Food Chemistry 121 (2010) 927–933

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

# Phenolic acids composition and antioxidant activity of canola extracts in cooked beef, chicken and pork

Amandine Brettonnet [a], Amitha Hewavitarana [b], Sharon DeJong [b], Maria Cecilia Lanari [c,*]

[a] Ecole Nationale Supérieure de Biologie Appliquée á la Nutrition et á la Alimentation, Dijon, Bourgogne, France
[b] CSIRO Food Science, Australia Cannon Hill, Queensland 4170, Australia
[c] Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), Facultad de Ciencias Exactas Universidad Nacional de La Plata (UNLP), Centro Científico Tecnológico CCT La Plata, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Calle 47 y 116 S/N°, La Plata (B1900AJJ), Buenos Aires, Argentina

ARTICLE INFO

*Article history:*
Received 16 June 2009
Received in revised form 3 November 2009
Accepted 10 November 2009

*Keywords:*
Lipid oxidation
Warmed-over favour
Antioxidants
Canola
Polyphenol
Beef
Pork
Chicken

ABSTRACT

Crude polyphenol extracts (15 or 100 mg gallic acid equivalents (GAE)/kg meat) from canola meal reduced the formation of 2-thiobarbituric acid-reactive substances (TBARS) in pre-cooked beef (66–92%), pork (43–75%) and chicken (36–70%). The canola extract contained sinapic (99.7%), ferulic (0.28%) and *p*-hydroxybenzoic acids (0.07%).

The relationship between polyphenol composition and the antioxidant activity of a blend containing of caffeic, cinnamic, *p*-coumaric, ferulic, gentisic, *p*-hydroxybenzoic, salicylic, sinapic and syringic acids was described by a first order polynomial model with 2 and 3 way interactions. Sinapic, caffeic and ferulic acids were the highest contributors to the linear term, followed by gentisic, syringic and cinnamic acids. Although the individual activities of salicylic, *p*-hydroxybenzoic and *p*-coumaric acids were marginal, all significant synergisms included them.



## 1. Introduction

There is an ever increasing trend towards the consumption of healthy, ready prepared meals, both in the food service industries and for home consumption. This has enhanced consumer demand for single serving, quick heat and serve meat products containing a high ratio of polyunsaturated (PUFA) to saturated fats. An inherent disadvantage of pre-cooked meats is the formation of undesirable flavours, known as warmed-over flavour (WOF), during chilled storage. Consumer detection of WOF and subsequent rejection of products have slowed the development and acceptance of these products.

Lipid oxidation is the main cause in the development of WOF and of toxic carcinogenic compounds in meat products. Consumer preference for meats with a high PUFA content has worsened this problem. For many years, meat processors have used synthetic antioxidants, such as butyl hydroxyanisol (BHA) or butyl hydroxytoluene (BHT), to prevent, or reduce, flavour deterioration. However, concerns about their safety and consumer preference for more natural foods have resulted in a high demand for "natural" additives that can extend the shelf life of both processed and unprocessed meat products.

Canola (*Brassica napus*) seeds, canola oil and their by-products are rich in polyphenols, including caffeic, cinnamic, *p*-coumaric, ferulic, gentisic, *p*-hydroxybenzoic, salicylic, sinapic and syringic acids (Shahidi & Naczk, 2003) with high radical-scavenging activities (Karamac, Kosinska, & Pegg, 2005). Shahidi, Wanasundara, Amarowicz, and Naczk (1995) demonstrated that addition of canola flour to meat resulted in 73–97% inhibition of lipid oxidation, as determined by the 2-thiobarbituric acid test (TBA). Vuorela et al. (2005) reported that phenolic extracts from rapeseed meal inhibited lipid oxidation in cooked pork.

Antioxidant efficacy in cooked meat is strongly affected by animal species. Previous studies showed that chicken and beef were more susceptible to oxidation than was pork (Wilson, Pearson, & Shorland, 1976). Therefore, analysing the efficacy of crude extracts of canola meal polyphenols for preventing or inhibiting

*Abbreviations:* ABTS, 2-2′ azinobis(3-ethylbenxothiazoline-6-sulphonic acid); AOA, antioxidant activity; BHA, butyl hydroxyanisole; BHT, butyl hydroxytoluene; caf, caffeic acid; cou, *p*-coumaric acid; fer, ferulic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical; GAE, gallic acid equivalents; HPLC, high performance liquid chromatography; pOHb, *p*-hydroxybenzoic acid; PUFA, polyunsaturated fatty acids; sin, sinapic acid; SM, semimembranosus; syr, syringic acid; TBARS, 2-thiobarbituric acid-reactive substances; TP, total polyphenol; WOF, warmed-over flavour.

* Corresponding author. Tel.: +54 221 424 9287/425 4853.
*E-mail address:* clanari@cidca.org.ar (M.C. Lanari).

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doi:10.1016/j.foodchem.2009.11.021

lipid oxidation and off-flavour development in these products is warranted.

The commercial use of crude extracts as natural antioxidants requires products of consistent activity and composition. It is well established that the antioxidant activity of a mixture depends on the identity and concentration of the active components (Evans, 1997). Naczk, Pegg, Zadernowski, and Shahidi (2005) reported significant correlations between total phenol content and antioxidant activity determined by DPPH and ABTS assays. Although there are numerous reports identifying phenolic antioxidants in canola seed, oil and meal (Shahidi & Naczk, 2003), the relationship between antioxidant activity and phenolic composition in cooked meat has not been clearly determined.

The objectives of this study were:

(a) to analyse the efficacy of crude polyphenol extracts from canola meal to reduce lipid oxidation in pre-cooked beef and chicken and pork.
(b) to determine the relationship between the composition of a mixture of selected polyphenols commonly found in canola meal and total antioxidant activity in ground beef.

## 2. Materials and methods

### 2.1. Reagents

2-Thiobarbituric acid (TBA), 1,1,3,3-tetraethoxypropane, Folin–Ciocalteau reagent and caffeic (caf), *p*-coumaric (cou), ferulic (fer), gallic (gal), *p*-hydroxybenzoic (pOHb) sinapic (sin) and syringic (syr) acids were supplied by Sigma Chemical (Perth, Australia). *t*-Cinnamic (cin), gentisic (gen) and salicylic (sal) acids were from ICN Biochemicals (Aurora, USA).

### 2.2. Extract

The canola (*B. napus)* seeds, Surpass 600 var; (Pacific Seeds Toowoomba QLD, Australia) were ground with a Waring commercial blender (New York, USA) using 2 cycles, each of 2 min. Portions (12 g) of the powder were wrapped with filter paper Whatman No.1, defatted with hexane 95% using a Soxhlet apparatus and air-dried overnight. The defatted canola meal was extracted 3 times with acetone 70% (8 g/50 ml); the residual meal was separated from the extract by centrifugation (10 min, 5000*g*). The extracts were combined, evaporated to dryness under vacuum at 40 °C and freeze-dried for 12 h at −35 °C to eliminate the remaining water.

### 2.3. Effect of extract concentration on lipid stability of pre-cooked ground beef, chicken and pork

The beef samples were obtained from the semimembranosus (SM) muscle of 5 steers from a local abattoir. At 48 h *post mortem*, each muscle was cut into sections, vacuum-packaged and stored at −20 °C in the dark until required for analysis (within 30 days). After removing the subcutaneous fat, sections of each muscle were ground with an Oskar mincer (Brisbane, Australia), using 4 cycles each of 5 s, and divided into 20 g patties The canola extract was dissolved in 2 ml of ethanol and added to each patty at a final concentration of 15 or 100 mg gallic acid equivalents/kg meat (GAE/kg meat). The control samples had 2 ml of ethanol with no antioxidant. Samples were thoroughly mixed and cooked in a water bath at 76 °C for 40 min. Marksberry, Kropf, Hunt, Hague, and Warren (1993) recommended 76 °C as the final internal temperature for cooking beef patties to a "well done" degree of doneness. After cooking, the samples were stored at 4 °C for 0, 3 or 6 days. Pork (SM, 8 animals) and chicken (breast, 12 birds) samples were processed similarly. At each storage period, lipid oxidation was determined using the 2-thiobarbituric acid-reactive substances (TBARS) method (Vynke, 1975).

### 2.4. Antioxidant activity and polyphenol composition in ground beef

SM samples from the 5 steers used in the previous experiment were ground and treated with different combinations of caffeic, cinnamic, *p*-coumaric, ferulic, gentisic, *p*-hydroxybenzoic, salicylic, sinapic and syringic acids. The different polyphenol blends were dissolved in 2 ml of ethanol and added to each patty at a final total concentration of 0.05 mmol /kg meat. Within each mixture, the relative amounts of the individual components were similar. The control samples had 2 ml of ethanol with no antioxidant. Meat samples were cooked under conditions similar to those described in the previous experiment and stored at 4 °C for 0 and 6 days. Lipid oxidation was determined at each storage time by the TBARS technique (Vynke, 1975).

### 2.5. Chemical analyses

#### 2.5.1. Total phenol concentration

The total phenolic content of the extracts was determined by the Folin–Ciocalteau method (Singleton & Rossi, 1965) and expressed as mg of gallic acid equivalents (GAE)/g extract. All tests were run in triplicates.

#### 2.5.2. Identification of major phenolic acids in the extract

To determine the phenolic acid composition of the extract, free and esterified phenolic acids were separated using the technique described by Zadernowski and Kozlowska (1983). After this step, phenolic acids were identified by HPLC, comparing their retention times with those from nine commercial standards (caf, cou, fer, sin, syr, cin, gen, sal, and pOHb).

The analysis was done with a Waters instrument (Milford, MA, USA) fitted with a gradient pump (Waters 501), column thermoregulator, autosampling injector (Waters TM717) and a photo diode array (PDA). The column was an Ultrasphere C18 (5 μm particle size, 250 × 4.6 mm ID; Alltech Assoc. Brisbane, Australia). Chromatographic separations were carried out using a phosphate buffer solution (A) ($NaH_2PO_4/Na_2HPO_4$ pH 7) and absolute methanol (B) as mobile phase with a flow rate was 1.0 ml/min. The gradient programme was: 0–20 min 100% A; 20–25 min 65% A/35% B; 25–35 min 100% A.

#### 2.5.3. Lipid oxidation

Lipid stability was monitored with the TBARS method (Vynke, 1975). Tests were performed in triplicate and the results were expressed as mg malondialdehyde/kg meat (mg MDA/kg meat).

Antioxidant activity ($AOA_t$) of each treatment was calculated using the TBARS values of control and treated meat samples at each time period according to the following equation:

$$AOA_t = 100 * ((TBARSc_t - TBARStrt_t)/TBARSc_t) \quad (1)$$

$TBARSc_t$ and $TBARStrt_t$ are the TBARS values of control and treated samples at time *t*, respectively.

### 2.6. Statistical analysis

The antioxidant effect of the canola extract on cooked beef, chicken and pork was analysed using the Proc Mixed procedure from SAS (1998). Significant differences amongst least square means were determined with the Student '*t*" test.

The experimental design adopted to determine the relationship between the polyphenol composition and antioxidant activity in

cooked ground beef stored for 6 days consisted of a simplex lattice mixture design (Cornell, 1990), with 9 factors (caf, cou, fer, sin, syr, cin, gen, sal, and pOHb) at 4 levels each, run in five blocks corresponding to five animals. The design was generated by the SAS software (1998) and resulted in a total of 300 runs per time point.

The analysis was performed with the following model:

$$AOA_{tpred} = \sum_{i=1}^{n} \beta i \times i + \sum \sum_{i<j=1}^{n} \beta ij \times i \times j \quad (2)$$

where, $\beta i$ and $\beta ij$ are the coefficients for the linear and interaction terms, respectively, and $_t$pred is the antioxidant activity predicted by the model at time $t$. Treatment effects and estimates of the model parameters were determined using the general linear model and regression procedures from SAS (1998). Model validation was carried out using combinations of the variables at different levels within the experimental range.

## 3. Results and discussion

### 3.1. Total polyphenol content and phenolic acid composition of the extract

The total polyphenol content of the canola extract, determined by the Folin–Ciocalteau method, was 106 mg GAE/g ext. Fig. 1 presents the HPLC chromatogram of the phenolic acids present in the extract after saponification and extraction. The major phenolic acids identified were sinapic (99.7%), ferulic (0.28%) and *p*-hydroxybenzoic (0.07%) acids.

### 3.2. Total polyphenol concentration and antioxidant activity of the extracts

Fig. 2a and b and c show the effect of canola extract concentration (0, 15 and 100 GAE mg/kg meat). In all treatments, lipid oxidation was highest in chicken breast ($P < 0.05$), followed by pork and beef samples. Previous reports also found that chicken breast oxidised faster than pork and beef (Wilson et al., 1976) and that pork was more prone to oxidation than was beef (Jayathilakan, Sharma, Radhakrishna, & Bawa, 2007).

All treatments significantly increased ($P < 0.05$) lipid stability; however, the effect was stronger in beef, followed by pork; the chicken patties were the least stable. The $AOA_6$ (antioxidant activity after 6 days storage) values were 66–92%, 43–75% and 36–70% for beef, pork and chicken, respectively. A similar behaviour was observed when the extract level was enhanced from 15 to 100 mg GAE/kg; meat and beef TBARS were reduced by 77% whilst, in chicken and pork samples, the diminution was between 53 and 57%. Extending storage from 3 to 6 days increased lipid oxidation for all species and extract levels except for beef samples treated with 100 mg GAE/kg meat where no effect ($P > 0.05$) was detected.

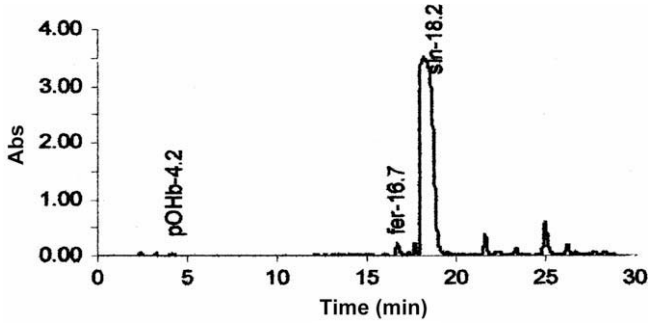


**Fig. 1.** HPLC chromatogram of the crude extract of phenolic compounds.

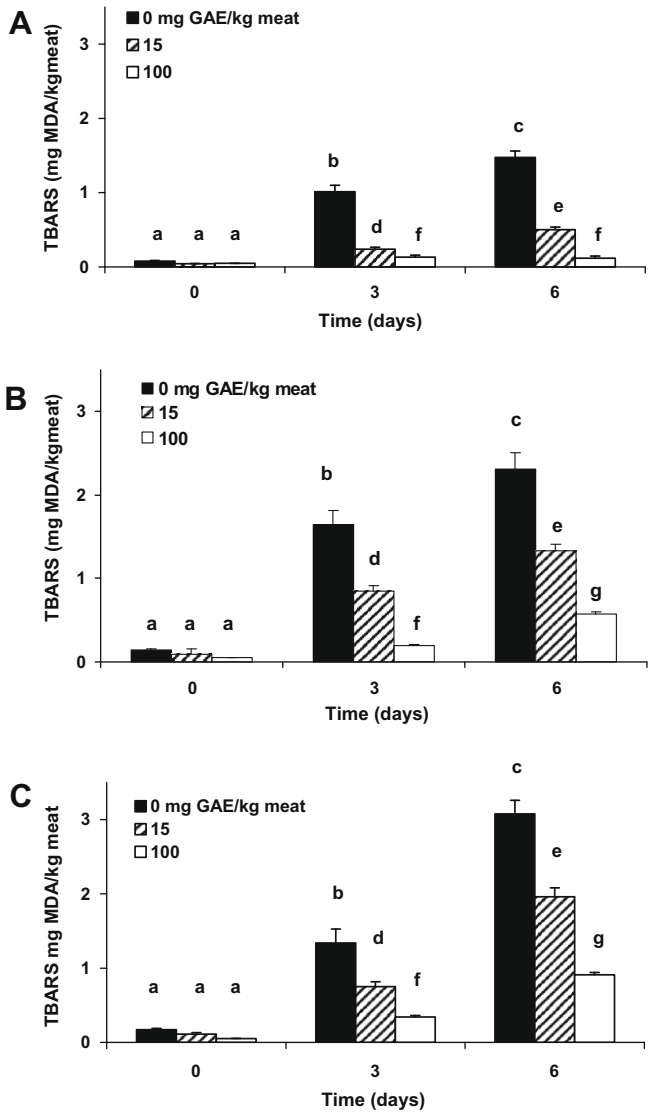


**Fig. 2.** Effect of different concentrations of the canola extract on the TBARS formation of cooked beef (A), pork (B) and chicken (C). $^{a-g}$Means within each storage period having the same letter are not significantly different ($P > 0.05$).

Vuorela et al. (2005) also showed that phenolic extracts from rapeseed meal inhibited lipid and protein oxidation in cooked pork.

The disparity in response to the antioxidant treatment across species detected in the current study can be attributed to differences in the polyunsaturated fatty acids (PUFA) content. Rhee, Anderson, and Sams (1996) concluded that, although PUFA concentration combined with haeme and free iron content significantly affected rancidity development in raw muscle, lipid oxidation potential in cooked meat was mainly determined by PUFA level. An increase in PUFA concentration enhances the muscle's susceptibility to oxidation and rancidity development (Channon & Trout, 2002; Tay, Aberle, & Judge, 1983) and, as a result, diminishes antioxidant activity. Higher doses of antioxidant will be necessary to achieve a similar effect.

### 3.3. Relationship between antioxidant activity and polyphenol composition

#### 3.3.1. Antioxidant effect of the individual polyphenols

The relationship between phenolic acid composition and antioxidant activity was focused on the action of five cinnamic (caffeic,

cinnamic, *p*-coumaric, ferulic and sinapic) and four benzoic acid derivatives (gentisic, *p*-hydroxybenzoic, salicylic and syringic) commonly found in canola meal (Shahidi & Naczk, 2003). Fig. 3 presents the chemical structures of the compounds used in this assay.

Fig. 4 shows the TBARS values of the different treatments in cooked beef patties. Sinapic and caffeic acids were the most effective antioxidants, followed by ferulic acid, their $AOA_6$ values were 88, 72 and 65%, respectively. Gentisic and syringic acids were less active, $AOA_6$ ranged from 45 to 32%. Although cinnamic acid's activity was only 21%, the effect was still significant. *p*-Coumaric and *p*-hydroxybenzoic acids were ineffective ($P > 0.05$) whilst salicylic acid was slightly pro-oxidative.

Antolovich et al. (2004) and Natella, Nardini, Di Felice, and Scaccini (1999) reported that the chemical structure of the side chain, as well as the number and position of free hydroxyl and methoxyl groups attached to the aromatic ring, played key roles in the antioxidant activity of the phenolic acids. Increasing the degree of hydroxylation and/or methoxylation of hydroxycinnamic and hydroxybenzoic derivatives resulted in higher levels of antioxidant effectiveness (Pekkarinen, Stokmann, Schwartz, Heinnonen, & Hopia, 1999).

In accordance with previous studies done in model systems (Antolovich et al., 2004; Natella et al., 1999), the presence of a single OH group in either the hydroxybenzoic (sal, pOHb) or hydroxycinnamic derivatives (cou) did not confer any antioxidant activity. However, elimination of the OH group of the *p*-coumaric to form cinnamic acid improved ($P < 0.05$) the activity from 6 to 22%. This result is in contrast with the report from Chaillou and Nazareno (2006) that showed that cinnamic acid's radical-scavenging activity and its capacity to inhibit lipid oxidation, measured by the β-carotene/linoleic acid test, were not significant.

The order of effectiveness with regard to aromatic substitution of the cinnamic acid derivatives (sin, caf, fer, cin, and cou) was *p*-hydroxydimethoxy > *o*-dihydroxy > *p*-hydroxymethoxy > *p*-hydroxy. Although antioxidant activity is strongly dependent on both the type of test and the substrate used, Natella et al. (1999) showed

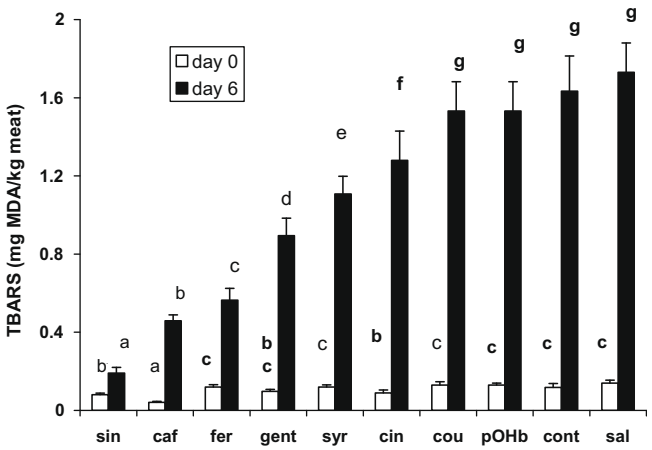


**Fig. 4.** Effect of individual polyphenols on the TBARS values of pre-cooked beef. [a–f]Means within each storage period with the same letter are not significantly different ($P > 0.05$).

that the reactivities of these compounds toward the peroxyl radical, measured by the kinetic competition test, followed a similar trend.

The addition of an *o*-hydroxy group to cou to form caf increased AOA 11 times; however, methoxylation of 3-OH of the caffeic acid molecule to form fer reduced AOA by 10%. The inclusion of an *o*-methoxyl group in cou, to form fer, increased AOA from 6 to 65%; in contrast, the addition of a second methoxyl to fer, to form sin, only increased AOA by 15%.

The effect of aromatic substitution in the AOA of the benzoic acid derivatives did not follow the same pattern as in the cinnamic acid derivatives. Syringic acid, with a *p*-hydroxy dimethoxy structure, was ineffective, whilst gentisic acid, an *m*-dihydroxylated compound, significantly reduced lipid oxidation in cooked patties.

The influence of –COOH substitution by a –CH=CH–COOH group on antioxidant activity was particularly strong for the syrin-

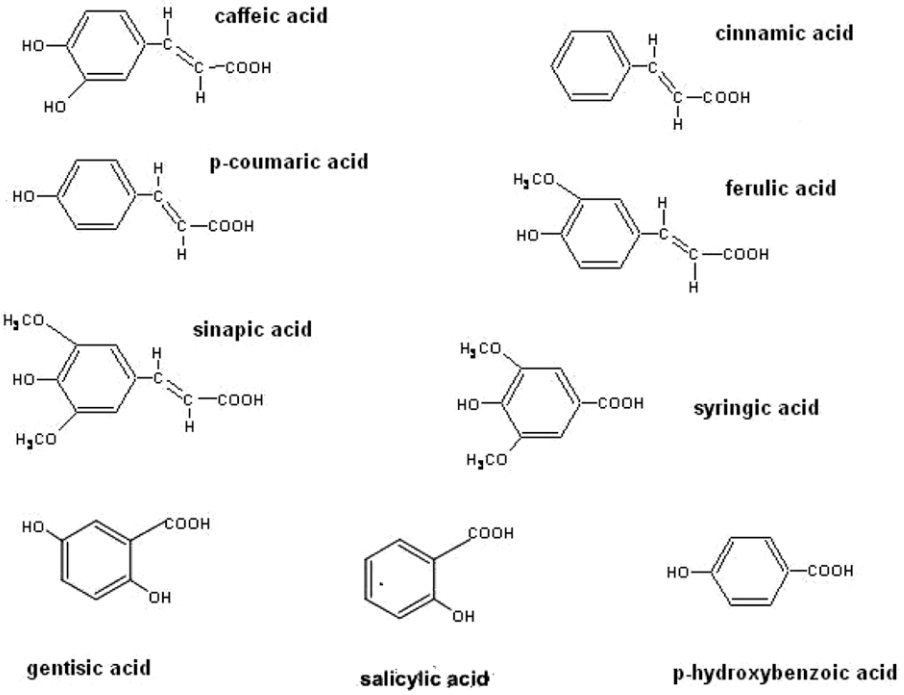


**Fig. 3.** Chemical structures of the phenolic acids.

gic/sinapic pair; $AOA_6$ increased from 32 to 88%. However, no difference in $AOA_6$ was detected between monohydroxylated compounds, e.g. *p*-coumaric or *p*-hydroxybenzoic acids. Cuvelier, Richard, and Berset (1992) reported that the presence of the –CH=CH–COOH group in the cinnamic acid derivative ensured greater antioxidant efficiency than the –COOH in the benzoic acid derivative molecules. The double bond of the propenoic side chain probably participated in stabilizing the radical by resonance. In addition, the electron withdrawing carboxylic group has a negative effect on the H donating ability of the phenolic ring (Natella et al., 1999).

### 3.3.2. Mathematical model

Experimental data were modelled by Eq. (2); the coefficient of determination ($R^2$) was 0.85. Table 1 presents the estimates of the coefficients of the linear ($\beta i$) and interaction ($\beta ij$) terms, as well as their standard error and probability value of the model's significant factors. $\beta i$ corresponds to the antioxidant activities of the individual compounds at the concentration used in this assay (0.05 mmol/kg meat).

From the $\beta i$ values, it is clear that sinapic, caffeic and ferulic acids were the highest contributors to the linear term, followed by gentisic, syringic and cinnamic acids. Although the salicylic, *p*-hydroxybenzoic and *p*-coumaric acids input to the linear term were marginal ($P > 0.05$), all the significant synergisms detected in the current study (cou∗sal, cou∗pOHb∗syr, cin∗gent∗pOHb) included them. $\beta ij$ values indicated that the interaction input from cou/pOHb/syr almost doubled the contribution from the cin/gent/pOHb combination.

The final model was:

$$AOA_6 = 0.77\ xcaf + 0.21\ xcin + 0.68\ xfer + 0.53\ xgent + 1.06\ xsin + 0.31\ xsyr + 1.56\ xcou\ xsal + 15.99\ xcou\ xpOHb\ xsyr + 8.03\ xcin\ xgent\ xpOHb \quad (3)$$

The validation tests done with different polyphenol blends (caf/sin 50:50%; cin/sin 50:50%; cin/fer 50:50%; cin/gent/pOHb 25:25:50%; cin/gent/sin 25:25:50%) showed that the experimental and predicted values were in good agreement; the correlation coefficient was 0.84.

Fig 5 presents the $AOA_6$ values predicted by Eq. (3) for mixtures with equimolar contents of the different polyphenols. The AOA value of a blend containing all components was 0.45 (Bar 1), 88% of the total AOA was due to the main effects whilst the 2 and 3 way interactions accounted for 4 and 8%, respectively.

Although removal of the non-significant contributors to the main effects (salicylic, *p*-hydroxybenzoic and *p*-coumaric acids), eliminated the interactions contribution to AOA, it also enhanced the concentration of the active components resulting in an AOA increment of 33% (Bar 2). Sequential elimination of the lowest contributors (cin, syr, gent, fer, and caf) increased the efficiency of the mixtures (Bar 3-Bar 7); the highest $AOA_6$ value corresponded to 100% sinapic acid (Bar 7).

**Table 1**
Coefficients of the linear and interaction terms predicted by the model.

| Effect | Coefficients | Standard error | *P* |
|---|---|---|---|
| CAF | 0.77 | 0.0 | 0 |
| CIN | 0.21 | 0.06 | 0.001 |
| FER | 0.68 | 0.06 | 0 |
| GENT | 0.53 | 0.06 | 0 |
| SIN | 1.06 | 0.06 | 0 |
| SYR | 0.31 | 0.06 | 0 |
| COU∗SAL | 1.55 | 0.36 | 0 |
| COU∗POHB∗SYR | 15.99 | 3.60 | 0 |
| CIN∗GENT∗POHB | 8.03 | 3.66 | 0.029 |

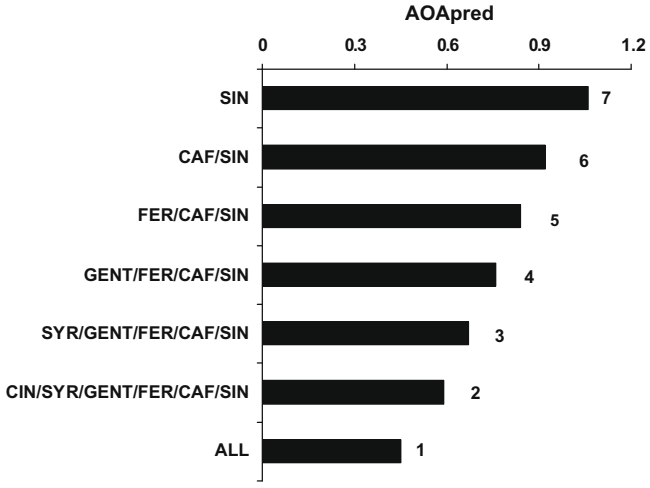


**Fig. 5.** Antioxidant activity of different combinations of phenolic acids predicted by the model in ground beef. Relative concentrations of each polyphenol within treatment are equal.

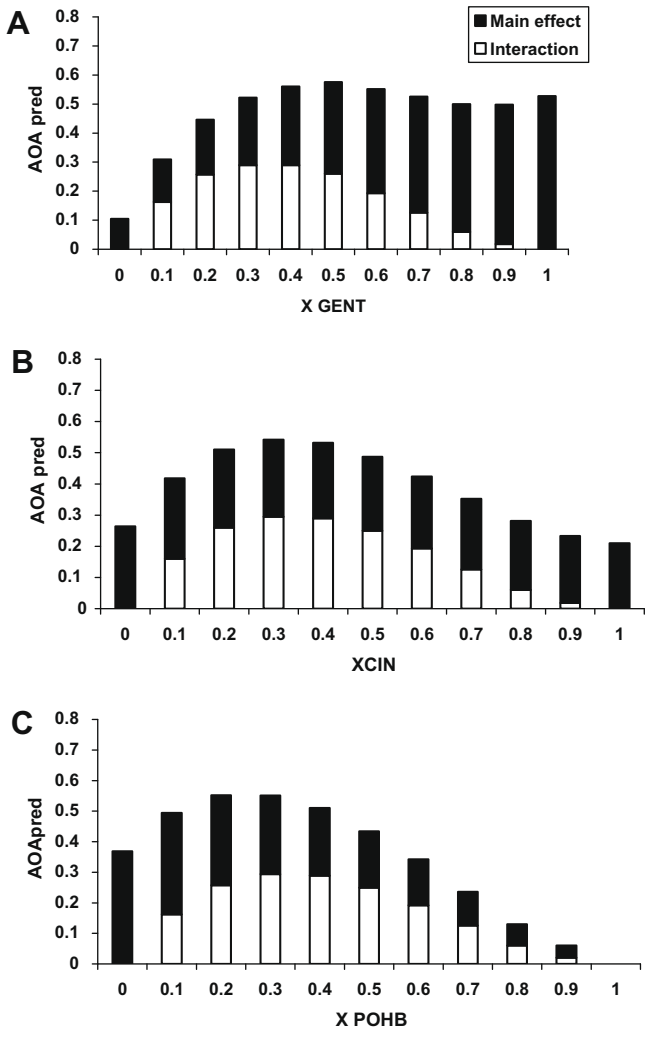


**Fig. 6.** Effect of gentisic (A), cinnamic (B) and *p*-hydroxybenzoic (C) acid concentrations on the predicted antioxidant activity and on the main and interaction effects of a blend of these compounds predicted by the model in ground beef.

To analyse the effects of cin, gent or pOHb concentrations on the predicted AOA of a mixture of the three compounds, we assumed a system where the molar fractions of one component varied from 0 to 1 (independent variable) and the contents of the other two polyphenols were equal (dependent variables). Fig. 6a–c depicts the effects of cin, gent or pOHb molar fractions on the predicted AOA, as well as the main effect and interaction inputs. The highest AOApred values corresponded to a blend of 50% gent and 25% cin or pOHb. In all cases, the contribution of the interaction term to the antioxidant activity of the blend was the same. Incorporation of the antioxidants at 0–33% enhanced the interactions term to a maximum level of 0.29, further accumulation diminished it to 0. The variations of the relationship between AOApred and the independent variable content observed across Fig. 6a–c depended only on the magnitude of the main effects, i.e. on the antioxidant activity and relative concentrations of the individual compounds. Addition of gentisic acid, the strongest antioxidant component, to a mixture of pOHb and cin, enhanced the main effect; AOApred of the system reached a maximum of 0.57 at xgent = 0.5. Further accumulation produced a continuous increment of the main effect which almost compensated the sharp drop of the interaction's contribution, resulting in a loss in AOApred of 7% (Fig. 6a). Although the addition of a less efficient antioxidant like cin marginally reduced the main effect, at levels higher than 50%, cin's antioxidant capacity was not high enough to balance the reduction of the interaction input and the AOApred values decreased by 49% (Fig. 6b). Incorporation of an inactive compound like pOHb ($\beta i = 0.06$) had a much stronger influence; the main effect term diminished from 0.37 to 0 (Fig. 6c) due to a reduction of the significant contributor relative concentrations. AOApred reached its highest level (0.55) at xpOHb = 0.25 and then fell to 0.06 with increasing pOHb content.

Fig. 7a and b show the effect of cou, syr or pOHb contents on the predicted AOA value of a mixture of these compounds. The assumptions regarding the system's composition were similar to those used for the previous blend. Variations of cou or pOHb resulted in similar AOA values; hence the results were pooled.

Although two of the three compounds, cou and pOHb, were inactive *per se*, the AOApred peak values were 27% higher than the results calculated for the cin/gent/pOHb combination. This was mainly due to the highly significant synergy of the components; a comparison between the $\beta ij$ values indicated that the cou/pOHb/syr interaction almost doubled that from the previous mixture. As in the first analysis, the interaction's input did not depend on which chemical species was considered as the independent variable. These results confirmed the conclusions obtained with the cin/gent/pOHb system regarding the influence of the antioxidant activity of the individual compounds on the AOApred values. The highest antioxidant activity corresponded to a blend with 40% syr and 30% cou or pOHb.

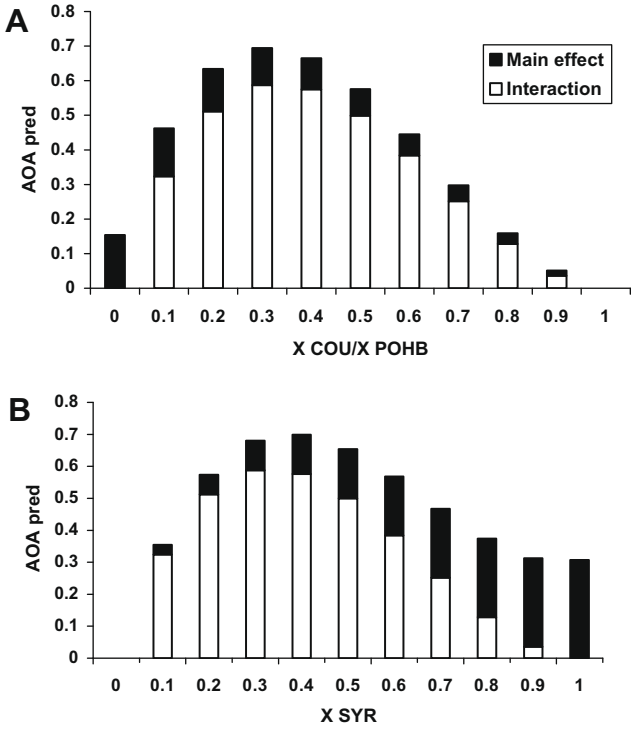


**Fig. 7.** Effects of coumaric/*p*-hydroxybenzoic (A) and syringic (B) acid concentrations on the antioxidant activity and on the main and interaction effects of a blend of these compounds predicted by the model in ground beef.

## 4. Conclusions

The crude polyphenol extract of canola meal inhibited lipid oxidation in cooked ground beef, chicken or pork stored for 6 days at 4 °C. The effect increased with the dose and was stronger in beef than in pork, the chicken samples were the least responsive to the treatment.

The relationship between polyphenol composition and antioxidant activity of a mixture of caffeic, cinnamic, *p*-coumaric, ferulic, sinapic, gentisic, *p*-hydroxybenzoic, salicylic and syringic acids in cooked ground beef was satisfactorily predicted with a polynomial model. Results showed that sinapic, caffeic and ferulic acids were the highest contributors to the linear term, followed by gentisic, syringic and cinnamic acids. Although salicylic, *p*-hydroxybenzoic and *p*-coumaric acids *per se* were ineffective; their contributions to the total antioxidant activity through synergism were significant. The model detected three significant positive interactions: cou∗sal, cou∗pOHb∗syr and cin∗gent∗pOHb. Statistical analysis showed that the synergy between the cou, pOHb and syr almost doubled that between cin, gent and pOHb.

As antioxidant activity is strongly affected by the animal species, the model is valid only for beef and within the experimental condition used in the present study. Similar analysis could be done for chicken of pork.

## Acknowledgements

We thank Ron Kuypers and Dr Robin Shorthose for their helpful suggestions and Peter Jones and Dr. Alicia Califano for their help with the statistical design. This research was funded by the Commonwealth Scientific and Industrial Research Organization(CSIRO). Ms. Amandine Brettonnet was funded by the Conseil Regional de Bourgogne (France).

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