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Analytical Methods

Extraction methods determine the antioxidant capacity and induction of quinone reductase by soy products *in vitro* ☆

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ABSTRACT

Gastrointestinal (GI) mimic and organic solvent extracts of whole soybean powder (WSP), soy protein concentrate (SPC), and soy protein isolate (SPI) as well as soy isoflavone concentrate (SIC) were analysed for total phenols; quinone reductase (QR) induction in hepa1c1c7 cells; antioxidant scavenging of DPPH, HOCl, $ONOO^-$, and $O_2^{\cdot}$; and total antioxidant capacity via FRAP and ORAC assays. GI extracts of all the soy products had higher concentrations of total phenols than from acidified methanol (MeOH) but lower antioxidant potency. The MeOH extract of SPC was most potent in quenching HOCl and $ONOO^-$ and increasing FRAP and ORAC, but did not induce QR. Despite weak antioxidant activity, hexane (HX) extracts induced QR more than GI and MeOH extracts with WSP > SPC > SPI > IC. Soy extracts were ineffective scavengers of DPPH and $O_2^{\cdot}$. Thus, extraction methods markedly affect the antioxidant profile and QR induction capacity of soy products.

1. Introduction

Soybean and its derived products are used extensively for food formulation, including meat analogues, infant formula, and soymilk, as well as dietary supplements. Soy is considered a desirable ingredient for health promotion, particularly because of its inverse association in observational studies with the risk of cardiovascular disease, some forms of cancer, menopausal symptoms, and osteoporosis (Omoni & Aluko, 2005). These health benefits have been ascribed substantially to the bioactivity of soy polyphenols, particularly the isoflavones, including their antioxidant, anti-proliferative, and hypocholesterolemic effects as well as binding to oestrogen receptors (Isanga & Zhang, 2008). Isoflavones have been shown in a variety of *in vitro* and animal studies to be potent antioxidant and chemopreventive flavonoids (Messina & Flickinger, 2002; Omoni & Aluko, 2005; Rufer & Kulling, 2006). The efficacy of isoflavones is dependent on their relative enrichment in soy products, which can vary as much as 50-fold due to processing methods (Wang & Murphy, 1994).

Rapid, *in vitro* screening methods have been employed to test the potential bioactivity of plant foods; e.g., an array of free radical scavenging assays has been used to identify and rank foods by their antioxidant capacity (Paganga, Miller, & Rice-Evans, 1999). Induction of phase II detoxification enzymes, such as quinine reductase (QR), has been employed to identify foods with chemoprotective potential (Zhang, Talalay, Cho, & Posner, 1992). Of course, the relevance of results from these *in vitro* tests to bioactivity *in vivo* is always limited due to their independence from nutrient factors such as bioaccessibility, bioavailability, and metabolism. However, a greater degree of predictability from *in vitro* antioxidant assays may be possible by more directly mimicking physiology and testing specific reactions found *in vivo*.

In screening plant foods for antioxidant activity, organic solvents are commonly employed to maximise the extraction of phytochemicals for testing against various chemical probes. For example, the *in vitro* antioxidant activity of soy has been assessed against the synthetic diphenyl-1-picryl-hydrazyl (DPPH) radical following extraction with acetone (Xu & Chang, 2007; Xu, Yuan, & Chang, 2007) and acetonitrile (Lee et al., 2004). However, this

Abbreviations: MeOH, acidified methanol; DHR 123, dihydrorhodamine 123; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalents; GI, gastrointestinal; HX, hexane; NBT, nitroblue tetrazolium; ORAC, oxygen radical absorbance capacity; SIC, soy isoflavone concentrate; SPI, soy protein isolate; SPC, soy protein concentrate; QR, quinone reductase; WSP, whole soybean powder; DMEM, Dubelco's modified eagle medium; TE, trolox equivalents; AAPH, 2,2′-azobis (2-amidinopropane) dihydrochloride; LSD, least significant difference.

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approach does not reflect the "extraction" or absorption processes in the gastrointestinal tract and the free radicals generated in the cell milieu.

Our hypothesis was that a gastrointestinal extraction method mimicking the "solvent" of the gastrointestinal lumen and organic solvent extracts of soy products have different *in vitro* antioxidant capacity and bioactivity. Therefore, the aim of this study was to determine the extent to which a gastrointestinal-simulated extraction (GI) differs from extraction with acidified methanol (MeOH) and hexane (HX) and affects the antioxidant capacity and QR induction *in vitro* of whole soy powder (WSP), soy protein concentrate (SPC), and soy protein isolate (SPI). We employed a soy isoflavone concentrate (SIC) as a reference standard to assess the potential contribution of isoflavones to bioactivity. The radical scavenging capacity of these soy extracts was measured *in vitro* against the physiologically relevant reactive species peroxynitrite ($ONOO^-$), superoxide anion ($O_2^{-\cdot}$), and hypochlorite (HOCl), as well as against the commonly used DPPH radical. In addition, the "total antioxidant capacity" of the extracts *in vitro* was determined by the ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) assays.

2. Materials and methods

Chemicals: Dubelco's modified eagle medium (DMEM) was obtained from American Type Culture Collection (Manassas, VA) and foetal bovine serum from Hyclone (Logan, UT). $ONOO^-$ in 0.3 N NaOH was purchased from Cayman (Ann Arbor, MI); 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) from Wako Chemicals USA (Richmond, VA); NaOH, methanol, and HCl from Fisher Scientific (Pittsburgh, PA). All other chemicals were acquired from Sigma Chemical (St. Louis, MO) or Sigma–Aldrich (St. Louis, MO). SPC (ARCON® S; containing 72% protein), SPI (PRO-FAM 955; containing 90% protein), WSP (NutriSoy®; containing 43% protein and 19.3% fat), and SIC (NOVASOY 400; containing 43.5% isoflavones) were generously provided by ADM (Decatur, IL).

Extractions: An accelerated solvent extraction system (ASE200, Dionex Corp., Sunnyvale, CA) was used to obtain MeOH and HX extracts of SPI, SPC and WSP, according to the method of Chen and Blumberg (2008). Briefly, powdered soy samples (3 g) were mixed with an equivalent mass of diatomaceous earth and loaded into 11 mL extraction cells topped off with sand. For both MeOH and HX extractions, the flush volume was set to 50%, purge at 180 s, pressure at 1500 psi, static cycle at 5 min, and temperature at 100 °C. MeOH extraction was performed using a sequential extraction with 90%, 60%, and 30% methanol solutions containing 5% acetic acid. HX extraction was run for four cycles with 100% HX. After the exact volume of extracts was measured and recorded, they were centrifuged for 5 min at 4000*g*, and divided into aliquots for subsequent assays.

GI extracts of soy products were performed to simulate the digestion process, according to the method of Chen and Blumberg (2008). Briefly, powdered soy sample (1 g) was mixed with a saline solution and digested with pepsin (pH = 2) in a shaking water bath at 37 °C for 1 h. The solution was then incubated with a pancreatin-bile solution at pH 6.9 for 2 h in a shaking water bath at 37 °C. Pepsin and pancreatin may interfere with antioxidant activity assays, so aliquots of the GI extracts were mixed with equal volumes of 100% methanol to precipitate protein, centrifuged, and the supernatant divided into aliquots for subsequent assays.

Aliquots of the three extracts were dried under N_2 gas at room temperature and stored at −20 °C. Prior to the assays, MeOH extracts were reconstituted in 60% methanol and 5% acetic acid, HX extracts in acetic acid, and GI extracts in water, respectively. SIC was not extracted, but rather dissolved directly in methanol.

Total phenols content: Total phenols of reconstituted extracts were determined according to the modified methods of Singleton, Orthofer, and Lamuela-Ravent (1999) with results expressed as μM gallic acid equivalents (GAE) or mg soy product/100 μmol GAE. For subsequent antioxidant and QR assays, doses of SPI, SPC, WSP, and SIC at 100, 10, and 0.1 μM GAE were used to reflect the range of concentrations potentially present in the gastrointestinal tract, plasma, and cells, respectively, following ingestion.

Radical scavenging activity: DPPH scavenging activity was performed according to Brand-Williams, Cuvelier, and Berset (1995). Briefly, DPPH in ethanol was mixed with an equal volume of different concentrations of the soy extracts and the absorbance at 520 nm was measured after 30 min incubation at room temperature in the dark. Intra- and inter-day assay coefficients of variation (CV) were 1.4% and 7.6%, respectively.

Scavenging activity against $ONOO^-$ was measured by monitoring the increase in fluorescence from the oxidation of dihydrorhodamine 123 (DHR123) according to a slightly modified method of Choi, Choi, Han, Bae, and Chung (2002). The concentration of $ONOO^-$ stock solution was determined by a spectrophotometry after alkalization using a cold 0.3 mol/L NaOH solution at a ratio of 1:40, and aliquots were stored at −80 °C. Immediately before use, $ONOO^-$ was diluted to a final concentration of 100 μM. Fluorescence at 485 nm excitation and 530 nm emission generated from DHR123 oxidation 5 min after the addition of $ONOO^-$ was recorded using a FLUOstar Optima multifunctional plate reader (BMG Labtech Inc., Durham, NC). Intra- and inter-day assay CV were 4.7% and 3.6%, respectively.

Scavenging activity against HOCl was assessed via the oxidation of ferrocyanide $[Fe(II)CN)_6]$ in a phosphate buffer as a reference reaction to investigate the stoichiometry of the reaction according to modification of the methods described by Zhu, Carr, and Frei (2002) and Prutz (1996). The concentration of HOCl stock solution obtained from Sigma was determined according to Hussain, Trudell, and Repta (1970). Briefly, the soy extracts were incubated with HOCl for 5 min at room temperature before the addition of $Fe(II)(CN)_6$ and then absorbance was monitored at 420 nm using a Shimadzu UV1601 spectrophotometer (Japan). Intra- and inter-day assay CV were 0.9% and 2.9%, respectively.

Scavenging activity against $O_2^{-\cdot}$ was measured in a xanthine/xanthine-oxidase system with spectrophotometric determination of the reduction product of nitroblue tetrazolium (NBT) according to a slight modification of the method described by Chun, Kim, and Lee (2003). Briefly, following 10 min of incubation of the soy extracts at room temperature with a reaction mixture of 50 μM NBT, 50 μM xanthine, and 0.05 U/mL xanthine oxidase (final concentrations), the change in absorbance of NBT was measured at 560 nm using a Shimadzu UV1601 spectrophotometer. Intra- and inter-day assay CV were 1.9% and 7.7%. Inhibition of xanthine oxidase activity by the extracts was monitored by the spectrophotometric determination of uric acid production.

Results of radical scavenging activity are expressed as a percentage of the control (no soy extract present) and the IC_{50} (concentration of the soy extract required to decrease absorbance by 50%) in μM GAE, calculated using a spline function.

Total antioxidant capacity assays: The "total antioxidant capacity" of the extracts was assessed using the ORAC and FRAP assays. The ORAC assay was conducted according to Ou, Hampsch-Woodill, and Prior (2001). The ORAC assay employs the area under the curve of the magnitude and time to the oxidation of fluorescein due to peroxyl radicals generated by the addition of AAPH. The assay was carried out on a FLUOstar OPTIMA plate reader utilising fluorescence filters with 485 nm excitation and 520 nm emission. All data are expressed as μmol trolox equivalents (TE)/μmol GAE. Intra- and inter-day assay CV were 3.0% and 7.3%, respectively.

The FRAP assay determines the capacity of antioxidants as reductants in a redox-linked colorimetric reaction of the reduction of Fe^{3+}-2,4,6-tri-pyridyl-S-triazine to a blue-coloured Fe^{2+} complex at low pH that is measured spectrophotometrically at 593 nm (Benzie & Strain, 1996). The extracts were incubated at room temperature with the FRAP reagent and the absorbance recorded after 1 h (Chen & Blumberg, 2008). The reducing power is expressed as μmol TE/μmol GAE. Intra- and inter-day assay CV were 0.7% and 4.2%, respectively.

Quinone reductase induction: The modulation of QR activity in murine hepatoma Hepa1c1c7 cells has been widely employed as a tool to examine the potential chemopreventive activity of phytochemicals (Kang & Pezzuto, 2004). Hepa1c1c7 cells were cultured until confluent in DMEM supplemented with 10% heat inactivated, charcoal-treated foetal bovine serum, 1% penicillin/streptomycin, and 1% L-glutamine in an incubator with 5% CO_2 at 37 °C. After confluence, cells were plated at a concentration of 2×10^4 cells/well in 96-well clear plates and allowed to settle for 24 h. After medium was aspirated, cells were treated with the soy extracts in the medium for 48 h. QR activity was measured by an NADPH-generating system, coupling the oxidation of menadione to the reduction of the dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide according to Kang and Pezzuto (2004). The resulting blue-brown colour was measured at 570 nm using a FLUOstar Optima plate reader. The protein content of cells in each well was determined with a BCA protein kit (Pierce, Rockford, IL). Following adjustment of protein content, QR activity was expressed as nmol/mg protein/min. β-Napthoflavone at concentration of 1 μM was employed as a positive control and increased QR activity 2.4 ± 0.5-fold of the negative control (absent soy extract).

Statistics: All results are expressed as mean ± SD. Statistical comparisons between extraction method and soy products were performed by two-factor ANOVA. Differences in antioxidant and QR activity amongst soy products were analysed by one-factor ANOVA, followed by *post hoc* analysis using Fisher's protected least significant difference (LSD) test. Pearson's correlation tests were performed to reveal possible associations between the antioxidant activity assays, using the JMP IN 4 statistical software package (SAS Institute Inc., Cary, NC). Differences with $P \leqslant 0.05$ were considered significant.

3. Results and discussion

DPPH and $O_2^{-\cdot}$: From 0.1 to 100 μM GAE, SPI, SPC, and WSP extracts and SIC had weak to no antioxidant activity in the DPPH and $O_2^{-\cdot}$ scavenging assays (data not shown). The antioxidant activity of a methanolic soy extract against DPPH has been reported to be 7 μmol TE/g (Xu & Chang, 2007). Gerhäuser et al. (2003) observed that IC_{50} of DDPH and $O_2^{-\cdot}$ scavenging activities for the isoflavone genistein was >250 μM and >100 μM, respectively. Therefore, it is likely in the present study that the soy extract doses approximating bioavailability were too low to scavenge DPPH and $O_2^{-\cdot}$.

Effect of extraction on antioxidant activity: Extraction methods had a significant effect on total phenols content of the soy products using two-factor ANOVA ($P = 0.02$), with the rank of total phenols being GI > MeOH > HX. A consistent relationship between the GI, MeOH, and HX extraction methods and the other assays utilised here was not apparent.

In contrast to the general understanding that extraction by organic solvents affords greater recovery of total phenols than aqueous solvents, the GI extract liberated more total phenols than MeOH and HX in all soy products (Table 1). Consequently, the GI extracts yielded products with the largest antioxidant quantity (i.e., antioxidant potency expressed on a mass basis) in the $ONOO^-$, ORAC, and FRAP assays than MeOH and HX extracts for all soy products tested (Table 2). Since GI extraction employed protease digestion, this finding is in agreement with previous studies in which hydrolysis of soy protein improved antioxidant efficacy due to liberation of bound phenols, release from chelating agents such as phytic acid, and production of antioxidant peptide se-

Table 1
Soy extracts and isoflavone antioxidant quality on a phenolic basis.

Extract	Soy product			
	SPI	SPC	WSP	SIC[e]
Total phenols (μM GAE[a])				
MeOH	163 ± 5	17.7 ± 5.7	805 ± 9	3980 ± 18
GI	1160 ± 8	1290 ± 7	990 ± 19	
HX	0.860 ± 0.010	0.317 ± 0.006	0.843 ± 0.020	
Phenolic equivalents[b] (mg soy/100 μmol GAE)				
MeOH	6.10 ± 0.01	56.6 ± 1.0	1.25 ± 0.00	0.0156 ± 0.0002
GI	0.131 ± 0.000	0.0994 ± 0.0003	0.146 ± 0.004	
HX	11.6 ± 0.0	31.6 ± 0.0	11.9 ± 0.0	
HOCl (IC_{50} in μM GAE)				
MeOH	3.41 ± 0.04	0.933 ± 0.067	2.98 ± 0.15	6.53 ± 0.19
GI	1.84 ± 0.03	2.84 ± 0.03	2.27 ± 0.04	
HX	NA[c]	NA	NA	
$ONOO^-$ (IC_{50} in μM GAE)				
MeOH	9.70 ± 0.11	2.36 ± 0.26	6.84 ± 0.29	3.51 ± 0.09
GI	30.9 ± 0.5	40.0 ± 2.0	12.7 ± 0.1	
HX	NA	NA	NA	
FRAP (μmol TE/μmol GAE)				
MeOH	0.267 ± 0.007	1.14 ± 0.06	0.475 ± 0.003	0.422 ± 0.006
GI	0.0524 ± 0.0	0.369 ± 0.004	0.263 ± 0.001	
HX	0.478 ± 0.004	0.570 ± 0.002	0.237 ± 0.000	
ORAC (μmol TE/μmol GAE)				
MeOH	6.24 ± 0.20	9.29 ± 0.62	2.85 ± 0.11[d]	8.96 ± 0.15
GI	2.72 ± 0.31	2.95 ± 0.04[d]	3.10 ± 0.02	
HX	0.069 ± 0.004	1.14 ± 0.03	NA	

Data are expressed as mean ± SD, $n = 3$.
[a] GAE – gallic acid equivalents.
[b] Phenolic equivalents are the dry weight of soy product in 100 μM GAE.
[c] NA, no measurable activity at 0.1 μM GAE.
[d] Means within antioxidant assays are not significantly different, by Fisher's protected LSD test, $P \leqslant 0.05$.
[e] The isoflavone concentrate was dissolved directly in methanol.

Table 2
Soy extracts and isoflavone antioxidant quantity on a mass basis.

Extract	Soy product			
	SPI	SPC	WSP	SIC[g]
HOCl (IC_{50} in g/100 mL)				
MeOH	10.0 ± 0.1[c]	52.8 ± 3.8[d]	3.74 ± 0.20[b]	0.102 ± 0.003[a]
GI	0.242 ± 0.004[a]	0.280 ± 0.003[a]	10.0 ± 0.2[c]	
HX	NA[f]	NA	NA	
$ONOO^-$ (IC_{50} in g/100 mL)				
MeOH	59.1 ± 0.7[c]	134 ± 15[d]	9.08 ± 1.21[b]	0.00548 ± 0.0014[a]
GI	4.06 ± 0.07[a]	3.97 ± 0.20[a]	1.86 ± 0.02[a]	
HX	NA	NA	NA	
FRAP (μmol TE/g)				
MeOH	0.438 ± 0.011[a]	0.195 ± 0.011[a]	3.79 ± 0.02[b]	271 ± 4[e]
GI	4.00 ± 0.0[b]	37.1 ± 0.4[d]	17.9 ± 0.1[c]	
HX	0.410 ± 0.003[a]	0.180 ± 0.000[a]	0.200 ± 0.000[a]	
ORAC (μmol TE/g)				
MeOH	10.2 ± 0.3[a]	1.64 ± 0.11[a]	8.14 ± 0.32[a]	5740 ± 90[d]
GI	207 ± 23[b]	296 ± 4[c]	9.63 ± 0.05[a]	
HX	0.060 ± 0.003[a]	0.362 ± 0.010[a]	NA	

Data are expressed as mean ± SD, $n = 3$.
[a–e] Means within antioxidant assays are not significantly different, by Fisher's protected LSD test, $P \leqslant 0.05$.
[f] NA, no measurable activity at 0.1 μM GAE.
[g] The isoflavone concentrate was dissolved directly in methanol.

quences (Chen, Muramoto, & Yamauchi, 1995; Saito et al., 2003; Yee, Shipe, & Kinsella, 1980).

Despite a higher antioxidant quantity, the antioxidant quality (i.e., potency expressed on the basis of total phenols) of GI extracts was moderate in the $ONOO^-$, ORAC, and FRAP assays relative to HX and MeOH. In contrast, GI extracts of SPI and WSP possessed high antioxidant quality toward HOCl, with a 24% greater potency than the MeOH extracts. These results demonstrate that antioxidant profiles of soy products are dependent upon the solvent used to extract them, an effect that is most likely due to the different phytochemical profiles of the respective extracts. The content of total phenols and flavonoids in soybeans and their effects on the FRAP, ORAC, and DPPH assays have been previously demonstrated to change with extraction solvent choice (Xu & Chang, 2007). Further, using extracts of almond skins, we found the flavonoid profiles of MeOH and GI extracts to be markedly different (Chen & Blumberg, 2008).

HX extracts were found to possess an antioxidant quality in the order of SPC > SPI > WSP in the FRAP assay. However, HX extracts of SPI, SPC, and WSP yielded lower concentrations of total phenols than GI or MeOH extracts, possessed weaker antioxidant quantity in the FRAP and ORAC assays, and did not exhibit antioxidant activity against $ONOO^-$ and HOCl.

The difference in assay results between the GI extracts compared to the organic solvent extracts highlights the substantial impact of this process on ranking the capacity of soy products with regard to their antioxidant quality and quantity. A limitation to this approach is that *in vivo* metabolism and biotransformation, which may have profound effects on the *in vitro* antioxidant activity of soy constituents (Rufer & Kulling, 2006), are not accounted for. Therefore, careful consideration is required in the design and interpretation of such studies to better allow an extrapolation of the results to potential *in vivo* bioactivity.

Effect of soy processing on antioxidant activity: The concentration of total phenols from the soy products provided from MeOH extracts ranked as WSP > SPI > SPC, reflecting an inverse association with the relative protein purity in these soy products. The production of SPI and SPC can include alcohol washing, protein denaturation, and/or precipitation plus concentration, processes that can enhance the loss of phenolic compounds. Consistent with our results, previous studies have reported decreased extractable phenols and isoflavones in SPI and SPC extracts (Pinto, Lajolo, & Genovese, 2005; Wang & Murphy, 1994).

MeOH extracts of SPC exhibited the greatest antioxidant quality against HOCl and $ONOO^-$ and possessed the highest FRAC and ORAC values. Similarly, the HX and GI extracts of SPC had greater antioxidant quality than SPI and WSP in the FRAP and ORAC assays. In contrast, the total phenols in SPC following MeOH extraction were ninefold less than from WSP and SPI. The total phenols concentration of GI extracts of SPC, SPI and WSP were not markedly different, varying $\leqslant$31%. Interestingly, whilst the antioxidant quantity of SPC was less than SPI and WSP, likely due to its manufacturing process, this same process appears to have enriched its antioxidant quality.

Although the processing did not exert a consistent impact on antioxidant activity measured by an array of assays ($P \geqslant 0.05$), the antioxidant quality of the MeOH extract of SPC was consistently greatest amongst the HOCl, $ONOO^-$, FRAP, and ORAC assays when compared to the other soy products, including SIC. Thus, new efforts to isolate and identify the constituents responsible for the enhanced antioxidant quality of MeOH extracts of SPC are warranted.

Contribution of isoflavones to antioxidant activity in soy products: Soybean extracts and individual isoflavones have been reported to inhibit DPPH, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ATBS) radical cation, and oxygen centred radicals as well as the *ex vivo* oxidation of low-density lipoprotein cholesterol (Lai & Yen, 2002; Rufer & Kulling, 2006; Ungar, Osundahunsi, & Shimoni, 2003; Xu & Chang, 2007). We found the antioxidant quality of purified soy isoflavones was lower than that of the soy extracts in the HOCl assay, but more effective than most of the extracts in the $ONOO^-$ and ORAC assays (except MeOH extracts of SPC). Whilst the contribution of isoflavones to the antioxidant activity of the soy extracts is greater in the $ONOO^-$ and ORAC assays, compared to the FRAP and HOCl assays, the importance of this observation to predicting *in vivo* bioactivity remains to be explored.

Quinone reductase: Determining the induction of QR activity provides an additional assessment of antioxidant function, as QR expression is induced via the redox-sensitive antioxidant response element (ARE) in the genome (Talalay, De Long, & Prochaska, 1988). QR also possesses a more direct antioxidant function by participating in the reduction of the quinone form of oxidised α-tocopherol (Siegel, Bolton, Burr, Liebler, & Ross, 1997). However, neither the soy extracts nor SIC induced QR in Hepa1c1c7 cells in the tested range of 0.1–1 μM GAE. At 100 μM GAE, cell viability was reduced $\leqslant$50% for all the extracts. The MeOH extract of WSP at 10 μM GAE induced QR activity by 31%, whereas the same extract of SPI and SPC had no effect in this assay (Table 3). Thus, the relatively higher antioxidant quality of the MeOH extract of SPC was not correlated with the induction of QR activity. HX extracts of the soy products induced QR activity from 42% to 66% at 10 μM GAE. Since HX extracts possess the lowest antioxidant quality, constituents other than the soy polyphenols appear to be contributing partly to QR induction.

The isoflavone genistein, and, to a lesser extent, daidzein and their glycosides have been found to significantly induce QR activity in Hepa1c1c7 and HepG2 cells (Chun, Chang, Choi, Kim, & Ku, 2005; Yannai, Day, Williamson, & Rhodes, 1998). We found SIC, containing 43.5% isoflavones, induced QR activity by 1.3-fold at 10 μM GAE. However, SIC was 14–44% less effective at inducing QR than the HX extracts of SPI, SPC, and WSP and the MeOH extract of WSP. Previous bioassay-guided fractionation of soy flour also found phenolic esters and other uncharacterized constituents were more potent QR inducers than isoflavones (Bolling & Parkin, 2008, 2009). Therefore, the capacity of the soy extracts to induce QR may be mediated via a synergistic interaction between their phenolic constituents or through other soy compounds in the extract that are more potent than isoflavones.

Correlations between antioxidant assays: Several significant correlations between the antioxidant assays were evident when their results were expressed as antioxidant quantity (Table 4). The content of total phenols correlated positively with the results of the FRAP, ORAC, and HOCl assays ($P \leqslant 0.05$). Notably, no correlations were found between the content of total phenols and antioxidant quality, suggesting that standardising these assays based on their content of total phenols may not provide a worthwhile approach for such comparisons. This apparent discordance may reflect contributions to antioxidant capacity from compounds other than iso-

Table 3
Quinone reductase induction in Hepa 1c1c7 cells by extracts of soy products.

Extract	Soy product			
	SPI	SPC	WSP	SIC[g]
QR induction (treated/control) at 10 μM GAE				
MeOH	1.00 ± 0.01[a]	1.00 ± 0.12[a]	1.31 ± 0.02[c]	1.15 ± 0.03[b]
GI	0.998 ± 0.04[a]	0.975 ± 0.10[a]	0.993 ± 0.07[a]	
HX	1.66 ± 0.06[f]	1.56 ± 0.07[e]	1.42 ± 0.03[d]	

Data are expressed as mean ± SD, $n = 3$.
[a–f]Means within antioxidant assays are not significantly different, by Fisher's protected LSD test, $P \leqslant 0.05$.
[g] The isoflavone concentrate was dissolved directly in methanol.

Table 4
Correlations between antioxidant activity assays of four soy products.[a]

Assay 1	Assay 2	Correlation	*P*-value
FRAP	ORAC	0.9945	<0.0001
Total phenols	FRAP	0.9942	<0.0001
Total phenols	ORAC	0.9895	<0.0001
$ONOO^-$	HOCl	0.9398	0.0017
FRAP	HOCl	0.8922	0.0069
Total phenols	HOCl	0.8734	0.0102

[a] Significant correlations were from antioxidant quantity (mass basis) only.

flavones and related polyphenols in soy extracts and/or variations in efficacy and potency of the soy polyphenols individually or in combination.

4. Conclusions

Extraction methods markedly affect the antioxidant profile and QR induction capacity of soy products. Measures of antioxidant quantity, with potency expressed on a mass basis, and antioxidant quality, with potency expressed per concentration of total phenols, appear to be useful measures for creating a more comprehensive profile of the antioxidant capacity of soy extracts *in vitro*. However, amongst the assays included in our screening panel for antioxidant capacity, no consistent effect of processing was apparent. This approach did reveal that amongst the SPC, SPI, and WSP extracts and SIC, the isoflavones contributed most importantly to the quenching of $ONOO^-$ and the increase in total antioxidant capacity measured by the ORAC assay, but not to the induction of QR activity. Results from the other assays in our panel of tests suggest that soy constituents other than the isoflavones may contribute to the antioxidant actions of soy extracts *in vitro*. For example, MeOH extracts of SPC provided the highest antioxidant quality but had no effect on the induction of QR, whilst the HX extract, with its low concentration of total phenols, was the most potent inducer of QR activity. These results suggest that a broader range of extracts and a more comprehensive set of *in vitro* tests than are typically conducted on plant food extracts may be necessary to provide better prediction of potential *in vivo* antioxidant actions. As GI extracts provided higher concentrations of total phenols than those from organic extracts, the use of extraction methods that more closely resemble the conditions found *in vivo* should prove an important part of these evaluations.

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