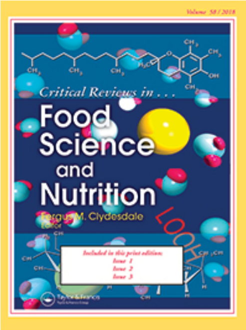

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Alterations in phenolic compound levels and antioxidant activity in response to cooking technique effects: A meta-analytic investigation

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ABSTRACT

The aim of this study was to review prior studies that have evaluated the effects of cooking techniques on polyphenol levels and antioxidant activity in vegetables and to release a meta-analysis of the findings. Meta-analysis with a random effect model was conducted using the weighted response ratios (R^*) that were calculated for each experiment. Baking ($R^* = 0.51$), blanching ($R^* = 0.94$), boiling ($R^* = 0.62$), microwaving ($R^* = 0.54$) and pressure cooking ($R^* = 0.47$) techniques precipitated significant reductions in the polyphenol levels. Significant decreases in the antioxidant activity levels were noted after baking ($R^* = 0.45$) and boiling ($R^* = 0.76$), while significant increases were observed after frying ($R^* = 2.26$) and steaming ($R^* = 1.52$).

KEYWORDS
Phenolic compound; polyphenol; antioxidant activity; cooking technique; cooked vegetable

Introduction

A diet that is rich in fruits and vegetables has been increasingly recommended, considering that they are sources of a wide variety of bioactive compounds that provide beneficial effects to human health. Bioactive compounds present anti-inflammatory, anticarcinogenic and antioxidative properties; thus, an elevated consumption of fruits and vegetables has been correlated with a delay in the aging process and a decreased risk of developing diseases, such as cancer, diabetes, cardiovascular disease, atherosclerosis, cataracts, cognitive function disorders, and neurological disease, including Alzheimer's (Wallace, 2011; Eliassen et al., 2012; Forester et al., 2012; Halliwell, 2012; Ros et al., 2012; Tanaka et al., 2012; Lim et al., 2013; Pojer et al., 2013).

An important group of bioactive compounds is the polyphenols, which comprise over 8000 already identified substances; they can be divided into groups according to their chemical structure, such as phenolic acids, stilbenes, coumarins, lignins and flavonoids (the largest group) (Ross and Kasum, 2002). The antioxidant properties of the polyphenols can be attributed to their increased ability to donate a hydrogen atom from the aromatic hydroxyl group to a free radical and/or to the capacity of their aromatic structures to support an unpaired electron (Nicoli et al., 1999). The phenolic compounds are produced as a result of the secondary metabolism of plants and are frequently found attached to sugars (glycosides); thus, they tend to be water-soluble (Ross and Kasum, 2002). This characteristic usually permits a strong correlation between the polyphenol and antioxidant activity; the majority of antioxidant activity methods, such as 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay, 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) assay, Total Radical-Trapping Antioxidant Parameter (TRAP), Ferric Reducing Antioxidant Power (FRAP), Cupric Reducing Antioxidant Capacity (CUPRAC), Oxygen Radical Absorbance Capacity (ORAC), Trolox Equivalent Antioxidant Capacity (TEAC), are based on aqueous buffers, hydrophilic probes and hydrophilic radical generators (Rodrigues et al., 2012). The methods used to evaluate the antioxidant activity can be classified according to the mechanism of deactivation of reactive species, which is based on either electron transfer (ET) or hydrogen atom transfer (HAT) (De Rosso, 2013). The ET-Based antioxidant capacity methods are the FRAP, DPPH, ABTS, TEAC, CUPRAC and Folin method; and the HAT-Based assays are TRAP, ORAC, PCL (photochemiluminescence), β-carotene bleaching and crocin-based assay (Apak et al., 2011).

However, similar to other bioactive compounds, polyphenols are also susceptible to losses; thus, it is important to consider a way to prepare the foods, especially vegetables, which are commonly consumed after having been submitted to some thermal processing. Thermal processing can induce losses of these compounds by the oxidation and leaching of water-soluble phenolics; on the other hand, high temperatures can imply inactivation of peroxidases that have pro-oxidant properties, thus increasing the antioxidant activity (Rodriguez-Amaya, 1997; Gazzani et al., 1998; Xu and Chang, 2008a; Xu and Chang, 2008b). Furthermore, the antioxidant potential of the vegetables could be enhanced due to an improvement in the antioxidant properties of naturally occurring compounds or the formation of novel compounds, such as Maillard reaction products (MRPs), which generally exhibit high antioxidant properties (Nicoli et al., 1999; Manzocco et al., 2000).

In fact, there is no consensus in the literature regarding the best way to prepare the vegetables and conserve their bioactive compounds. The effect of thermal processing is likely to depend on several factors, such as the cooking technique, degree of heating, leaching into the cooking medium, solvent used for extraction, and pH and surface area exposed to water and

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oxygen (Wachtel-Galor et al., 2008). Furthermore, each food matrix contains different compounds that are more or less thermally labile; consequently, the same cooking technique can imply different effects, depending on the vegetable (Bernhardt & Schlich, 2006). Burgos et al. (2013) investigated the effect of boiling on the total phenolic and antioxidant activity in purple-fleshed potatoes and found an increase in the content of total phenolic and antioxidant activity as determined by the ABTS and DPPH methods, on four different accessions of purple potatoes. On the other hand, Xu and Chang (2008a) found a decrease in the total phenolic content of yellow soybeans and black soybeans that were submitted to boiling as well as a decrease in the antioxidant activity; these findings were determined by the FRAP and ORAC assays for yellow soybeans and the DPPH, FRAP and ORAC assays for black soybeans.

Therefore, a systematic review with meta-analysis was chosen as the research method, which is similar to research performed previously in the investigation of the effects of cooking techniques on carotenoid and anthocyanin levels (Murador et al., 2014). Meta-analysis was primarily used to observe the effects of treatments and interventions in randomized clinical trials (Stroup et al., 2000), but it has also been used to observe the effects of food processing on pesticide residues (Keikotlhaile et al., 2010) and pasteurization on the vitamin levels in milk (MacDonald et al., 2011) as well as other studies that involve aspects of food technology (Black et al., 2012; Bucher et al., 2012).

In this context, the aim of this work was to review the studies that have evaluated the effects of cooking techniques on polyphenols and antioxidant activity in vegetables and to release a meta-analysis of the findings. The present study was performed by applying two different meta-analysis approaches: the first approach considered all of the found studies, and the second approach considered those previously selected studies that were on antioxidant effects and evaluated them according to the reaction mechanisms used in the different methods.

Methods

Literature search

A literature search was conducted to identify studies that evaluated the effects of home cooking techniques (specifically baking, blanching, boiling, frying, microwaving, pressure cooking and steaming) on the levels of polyphenol compounds and antioxidant activity in vegetables and fruits. Two strategies were used to search the relevant literature. First, searches on the Medline and Science Direct databases were performed using the following combination of keywords: antioxidants, antioxidant*, "antioxidant activity," phenolic compounds, polyphenol*, ORAC, ABTS, FRAP, DPPH, boiling, pressure, stir-frying, microwave*, baking, "pressure boiling", frying, steaming, stewing, process*, reduct*, "home process," "food processing," "domestic processing," vegetables and fruits. Second, a manual search was conducted, checking the references cited by the articles that were retrieved to maximize the results.

The data extracted included the following: study authors, year of the study, cooking techniques, evaluated polyphenols, evaluated methods for antioxidant activity analyses, pre- and post-processing levels of polyphenols and antioxidant activity, standard deviation (SD) and number of samples (n). Only studies that used at least three samples and that presented the standard deviation were included. Furthermore, only studies that evaluated cooking techniques that involved heat were chosen.

Meta-analysis calculation

Two meta-analysis approaches were designed. The first approach was developed to assess the effects of the cooking techniques on polyphenol compounds and antioxidant activity levels; the second approach was developed as a variation of the first meta-analysis. A new analysis was performed by stratifying the data according to the mechanism of each method that was used to quantify the antioxidant activity while considering two groups: ET-based assays (FRAP, DPPH, ABTS, TEAC, CUPRAC and Folin method) and HAT-based assays (TRAP, ORAC, PCL, β-carotene bleaching and crocin-based assay). This approach was used to investigate the differences between the analysis methods.

For both of the approaches, a response ratio was calculated as described by Borenstein et al. (2009a), Hedges et al. (1999) and used by Murador et al. (2014). The response ratio (R) was computed as $R = \overline{X}_{(proc)} \,/\, \overline{X}_{(unproc)}$ where *proc* is the value present in the vegetable after cooking, and *unproc* is the value from the raw vegetable). The value R was calculated for each experiment. This ratio was transformed by taking its natural logarithm, $L = \ln(R)$, to linearize the metric and normalize the distribution of R. The variance was calculated using the pooled within-group standard deviation $V_{lnR} = \left(SD_{pooled}\right)^2 \left(\frac{1}{n_{proc}\,\overline{X}^2_{proc}} + \frac{1}{n_{unproc}\,\overline{X}^2_{unproc}}\right)$, and the approximate standard error was calculated as $SE_{\ln R} = \sqrt{V_{\ln R}}$.

A random-effects model was selected for all of the analysis, which considered the different experimental conditions of the selected studies (Borenstein et al., 2009b) and the values of the Q-statistic and I^2 (Higgins et al., 2003). The L values of all of the studies received weights that were based on the random effect model and the variance between the individual experiments (L^*). The weighted mean response ratio (R^*) was given by converting the weighted L using $R^* = \exp\,(L^*)$.

For correlations between ratios and cooking time the Pearson's correlation was used.

Comprehensive Meta-Analysis Software Version 2 (BioStat®) was used for the data analysis.

Results and discussion

Literature search and data collection

First, a database search was conducted on the Medline database, to identify the literature that regards the effects of cooking techniques on polyphenol compounds and antioxidant activity. In this search, 1006 articles were found, but only 2 followed the inclusion criteria. Thus, a second search was performed in the Science Direct database, in which 612 articles were found, from which we selected 13 articles. Some other studies were included from a manual search of references that were cited in the articles. In total, 21 articles were selected as meeting the inclusion criteria, dating from 2003 to 2015.

Articles that did not present the mean values, standard deviations or raw data that could be used for further calculations and articles that only evaluated a single sample or that did not define the sample number were excluded. Furthermore, articles that evaluated the effects of industrial processes, such as canning, drying, freeze-drying and spray-drying, and articles that considered kinetic parameter values instead of absolute values were disregarded. The retained studies contained quantitative information about the effects of cooking techniques on polyphenol compounds and/or antioxidant activity in vegetables; no work about this theme in fruits was included. Summaries of the selected publications that involve polyphenol compounds and antioxidant activity are listed in Tables 1 and 2, respectively. The studies quantified the total or specific phenolic compounds and/or antioxidant activity levels in one type of vegetable or more, covering one or more cooking techniques.

Effects of cooking techniques on polyphenols

The results that are related to polyphenols are shown in Table 3. The R^* values of <1 indicate a reduction, and the R^* values of >1 indicate an increase in the polyphenol levels after using a specific cooking technique. A lower R^* value

Table 1. Summary of studies that address the effects of cooking techniques on polyphenol compounds.

Reference	Cooking technique	Vegetable	Phenolic compound
Dini *et al.* (2013)	Boiling Frying Microwaving Steaming	Pumpkin pulp	Total phenolics
dos Reis *et al.* (2015)	Boiling Microwaving Steaming	Organic broccoli, organic cauliflower	Quercetin, kaempferol
Ferracane *et al.* (2008)	Boiling Frying Steaming	Artichoke	5-*O*-caffeoylquinic acid, 1,5-di-*O*-caffeoylquinic acid, apigenin 7-*O*-glucuronide
Gahler *et al.* (2003)	Baking	Tomato	Total phenolics
Harakotr *et al.* (2014)	Boiling Steaming	Purple waxy corn	Total phenolics
Im *et al.* (2011)	Boiling	White onions, white lotus roots	Total phenolics, flavonoids, flavonols, tannins
Ismail *et al.* (2004)	Blanching	Cabbage, kale, shallots, spinach, swamp cabbage	Total phenolics
Lemos *et al.* (2012)	Baking	Baru nuts	Total phenolics
Lemos *et al.* (2015)	Baking Boiling Microwaving Steaming	Potato (Purple Majesty)	Total phenolics
Makris & Rossiter (2001)	Boiling	Onion bulbs, asparagus spears	Quercetin 3,4'-diglucoside, Quercetin 4'-glucoside, rutin
Miglio *et al.* (2008)	Boiling Frying Steaming	Broccoli, carrot, courgette	Total phenolics
Pellegrini *et al.* (2010)	Boiling Microwaving Steaming	Broccoli, Brussels sprouts, cauliflower	Total phenolics
Perla *et al.* (2012)	Baking	Potatoes (Mesa Russeta, CO99256-2R, Silverton Russeta, CO98012-5R, CO95172-3RU, Colorado Rosea, Russet Nuggeta, VC0967-2R/Y, CO99045-1W/Y, CO01399-10P/Y, AC99329-7PW/Y, Purple Majesty, CO97222-1R/R, CO97226-2R/R)	Total phenolics, total flavonoids, total flavonols
	Boiling Microwaving		
Roy *et al.* (2009)	Steaming	Broccoli	Total phenolics, total flavonoids
Sahlin *et al.* (2004)	Baking Boiling Frying	Tomatoes (Aranca, Excell)	Total phenolics
Turkmen *et al.* (2005)	Boiling Microwaving Steaming	Broccoli, green beans, leek, peas, pepper, spinach, squash	Total phenolics
Valdés *et al.* (2011)	Pressure cooking	Beans (IAPAR-81, Uirapuru, BAF 55)	Total phenolics
Wen *et al.* (2010)	Blanching	Beans (Four-angled, French, Long), peas (snow, snap)	Total phenolics
Xu & Chang (2009)	Boiling Pressure cooking Steaming	Black beans, pinto beans	Total phenolic acids, total flavonols
Zhang & Hamauzu (2004)	Boiling Microwaving	Broccoli	Total phenolics

Table 2. Summary of studies that address the effects of cooking techniques on antioxidant activity.

Reference	Cooking technique	Vegetable	Method
Amin *et al.* (2006)	Blanching	Spinach (Bayam itik, Bayam merah, Bayam panjang, Bayam putih)	Total Antioxidant Activity
Dini *et al.* (2013)	Boiling Frying Microwaving Steaming	Pumpkin pulp	DPPH, FRAP
dos Reis *et al.* (2015)	Boiling Microwaving Steaming	Organic broccoli, organic cauliflower	DPPH
Ferracane *et al.* (2008)	Boiling Frying Steaming	Artichoke	FRAP, TEAC, TRAP
Gahler *et al.* (2003)	Baking	Tomato	Photochemiluminescence (PCL), FRAP, TEAC
Harakotr *et al.* (2014)	Boiling Steaming	Purple waxy corn	FRAP, TEAC
Im *et al.* (2011)	Boiling	White onions, white lotus roots	Cupric reducing antioxidant capacity (CUPRAC), ABTS, DPPH, FRAP
Ismail *et al.* (2004)	Blanching	Cabbage, kale, shallots, spinach, swamp cabbage	β-carotene bleaching
Lemos *et al.* (2012)	Baking	Baru nuts	DPPH
Lemos *et al.* (2015)	Baking Boiling Microwaving Steaming	Potato (Purple Majesty)	FRAP
Miglio *et al.* (2008)	Boiling Frying Steaming	Broccoli, carrot, courgette	FRAP, TEAC, TRAP
Pellegrini *et al.* (2010)	Boiling Microwaving Steaming	Broccoli, Brussels sprouts, cauliflower	FRAP, TEAC, TRAP
Perla *et al.* (2012)	Baking	Potatoes (Mesa Russeta, CO99256-2R, Silverton Russeta, CO98012-5R, CO95172-3RU, Colorado Rosea, Russet Nuggeta, VC0967-2R/Y, CO99045-1W/Y, CO01399-10P/Y, AC99329-7PW/Y, Purple Majesty, CO97222-1R/R, CO97226-2R/R)	DPPH
	Boiling Microwaving		
Roy *et al.* (2009)	Steaming	Broccoli	ORAC, Cellular assay for oxidative stress
Sahlin *et al.* (2004)	Baking Boiling Frying	Tomatoes (Aranca, Excell)	ABTS
Turkmen *et al.* (2005)	Boiling	Broccoli, green beans, leek, peas, pepper, spinach, squash	DPPH
	Microwaving Steaming		
Valdés *et al.* (2011)	Pressure cooking	Beans (IAPAR-81, Uirapuru, BAF 55)	DPPH
Wen *et al.* (2010)	Blanching	Beans (Four-angled, French, Long), peas (snow, snap)	β-carotene bleaching, DPPH
Zhang & Hamauzu (2004)	Boiling Microwaving	Broccoli	DPPH

corresponds to a greater reduction in the compounds, and vice versa.

Some of the studies report increases on the phenolic content. Gahler et al. (2003) analyzed tomatoes that were baked for 15, 30 and 45 minutes and found increases that ranged from 5.2% ($R = 1.05$) to 44.4% ($R = 1.44$) for baking at 180°C degrees; from 5.4% ($R = 1.05$) to 40.4% ($R = 1.40$) at 200°C degrees; and from 19.4% ($R = 1.19$) to 37.4% ($R = 1.37$) at 220°C

Table 3. Response ratios and confidence intervals of polyphenol content in published studies on processed vegetables used in meta-analysis.

Cooking technique	n^*	Cooking time (mean minutes)	Q	R^*	Lower	Upper	p
Baking	55	53.4	23486.78	0.515	0.474	0.561	<0.001
Blanching	16	7	14132.53	0.937	0.879	0.999	0.046
Boiling	147	35.5	400998.04	0.625	0.570	0.684	<0.001
Frying	9	5.1	265205.8	0.716	0.501	1.024	0.06
Microwaving	68	14.2	168282.06	0.539	0.476	0.610	<0.001
Pressure cooking	13	26.6	901.15	0.468	0.390	0.561	<0.001
Steaming	31	13.4	483823.29	0.950	0.784	1.152	0.604

* Sum of the samples used in each selected study; Q = Q-statistic value; R^* = weighted response ratio.

degrees. Dini et al. (2013) evaluated the effect of various cooking techniques on pumpkin pulp and found an increase of 14% ($R = 1.14$) for boiling, 17% ($R = 1.17$) for steaming, 18% ($R = 1.18$) for microwaving, and 6% ($R = 1.06$) for frying.

The authors justified these results by the fact that heat treatment might cause cellular disruption and dissociation of some of the phenolic compounds from the biological structures, releasing the phenolics from the food matrix, and it could also cause an alteration in their chemical structure, enabling the conversion of insoluble phenolics into more soluble forms (Gahler et al., 2003; Dini et al., 2013). According to Burgos et al. (2013), cooking could cause hydrolysis of different components releasing phenolic compounds and making them more available for extraction.

On the other hand, Pellegrini et al. (2010) studied broccoli, Brussel sprouts and cauliflower, and found various results: boiled broccoli presented an increase of 15% ($R = 1.15$), boiled Brussels sprouts had an increase of 48% ($R = 1.48$), microwaved Brussels had a 90% increase ($R = 1.90$), and steamed Brussels sprouts a 56% increase ($R = 1.56$). Microwaved broccoli presented a reduction of 67.1% ($R = 0.33$), steamed broccoli had a decrease of 33.1% ($R = 0.67$), boiled cauliflower a 45.5% decrease ($R = 0.54$), microwaved cauliflower a 29.6% decrease ($R = 0.70$), and steamed cauliflower a 32.6% decrease ($R = 0.67$). The effect of cooking is likely to depend on several factors, such as the cooking technique, heating degree, solvent used for extraction, pH, surface area exposed to water and oxygen, and mainly, the food matrix, which implies varying interactions in different compounds; thus, the same cooking technique could present different effects in different types of food (Bernhardt and Schlich, 2006; Wachtel-Galor et al., 2008). Burgos et al. (2013) and Perla et al. (2012) evaluated total phenolics in raw and cooked potatoes, but the different concentrations of methanol used in the extraction phase could implied on opposite results.

The cooking time also can be an influence factor on polyphenol contents. The baking ($r = -0.74$; $p < 0.001$), boiling ($r = -0.67$; $p < 0.001$) and microwaving ($r = -0.43$; $p < 0.001$) techniques presented negative correlations statistically significant, indicating that the longer the cooking time, the lower the ratio, so higher the losses on polyphenol contents. The phenolic compounds are susceptible to chemical or enzymatic oxidations that have been widely proved to cause a progressive decrease in polyphenol antioxidant properties (Nicoli et al., 1999), so that a longer cooking time can reflect in a higher expose of the phenolic compounds to oxidations. The cooking time showed high variation between experiments. Therefore, the correlation analysis between cooking time and ratios should be interpreted as indicative rather than conclusive. Moreover, some cooking techniques presented low number of samples and studies, limiting such correlations.

According to the results of this meta-analysis, the cooking process implied a reduction in the polyphenol levels compared to the raw vegetables. However, these results were significant only for baking ($R^* = 0.51$; $p < 0.001$), blanching ($R^* = 0.94$; $p = 0.046$), boiling ($R^* = 0.62$; $p < 0.001$), microwaving ($R^* = 0.54$; $p < 0.001$) and pressure cooking ($R^* = 0.47$; $p < 0.001$); no significance was noted for frying ($R^* = 0.72$; $p = 0.06$) and steaming ($R^* = 0.95$; $p = 0.604$). One reason for the reduction in the polyphenol levels after cooking is related to their release from the food matrix; cutting the vegetable tissue and exposure to higher temperatures can lead to cellular disruption and the disassociation of some phenolic compounds from the cellular structures, such as lignin and polysaccharides (Bernhardt and Schlich, 2006; Faller and Fialho, 2009). Moreover, some vegetables, as mushrooms, exhibit high phenol oxidase and phenol peroxidase activity, which causes polyphenols to oxidize when vegetable tissue is broken (Jaworska et al., 2014). Besides, when considering the cooking techniques that involve water, the phenolics are largely lost by leaching into the cooking water (Wachtel-Galor et al., 2008).

Furthermore, during processing, the chemical structures of the polyphenols suffer changes, such as isomerization and hydrolysis events, which lead to a substantial redistribution of the phenolic concentrations due to a massive transesterification phenomenon (Ferracane et al., 2008). Data in the literature about the effect of cooking on the polyphenol content are scarce and often limited to only the total phenolic compound concentration, as measured by the Folin-Ciocalteu method (Ferracane et al., 2008). However, polyphenols are a broad group and include many compounds, which present different chemical structures and some particularities and, consequently, different behavior; thus, it is difficult to evaluate the effect of cooking on polyphenols in general rather than on a specific phenolic compound.

Effects of cooking techniques on antioxidant activity

The results that are related to antioxidant activity are shown in Table 4. First, when considering the effect of the cooking process on the antioxidant activity, it is fundamental to regard the many methodologies that are related to the antioxidant activity, beyond other factors already mentioned before, such as the food matrix and extractability, cooking and environmental conditions.

According to the selected studies, frying ($R^* = 2.26$; $p < 0.001$) and steaming ($R^* = 1.52$; $p < 0.001$) techniques resulted in an increase in the antioxidant activity levels. Miglio et al. (2008) also found increases in these levels as measured by TEAC, FRAP and TRAP when evaluating carrots, zucchini and broccoli that were submitted to frying, steaming and boiling. For frying, the increases ranged from 49.6% ($R = 1.50$) to 1767% ($R = 18.67$), including all vegetables and methodologies; for steaming, the increases ranged from 33.3% ($R = 1.33$) to 219% ($R = 3.19$); finally, for boiling, the increases ranged from 21.1% ($R = 1.21$) to 567% ($R = 6.67$). During intense heat treatment or prolonged storage, MRPs can be formed, and generally, they exhibit strong antioxidant properties; as a result, MRPs can contribute to an increase in the antioxidant activity levels after the cooking process (Eichner, 1980; Nicoli et al., 1997). Thermal treatment does not always result as the destruction of bioactive compounds; in some cases, cooking can induce the formation of novel compounds and improve antioxidant properties (Xu & Chang, 2008a; Harakotr et al., 2014).

Moreover, the positive effect on antioxidant activity may also be due to breakdown compounds, which leads to a

Table 4. Response ratios and confidence intervals of antioxidant activity levels in published studies on processed vegetables used in meta-analysis.

Cooking technique	n^*	Cooking time (mean minutes)	Q	R^*	Lower	Upper	p
Baking	46	39.6	4916.14	0.454	0.400	0.515	<0.001
Blanching	34	9.2	2530.97	0.900	0.809	1.001	0.052
Boiling	124	27.9	53943.39	0.762	0.734	0.791	<0.001
Frying	16	5.5	12534.78	2.256	1.68	3.017	<0.001
Microwaving	44	12.4	892919.33	0.677	0.455	1.006	0.054
Pressure cooking	9	26	0.02	1.279	0.000	4846.8	0.95
Steaming	42	13.6	17373.62	1.516	1.428	1.609	<0.001

* Sum of the samples used on each selected study; Q = Q-statistic value; R^*=weighted response ratio.

variation in the phenolic content and the composition (Makris and Rossiter, 2001). Chemical or enzymatic oxidations have been widely proved to cause a decrease in polyphenol antioxidant properties; however, polyphenols with an intermediate oxidation state can exhibit higher radical scavenging efficiency than the non-oxidized ones (Nicoli et al., 1999).

The present meta-analysis study showed that baking ($R^* = 0.45$; $p < 0.001$) and boiling ($R^* = 0.76$; $p < 0.001$) led to a significant decrease in the antioxidant activity levels. Sahlin et al. (2004) evaluated the antioxidant activity of two tomato cultivars using the ABTS assay. They found decreases on the antioxidant activity levels that ranged from 7.8% ($R = 0.92$) to 17.8% ($R = 0.82$) after baking; from 9.8% ($R = 0.90$) to 10.5% ($R = 0.89$) after boiling; and from 36.2% ($R = 0.64$) to 37.1% ($R = 0.63$) after frying. The Maillard reaction occurs as a consequence of the heat treatment; however, despite the well-documented antioxidant properties of the MRPs, during the early stages of the Maillard reaction, highly reactive radicals can be formed that exhibit pro-oxidant properties (Roberts and Lloyd, 1997). The formation of these pro-oxidant radicals during the early phases of the Maillard reaction may depend on the intensity and duration of the heat treatment: the phases that contribute to the formation of compounds with pro-oxidant properties last longer when low-temperature heating is applied than in the cases of high-temperature treatments (Nicoli et al., 1999). Furthermore, according to Harakotr et al. (2014), who analyzed purple waxy corn, the synergistic combinations or interactions of several types of chemical reactions, diffusion of water soluble compounds, and the formation or breakdown of them can also contribute to losses in antioxidants from the cooked vegetable. The boiling technique led to the loss of phenolic compounds by leaching, which could reflect a reduction of the antioxidant activity. Thus, it is clear that the conditions of cooking influence the antioxidant activity levels of foods that are submitted to heat treatments.

Similarly to founds for the phenolic compounds, the cooking time exhibit negative correlations for the baking ($r = -0.53$; $p < 0.001$), boiling ($r = -0.28$; $p = 0.002$ and microwaving ($r = -0.32$; $p = 0.02$) techniques, indicating once more, that the longer the cooking time, the lower the ratio, so higher the decreases on antioxidant activity levels.

Finally, with respect to the present results, some techniques increased or reduced the antioxidant activity levels, mainly due to heterogeneous results that had high response ratios to both increases and reductions of these levels, such as blanching ($R^* = 0.90$; $p = 0.052$) and microwaving ($R^* = 0.68$; $p = 0.054$). For the pressure cooking ($R^* = 1.28$; $p = 0.95$) technique, the low number of samples ($n = 9$) likely reduced the statistical power to determine its effects. Yang et al. (2014) performed a study on the effect of cooking on Zhejiang pecan and concluded that DPPH radical-scavenging activity revealed a rising trend at lower temperatures or over shorter heating durations, while a declining trend was noted at higher temperatures or over longer heating durations. In addition, with respect to the antioxidant activity, it is fundamental to consider that there are hundreds of antioxidant compounds in most foods; thus, the total antioxidant capacity of a given food may represent the integrated action of multiple compounds rather than a single compound. Therefore, to evaluate the total antioxidant activity of a given food, it is necessary to consider the possible interaction of components in their contribution to the antioxidant activity (Wu et al., 2004).

Effects of cooking techniques on antioxidant activity according to the mechanisms of action that the methods are based on

The results of the second meta-analysis are presented in Table 5. This meta-analysis evaluated the effects of the cooking techniques that were statistically significant in the first meta-analysis (boiling, steaming, microwaving and baking) but considered the mechanism of each method that was used to quantify the

Table 5. Response ratios and confidence intervals of antioxidant activity levels in published studies, according to the method used (ET-Based assays and HAT-Based assays).

	ET-Based assays					HAT-Based assays				
Cooking technique	n^*	R^*	Lower	Upper	P	n^*	R^*	Lower	Upper	p
Boiling	117	0.728	0.721	0.776	<0.001	7	1.83	0.88	3.92	0.11
Steaming	28	1.236	1.191	1.284	<0.001	14	2.142	1.746	2.628	<0.001
Microwaving	41	0.673	0.447	1.013	0.057	3	0.822	0.509	1.326	0.422
Baking	37	0.454	0.399	0.515	<0.001	9	0.855	0.000	5568.0	0.97

* Sum of the samples used in each selected study; R^*=weighted response ratio.

antioxidant activity. Thus, two groups were considered: ET-based assays (FRAP, DPPH, ABTS, TEAC and CUPRAC) and HAT-based assays (TRAP, ORAC, PCL and β-carotene bleaching). This approach is interesting because there are many methodologies to evaluate antioxidant activity, which hampers the comparison between the results. According to the selected studies for this meta-analysis, with respect to the ET-Based assays, boiling ($R^* = 0.728$; $p < 0.001$) and baking ($R^* = 0.454$; $p < 0.001$) techniques were associated with decreases of antioxidant activity levels, while steaming ($R^* = 1.236$; $p < 0.001$) resulted in an increase in these levels.

These results reflected the foundations of the first meta-analysis because most of the evaluated studies considered ET-Based assays. In contrast, the number of studies that used HAT-Based assays is very limited; thus, the largest number of results had no statistical power to determine their effects, with the exception of a steaming technique that reflected an increase in the antioxidant activity levels. Thus, the steaming technique was the only technique that presented statistically significant results in both groups (the ET and HAT-Based assays), making it possible to correlate those results. As can be noted in **Table 2**, many studies used more than one antioxidant activity assay; however, if the results cannot be correlated, then the reason for performing more than one assay is not clear, not least because some studies used more than one method based on the same mechanism of action (Gahler et al., 2003; Ferracane et al., 2008; Miglio et al., 2008; Pellegrini et al., 2010; Dini et al., 2013). The utility of the ORAC method should be noted, given that the peroxyl radical is present in biological and food resources. In a study, the ORAC assay and another interesting method that can provide evidence (the cellular antioxidant activity assay) were considered for evaluating the effect of the steaming technique on broccoli (Roy et al., 2009). However, the researchers used the ORAC assay for lipophilic extracts, when in fact this method is appropriate only for hydrophilic extracts. This meta-analysis once again reflects the scarcity of literature on this theme, consisting mainly of studies that use HAT-Based assays, which makes it very difficult to evaluate these results.

Meta-analysis considerations

One of the most challenging aspects of this work was to find articles that presented the prerequisites that would be included in a meta-analysis. In several cases, the number of samples was less than three. A large number of studies were excluded because of this aspect alone. A lack of information about the cooking method (e.g., temperature, cooking time, addition or not of water) was also observed in several cases. Our study design allowed us to analyze the effects of different cooking techniques in a generic way. However, it was not possible to fully analyze and describe the effects of these techniques while considering only ET-based essays. It is possible that the effect measured by HAT-based essays would be similar for ET-based essays, but this prospect is only a hypothesis.

The antioxidant activity and phenolic compound quantifications are true ratio measures, unlike the variables used in social sciences studies. This fact allows the use of response ratios such as those used in the present study (Borenstein et al., 2009a). However, considering that each study used different conditions, equipment and techniques to quantify the phenolic compounds and measure the antioxidant activity, and considering the values of the Q-statistic and I^2, random effect sizes were used in the meta-analysis (Higgins et al., 2003). Therefore, it would be foolhardy to establish quality scores for each study with the intent of using those scores to assign the fixed weights that are required when performing fixed effect analysis.

As a final consideration, new studies on this topic should adopt quality controls, such as those discussed in this review, to analyze their experiments and present their results and to provide reliable data that can be understood and analyzed in the future.

Conclusions

Cooking techniques influence the polyphenol content and antioxidant activity levels in vegetables. Heat treatment can soften the vegetable tissue, facilitating the extraction of the phenolic compounds from the cellular matrix, but it can also cause cellular disruption and disassociation of some polyphenols from cellular structures. Phenolic compounds are water soluble; thus, cooking techniques that involve water usually lead to phenolic losses by leaching. Thus, not accidentally, the present study showed that the polyphenol content decreased most significantly after the boiling technique, and no technique led to a significant increase. In contrast, the antioxidant activity levels decreased most during the baking technique, while frying precipitated the greatest increase. Considering the mechanism of the used method, steaming was the only technique that presented significant increases for both groups (ET-based assays and HAT-based assays). During the cooking process, the Maillard reaction occurs, and the intensity and duration of the heat treatment appears to determine the formation of compounds that have pro-oxidant properties or the formation of Maillard-reaction products that exhibit strong antioxidant properties. Moreover, the food matrix that is involved directly influences the levels of polyphenols and antioxidant activity because it determines what compounds are presented and the interactions that determine the total antioxidant capacity of a particular food. Thus, the effects of the cooking technique on phenolic compound content and antioxidant activity levels depend on several factors, such as the cooking time, heating degree, solvent used for extraction, pH, and surface area exposed to water and oxygen, but mainly the food matrix.

From the nutritional point of view, the steaming technique can be considered to be the best recommendation for increasing the antioxidant activity levels in a cooked vegetable. In this technique, despite being a moist-heat cooking technique, the water-soluble compounds are not lost by leaching because the vegetable does not remain in contact with the water; furthermore, the technique does not involve the addition of oil. The steaming technique presented significant increases in antioxidant activity levels against both mechanisms of action considered by the analysis methods, which shows it to be the best cooking technique to prepare vegetables that have a higher antioxidant power.

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