Inhibitory Effects by Soy Antioxidants on the Oscillations of the *Briggs-Rauscher* Reaction

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Inhibitory effects by addition of aqueous extracts of soy flour to an active *Briggs-Rauscher* mixture are reported. The effect consists of an immediate cessation of oscillations, but, after some time, the oscillatory behavior is regenerated with amplitude and frequency different from those observed in a reference mixture. The inhibition time depends linearly on the concentration of substances contained in the extract in a wide range of concentration. The inhibitory effects are due to the high free-radical scavenging activity of substances contained in the soy flour. Two preponderant products contained in the soy flour extract were identified and characterized as malonyldaidzin and malonylgenistin. The antioxidant activity of these isoflavones contained in the extracts was determined on the basis of the inhibition time. A qualitative mechanistic explanation of the inhibitory effects is given. Our findings are decisive indirect evidence of involvement and important role played by $HOO^{\cdot}$ radicals in establishing oscillations in the *Briggs-Rauscher* (*BR*) system. The linear relationship between the inhibition time and the whole-mass concentration of antioxidant contained in soy extracts added to a *BR* mixture is an indication of the possibility to develop and implement an analytical procedure for monitoring the activity of antioxidant scavengers of free radicals based on the oscillating *Briggs-Rauscher* reaction.

1. Introduction. – As stated by *Furrow* and *Noyes* [1], the most dramatic oscillating reaction in solution is probably that discovered by *Briggs* and *Rauscher* [2], now referred to as the *BR* reaction. When appropriate amounts of H_2O_2, acidic iodate, manganous salt, malonic acid (MA), and starch indicator are mixed in aqueous solution, the system repeats the sequence colorless → yellow → blue several times.

The mechanism of the *BR* reaction is quite complex; a skeleton mechanism, proposed in 1982 by *Noyes* and *Furrow* [3], reproduces some of the basic features of the oscillations in the system in closed reactors (batch conditions). At the same time, *De Kepper* and *Epstein* [4] developed a qualitatively similar mechanism that, taking into account flow terms, is able to model, besides oscillations, a variety of phenomena that appear in continuously stirred tank reactors (open conditions).

The main intermediates in these mechanisms are: iodine, iodide ion, the oxyiodine species HOI, HOIO, and $IO_2^{\cdot}$, and the hydroperoxyl radical $HOO^{\cdot}$. It is to be noted that $IO_2^{\cdot}$ and $HOO^{\cdot}$ radicals were not detected in *BR* mixtures: their presence and the important role they play in the onset of oscillations were proposed in analogy with the well-established mechanism for the *Belousov-Zhabotinsky* (*BZ*) reaction [5] and with a mechanism proposed for the *Bray-Liebhafsky* (*BL*) reaction [6].

In a qualitative study, *Franz* [7] found that the addition of a crumbled superoxide dismutase pellet (health food, 2000 SOD units per pellet) to an actively reacting *BR* mixture caused an immediate cessation of oscillations (initial concentrations in the

mixture: $[H_2O_2] = 1.20$M, $[KIO_3] = 6.67 \times 10^{-2}$ M, $[H_2SO_4] = 2.67 \times 10^{-2}$ M, $[MA] = 0.05$M, $[MnSO_4] = 6.67 \times 10^{-3}$ M; 0.01% *w*/*w* starch). We concur with this observation, but *Franz* also claimed that the same amount of pure SOD (superoxide dismutase; from bovine livers) has the same effect, and concluded that, since SOD is a scavenger of $HOO^{\bullet}$ radicals, this inhibitory effect strongly suggests involvement of $HOO^{\bullet}$ in the *BR* system. We performed a number of experiments on the same mixture and found that the addition of pure SOD at up to 11600 units causes only a very small effect [8]. Only a slight lowering of the oscillations' amplitude (monitored potentiometrically with a iodide-ion-selective electrode) was observed [8]. Then, we decided to perform a study to investigate the real SOD effect and to identify some other possible inhibitory agents contained in the SOD pellets. For this purpose, we used SOD pellets from *GNC* (*General Nutrition Corporation*, Pittsburgh, PA, 2000 units per pellet). We prepared an aqueous extract of two finely crumbled pellets (*ca.* 1.2 g) by adding the powder to 10 ml of double distilled H_2O, stirring for *ca.* 15 min at room temperature. Finally, the slurry was filtered. After adding 1.0 ml of a 10-fold diluted extract to 30 ml of an active *BR* mixture (initial composition: $[H_2O_2] = 1.30$M, $[KIO_3] = 6.67 \times 10^{-2}$ M, $[H_2SO_4] = 2.60 \times 10^{-2}$ M, $[MA] = 0.051$M, $[MnSO_4] = 6.67 \times 10^{-3}$ M), we observed dramatic effects with respect to a reference mixture of the same initial composition, to which 1.0 ml of distilled H_2O was added. At first, oscillations were damped, then ceased, but, after some time (in the reported example, *ca.* 2 min), the oscillatory behavior was regenerated with small amplitude, then the amplitude increased as far as the number of oscillations with respect to the reference mixture [8]. This behavior indicates that, in the aqueous extract of SOD pellets, not only the SOD is present, but also another (or more) substance(s) that initially acts (probably with a synergistic effect by SOD) as an inhibitor, and, subsequently, perhaps owing to some structural modifications by reaction with radicals, acts as an 'activator'. The SOD pellets have the following composition (apart the enzyme) declared in the label of the bottle: dicalcium phosphate and soy fiber as fillers, cellulose as binder, food glaze as coating, and stearic acid as excipient. Since it has been reported that soybeans contain a number of glycosides that show strong free-radical scavenging activity [9][10], we decided to perform a detailed investigation of inhibitory effects of aqueous extracts of soy wholeflour on the oscillations of the *BR* reaction in order to obtain reasonable indirect evidence of the involvement of hydroperoxyl radicals. Another aim of this research work is the identification, chemical-structure determination, and radical-scavenging-activity measurement of some glycosides contained in the soy.

2. Experimental. – 2.1. *Materials and Apparatus.* Malonic acid (*Merck*, reagent grade, >99%), manganese(II) sulfate monohydrate (*Merck*, reagent grade, >99%), $NaIO_3$ (*Merck*, reagent grade, >99.5%) were used without further purification. $HClO_4$, H_2O_2, and other chemicals were of anal. grade. All stock solns. were prepared from doubly distilled, deionized H_2O. Perchloric acid was analyzed by titration *vs.* a standard 0.1M NaOH soln. (from *Merck*). H_2O_2 was standardized daily by manganometric analysis. Soy wholeflour was purchased from a health food shop.

Oscillations in the *BR* mixtures were followed potentiometrically by recording the potential of a iodide-ion-selective electrode (*Orion*, model *9453*). As reference electrode, we used a double-junction Ag/AgCl electrode (*Ingold*, model *373-90-WTE-ISE-S7*). Electrodes were connected to a homemade multimeter controlled by an *IBM*-compatible PC. The accuracy of this multimeter is ± 1 mV. A suitable data-acquisition program has been written in *Borland C++* language to attain a time-sampling accuracy of ± 1 ms. All solns. and mixtures were maintained by at 25.0°. Chromatography columns used to fraction aqueous soy extracts will be described below,

pump for chromatography at middle pressure was a *Duramat* model *C.F.G. Ultraturrax* used to homogenize soy extracts was an *Ika-Werk* model *MI/26*. HPLC Analyses were performed with a *Shimadzu HPLC-DAD* model *SPD-M10A-VP*.

2.2. *Preparation of the Aqueous Soy Extract Used as 'Standard' and Inhibitory Effects.* Soy flour (4 g) was added to 100 ml of doubly distilled H_2O. The slurry was homogenized for 30 s, then centrifuged at 5000 × g for 6 h. Finally, the supernatant was allowed to stand and decanted. An aliquot of this extract was used to determine the weight of the dry residue per ml of extract, and the value of 25.6 mg/ml was obtained. This extract (1.0 ml), added to 30 ml of an actively well-stirred *BR* mixture (initial composition: $[H_2O_2] = 1.20$M, $[NaIO_3] = 6.67 \times 10^{-2}$ M, $[HClO_4] = 1.00 \times 10^{-2}$ M, $[MA] = 0.05$M, $[MnSO_4] = 6.67 \times 10^{-3}$ M) after the third oscillation, causes an immediate cessation of the oscillatory regime. Oscillations restart after 1440 s. We define the *inhibition time* as the time elapsed between the end of the addition of the extract (immediate cessation of oscillations) and the first regenerated oscillation. A number of similar experiments were performed adding 1.0 ml of the extract at different dilutions. The results are reported in *Table 1*.

Table 1. *Inhibitory Effects on the* BR *Mixture by Addition of 1.0 ml Aliquots of the Aqueous Soy Flour Extract at Different Concentrations*

Inhibition time [s]	Mass concentration of the extract added to the *BR* mixture [mg/ml]
20	3.4
50	3.6
84	3.9
112	4.6
120	5.1
160	5.7
210	6.4
225	7.3
320	8.5
370	10.2
520	12.8
1440	25.6

Typical recordings are shown in *Fig. 1,a–d*. *Fig. 1,a*, shows the oscillation behavior of a reference mixture (to which 1.0 ml of doubly distilled H_2O was added after the third oscillation), *Fig. 1,b–d*, show the behavior when different amounts of substances contained in 1.0 ml of suitably diluted extract were added.

Data reported in *Table 1* are satisfactorily fitted to a straight line in the range 3.6–12.8 mg/ml of substances added, as shown in *Fig. 2*.

The equation of the straight line is

$$t_{\text{inhib.}}\,[\text{s}] = (50.7 \pm 1.7)C\,[\text{mg/ml}] - (131 \pm 12)\,[\text{s}] \quad (1)$$

where C is the mass concentration of substances in 1.0 ml of added extract. Quoted errors on slope and intercept have been calculated by the procedure suggested by *Harris* [11].

Below 3.6 mg/ml of substances added, the behavior deviates from linearity. In fact, at low concentrations of substances added, the inhibition times become too low to be measured. As an example, *Fig. 3* shows the behavior of a 30-ml *BR* mixture, when 1.0 ml of the extract diluted to 1:100 (0.1 mg/ml of substances) was added.

Even if the oscillatory behavior is different from that of the reference mixture (see *Fig. 1,a*), the inhibition time is not evident. Then, there is a threshold under which inhibition time cannot be detected. Since the extract contains other substances (starch, proteins, *etc.*) besides active glycosides, it seems not correct to speak in terms of LOD (limit of detection), but we can calculate a value for the threshold as 3.6 mg/31 ml, or *ca.* 0.2 mg/ml. We believe that, under this lower limit, the straight-line curves towards 0 (dashed line in *Fig. 2*).

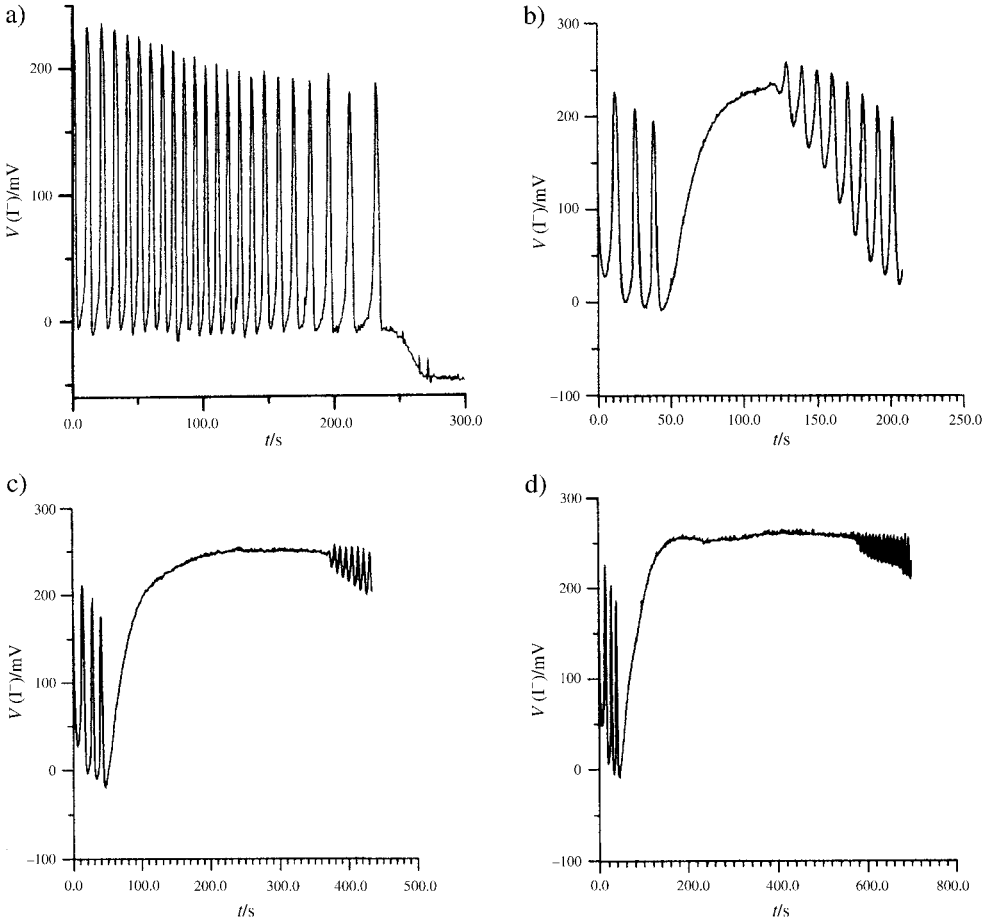

Fig. 1. *Recordings of the potential of the iodide-selective-electrode* vs. *time. a*) Reference mixture, initial conditions: $[H_2O_2]_o = 1.20$M, $[IO_3^-]_o = 6.67 \times 10^{-2}$ M, $[HClO_4]_o = 1.00 \times 10^{-2}$ M, $[MA]_o = 5.00 \times 10^{-2}$ M, $[Mn^{2+}]_o = 6.67 \times 10^{-3}$ M; *b*), *c*), and *d*): effect of the addition of 3.9 mg, 8.5 mg, and 12.8 mg of substances, resp.

2.3. *Extraction Procedure: Relative-Activity Measurements.* Four different extractions were performed in the following ways: *1*) soy flour (4 g) was added to 100 ml of H_2O. The slurry was stirred for 10 min, then centrifuged at 5000 × g for 5 h. The supernatant was allowed to stand and was decanted. *2*) Soy flour (4 g) was added to 100 ml of H_2O. The slurry was homogenized for 30 s, and processed as described in *1*. *3*) Soy flour (4 g) was added to 100 ml of a mixture H_2O/MeOH 1:1 (*v*/*v*), and processed as described in *2*; *4*) Soy flour (4 g) was added to 100 ml of a mixture H_2O/MeOH 1:1 (*v*/*v*), and processed as described in *1*. The dry weight of each extract was determined.

To measure the activity of an extract, suitable dilutions were performed to obtain inhibition times falling in the range of linearity of the line of *Fig. 2*, when 1.0 ml of the diluted extract was added to 30 ml of the active *BR* mixture.

The activity of an extract relative to that chosen as 'standard' was determined in the following way: from *Eqn. 1*, the mass concentration *C* of the standard that should give the same inhibition time of the sample was calculated. The ratio between this value and the mass concentration of the sample gives the activity of the sample referred to the 'standard'. The results of the *Extracts 1–4* are listed in *Table 2*.

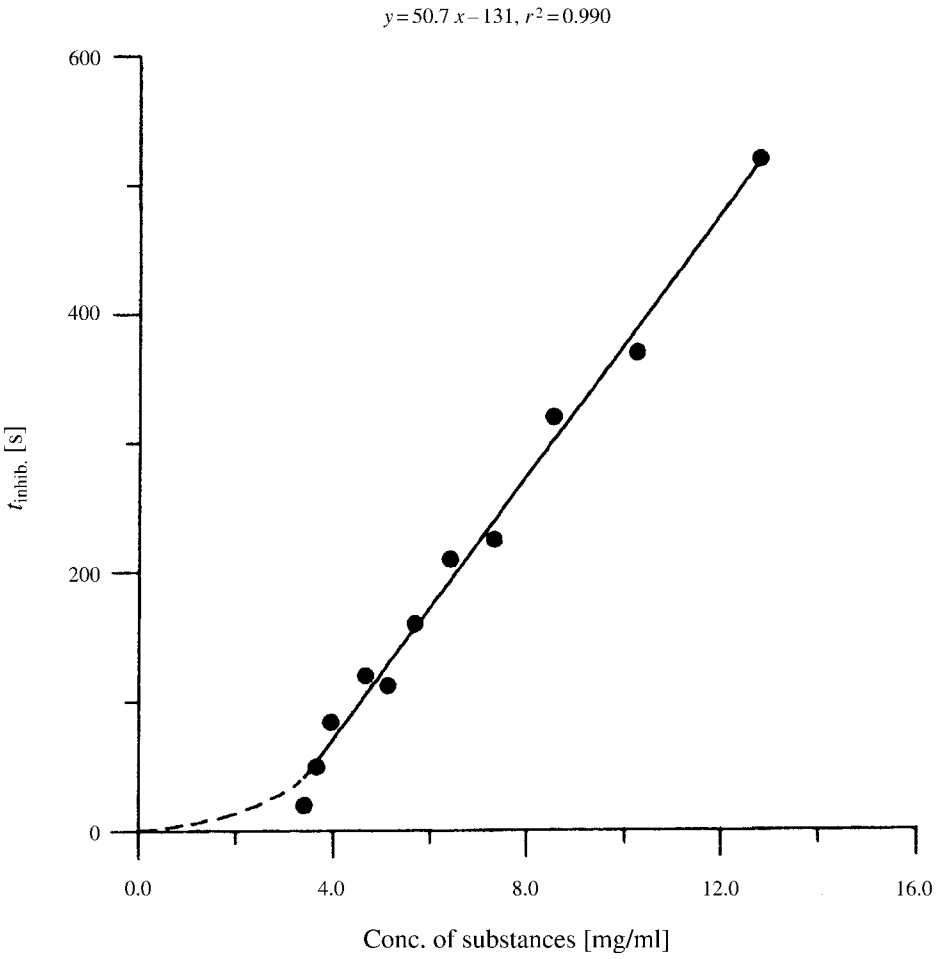

Fig. 2. *Graph of the inhibition time* vs. *concentration of substances added to 30 ml of the* BR *mixture*

From *Table 2*, it can be seen that: *i*) *Extract 2*, obtained by the same procedure as the 'standard', shows unitary activity; this means that either the extraction procedure or the method of determination of the activity shows a very good reproducibility; *ii*) the extract obtained by *Method 1* shows an activity more than twice than extract obtained by *Method 2*. The dry weight of *Extract 2* is higher than that of *Extract 1*, but its activity is lower; it means that by *Method 2* a larger amount of substances without antioxidant activity (probably starch and

Table 2. *Data of the Extracts Obtained by Different Procedures* (see text)

Extract	Dry weight [mg/ml]	mg added to the *BR* mixture	Inhibition time [s]	Activity referred to the 'standard'
1	16.2	4.6	415	2.3 ± 0.2
2	21.4	6.1	175	1.0 ± 0.1
3	8.8	2.5	365	3.9 ± 0.3
4	11.1	3.2	500	3.9 ± 0.2

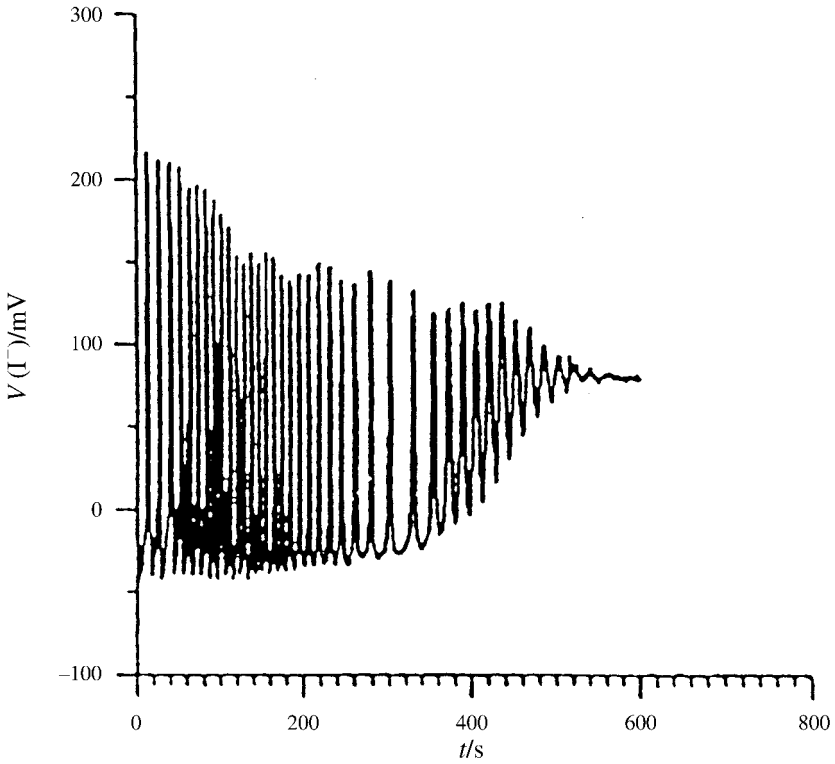

Fig. 3. *Recording of the potential of the iodide-selective-electrode* vs. *time when 1.0 ml* of an *aqueous soy flour extract diluted to 1:100 was added to 30 ml of the* BR *mixture*

proteins) was extracted; *iii*) both hydroalcoholic *Extracts 3* and *4* show an activity four times higher than that of the standard; in the *Extract 4* a larger amount of substances without activity is present.

2.4. *HPLC Chromatographic Conditions.* Chromatographic separations were performed on a *Phenomenex Luna* column (15 × 4.6 mm i.d.) filled with *C18(2)* (3 μ) and a guard column of the same material. The mobile phase consisted of solvent *A* (0.1% *ortho*-phosphoric acid in H_2O (*v*/*v*)) and solvent *B* (MeCN/MeOH 60:40). The elution profile was: 0 min, 5% *B* in *A*; 0–40 min, 5–66% *B* in *A* (linear gradient). The flow rate was set to 1.0 ml/min, and the injection volume was 20 μl. Peak identifications were based on retention times, and UV spectra (photodiode-array detector (DAD)) of authentic reference compounds to sample solns.

2.5. *Extraction and Fractionation by SPE (Solid-Phase Extraction).* On the basis of the results reported in *Table 2*, 200 g of soy flour were added to 5 l of H_2O/MeOH 1:1 (*v*/*v*). The slurry was stirred for 30 min then centrifuged at 10000 × g for 1 h. The clear supernatant was concentrated under reduced pressure to a volume of 800 ml in order to remove org. solvent. The dry weight of the soln. corresponded to 41.4 mg/ml.

A series of activity measurements were performed with this extract, and a mean value of 4.1, in agreement with the value (3.9) reported in *Table 2*, was obtained.

After addition of NaCl (3%), the extract was fractionated over *XAD-4* by successive elutions with distilled H_2O (*Fraction 1*; 2 l), H_2O/MeOH 1:1 (*v*/*v*; *Fraction 2*, 1 l), and finally with MeOH (*Fraction 3*; 1 l). The dry weight and the relative activity of each fraction are reported in *Table 3*.

HPLC Analysis (DAD) of *Fraction 2* revealed the presence of two main peaks, *A* and *B* (see *Fig. 4*) besides two minor ones, *C* and *D*. All these products showed the characteristic UV spectra of isoflavones, in particular products *B* and *D* exhibited profiles identical with an authentic sample of genistein.

Table 3. *Data of the Extract and Fractions Eluted from the SPE Column*

Fractions	Dry weight [mg/ml]	mg added to the *BR* mixture	Inhibition time [s]	Activity referred to the 'standard'
Crude extract	41.4	1.0	59	3.7 ± 0.6
		2.4	410	4.5 ± 0.3
Effluent	[a])	[a])	[a])	[a])
Fraction 1	[a])	[a])	[a])	[a])
Fraction 2	2.1	0.5	1400	60 ± 3
		0.8	1820	51 ± 3
Fraction 3	1.3	1.0	195	6.4 ± 0.6
		1.3	255	5.9 ± 0.5

[a]) Data not reported due to the presence of NaCl, since chloride ions inhibit the *BR* reaction [12].

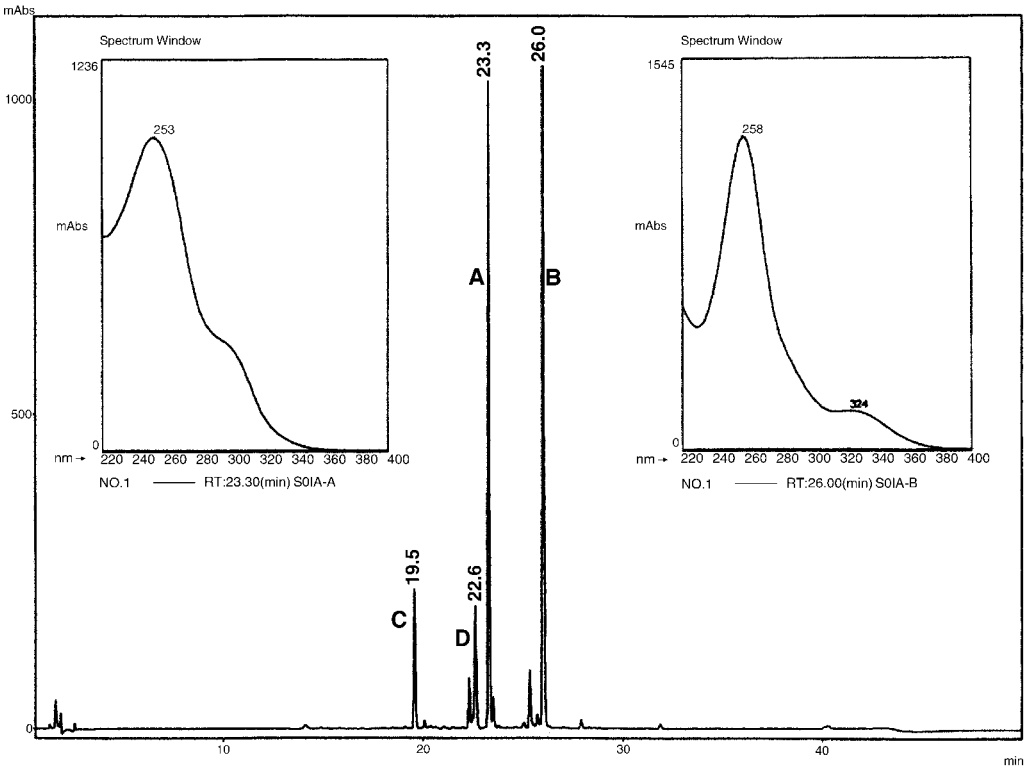

Fig. 4. *Chromatogram of* Fraction 2 *eluted from the SPE column and UV spectra of the two main compounds*

Fraction 3 contained the same products, but in smaller amounts. Since, during the storage of *Fraction 2*, a decrease of the peaks *A* and *B*, corresponding to an equal increase of the peaks *C* and *D*, was observed, the latter were considered degradation products of *A* and *B*, resp. To confirm this hypothesis, a portion of the *Fraction 2* was hydrolized in 1M HCl at 100° for 1 h. A HPLC analysis (DAD) performed in the same solvent system showed the presence of only two main peaks corresponding to daidzein and genistein (see *Fig. 5*).

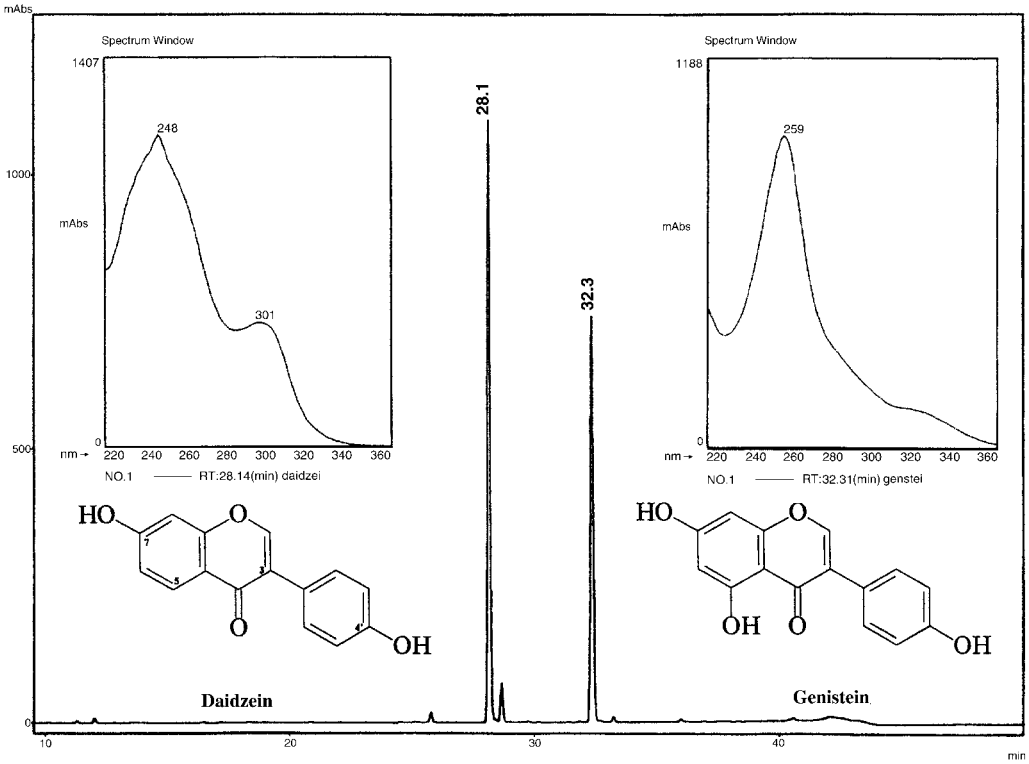

Fig. 5. *Chromatogram of* Fraction 2 *from the SPE column after acidic hydrolysis and UV spectra of compounds*

The content of isoflavones in the *Fraction 2* was calculated: *A:* 213.4 mg, *C:* 67.5 mg (expressed as daidzein), *B:* 281.9 mg, *D:* 90.0 mg (expressed as genistein).

2.6. *Isolation and Purification of the Compounds* A *and* B. *Fraction 2* from the *XAD-4* column was chromatographed over a *C18* reversed-phase column (3 × 25 cm) and eluted with increasing amounts of MeOH in H_2O. *Fractions 1–16* (1 l each) were eluted and analyzed. Products *A* and *B* were present in *Fractions 6–9*.

Fraction 7 eluted with 10% MeOH, containing 95.4 mg of *A* and 110 mg of *B* expressed as daidzein and genistein, respectively, was chromatographed again over a *C8* reversed-phase column (5 × 25 cm). Elution was performed with increasing amounts of MeOH in H_2O, and 45 fractions (300 ml each) were collected. Separation of *A* (*Fractions 3–7*) and *B* (*Fractions 17–38*) in pure form was accomplished. *Fractions 3–7* pooled and dryed afforded 56.2 mg of compound *A* and *Fractions 17–38* 61.0 mg of compound *B*.

2.7. *Structural Characterization of Compounds* A *and* B. The structural characterization was achieved by NMR (^{1}H and ^{13}C) and mass spectrometry. From the results of these analyses, compound *A* was identified as malonyldaidzin (*= 3-(4-hydroxyphenyl)-7-(6-malonyl-β-*D*-glucopyranosyloxy)-4*H*-1-benzopyran-4-one)* and compound B as malonylgenistin (*= 5-hydroxy-3-(4-hydroxyphenyl)-7-(6-malonyl-β-*D*-glucopyranosyloxy)-4*H*-1-benzopyran-4-one)*[1]). The structures of these well-known isoflavones contained in the soy [9] are shown below.

2.8. *Scavenging Activity of Malonyldaidzin and Malonylgenistin.* Relative-activity determinations were performed by measuring the inhibition time of the oscillatory regime in a *Briggs-Rauscher* mixture as described in *Sect. 2.3.* The results are summarized in *Table 4*.

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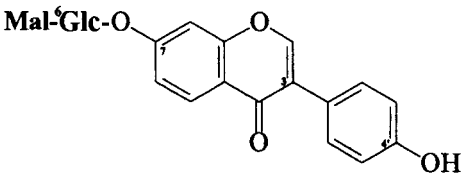
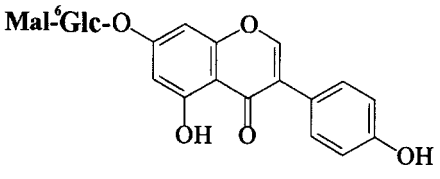
[1]) These analyses were performed by Prof. *Bruno Danieli*, Dipartimento di Chimica Organica, Università Statale di Milano, with a mass spectrometer *VG-7070-EQ-HF* with FAB source and an NMR *Bruker* spectrometer model *300 EC*.



Malonyldaidzin



Malonylgenistin

Table 4. *Data of Malonyldaidzin and Malonylgenistin*

Compound	mg added to the *BR* mixture	Inhibition time [s]	Activity referred to the 'standard'
Malonyldaidzin	1.5	355	6.4 ± 0.4
Malonylgenistin	1.0	108	4.7 ± 0.6
Malonylgenistin	1.5	230	4.7 ± 0.4

For malonylgenistin, two different measurements at different mass concentrations were possible: as can be seen from *Table 4*, results are identical, and this is another proof of the good reproducibility of the procedure based on the *BR* reaction.

Both compounds show a good free-radical-scavenging activity; however, their activity values did not account for the whole activity showed by *Fraction 2* collected from the SPE column (see *Table 3*). The activity of this fraction is in fact an order of magnitude higher than that of malonyldaidzin and malonylgenistin tested separately. Then, we decided to perform activity measurements on all the fractions from *C18* column. Most of these fractions showed low or no activity, but we found a relative activity of 12 in *Fraction 2*. *Fraction 1* showed an activity comparable to that of malonylgenistin. An HPLC analysis of these fractions showed the presence of a group of compounds with characteristic UV spectra, but, among them, there were not preponderant ones. Moreover, these compounds with high antioxidant activity are present only in traces, so in order to isolate and characterize them it would be necessary to use some Kg of soy flour! Also *Fraction 12* showed a remarkable activity: its chromatogram indicated the presence of compounds previously named *C* and *D*, and traces of daidzein and genistein.

3. Discussion and Final Remarks. – To interpret the inhibitory effects observed, since we worked in batch conditions, it seems appropriate to refer to the *Noyes-Furrow* skeleton mechanism (*NF* model) that we sketch here briefly. For sake of clarity, we have subdivided the 11 pseudo-elementary processes into four groups, maintaining the original notation [3].

Oxyiodine Reactions:

$$I1 \quad HOI + I^- + H^+ \rightleftharpoons I_2 + H_2O$$

$$I2 \quad I^- + HOIO + H^+ \rightarrow 2\,HOI$$

$$I4 \quad 2\,HOIO \rightarrow IO_3^- + HOI + H^+$$

$$I3 \quad I^- + IO_3^- + 2\,H^+ \rightleftharpoons HOI + HOIO$$

$$I5 \quad IO_3^- + HOIO + H^+ \rightleftharpoons 2IO_2^{\bullet} + H_2O$$

These reactions are analogous to oxybromine reactions in the *Field-Kőrős-Noyes* (*FKN*) mechanism of *BZ* reaction [13].

Reactions Involving the Catalyst:

$$M1 \quad IO_2^{\bullet} + Mn^{2+} + H_2O \rightarrow HOIO + Mn(OH)^{2+}$$
$$M2 \quad Mn(OH)^{2+} + H_2O_2 \rightarrow Mn^{2+} + H_2O + HOO^{\bullet}$$

Step *M1* represents the oxidation of species Mn^{II} by the $IO_2^{\bullet}$ radicals. $Mn(OH)^{2+}$ is written as the dominant Mn^{III} species at the pH usual in the *BR* reactant mixtures [14]. Step *M2* represents the reduction of species Mn^{III} by H_2O_2, accompanied by the formation of radicals that will rapidly decay *via* step *O2* (see below).

Organic Substrate/Iodine Reactions:

$$C3 \quad CH_2(COOH)_2 \text{ (diacid)} \rightarrow (HOOC)CH{=}C(OH)_2 \text{ (enol)}$$
$$C4 \quad (HOOC)CH{=}C(OH)_2 \text{ (enol)} + I_2 \rightarrow IHC(COOH)_2 + I^- + H^+$$

As can be seen, in this skeleton mechanism the organic substrate acts only as a sink for I_2 and a source of I^- ion. The *BR* oscillator is, in fact, considered as a halate-driven oscillator controlled by halide ions in the sense that oscillations of all intermediates arise because of the periodic consumption and formation of I^- ions.

Reactions Involving Oxygen:

$$O2 \quad 2\,HOO^{\bullet} \rightarrow H_2O_2 + O_2$$
$$D1 \quad HOI + H_2O_2 \rightarrow I^- + O_2 + H^+ + H_2O$$

Finally, steps *O2* and *D1* involve reactions that lead to the formation of O_2. In fact, the formation of O_2 gas bubbles can be observed during the course of the reaction. The generation of O_2 increases towards the end of the oscillatory regime and continues to increase after the cessation of the oscillations.

This skeleton mechanism has been investigated by *Turànyi* [15] by means of principal-component analysis of the rate-sensitivity matrix. His results showed that steps *O2* and *M2* are mechanistically unimportant, since they are the sole reactions of species $Mn(OH)^{2+}$ and $HOO^{\bullet}$ within the mechanism, so $HOO^{\bullet}$ is an end product not involved in propagation of radicals. Very recently, *Furrow et al.* [16] have modified the original *NF* skeleton mechanism on the basis of experimental evidence. The following process (not an elementary mechanistic step) was proposed to produce radicals:

$$M \quad HIO_2 + 2\,H_2O_2(+\,Mn^{2+}) \rightarrow 2\,HOO^{\bullet} + HOI(+\,Mn^{2+}) + H_2O$$

This process preserves the autocatalytic production of HOIO when combined with steps *D1′* and *D2* below [16]:

$$D1' \quad H^+ + IO_3^- + HOO^{\bullet} \rightarrow IO_2^{\bullet} + H_2O + O_2$$
$$D2 \quad IO_2^{\bullet} + H_2O_2 \rightarrow HOIO + HOO^{\bullet}$$

If the forward of step *I5* is eliminated, some reduction process is necessary to bring I^V to a lower state. *Furrow et al.* [16] added step *D1* for this purpose. Step *M2* may be a substep of process *M*. With these modifications, *Furrow et al.* [16] obtained not only a better agreement between experimental results and simulations for malonic acid derivatives, but the modified mechanism is able to simulate oscillations in *BR* systems with substrates that are iodinated in a different way than malonyl derivatives, as crotonic and acrylic acids, or anisole and *p*-nitrophenol [17].

It is reasonable to assume that an antioxidant or a mixture of antioxidants contained in the soy, added to an active *BR* mixture, subtract $HOO^{\bullet}$ radicals *via* such a reaction as:

$$D3 \quad \text{Antioxidant} + HOO^{\bullet} \rightarrow \text{Radical} + H_2O_2$$

This reaction, being faster than *D1′* step, stops the oscillatory regime, because radicals formed *via* process *M* are subtracted rather *via D3* than *via D1′*. Then, radicals from the antioxidant can decay to end products. When the amount of antioxidant added has completely reacted *via D3* and subsequent decay, process *M* and step *D1′* become predominant and oscillations can restart. The particular structure of the two isolated and characterized isoflavones, active as scavengers of $HOO^{\bullet}$ radicals, can account for the fact that oscillations restart with period, amplitude, and duration different from those of a *BR* mixture like that used as reference. In fact, as malonyldaidzin and malonylgenistin contain a malonyl group, we assume that, in the reaction between $HOO^{\bullet}$ radicals and these isoflavones, some derivatives of malonic acid are formed. These derivatives seem to be good substrates for the *BR* reaction.

In any case, our findings render decisive indirect evidence of involvement of and the important role played by $HOO^{\bullet}$ radicals in establishing oscillations in the *BR* systems.

Finally, the linear relationship between the inhibition time and the whole-mass concentration of antioxidants contained in soy extracts added to a *BR* mixture in a wide range of concentrations is an indication of the possibility to develop and implement a simple, reproducible, rapid, and inexpensive analytical procedure for monitoring the activity of antioxidant scavengers of free radicals, based on the oscillating *BR* reaction. For this purpose, a suitable choice of a standard substance with known activity and structure, *e.g. Trolox*® or caffeic acid, will be necessary.

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