et al., 2000; Bruno et al., 2006; Ajuwon et al., 2013). A recent review by Wang and Zhu (2015) discussed antioxidant synergy in these systems. Although *in vitro* biological assays are more effective than heterogeneous chemical assays in mimicking *in vivo* conditions, their uses in phytochemical interaction studies are less common because of the high cost and the complexity of the analysis. *In vivo* assays are even more costly and complicated. To date, there is no model optimal for the study of antioxidant interactions *in vivo*(Wang and Zhu, 2015).

Antioxidant interactions in simple and complex mixtures

Changes of antioxidant capacities when combining pure antioxidants

Combining different pure or isolated phytochemicals could produce nonadditive anti-oxidative effects. Lists of antioxidant mixtures that exert antagonistic and synergistic effects on anti-oxidation are shown in Table 1 and Table 2, respectively. Interactions can occur between hydrophilic antioxidants. Polyphenolic compounds in green tea extracts have mutual interactions on antioxidant capacity when tested for DPPH$^{\bullet}$ radical scavenging activity. Combinations containing gallocatechin gallate (GCG), epigallocatechin gallate (EGCG), catechin gallate (CG) and epicatechin gallate (ECG) show synergy (Colon and Nerín, 2016). Hyperoside, one of the major phenolic compounds in *Potentilla fruticosa* L. leaves (a traditional Chinese tea), acts in synergy with ECG in green tea on ABTS$^{\bullet}$ and DPPH$^{\bullet}$ radical scavenging activities (Liu et al., 2016). The combination of the anthocyanins malvidin-3-glucoside or peonidin-3-glucoside with the flavan-3-ol catechin increases the inhibition of linoleic acid oxidation initiated by free radicals (Rossetto et al., 2002). Hidalgo et al.(2010) observed potential synergistic interactions on DPPH$^{\bullet}$ radical scavenging between cyanidin-3-glucoside and kaempferol, delphinidin-3-glucoside and kaempferol, and

Table 1. List of mixtures of bioactive compounds that exert antioxidant antagonism.

Mixtures of antioxidants	Ratio, total concentration	Type of assay	Proposed mechanisms	References
Combination of hydrophilic antioxidants				
Interaction among five major anthocyanin (ACNs) compounds (CY, DP, MV, PE, PG):				
PN + DP	1:1, 200 μM	DPPH, FRAP	Antagonistic interactions could be explained by the formation of intermolecular hydrogen bonds between two different compounds that reduced the availability of the active hydroxyl groups for radical scavenging activities.	Hidalgo et al. (2010)
PN + CY		FRAP	The interactive effects could also depend upon the compatibility of the compounds to the reaction mechanism of the antioxidant assays	
PN + MV				
ACNs and flavan-3-ols:				
PN + ECAT	1:1, 200 μM	DPPH, FRAP		
PN + CAT		DPPH		
CY + CAT				
DP + CAT				
DP + ECAT				
MV + CAT				
PG + CAT				
PG + ECAT				
ACNs and flavonols:				
PN + MYR	1:1, 200 μM	DPPH		
PN + QUE				
PN + Q3G				
DP + QUE				
DP + Q3G				
MV + KAEM				
PG + Q3G				
CY + Q3G		DPPH		
MV + QUE				
MV + Q3G				
PG + QUE				
PN + KAEM		FRAP		
Flavan-3-ols and Flavonols				
MYR + QUE	1:1, 200 μM	DPPH, FRAP		
MYR + Q3G		DPPH		
QUE + Q3G		DPPH		
Flavan-3-ols and Phenolic acid				
CAT + EGA	1:1, 5 μM	Copper-catalysed human LDL oxidation	The ability of catechin to donate hydrogen was disabled by the formation of hydrogen bonds between the carbonyl groups in ellagic acid and the *o*-dihydroxyl groups in catechin	Meyer et al. (1998)
Ascorbate and Flavonols				
ASC + RUT	2.9 μM + 4.1 μM	DPPH	Not reported	González and Nazareno (2011)
ASC + HESD	2.9 μM + 400 μM	DPPH	Not reported	González and Nazareno (2011)
ASC + NCH	2.9 μM + 25.9 μM			
ASC + NDCH	2.9 μM + 29.2 μM			
ASC + NARG	2.9 μM + 672 μM			
Combination of lipophilic antioxidants				
ASTA + ZEA	1:1, 9 μM		Similar orientation in the membrane, so not able to synergize.	Liang et al. (2009b)
LYC + γ-TOC	1:1, 10 μM	- Human LDL oxidation was initiated by copper ions ($CuSO_4$) at 37°C and assessed by the formations of TBARS - AAPH-induced LDL oxidation	LYC might cause pro-oxidative effect to γ-tocotrienol	Fuhrman et al. (2000)

(*Continued on next page*)

Table 1. (*Continued*)

Mixtures of antioxidants	Ratio, total concentration	Type of assay	Proposed mechanisms	References
Combination of lipophilic and hydrophilic antioxidants				
Carotenoid and Flavones				
β-CAR + DAI β-CAR + BAI	1:1, 3 μM 1:1, 3 μM	Liposome oxidation was initiated by AMVN and assessed by the formation of conjugated dienes at 43°C	β-CAR radicals reacted with the flavones to form β-CAR/flavone adducts leading to decreases of active antioxidant concentration in lipid phase	Liang et al. (2010)
Carotenoid or α-tocopherol and Flavan-3-ols				
β-CAR + ECAT β-CAR + EGC β-CAR + ECG β-CAR + EGCG	1:1, 3 μM	Liposome oxidation was initiated by AMVN and assessed by the formation of conjugated dienes at 45°C	β-CAR radicals reacted with the flavan-3-ols to form β-CAR/flavan-3-ols adducts leading to decreases of active antioxidant concentration in lipid phase	Song et al. (2011)
α-TOC + CGA	1:1, 5 μM		Chlorogenic acid has a steric structure and low affinity to membrane lipids, so low concentration presenting in the membrane did not show synergy with α-TOC	Neunert et al. (2015)

CY: Cyanidin-3-glucoside; DP: Delphinidin-3-glucoside, MV: Malvidin-3-glucoside, PE: Peonidin-3-glucoside, PG: Pelargonidin-3-glucoside; CAT: catechin; ECAT: epicatechin; MYR: myricitin; QUE: quercetin; Q3G: quercetin-3-glucoside; KAEM: kaempferol; EGA: ellagic acid; DDPH: 2,2-Diphenyl-1-picrylhydrazyl assay; FRAP: ferric ion reducing antioxidant power; LDL: low-density lipoprotein

RUT: rutin; ASC: ascorbic acid; HESD: hesperidin; NCH: naringenin chalcone; NDCH: naringin dihydrochalcone; NARG: naringenin; ASTA: astaxanthin; ZEA: zeaxanthin; LYC: lycopene; γ-TOC: γ-tocotrienol; DAI: daidzein; BAI: baicalein; β-CAR: β-carotene; ECAT: epicatechin; EGC: (−)-epigallocatechin, ECG: (−)-epicatechin gallate; EGCG: (−)-epigallocatechin gallate; α-TOC: α-tocopherol; CGA: chlorogenic acid; AMVN: 2,2′-azobis(2,4-dimethylvaleronitrile; DDPH: 2,2-Diphenyl-1-picrylhydrazyl assay; TBARS: thiobarbituric acid reactive substances; LDL: low-density lipoprotein

cyanidin-3-glucoside and myricetin although the interpretation of this study may not be valid because the authors did not use appropriate methods (isobologram or combination index) to determine the interactive effects.

Lipophilic antioxidants also interact. Several mixtures of carotenoids are more efficient than a single compound in oxidative inhibition (Shi et al., 2004; Shixian et al., 2005; Han et al., 2012). Binary mixtures of carotenoids: lycopene-β-carotene, lycopene-lutein and β-carotene-lutein (Zanfini et al., 2010), or the combination of α-tocopherol and lycopene (Zanfini et al., 2010; Stinco et al., 2016) show stronger ABTS radical scavenging activity than the sum of individual compounds, indicating potential synergy. Lycopene combined with α-carotene, β-carotene or lutein increases the inhibition of lipid peroxidation (Stahl et al., 1998; Shi et al., 2004), among which lycopene-lutein interaction is strongest (Stahl et al., 1998). A mixture of lycopene and astaxanthin enhances the inhibition of liposome oxidation initiated by 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN). Such cooperative antioxidant effect, however, does not occur in the combination of the xanthophylls, astaxanthin and zeaxanthin. Proper spatial orientation of the antioxidants in the lipid phase that could facilitate electron / hydrogen transfer for antioxidant regeneration is crucial for synergy in these systems (Han et al., 2012).

Synergy also occurs with combinations of lipophilic and hydrophilic bioactive compounds: carotenoids and flavonoids; carotenoids and phenolic acids; or carotenoids/tocopherols and water-soluble vitamins. These combinations synergistically improve the antioxidant capacity. For instance, when β-carotene is paired with the flavonoids puerarin, quercetin or rutin, the antioxidant capacity increases by up to 50% (Han et al., 2010; Han et al., 2011a). When lycopene is mixed with the polyphenols glabridin, rosmarinic acid or carnosic acid, oxidation of low-density lipoprotein is retarded (Fuhrman et al., 2000). The antioxidant capacity of lycopene together with glabridin is nearly twice as strong as that attained from the sum of the individual activities. Rosmarinic acid or carnosic acid combined with lycopene increases the effect by 32% and 15%, respectively (Fuhrman et al., 2000; Shi et al., 2004). α-Tocopherol paired with quercetin, caffeic acid or ferulic acid provides stronger inhibitory effects against lipid peroxidation (soybean phosphatidylcholine liposome model) than the single compounds (Becker et al., 2007; Neunert et al., 2015). Regeneration of α-tocopherol by the antioxidant partner maintains the radical scavenging activity in these systems (Becker et al., 2004).

Some combinations of phytochemicals reduce antioxidant capacity. Although α-tocopherol with caffeic acid or ferulic acid shows synergy against lipid oxidation, its combination with chlorogenic acid (2.5 μM) shows antagonism, possibly, because the steric structure of chlorogenic acid makes it unable to interact with α-tocopherols (Neunert et al., 2015). When β-carotene is combined with the flavonoids daidzein, baicalein or with green tea polyphenols ((−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG) and (−)-epigallocatechin gallate (EGCG)), the antioxidant activity is significantly decreased (Song et al., 2011).

A number of factors are essential for the combination of lipophilic and hydrophilic antioxidants to show synergy in lipid anti-oxidation. These factors include: (i) the ability of the compounds to interact at water/lipid interface, (ii) the differences in the standard redox potential between the two antioxidants, and (iii) the ability to rapidly transfer electrons from one compound to another (Liang et al., 2009b; Liang et al., 2010). Among these factors, the structure and the ability of the hydrophilic compound to orientate and position appropriately at the

Table 2. List of mixtures of bioactive compounds that exert antioxidant synergy.

Mixtures of antioxidants	Ratio, total concentration	Type of assay	Proposed mechanisms	References
Combination of hydrophilic antioxidants				
ACNs and Flavonols				
CY + Q3G	1:1, 200 μM	FRAP	Synergistic effects have resulted from the cooperative interactions between individual compounds, in which one could act as a hydrogen/electron donor to regenerate the partner.	Hidalgo et al. (2010)
MV + QUE			The interactive effects could also depend upon the compatibility of the compounds to the reaction mechanism of the antioxidant assays	
MV + Q3G				
PG + QUE				
Flavan-3-ols and Flavonols				
ECAT + MYR	1:1, 200 μM	FRAP		
ECAT + QUE				
ECAT + Q3G				
CAT + QUE				
CAT + Q3G				
Flavonols and Flavonols				
KAEM + MYR	1:1	DPPH		
KAEM + QUE				
KAEM + Q3G				
QUE + Q3G	1:01	FRAP		
QUE + RUT	1:1, 0.15 mol%	- Liposome oxidation was initiated by AAPH and assessed by the formation of conjugated dienes - Oxidation of methyl linoleate emulsion		Becker et al. (2007)
ACNs and Flavan-3-ols				
MV + CAT	1:1, 12 μM	Linoleic acid oxidation initiated by AIBP	CAT recycled MV and PE	Rossetto et al. (2002)
PE + CAT				
Tea polyphenols				
GCG + EGCG; GCG + ECG; CG + GCG; CG + ECG; EGCG + CG; EGCG + ECG	1:01	DPPH	The galloyl fragment on the C rings of these compounds has been suggested to play an important role on the antioxidant synergy	Colon and Nerin (2016)
Ascorbate and Flavonols				
ASC + QUE	2.9 μM + 3.8 μM	DPPH	QUE or NAR reacted with dehydroascorbate (DHA) to regenerate semiascorbyl radical ($SAsc^{\bullet}$) as the following equations: $Asc^{-} + DPPH^{\bullet} \rightarrow SAsc^{\bullet} + DPPH - H$ $SAsc^{\bullet} + DPPH^{\bullet} \rightarrow DHA + DPPH - H$	González and Nazareno (2011)
Ascorbate and Flavanones				
ASC + NAR	2.9 μM + 606 μM	DPPH	$FOH + DHA \rightarrow FO^{\bullet} + SAsc^{\bullet}$	González and Nazareno (2011)
Combination of lipophilic antioxidants				
β-CAR + LYC	1:1, 50 μM 1:1, 3 μM	Liposome oxidation was initiated by AMVN and assessed by the formation of TBARS	Regeneration activity Impact on signal transduction pathway	Stahl et al. (1998), Shi et al. (2004)
LUT + LYC	1:1, 50 μM 1:1, 3 μM		Differences in physiochemical properties and spatial compartmentation of the carotenoids in the membrane	Stahl et al. (1998), Shi et al. (2004)
β-CRYP + LYC	1:2, 3 μM			Stahl et al. (1998), Shi et al. (2004)
ZEA + LYC	1:2, 3 μM			
LUT + β-CAR	2:1, 3 μM			
β-CAR – α-CAR	2:1, 3 μM			
α-CAR + LYC	1:2, 3 μM			
LYC + α-CAR + β-CAR + β-CRYP + ZEA + LUT	2:1:2:1:1:2, 3 μM			
α-CAR + β-CAR + β-CRYP + ZEA + LUT	1:2:1:1:2, 3 μM			
LYC + α-CAR + β-CAR + β-CRYP + ZEA	2:1:2:1:1, 3 μM			
α-CAR + β-CAR + β-CRYP + ZEA	1:2:1:1, 3 μM			
LYC + α-CAR + β-CRYP + ZEA	2:1:1:1, 3 μM			
LYC + α-CAR + β-CAR + β-CRYP + ZEA + LUT + α-TOC	2:1:2:1:1:2:90, 3 μM			
LUT + α-TOC	1:1, 50 μM			

(*Continued on next page*)

Table 2. (*Continued*)

Mixtures of antioxidants	Ratio, total concentration	Type of assay	Proposed mechanisms	References
ASTA + LYC	1:1, 9 μM	Liposome oxidation was initiated by AMVN and assessed by the formation of conjugated dienes at 43°C. Lag phase (minutes) of liposome oxidation was calculated	Different spatial distribution in liposome: ASTA anchored to the interface while LYC and β-CAR stayed in the centre.	Liang et al. (2009b)
ASTA + β-CAR	1:1, 9 μM		Differences in E_0: LYC and β-CAR are more reducing, so able to transfer electron to regenerate ASTA.	
LYC + α-TOC	5:1, 6 μM 2:1, 7.5 μM 1:1, 10 μM 1:1, 10 μM	Human LDL oxidation was initiated by coper ions ($CuSO_4$) at 37°C and assessed by the formations of TBARS AAPH-induced LDL oxidation	Different spatial distribution in LDL: α-TOC at the surface while LYC at the core, so acted at different sites of LDL and synergistically prevent LDL oxidation α-TOC scavenged unstable lycopene free radicals which were formed when lycopene quenched oxygen molecules	Fuhrman et al. (2000)
Combination of lipophilic and hydrophilic antioxidants				
Carotenoid or α-tocopherol and Flavonols				
β-CAR + PUE		Liposome oxidation was initiated by AMVN and assessed by the formation of conjugated dienes at 43°C	Spatial distribution of the compounds facilitated the regeneration of β-CAR by the flavonols	Han et al. (2007)
β -CAR + 4′-PROP				
β -CAR + QUE	1:1, 3 μM			Liang et al. (2010)
β -CAR + RUT	1:1, 3 μM			
α-TOC + QUE	1:4, 0.15 mol%	Liposome oxidation was initiated by AAPH and assessed by the formation of conjugated dienes	High concentration of QUE at interfaces scavenged radicals initiated in aqueous phase and protected α-TOC from oxidation	Becker et al. (2007)
			Synergy was mostly because QUE trapped initiating radicals, and partly because QUE regenerated α-TOC	
	1:1, 40 μM	AAPH-induced oxidation of linoleic acid in tBuOH/water (3:2) or micelles		Zhou et al. (2005b)
α -TOC + MYR	1:1, 40 μM	AAPH-induced oxidation of linoleic acid in tBuOH/water (3:2) or micelles	Synergy was mostly because the flavonols trapped initiating radicals, and partly because the flavonols regenerated α-TOC	Zhou et al. (2005b)
α-TOC + RUT				
α-TOC + KAEM				
α-TOC + MOR				
α-TOC + QGP				
α-TOC + QRP				
α-TOC + KGP				
α-tocopherol and Flavan-3-ols				
α-TOC + CAT	1:1, 1:2, 2:1	Rancidity of sunflower oil AAPH-induced liposome oxidation	Different phase partition of antioxidants protected lipid from oxidation by radicals initiated in both phases	Yin et al. (2012)
α-TOC + ECAT	0.05 or 0.1 mmol/g***0.5% or 0.1% mol of lipid for each antioxidant			
α-TOC + ECG	1:1, 2 μM	Liposome oxidation catalyzed by iron metals	α-TOC located on membrane surface enabled synergy with the aqueous polyphenols	Murakami et al. (2003)
α-TOC + EGCG				
Combination of lipophilic and hydrophilic antioxidants				
α-tocopherol and ascorbic acid and flavan-3-ols				
α-TOC + ASC + EGCG	7.5:10:10, 27.5μM	AAPH-induced oxidation of linoleic acid micelles	ASC recycled flavan-3-ols and the latter recycled α-TOC	Dai et al. (2008)
α-TOC + ASC + ECG				
α-TOC + ASC + EGC				
α-TOC + ASC + EC				
α-TOC + QUE + ASC	1:1, 2 μM	Liposome oxidation catalyzed by iron metals		Murakami et al. (2003)
Carotenoid and ***Isoflavanes***				
LYC + GLB	5:1, 6 μM	AAPH-induced human LDL oxidation Cu^{2+}-induced human LDL oxidation	Spatial distribution of the compounds in LDL facilitated the cooperation of radical scavenging	Fuhrman et al. (2000)
β-CAR + GLB	5:1, 6 μM			
Carotenoid / α-tocopherol – phenolic acids				

(*Continued on next page*)

Table 2. (*Continued*)

Mixtures of antioxidants	Ratio, total concentration	Type of assay	Proposed mechanisms	References
LYC + RMA	1:5, 30 μM	AAPH-induced human LDL oxidation	Spatial distribution of the compounds in LDL facilitated the cooperation of radical scavenging	Fuhrman et al. (2000)
LYC + CNA	1:5, 30 μM	Cu^{2+}-induced human LDL oxidation		
β-CAR + RMA	1:5, 30 μM			
β-CAR + CNA	1:5, 30 μM			
β-CAR + CGA	1-10$\times10^{5}$ (mol/dm^3) + 2×10^{-5} (mol/dm^3)	Irradiation using *t*-BuOH/water (4:1) in quantum yield reactor	CGA regenerated β-CAR due to its lower redox potential and bond dissociation energy	Vijayalakshmi et al. (2014)
α-TOC + CFA	1:1, 5 μM or 10 μM	AAPH-induced liposome oxidation, assessed by the changes in the fluorescence intensity of C11-BODIPY 581/591	The ability of the phenolic acids to penetrated into membrane enabled them to regenerate or protect α-TOC from radicals	Neunert et al. (2015)
α-TOC + FRA	1:1, 5 μM or 10 μM			
α-TOC + CGA	1:1, 10 μM		Chlorogenic acid has low affinity to membrane lipids. High concentration in the membrane was crucial for synergy	

CY: Cyanidin-3-glucoside; MV: Malvidin-3-glucoside, PE: Peonidin-3-glucoside, PG: Pelargonidin-3-glucoside; MYR: myricetin; QUE: quercetin; Q3G: quercetin-3-glucoside; KAEM: kaempferol; RUT: rutin; CAT: catechin; GCG:gallocatechin gallate; EGCG: epigallocatechin gallate; CG: catechin gallate; ECG: epicatechin gallate; ASC: ascorbic acid; NAR: naringin; AAPH: 2,2′-azobis(2-amidinopropane) dihydrochloride; ABIP: 2,2′-azobis[2-(2imidazolin-2-yl)propane] dihydrochloride; DDPH: 2,2-Diphenyl-1-picrylhydrazyl assay; FRAP: ferric ion reducing antioxidant power

β-CAR: β-carotene; LYC: lycopene; LUT: lutein; α-TOC: α-tocopherol; β-CRYP: β-cryptoxanthin; ZEA: zeaxanthin; α-CAR: α-carotene; ASTA: astaxanthin; AMVN: 2,2′-azobis (2,4-dimethylvaleronitrile; TBARS: thiobarbituric acid reactive substances; LDL: low-density lipoprotein

β-CAR: β-carotene; PUE: puerarin; 4′-PROP: 4′-propylpuerarin; QUE: quercetin; RUT: rutin; α-TOC: α-tocopherol; MYR: myricetin; KAEM: kaempferol; MOR: morin; QGP: quercetin galactopyranoside; QRP: quercetin rutinpyranoside; KGP: kaempferol glycopyranoside; CAT: catechin; ECAT: epicatechin; ECG: (−)-epicatechin gallate; EGCG: (−)-epigallocatechin gallate; AMVN: 2,2′-azobis(2,4-dimethylvaleronitrile; AAPH: 2,2′-azobis(2-amidinopropane) dihydrochloride

α-TOC: α-tocopherol; QUE: quercetin; ASC: ascorbic acid; EGC: (−)-epigallocatechin, ECG: (−)-epicatechin gallate; EGCG: (−)-epigallocatechin gallate; α-TOC: α-tocopherol; β-CAR: β-carotene; GLB: glabridin; RMA: rosmarinic acid; CNA: carnosic acid; CFA: caffeic acid; FRA: ferulic acid; CGA: chlorogenic acid; AAPH: 2,2′-azobis(2-amidinopropane) dihydrochloride; LDL: low-density lipoprotein

water/lipid interface is the most important for high anti-oxidative synergy (Han et al., 2012).

Changes of antioxidant capacities in complex phytochemical mixtures

The addition of isolated bioactive compounds to fruit and/or vegetable extracts can improve the antioxidant efficiency. The lipid inhibitory effect of black chokeberry juice significantly increases when added with α-tocopherol (Graversen et al., 2007). The addition of α-tocopherol to the extracts of açaí seed and grape rachis improves the protective efficiency against lipid peroxidation (Melo et al., 2016). α-tocopherol or quercetin added to lettuce extract improves the delaying of the onset of L-α–phosphatidylcholine liposome oxidation induced by either hydrophilic or lipophilic radicals (Altunkaya et al., 2009; Altunkaya et al., 2016).

Combinations of various fruits, vegetables and/or legumes bring significant changes in the antioxidant efficiency. Combining foods within and across categories results in different degrees of synergy, addition, and antagonism. Wang et al. (2011) examined the antioxidant capacity and the combined effects of a number of mixed extracts made from fruits, vegetables and legumes. Within the same food category, only 13% of the tested combinations showed synergy; 68% addition and 21% antagonism. In mixtures across food categories, 21% showed synergy; 54% addition and 25% antagonism (Wang et al., 2011).

Table 3 presents a list of fruit and/or vegetable mixtures that show synergy on anti-oxidation. Several vegetable binary mixtures: tomato-lettuce, tomato-onion (Gawlik-Dziki, 2012), eggplant-tomato, and purple potato-tomato (Jiang et al., 2015) produce relatively high synergistic effects in an ABTS$^{\bullet}$ radical scavenging assay. Other vegetable combinations: carrot-eggplant, carrot-purple potato, and eggplant-purple potato (Jiang et al., 2015) show synergy in DPPH$^{\bullet}$ radical scavenging activity. Some pairs of fruit-vegetable mixtures: apple-purple cauliflower, raspberry-mushroom; fruit-legume mixtures: raspberry-adzuki beans, apples-black beans; or vegetable-legume mixtures: tomatoes-soybean, broccoli-adzuki bean show synergy in anti-oxidation (Wang et al., 2011). Lettuce extract mixed with green tea or grape seed extracts demonstrate additive or slightly synergistic effects in the inhibition of liposome peroxidation (Altunkaya et al., 2016). Wang et al. (2011) observed the percentage of synergy produced by different patterns of food combinations to evaluate which food category combinations were more likely to cause anti-oxidative synergy. The combinations of fruits and legumes are most likely to provide synergistic anti-oxidation (28%), followed by fruit and vegetable mixtures, 22%. Legume combinations are the least effective mixtures in providing anti-oxidative synergy. Among the extract mixtures of fruits, vegetables and legumes analyzed in the study, the combination of raspberry and adzuki bean was the only one that exhibited multiple synergistic interactions in all antioxidant assays (DPPH, FRAP, and ORAC) (Wang et al., 2011). In that study, all of the food mixtures used were the hydrophilic extracts of fruits, vegetables and legumes, therefore only the combined anti-oxidative effects resulting from the interactions among water-soluble phytochemicals were investigated. Similarly, in the study of Gawlik-Dziki (2012), the author used only the water-soluble extracts of tomato, onion, garlic, and lettuce for the evaluation of anti-oxidative interactions. Some food materials, such as tomato and carrot, used in these studies, however, contain mainly lipophilic phytochemicals: lycopene, α-carotene and β-carotene which were excluded by the interaction tests. Thus, the combined effects on

Table 3. Combination of fruit and/or vegetable extracts showing synergy in antioxidant activities.

Extract combinations (1:1, v/v ratio)	Antioxidant assay used	Synergistic rate* (%)	References
Tomato + onion	ABTS	32	Gawlik-Dziki (2012)
	Xanthine oxidase inhibitory	11	
Tomato + Garlic	ABTS	50	
	Xanthine oxidase inhibitory	35	
Tomato + Lettuce	ABTS	5	
	Xanthine oxidase inhibitory	15	
	Lipoxygenase inhibitory	23	
Tomato + Garlic + Lettuce	ABTS	30	
	Xanthine oxidase inhibitory	22	
Lettuce + Green tea	Liposome oxidation	16[a]	Altunkaya et al. (2016)
Lettuce + Grape seed extract	Liposome oxidation	17[a]	
Eggplant – Purple potato	DPPH	46.6	Jiang et al. (2015)
	ABTS	47.9	
Carrot – Purple potato	DPPH	73.4	
	ABTS	82.1	
Carrot – Eggplant	DPPH	87.4	
	ABTS	81.8	
Tomato – Purple potato	DPPH	35.5	
	ABTS	45.5	
Tomato – Eggplant	DPPH	71.4	
	ABTS	80.6	
Tomato + Purple cauliflower	ORAC	20.3	Wang et al. (2011)
Soybean + Adzuki bean	ORAC	14	
Apple + Purple cauliflower	DPPH	15	
Raspberry + Mushroom	ORAC	12.2	
Apple + Tomato	ORAC	7.5	
Raspberry + Adzuki bean	FRAP	19.5	
	DPPH	31.5	
	ORAC	8.2	
Raspberry + Soybean	ORAC	10.1	
Apple + Black bean	FRAP	7.6	Wang et al. (2011)
Raspberry + Black bean	ORAC	14.1	
Apple + Adzuki bean	ORAC	9.3	
Apple + Black bean	ORAC	8.5	
Tomato + Soybean	DPPH	5.2	
Broccoli + Adzuki bean	ORAC	13.7	
Tomato + Adzuki bean	ORAC	8.3	
Purple cauliflower + soybean	ORAC	7.2	
Purple cauliflower + Black bean	ORAC	8.4	
Sumac + Raspberry	ORAC	10	Wang et al. (2015)
	FRAP	15	
	DPPH	45	

DDPH: 2,2-Diphenyl-1-picrylhydrazyl assay; FRAP: ferric ion reducing antioxidant power; ORAC: Oxygen radical absorbance capacity; ABTS/Trolox equivalence antioxidant capacity

*Synergistic rate (%) = (difference between theoretical effect and experimental effect / theoretical effect)*100 (Jiang et al., 2015). Some of the percentage values might not be directly presented in the original papers, but were calculated using the reported results for comparison purpose in this review.

[a]Reported value of synergy at pH 6

anti-oxidation of the food mixtures containing high content of lipid-soluble antioxidants may be incorrect because these compounds were excluded from possible interactions with water-soluble phytochemicals. When the combinations of food extracts containing hydrophilic antioxidants and those containing lipophilic antioxidants were tested, they showed very high synergistic effects on free radical scavenging activities. For instance, the combinations of the lipophilic extracts of tomato or carrot with the hydrophilic extracts of eggplant or purple potato provide high percentage of anti-oxidative synergy (50–80%), and the synergistic rate is enhanced with the increase of lipophilic extract ratios (Jiang et al., 2015). In comparison, when the tomato hydrophilic extract is mixed with the water-soluble extract of purple cauliflower, the synergy is as low as 20% (Wang et al., 2011).

Antioxidative synergy: mechanism and influencing factors

Mechanism of synergistic anti-oxidation

Different mechanisms of antioxidant synergy have been proposed:

- *Regeneration:* one antioxidant is oxidized and becomes a free radical which can receive electrons or hydrogen atoms donated by the other antioxidant to regenerate itself. Generally, in a binary mixture of antioxidants, the compound whose antioxidant capacity is weaker regenerates the stronger one (Becker et al., 2004; Shi et al., 2004; Becker et al., 2007; Dai et al., 2008; Vijayalakshmi et al., 2014).
- *Spatial distribution:* the two antioxidants have different orientation or position at the water/lipid interface or within the membrane that facilitates synergistic

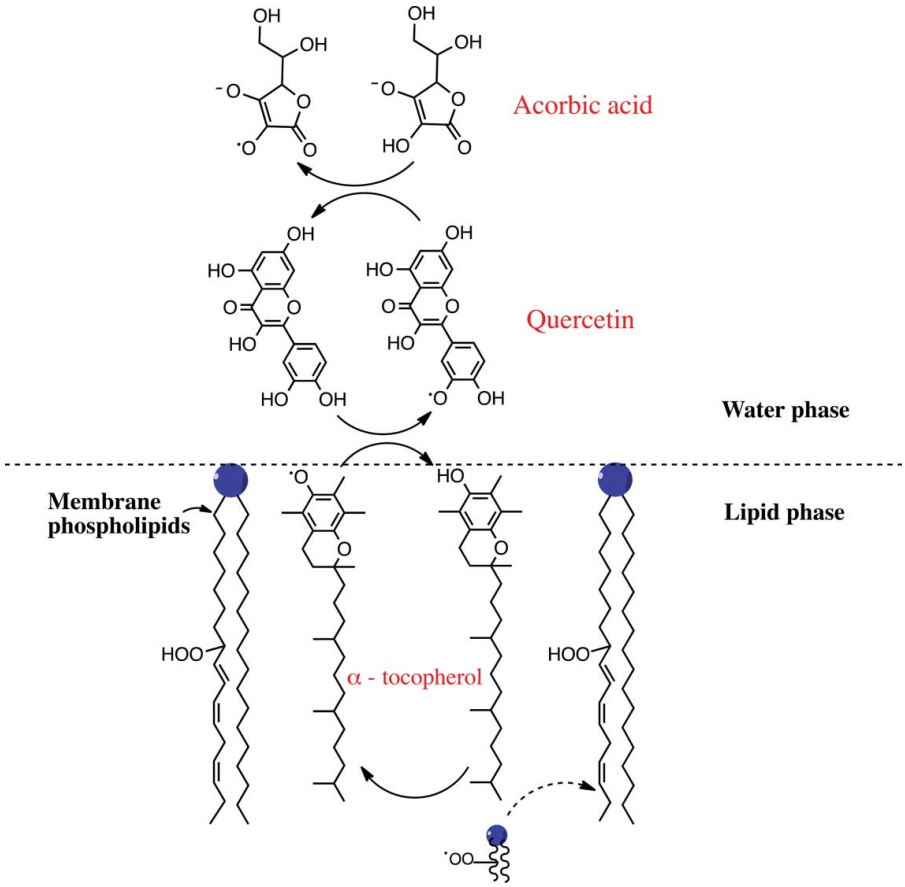

Figure 2. The mechanism of synergy in anti-oxidation of the mixture α-tocopherol, quercetin, and ascorbic acid at the water–lipid interface in a membrane model. In this system, α-tocopherol locates near the water–lipid interface enabling it to interact with quercetin in the aqueous phase. Quercetin regenerates α-tocopherol and is subsequently regenerated by ascorbic acid.

interactions (Fuhrman et al., 2000; Murakami et al., 2003; Becker et al., 2004; Han et al., 2007; Liang et al., 2009b).

- *Sacrificial oxidation:* one antioxidant reacts with free radicals or singlet oxygen to protect the partner from oxidation (Neunert et al., 2015).
- *Metal chelation:* one component chelates metal ions to allow the partner to remain active (Becker et al., 2004).
- *Mutual protection:* the combined antioxidants act in different ways of anti-oxidation that enable them to protect each other from oxidative agents (e.g. a chain breaking antioxidant scavenges free radicals to protect a singlet oxygen quencher from oxidation and that enables the latter to stay active longer to protect the former against singlet oxygen oxidation) (Becker et al., 2004; Becker et al., 2007).

Among the proposed mechanisms, regeneration of antioxidant has been mostly used to explain synergistic interactions between antioxidants, such as the synergy between α-carotene and lycopene; α-tocopherol and lycopene; lycopene and glabridin; lycopene and rosmarinic acid. In these binary mixtures of antioxidants, the less active antioxidant regenerates the more active compound. The regeneration activity is determined by the differences in the standard reduction potential (E^0), which demonstrates the ability of the antioxidants to donate hydrogen atoms or electrons, under standard conditions. For example, the regeneration of α-tocopherol by ascorbic acid in membranes and low-density lipoprotein systems is consistent with E^0 of the half-equation of the reduction of ascorbic acid (0.28 V) being lower than that of α-tocopherol ($E^0 = 0.5$ V) (Buettner and Jurkiewicz, 1996). Similarly, in the ternary mixture of α-tocopherol, quercetin and ascorbic acid (listed in descending order of the reduction potential), α-tocopherol is regenerated by quercetin, which is subsequently regenerated by ascorbic acid (Figure 2) (Murakami et al., 2003). E° can be no more than a guide to antioxidant regeneration activity when temperature, solvent type, phase separation, and concentrations are not standard (Becker et al., 2004). The mixture of lycopene and astaxanthin is a typical example of the importance of proper phase distribution for the exertion of antioxidant synergy (Han et al., 2012). The lipophilic centre of astaxanthin (and other xanthophyll) molecules remains in a membrane with the two polar end groups attached to the lipid/water interface (El-Agamey et al., 2006; Polyakov et al., 2010). Because of this orientation, astaxanthin is initially oxidized by free radicals to become a radical cation. In contrast, lycopene molecules, with no hydrophilic sites, located within the inner membrane act as electron donors to the astaxanthin radical cations resulting in the regeneration of the partner (El-Agamey et al., 2006; Polyakov et al., 2010).

One antioxidant in a binary mixture can also protect another from oxidative degradation or isomerization by sacrificial oxidation**.** Lycopene protects all-trans β-carotene against isomerization induced by singlet oxygen and free radicals. The isomerization of β-carotene is retarded and is only triggered once lycopene has been completely depleted (Heymann et al.,

Table 4. Factors that affect antioxidant interactions in chemical assays.

Factors		Influences on	References
Reaction medium	Homogeneous solutions (single-phase systems)	Hydrogen / electron transfer activities	Becker et al. (2007), Zhou et al. (2005)
	Heterogeneous solution (multi-phase systems)	Solvation and interfacial phenomena	
Solvent	Compositions	Compatibility between sample preparation solvent and reaction medium	Celik et al. (2010), Serrano et al. (2007), Hidalgo et al. (2010)
	Polarity		
Interacting antioxidants	Structural features	Orientation of AOXs at interface	Hidalgo et al. (2010), Liang et al. (2009a), Jiang et al. (2015)
	Concentration	Interaction of AOXs with membrane lipids	
	Ratio	Regeneration of AOXs	
Nature of radical initiators	Hydrosoluble, e.g. AAPH, AIPH	Participation of AOXs in radical scavenging	Beretta et al. (2006), Yeum et al. (2009); Frankel and Meyer (2000)
	Liposoluble, e.g. AMVN, MeO-AMVN		
Interfering substances	Food matrix components, e.g. proteins, amino acids	Performance of AOXs	Pérez-Jiménez and Saura-Calixto (2006),
	Pro-oxidant agents, e.g. metal ions, free fatty acids		Aubourg (2001), Çelik et al. (2015)

AOXs: antioxidants; AAPH: 2,2′-azobis(2-amidinopropane) dihydrochloride; AIPH: 2,2′-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride; AMVN: 2,2′-azobis(2,4-dimethylvaleronitrile); MeO-AMVN: 2,2′-azobis(4-methoxy-2,4-dimethylvaleronitrile)

2015). The ability of lycopene to protect β-carotene against singlet oxygen- and free radical-induced isomerization results from its higher anti-oxidative scavenging activities (Di Mascio et al., 1989; Clinton, 1998; Wang, 2012b). Lycopene acted as a quenching shield that is initially isomerized and degraded in the reaction preventing the accompanying carotenoid from isomerization (Namitha and Negi, 2010).

Factors influencing antioxidant synergy

The types of models used to study the antioxidant interaction: *in vitro* (chemical-based or cell-based) and *in vivo* (animal- or human-based) affect the observation of synergy. Synergistic effects on bioactivities seen in chemical systems (e.g. FRAP, DPPH, ORAC) might not be shown in biological systems (e.g. cancer-cell lines) and vice versa, as these two approaches are totally different in assay conditions and ways to evaluate the effects (Wang and Zhu, 2015). On the other hand, the interactive effects of antioxidant mixtures may also be seen differently in different chemical assays. For instance, positive interaction between some anthocyanins (cyanidin-3-glucoside, malvidin-3-glucoside or pelargonidin-3-glucoside) and quercetin appear in FRAP assay but not in DPPH assay (Hidalgo et al., 2010). Although the mixture of malvidin-3-glucoside and catechin lowers the DPPH scavenging activity (Hidalgo et al., 2010), it increases the inhibition of the oxidation of linoleic acid micelles initiated by ABIP (2,2′-azobis[2-(2imidazolin-2-yl)propane] dihydrochloride) (Rossetto et al., 2002). As chemical methods are widely used in studying phytochemical interactions they are worthy of further discussion on factors that may influence the interactive effects observed in these systems.

A number of factors affect the interactions between antioxidants in chemical systems (Table 4). A recent review by Wang and Zhu (2015) has described in detail reaction medium and solvent effects. The following section summarizes important aspects of these and others to provide a comprehensive overview.

Antioxidant interactions can show differently in different reaction media. The mixture of α-tocopherol and quercetin in methyl linoleate emulsion or liposome shows synergy in lipid oxidation inhibition, but in bulk sunflower oil shows addition (Becker et al., 2007). Antioxidants may perform differently in homogeneous- and heterogeneous-reaction-media (Zhou et al., 2005b; Becker et al., 2007). For instance, α-tocopherol combined with the flavonols quercetin or myricetin inhibits AAPH-induced linoleic acid peroxidation more in single-phase (tBuOH/H_2O 3:2) than in multi-phase systems (sodium dodecyl sulfate and acetyl trimethylammonium bromide micelles) (Zhou et al., 2005b). In the single-phase system, the combined antioxidants scavenge the initiating AAPH radicals. In the multi-phase system, they trap the propagating lipid peroxyl radicals on micelle surface and reduce α-tocopheroxyl radicals to regenerate α-tocopherol (Zhou et al., 2005b).

Solvent compositions can affect phytochemical interactions (Pérez-Jiménez and Saura-Calixto, 2006; Serrano et al., 2007; Celik et al., 2010; Hidalgo et al., 2010). The impacts of solvents are highest in ORAC assay, less in ABTS and DPPH assays, and least in FRAP assay (Pérez-Jiménez and Saura-Calixto, 2006). When any solvent components used for sample preparation interfere with assay reagents, the interactions between phytochemicals may not appear (Serrano et al., 2007). The mixture of BHA and BHT prepared in dichloromethane (DCM)/ethanol (EtOH) 9:1 shows synergy in CUPRAC and ABTS assays. This mixture, however, does not show synergy in FRAP assay because of the difference in solvent polarity: the sample preparation solvents (DCM) is less polar, whereas the FRAP reagent solution ($Fe(TPTZ)_2^{2+}$ chromophore solution) is high polar (Celik et al., 2010).

The combined effects of a phytochemical mixture depend upon the antioxidant potency of each component, which is determined by the structural characteristics of the compound: the presence of glycosidic moieties, and the number and position of hydroxyl and methoxy groups (Cao et al., 1997; Azevedo et al., 2010; Zhao et al., 2014). When two phytochemical compounds interact and form hydrogen bonds at their active hydroxyl groups, the free-radical-scavenging capacities of the compounds are decreased and antagonism appears (Hidalgo et al., 2010). In multi-phase reaction systems, interaction between a phytochemical in water-phase with the one in lipid-phase depends on the polarity of the hydrophilic compound. The less polar the compound, the higher affinity the compound has to the lipid membrane interior, and the stronger the

synergy is. For example, synergy in inhibiting AAPH-induced liposome oxidation appears stronger when α-tocopherol combines with ferulic acid than when it combines with caffeic acid. Ferulic acid is less polar than caffeic acid resulting in stronger interaction with α-tocopherol (Neunert et al., 2015). Specific functional groups on the structure of the hydrophilic compound also determine the occurrence of synergy (Han et al., 2011b). When β-carotene combines with puerarin or its derivatives in a liposome model, synergy in anti-lipooxidation shows only in combinations where puerarin component has a free 7-phenolate group on the A ring (puerarin and 4′-propylpuerarin). The A-ring phenolate group functions to regenerate β-carotene.

Antioxidant concentration and ratio in the mixtures affect the interactive effect. In a synergy system with two components, one acts as protector or regenerator of the other by donating its electrons or hydrogen atoms. The alterations of concentration or ratio of each component affect these actions leading to changes in the interactive effect. Tocopherols combined with carotenoids show synergy only when tocopherols are present at higher ratios than carotenoids (Palozza and Krinsky, 1992; Mortensen and Skibsted, 1997; Wrona et al., 2003). In these mixtures, tocopherols regenerate carotenoids.

The type of free radical impacts the observation of interactions between antioxidants. In heterogeneous systems, the contribution of each phytochemical component in radical scavenging activity depends on which phase the initiating radicals are generated in (Beretta et al., 2006). When radicals are induced in aqueous phase by hydrophilic radical initiators (e.g. AAPH), water-soluble antioxidants mainly contribute to scavenge the free radicals. Fat-soluble antioxidants, especially the ones located far from the interface, cannot not approach the initiating radicals (Beretta et al., 2006; Han et al., 2007). Antioxidant interactions, therefore, may not appear in this system. In contrast, when lipophilic azo compounds: 2,2′-azobis(2,4-dimethylvaleronitrile (AMVN) or 2,2′-azobis(4-methoxy-2,4- dimethylvaleronitrile) (MeO-AMVN) are used for radical initiation in lipid phase, lipophilic antioxidants can scavenge the radicals and interact with hydrophilic antioxidants at the interface (Beretta et al., 2006; Han et al., 2007; Yeum et al., 2009; Liang et al., 2010). For instance, the mixtures of α-tocopherol and ascorbic acid (Yeum et al., 2009), or β-carotene and puerarin (Han et al., 2007; Liang et al., 2010) provide synergy in inhibition against AMVN-induced liposome oxidation, but does not show in AAPH-induced system. The lipophilic antioxidant (α-tocopherol or β-carotene) scavenge AMVN radicals in the lipid phase, and is subsequently regenerated by the hydrophilic antioxidant (ascorbic acid or puerarin) at the interface.

The presence of nonantioxidant compounds in the reaction can influence phytochemical interactions because these compounds can interfere with the antioxidant activities of phytochemicals. Interfering compounds include food matrix compositions: proteins and amino acids (Pérez-Jiménez and Saura-Calixto, 2006); and pro-oxidant agents: metal ions (Celik et al., 2010) and free fatty acids (Aubourg, 2001; Kamal-Eldin and Budilarto, 2014). These compounds affect the performance of antioxidants resulting in incorrect estimation of the antioxidant synergy.

Effects of phytochemical interactions on anti-carcinogenic activities

The consumption of whole fruits and vegetables rather than dietary supplements of single bioactive components is recommended (Liu, 2013; Rodriguez-Casado, 2016). Biological effects of a whole food are the combined effects of different bioactive components presenting in the food (Lila, 2007; Liu, 2013; Singh et al., 2016). Consuming dried tomato powder reduces the mortality from prostate cancer in human more effectively than diets supplemented with lycopene at equivalent concentrations (Campbell et al., 2004). Lycopene, the major phytochemical component in tomato, and other carotenoids and polyphenolic components contribute to the biological activities of tomato (Boileau et al., 2003; Campbell et al., 2006). Several comprehensive reviews have discussed the role and the mechanisms of phytochemical interactions in the prevention of different cancer diseases (de Kok et al., 2008; DiMarco-Crook and Xiao, 2015). The following section presents some recent findings and highlights some phytochemical combinations showing synergy in inhibiting the development of cancers.

Curcumin is the major active component of turmeric and is known for its high anti-proliferative activities (Basnet and Skalko-Basnet, 2011; Metzler et al., 2013). The mixtures of curcumin with other bioactive compounds show high synergistic effects on cancer chemoprevention *in vitro*(Altenburg et al., 2011; Iwuchukwu et al., 2011; Montgomery et al., 2016; Wang et al., 2016). Tumeric curcumin (12.5 μM) combined with milk thistle silymarin at various concentrations: 3.125, 6.25, 12.5, 25, 50, and 100 μM synergistically inhibits the growth of three colon cancer cell lines (DLD-1, LoVo, HCT116) (Montgomery et al., 2016). The combination of curcumin (12.5 μM) and sylimarin (12.5 μM) shows synergy in inducing apoptosis of DLD-1 colorectal cancer cells by suppressing nuclear factor – kappa B (NF-κB) activity (Montgomery et al., 2016). Curcumin mixed with berberine provides synergy in inhibiting the growth of MCF-7 and MDA-MB-231 breast cancer cell lines by elevating the induction of apoptosis and autophagic cell death, and by modulating different pathways (JNK, Beclin1, and Bcl-2) (He et al., 2016). Curcumin combined with epigallocatechin gallate (ECGC) provides chemopreventive synergy (Khafif et al., 1998; Balasubramanian and Eckert, 2004; Eckert et al., 2006). This treatment synergistically inhibits the growth of carcinoma cells by blocking the cell cycle at G_1 and S/G_2M phase (Khafif et al., 1998). When curcumin and ECGC are used in combination, the required, efficacious dose is diminished (to 12.5 to 25% for EGCG, and to 33 to 50% for curcumin (Balasubramanian and Eckert, 2004). Different mixtures of ECGC with the other tea polyphenols epicatechin (EC), epigallocatechin (EGC) or epicatechin gallate (ECG), or the combination of all four compounds provide synergy in suppression of lung tumor and gastric carcinoma cell growths *in vitro* (Suganuma et al., 1999; Williams et al., 2000; Williams et al., 2003; Horie et al., 2005). The mechanisms behind the synergy of tea polyphenol mixtures could be: increase of EGCG cellular uptake, enhancement of apoptosis induction, and/or modulation of transcription of human

CYP1A1 (Suganuma et al., 1999; Williams et al., 2000; Williams et al., 2003; Horie et al., 2005). In addition, synergy in *in vitro* anti-proliferation of cancer cells occurs when EGCG is combined with drugs: 4-methylumbelliferone (4-MU) for suppressing the growth of human breast cancer cells (MDA-MB-231 line) and human microvascular endothelial cells (HMECs); and Paclitaxel (a common prescribed drug in cancer treatment) for inhibiting the proliferation of HMECs (García-Vilas et al., 2016). The combinations of green tea or black tea extract with soy phytochemicals provide synergy in suppressing the development of human prostate and breast tumors in mice (Zhou et al., 2003; Zhou et al., 2004). Decaffeinated green tea extracts combined with grape skin extracts synergistically inhibit the growth of human cervical carcinoma cells *in vitro*, and combined with grape pomace powder at 25:1 ratio show synergy in inhibiting the growth of 4T1 mammary tumors in mice (Morré and Morré, 2006). Other combinations of dietary phytochemicals providing synergy in *in vitro* anti-proliferation of cancer cell lines (presented in brackets) are: resveratrol-chrysin-curcumin (Caco-2 colon carcinogenic cells) (Iwuchukwu et al., 2011); daidzein-genistein (LNCaP and C4-2B prostate cells) (Dong et al., 2013); genistein-quercetin-biochanin A (PC-3, LNCaP and DU-145 prostate cancer cells) (Kumar et al., 2011); quercetin-kaempferol and/or naringenin (Hepa-1c1c7 mouse liver cancer cells and the LNCaP human prostate cancer cells) (Campbell et al., 2006); quercetin-resveratrol, and quercetin-resveratrol-ellagic acid (human leukemia cells) (Mertens-Talcott et al., 2003); quercetin-EGCG (PC-3 and LNCaP prostate cells); and docosahexanenoic acid-curcumin (SK-BR-3 breast cancer cells) (Altenburg et al., 2011).

Synergy in inhibition of cancer cell growth *in vitro* occurs when isolated bioactive compounds are added with food extracts. For example, the blends of quercetin-3-glucoside and apple extract (Yang and Liu, 2009), or genistein and pomegranate extract (Jeune et al., 2005) synergistically inhibit MCF-7 human breast cancer cells. Grapeseed extracts added with resveratrol enhance the suppression of HCT116 colon carcinogenic cells (Radhakrishnan et al., 2011). The anti-carcinogenic synergy of these combinations possibly results from phytochemical interactions in different biological activities: anti-oxidation, apoptosis induction, cell cycle arrest, enzyme modification, or gene-transcription modulation (Knowles et al., 2000; Mertens-Talcott et al., 2003; Campbell et al., 2006; de Kok et al., 2008). Various mixtures of plant-based extracts: tomato and broccoli (Canene-Adams et al., 2007), or tomato and garlic (Sengupta et al., 2004) fed to rats, or peppermint and rosemary (Yi and Wetzstein, 2011) treated to SW-480 colon cancer cells *in vitro*, show greater anti-tumor and anti-proliferative effects than each single extract alone (no synergy analysis).

Effects of phytochemical interactions on anti-inflammatory activities

Inflammation is a response of the immune system to protect the cells or tissues from foreign agents. Prolonged inflammation contributes to develop several chronic disorders: diabetes, atherosclerosis and cancers (Libby et al., 2010). Diet-based strategies with regular consumption of fruits and vegetables reduce the risk of inflammation because these foods contain a wide variety of bioactive compounds with antioxidant and anti-inflammatory properties (Vainio and Weiderpass, 2006). The efficiency of inflammatory treatments can be enhanced by combination approaches: drug-phytochemical, or mixed phytochemicals. The combinations of anti-inflammatory drugs with naturally occurring phytochemicals allow the drugs use at lower doses for stronger effects. For instance, atorvastatin (0.1 μM), a common drug for atherosclerosis treatment, in conjunction with cyanidin-3-glucoside (2 μM) provides synergistic suppression of the proliferation of human aortic smooth muscle cells (HASMCs) induced by angiotensin II *in vitro*. When combining with cyanidin-3-glucoside, atorvastatin can be used at a dose lower than when it is used alone, which reduces its adverse effects (Pantan et al., 2016). Several combinations of dietary phytochemicals provide synergy to inhibit inflammation. When luteolin is combined with tangeretin, synergy occurs in protecting RAW 264.7 cells against inflammation stimulated by lipopolysaccharide (LPS) *in vitro* (Funaro et al., 2016). Different combinations of polyphenolic compounds: (1) 4′-hydroxymandelic acid, 4-hydroxyphenylacetic acid, 5-(3′-hydroxyphenyl) propionic acid and 3-(4′-hydroxyphenyl) lactic acid; (2) (–)-epigallocatechin-3-O-gallate, pelargonidin-3-O-glucoside, cyanidin-3-O-glucoside and punicalagin; (3) dihydroferulic acid, feruloylglycine, quercetin and 3-O-methylquercetin; (4) caffeic acid, ferulic acid, isoferulic acid and isoferuloylglycine; (5) hippuric acid, tyrosol, 4′-hydroxyhippuric acid and chlorogenic acid, show synergy in modulating the release *in vitro* of pro-inflammatory cytokines by Jurkat T-lymphocites (Ford et al., 2016). The combinations of coffee extract with the extracts of cinnamon (Durak et al., 2014); ginger (Durak et al., 2015); or dried coconut meat (Gawlik-Dziki et al., 2016) synergistically inhibit *in vitro* lipoxygenase (LOX-1), which is one of the pro-inflammatory factors. An oral nutraceutical mixture of berberin, red yeast rice, policosanol, astaxanthin, folic acid and coenzyme Q10 enhances anti-inflammatory effects *in vivo*: lowering LDL cholesterol level, and reducing systemic inflammation and endothelial injuries in patients with low-grade systemic inflammation (Pirro et al., 2016).

Inflammation can occur when there is a persistent presence of high concentrations of reactive oxygen species (ROS) (Hsu et al., 2010), which can activate intracellular signaling pathways such as NF-κB and mitogen-activated protein kinase (MAPK) pathways (Hsu et al., 2010; Fukumitsu et al., 2016; Pantan et al., 2016). The activated pathways trigger the expressions of several pro-inflammatory cytokines or chemokines. The potential mechanisms underlying the synergy of phytochemical combinations in anti-inflammation and anti-cancer are related to the synergistic multi-target effects of the combinatory components: single constituents can direct to several targets (e.g. enzymes, or activators) (Williamson, 2001; Imming et al., 2006; Wagner and Ulrich-Merzenich, 2009). Luteolin and tangeretin provide synergistic effects in inhibiting the formation of nitric oxide free radicals produced by nitric oxide synthase (iNOS), which is one of the pro-inflammatory enzymes mediating inflammatory processes (Funaro et al., 2016). This mixture also potentiates the suppression of several pro-inflammatory mediators such as PGE_2, IL-1β and IL-6 (Funaro et al., 2016). The synergy between atorvastatin and cyanidin-3-glucoside in the

protection of HASMCs against angiotensin II-induced inflammation *in vitro* relies on the ability of the two compounds to synergistically affect different pro-inflammatory pathways: inhibiting NF-κB activity leading to down-regulating iNOS and reducing NO production; and suppressing the expression of NADPH-oxidase resulting in diminishing ROS formation. The two compounds also elevate Nrf2 transcription leading to the activation of several cytoprotective enzymes (Pantan et al., 2016).

Other biological effects of phytochemical combinations

Synergy or potentiation of dietary phytochemical combinations in treatments of other oxidative stress and metabolic disorders is reported. The combinations of resveratrol with quercetin and/or genistein enhance the suppression of adipogenesis. The inhibitory effects of the mixtures of resveratrol (12.5 μM), quercetin (12.5 μM), and genistein (6.5 μM) on lipid accumulation in both human adipocyte and 3T3-L1 mouse cell lines are higher than the calculated additive effects of individual components (Park et al., 2008). Rats fed either resveratrol or quercetin shows no significant reduction in adipose tissue weights. When they are fed a diet supplemented with a mixture of resveratrol (15 mg/kg/day) and quercetin (30 mg/kg/day), their body fat accumulation and triacylglycerol metabolism in white adipose tissue are remarkably reduced indicating *in vivo* potentiation in anti-obesity of these two compounds (Arias et al., 2016). The effects of phytochemical combinations on anti-diabetes and neuroprotection are reported. Potentiation in anti-hypoglycemic activities shows in the mixture of loganin and ursolic acid isolated from *Cornus officinalis* fruits (He et al., 2016), or in the blend of 80% apple cider and 20% whole blueberry juice (Agustinah et al., 2016). Fermented soybeans added with sprouted garlic are more effective in inhibition of oxidation and protection of rat neurons against cognitive dysfunction induced by glutamate (Woo et al., 2016). The mechanisms underlying these combined biological effects of phytochemicals are complicated or unknown in most cases beyond the scope of this review.

Effects of dietary phytochemical interactions on bioavailability

Bioavailability interferences between hydrophobic phytochemicals

Interactions on intestinal absorption occur when different carotenoids are co-ingested. The carotenoid interactions during absorption and post-absorptive metabolism have been comprehensively reviewed by van den Berg (1999). α-Carotene absorption in humans is decreased by 38% when lutein supplementation is increased from 18 mg to 36 mg (Reboul et al., 2007a). Absorption interferences occur both *in vitro* and *in vivo* when any two carotenoids: β-carotene, lycopene, lutein or cathaxanthin are co-supplemented. There are inconsistent findings on the direction and magnitude of the interactions. For instance, an improvement or impairment of lycopene absorption by β-carotene are both reported (Prince et al., 1991; White et al., 1993; Wahlquist et al., 1994; Gaziano et al., 1995; Johnson et al., 1997). β-carotene absorption improved by lycopene is also reported (White et al., 1993). Different findings about carotenoid absorption interferences are due to the differences in study protocols: quantitative methods; dose usage (single-dose, short-term- or long-term supplementation); research models or species used (animals, human or *in vitro* models) (van den Berg, 1999).

Carotenoid interactions may occur at different stages of digestion, metabolism and distribution. Several mechanisms are proposed to explain bioavailability interactions of carotenoids. Lycopene can scavenge singlet oxygen and free radicals, which protects β-carotene from isomerization resulting in more active all-trans β-carotene remaining for absorption into the plasma (Heymann et al., 2015). An increase in absorption of one carotenoid occurs when the other one can inhibit the activity of carotenoid cleavage enzymes in the intestinal mucosa. For example, the oxycarotenoid lutein or cataxanthin inhibits the cleavage enzyme of β-carotene resulting in an increase of β-carotene in the serum (Kostic et al., 1995). The absorption of a carotenoid is decreased when the co-consumed compound interferes with the carotenoid micellarization in intestinal lumen or with the uptake in intestinal chylomicrons. In systemic circulation, carotenoid compounds may compete to each other for binding with appropriate plasma proteins (van den Berg, 1999; Hidalgo et al., 2010).

Carotenoids can impair the absorption of α-tocopherol. The carotenoids β-carotene, lycopene or lutein, at dietary levels, decrease α-tocopherol absorption in Caco-2 TC7 cell line (Reboul et al., 2007a). Lycopene induces highest impact on α-tocopherol absorption, followed by lutein, and β-carotene. γ-tocopherol, another vitamin E species, can interfere with the absorption of α-tocopherol. Each of these compounds competes with α-tocopherol for uptake at the apical compartment of the Caco-2 cell monolayers and reduces the absorption of α-tocopherol (Reboul et al., 2007a).

Bioavailability interferences between hydrophilic phytochemicals

Hydrophilic compounds can interfere with the absorption of each other. Absorption interferences occur between flavonoids, and between flavonoids and phenolic acids. The mucosal absorption of cyanidin-3-glucoside, in the presence of 50 μmol/L of quercetin-3-glucoside, is significantly decreased by 73.9% ($p < .001$) over 2 hours, while the absorption of quercetin-3-glucoside is increased over time (Walton et al., 2006). The competition of these compounds for cellular uptake, or for cellular transportation contributes to the changes in their absorption. These two compounds can be transported to cells by the same transporter, which has higher affinity to flavonols than to anthocyanins, thus facilitating the absorption of quercetin-3-glucoside. An increase on the absorption of rosmarinic acid in Caco-2 cells occurs when the flavonoids luteolin and apigenin are present (Fale et al., 2013). The absorption rate of rosmarinic acid in Caco-2 cells is significantly boosted in the presence of increasing concentrations of luteolin and apigenin. The simultaneous presence of the flavonoids inhibits the efflux

of rosmarinic acid by inhibiting ABC transporters resulting in the increase of rosmarinic acid bioavailability (Fale et al., 2013).

Bioavailability of a bioactive compound can be increased when its stability and solubility are improved. Phytochemical interactions can result in these improvements. The bioavailability of quercetin is increased in the presence of proanthocyanidins (*Pyracantha fortuneana* fruit) (Zhao et al., 2015). These compounds improve the bioavailability of quercetin by improving its solubility and stability. Proanthocyanidins form hydrogen bonds with quercetin, which possibly contribute to improve quercetin solubility. They improve chemical stability of quercetin by preventing it from oxidation and improving reducing ambient of solvent systems (Zhao et al., 2015).

Bioavailability interferences between hydrophobic and hydrophilic phytochemicals

Hydrophilic compounds can impair the absorption of lipophilic ones. Naringenin can interfere with the absorption of α-tocopherol, lutein or the mixture of β-carotene and β-cryptoxanthin in Caco-2 cells (Reboul et al., 2007a; Reboul et al., 2007b; Claudie et al., 2013). The cellular concentrations of the carotenoid mixture β-carotene-β-cryptoxanthin are increased by 30% when naringenin is present, and by 60% in the presence of hesperidin or hesperetin (aglycone) (Claudie et al., 2013). The cellular uptake of β-carotene or β-cryptoxanthin increases by 150% in the presence of hesperetin (Claudie et al., 2013). Absorption interferences do not occur between ascorbic acid and the carotenoids β-carotene, β-cryptoxanthin or lutein (Reboul et al., 2007a; Reboul et al., 2007b), or between α-tocopherol and vitamin C, caffeic acid, gallic acid or (+)-catechin (Reboul et al., 2007a).

Different explanations for the absorption interferences between hydrophilic and lipophilic phytochemicals have been proposed. Naringenin interferes with the absorption of the lipophilic compounds by interacting with the lipophilic transporter (scavenger receptor class B type I), or with cell membrane lipids, which lead to the invagination of lipid raft domains containing the carotenoids or α-tocopherol receptors (Reboul et al., 2007a; Reboul et al., 2007b). The affinity to cell membrane lipids of water-soluble phytochemicals determines the interfering effects on bioavailability of fat-soluble compounds. The absorption of the carotenoid mixture β-carotene-β-cryptoxanthin is more enhanced by the flavonone glucoside hesperidin than by the aglycone hesperetin. Hesperidin is poorly bioavailable because of its high affinity to membrane lipids. The sugar moiety of hesperidin interacts with the polar head of the lipids and alters the barrier function of the double-layer membrane facilitating the cellular uptake of the carotenoids (Claudie et al., 2013). The aglycone hesperetin, which is more liposoluble, interacts with the acyl chains of the membrane lipids. Among the three flavonones hesperetin, hesperidin and naringenin, the last has lowest affinity to the membrane lipids and shows the least effect on the carotenoid uptakes (Claudie et al., 2013). Therefore, the stronger the affinity of the flavanones to biological membranes, the greater is the enhancing effects on carotenoid absorption. More work is required to understand the mechanisms of bioavailability interferences between bioactive compounds.

Research gaps for future studies

The number of studies on bioavailability interferences between bioactive compounds is very limited. Only few phytochemical mixtures are investigated for their interferences on intestinal uptake. The mechanisms of the absorptive interactions between phytochemicals are not fully understood. In addition, there is limited attention given to studying the impacts of bioaccessibility and bioavailability interferences on bioactivity interactions between phytochemicals. Synergy in biological activities can result only from phytochemical combinations that can promote "solubility, safety, absorption, stability or bioavailability of the principal active compounds" (Kirakosyan et al., 2010). Gawlik-Dziki (2012) measured the antioxidant activities of various vegetable mixtures including raw extracts and their *in vitro* digested and absorbed extracts. If the digested extracts of all vegetable binary mixtures demonstrate higher antiradical activities than their raw combinations, the simulated gastrointestinal digestion must have released more bioaccessible antiradical components from the raw materials. For some vegetable blends such as tomato and onion, or tomato and lettuce, synergy of antiradical activity is seen in the raw and digested extracts, but not in the absorbed extract. Only the combination of tomato and garlic shows synergistic bioactivities in all forms tested (raw, digested and absorbed), probably because tomato and garlic contain highly bioaccessible and bioavailable active constituents. The mode of interaction and the intensity of the interactive effect in some food combinations can change after the foods undergo gastrointestinal digestion. For instance, a raw water-soluble extract mixture of coffee and cinnamon shows synergy in the inhibition of lypoxygenase activity but changes to antagonism after digestion (Durak et al., 2014). In contrast, nondigested extracts of coffee and ginger act antagonistically but their digested bioaccessible constituents synergistically inhibit lypoxygenase (Durak et al., 2015). The degree of antagonism of the coffee-coconut mixture is higher in its digested extracts than in the combined raw materials (Gawlik-Dziki et al., 2016). These results imply that bioaccessibility and bioavailability of bioactive components should be considered when evaluating the bioactivity interactions. Several factors can contribute to the changes of the interaction between phytochemicals after digestion. They include: the chemical properties of the bioactive components, the ratios of different phytochemicals in the raw extracts and in the digested mixture, and the possible formation of complexes among the compounds and /or with food matrix during digestion (Gawlik-Dziki et al., 2016). Further studies are required to understand these intricate issues.

Isolated compounds, or fruit/vegetable extracts are used more often than food products in interaction studies. These approaches do not consider the interferences of food matrix components and/or the impacts of food processing on the bioaccessibility and bioactivities of the active compounds. In fact, the bioaccessibility of bioactive compounds can be different in different food matrices. The highest bioaccessibility of vitamin C shows in soy-containing-fruit-juice blends; and of phenolic compounds in water-based fruit-juice blends (fruit juices blended with water) (Rodríguez-Roque et al., 2015). When whole milk is blended with the fruit juices, the bioaccessibility

of vitamin C or phenolic compounds is lowest. Milk proteins can interact with vitamin C and polyphenols; metal ions (Fe, Cu, Zn) and other vitamins (B_1, B_2, B_{12}) in milk can interact with vitamin C. These interactions result in the degradation of vitamin C and/or the formation of protein-vitamin and protein-phenol complexes, which cause decreases in bioaccessibility of these compounds. The bioaccessbility of bioactive compounds in foods can be changed after food processing (Rodríguez-Roque et al., 2015; Swada et al., 2016). Applying nonthermal processes: high-intensity pulsed electric fields or high-pressure processing to fruit juice beverages can increase by up to 38% of the bioaccessibility of several phenolic compounds (e.g. hesperidin, rutin), but can also reduce that of the others (ferulic acid, chlorogenic acic and *p*-hydroxybenzoic acid from water-based beverages) (Rodríguez-Roque et al., 2015). This phenomenon occurs because processing can induce changes in physicochemical properties of phenolic compounds (structure changes: hydroxylation, glycosylation, methylation or dimerization, or formation of phenolic derivatives) resulting in modification (increase or decrease) of the compound bioaccessibility (Dugo et al., 2005). The effects of processing depend on the type of phytochemical substances and of compound-containing food matrices (Rodríguez-Roque et al., 2015); and the duration and intensity of the treatment (Chandrasekara and Shahidi, 2012). Food processing can also affect phytochemical interactions. Different blends of strawberry and papaya nectars (25:75, 50:50, and 75:25) show synergistic effect in anti-oxidation after ultra-high temperature treatments (80–135°C) (Swada et al., 2016). Nonheat treated blends show additive effect. Heating at high temperature causes cell rupture that releases more antioxidants trapped in the cell membrane of the fruits to interact with each other. Combining the two fruit nectars followed by heat processing is therefore important for anti-oxidative synergy (Swada et al., 2016).

Another concern in most of the *in vitro* studies on phytochemical interactions is the use of raw food extracts or isolated bioactive compounds at concentrations higher than their physiological ones. In *in vivo*, however, bioactive compounds are present in plasma or tissues at smaller concentrations. They can be structure-modified or metabolized into other compounds following oral consumption. The active metabolites can interact and produce changes in bioactivities. For example, synergy in radical scavenging activities occurs between the gastric and intestinal metabolites of purple rice anthocyanins (Sun et al., 2015).

Knowing the gaps, future studies, therefore, should be designed to increase more knowledge on:

- The interactive pattern, direction and mechanisms of bioavailability interferences;
- The impact of bioaccessibility and bioavailability on bioactivity interactions between phytochemicals: how and why the interactive effects on biological activities of phytochemical mixtures would change after intestinal digestion, absorption and metabolism;
- Phytochemical interactions in bioactivity and bioavailability considering the physiological concentrations of phytochemicals and the effects of complex food matrices and food processing;

Conclusion

The combinations of bioactive compounds could result in changes in biological properties and bioavailability of the compounds. A number of phytochemical mixtures and food combinations provide synergistic effects on inhibiting oxidation, inflammation and cancer cell proliferation. These biological effects of phytochemicals depend on the bioaccessibility and bioavailability of the compounds, which can be impaired with by the co-digestion of other phytochemicals. More research should be conducted to completely understand the mechanism of bioavailability interferences and the inter-relationship between bioavailability and bioactivity as the result of interactions between bioactive compounds.

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