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FLAVONOIDS IN CULTIVARS OF SOYBEAN: ANTIOXIDANT ACTION

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ABSTRACT

The purpose of this investigation was the identification, quantification and determination of antioxidant activity of flavonoids of soybean. Examination of nine cultivars was carried out. All of them had the same stage of maturity and were grown on the same soil. The results showed the presence of genistein, genistin, daidzein, daidzin, naringenin, kaempherol, formonometin and biochanin A in all cultivars. In terms of antioxidant activity, the best results were obtained with genistein and genistin.

KEYWORDS: Flavonoids, soybean, antioxidant action.

RESUMO

O propósito do presente estudo foi a identificação, quantificação e determinação da ação antioxidante de compostos flavonóidicos em sementes de soja. Foram examinados nove cultivares, todos no mesmo estágio de desenvolvimento e cultivados no mesmo solo. Os resultados experimentais mostraram a presença de genisteína, genistina, daidzeiona, daidzina, naringenina, kaempherol, formonometina e biochanina A em todos os cultivares. Os melhores resultados de ação antioxidante foram obtidos com genisteína e genistina.

INTRODUCTION

Flavonoid compounds have been studied for many years, particularly because of their use in a variety of areas including food industry[1-3], mycology and plant physiology[4], phytopathology[4-6], microbiology[7], biochemical ecology[7-10], entomology[8] and chemistry[11].

We decided to identify and study this type of compounds and consider some of their applications, because of their world wide economic importance. The use of flavonoid compounds

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as antioxidant agents[1-3], antifungal[4-6], antibacterial agents[7], as well as insect repellents[8], sweeteners[9], estrogenics[10,11] and phytoalexins[4] has been described in the literature. The purpose of this work was to isolate, identify, quantify and above all test these secondary matabolites as antioxidizing agents.

MATERIALS AND METHODS

Flavonoid compounds were extracted from soybean seeds obtained from nine cultivars at the same stage of maturity and grown on the same soil. The procedure described by Naim[12] was used for genistein (I), genistin (II), daidzein(III) and daidzin (IV). The soybean seeds, ground (40 mesh),were extracted exhaustively with hexane and 60% ethanol. After evaporation of the solvent, the extract was treated with acetone (2 to 1) and filtered. After evaporation of the solvent, the soluble portion was adsorbed on silica gel 200 G, followed by exhaustive extraction with acetone for three days. The residue obtained after evaporation of the solvent was crystallized from 80% ethanol and washed with chloroform.

The compounds naringenin (V), kaempherol (VI), formonometin (VII) and biochanin A (VIII) were extracted according to the method described by Murphy[13]. The sample was heated in acetic acid for 10 minutes using a water bath. The filtered product, after 1 hour hydrolysis with 1,00 N HCl was extracted with anhydrous diethyl ether and the solvent was evaporated.

The flavonoid compounds genistein, genistin, daidzein, daidzin, kaempherol, formonometin, biochanin A were isolated by thin layer chromatography and identified by ultraviolet and infrared spectroscopy, proton nuclear magnetic resonance and melting point. Comparison with standars available in our laboratory was also done.

The quantification was done by HPLC according to the method described by Eldrige[16]. A Corasil C-18 30cm x 30mm ID column was employed.Polar eluents such as water-methanol (25-50%) for 20 min and a flux ratio of 1 ml/min (with N-butyrophenone as internal standard) were used.

The antioxidant activity of the flavonoid compounds mixed with soybean oil was obtained by determining the acidity, iodine and peroxide indexes using the method described by Pregnolatto[14]. The determination of the acidity index involved the titration with 0,1 N NaOH using phenolphthalein as an indicator. The iodine index was determined by thiosulfate titration of the excess iodine of the sample, previously dissolved in chloroform and treated with fresh 15% KI solution.

The peroxide index was determined by dissolving the sample in acetic acid-chloroform solution (3to2), addition of 1% starch solution (0,5 ml) , titration of the excess iodine with 0,01 N sodium thiosulfate and comparison to a blank solution under the same conditions.

The quantification of the protein in the soybean seeds was performed using the method of Bradford[15] and the determination of lipid content was done according to the procedure described by Pregnolatto[14].

RESULTS AND DISCUSSION

The identification of the flavonoid compounds was done using ultraviolet, infrared and proton nuclear magnetic resonance spectroscopy and comparison to standard samples available in the laboratory. Tables I,II,III and IV sumarize the experimental results obtained by the different tecnhiques. Figure 1 illustrates the chemical structures of the flavonoids studied.

Table V shows the average values obtained for protein, oil and flavonoid compounds extracted from the nine cultivars studied. As can be seen, Cultivar UFV-5' showed the highest contents for all the parameters determined.

It is well known that great part of the phenolic compounds is found in plants bound to proteins[17]. In fact, in *Cicer arietanum*, member of the Leguminosae, the vast majority of protein is found along with a large quantity and a variety of flavonoid compounds[17].

These results are wholly justifiable, if one considers that flavonoid compounds are involved in nitrogen fixation in plants[23-26]. This process, that begins with the capture of nitrogen,involves the synthesis of phenylalanine and thyrosine in one of the metabolic pathways. Both amino acids are precursors of flavonoid compounds in plants. Of course, the fixation of nitrogen also leads to the biosynthesis of proteins.

Analysis of the results shown in Table V also shows that the average values obtained for daidzin (IV) are higher than those for daidzein (III) and that the values obtained for genistin (II) are higher than those of genistein (I). This is in acoordance with the results described by Farmalidis[18]. This may be attributed to the need of glucose/sulfate/glucuronate incorporation in the detoxification process of these secondary metabolites in plants. The presence of glucose units in genistin (II) and daidzin (IV) is supposed to facilitate hydrosolubility and transport by water during the elimination[9].

The results obtained in this experiment also point out that the differences observed in all parameters studied for genetically different cultivars are in agreement with results described by other workers with genetically different cultivars of *Trifolium subterraneum* [11,20] *and Ononis Spinosa L.*[19].

Further analysis of Table V also permits the identification of the cultivar with the highest concentration of flavonoid compounds. This may facilitate future studies.

Table VI points out the high antioxidizing power of flavonoid compounds. As can be seen, genistein (I) and genistin (II) exhibited the best acidity, iodine and peroxide indexes, when compared to normal values-CNNPA (ABIA)[21]. These values point an acidity index of $\pm$ 0,423 percent oleic acid; iodine index of 188-198 in mg/100g and a peroxide index of 20 meq/kg.

It is interesting to note that results similar to those obtained by us with soybean oil have also been observed by other

TABLE I. SOME PHYSICAL PROPERTIES OF THE FLAVONOID COMPOUNDS STUDIED.

COMMON NAME	OFFICIAL NAME	Molecular Weight	Literature m.p. ºC	Melting Point Found ºC
Genistein	4',5,7-trihydroxy-isoflavone	270,25	302	300 - 302
Genistin	4',5-dihydroxy-7-0-glucosil isoflavone	-	253	251 - 253
Daidzein	4',7-dihydroxy-isoflavone	254,25	323	321 - 323
Daidzin	4'-hydroxy-7-0-glucosil isoflavone	-	238	236 - 238
Naringenin	4',5,7-trihydroxy-flavonone	272,25	251	249 - 251
Kaempherol	3,4',5,7-tetrahydroxy-flavone	286,23	277	274 - 276
Formonometin	7-hydroxy-4'-methoxy-isoflavone	268,26	212	210 - 212
Biochanin A	5,7-dihydroxy-4'-methoxy-isoflavone	284,3	284	281 - 283

TABLE II. ULTRAVIOLET SPECTRA OF FLAVONOID COMPOUNDS (WAVELENGTH IN NANOMETERS).

COMPOUND	MeOH	NaOMe	$AlCl_3$
Genistein	260, 327	275, 328	271, 307, 372
Genistin	260, 330	270, 353	270, 306, 374
Daidzein	238, 248, 257 309	253, 271, 333	236, 248, 260, 300
Daidzina	255, 312	255, 270, 319	257, 302
Naringenin	289, 325	242, 320	310, 373
Kaempherol	251, 262, 293 322, 367	278, 318, 415	260, 265, 302 349, 423
Formonometin	236, 247, 258, 301	258, 288, 327	240, 248, 259, 300
Biochanin A	259, 329	248, 272, 326	270, 309, 371

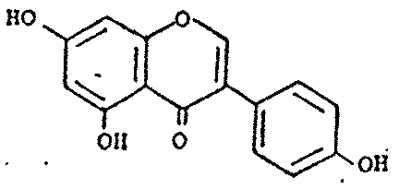
COMPOUND	$AlCl_3$/HCl	NaOAc	NaOAc/H_3BO_3
Genistein	270, 308, 371	270, 323	260, 336
Genistin	271, 305, 371	260, 329	260, 326
Daidzein	239, 248, 260, 301	253, 310, 332	260, 301
Daidzina	255, 301, 260	256, 320	254, 316
Naringenin	309, 370	284, 321	290, 331
Kaempferol	256, 269, 303 348, 423	271, 302, 387	266, 298, 320, 371
Formonometin	241, 247, 260 300	253, 272, 309 329	261, 303
Biochanin A	273, 309, 374	271, 326	261, 331

TABLE III. INFRARED SPECTRA OF FLAVONOID COMPOUNDS.

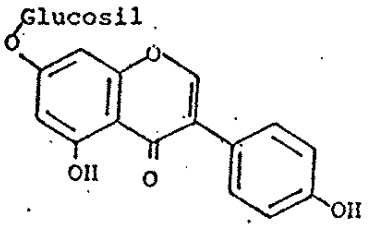
COMPOUND	WAVELENGTH (cm^{-1})
Genistein	3650-3000,1650,1620,1570,1520,1500,1420,1360,1320,1280,1260,1210,1180,1150,1050
Genistin	3600-3000,1640,1620,1500,1360,1280,1260,1210,1200,1180,1150,1100,1050
Daidzein	3550-3000,1640,1620,1600,1530,1470,1390,1310,1280,1240,1200,1100,1050,890,850
Daidzin	3500-3000,1640,1600,1530,1480,1390,1300,1280,1240,1220,1200,1150,1130,1050
Naringenin	3500-3000,1640,1600,1520,1500,1460,1380,1330,1320,1250,1180,1150,1080,1060,890,830
Kaempherol	3500-3000,1640,1600,1520,1500,1460,1300,1250,1150,1100,1050
Formonometin	3500-3000,1640,1600,1570,1515,1460,1390,1320,1290,1270,1250,1200,1180,1100,1040,880
Biochanin A	3400-3000,1650,1620,1570,1520,1440,1360,1290,1250,1190,1150,1050,1030,830

TABLE IV. PROTON NUCLEAR MAGNETIC RESONANCE SPECTRA OF FLAVONOID COMPOUNDS (CHEMICAL SHIFTS IN PPM).

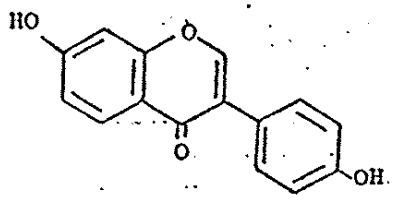
COMPOUND	SOLVENT	H-2	H-3	H-5	H-6	H-7	H-8'	H-2'	H-3'	H-5'	H-6	CH' OCH_3	Glucosil
Genistein	δ CCl_4	7,60	-	-	5,8	-	6,85	7,50	6,85	6,85	7,50	-	
Genistin	δ CCl_4	7,80	-	-	6,3	-	7,10	7,50	6,85	6,85	7,50	-	H1-Glucosil 5,0/ 3,90-3,3 (6H)
Daidzein	δ CCl_4	7,70	-	8,0	6,50	-	6,75	7,50	6,85	-	6,85	7,50	
Daidzin	δ CCl_4	7,90	-	8,2	7,1	-	7,0	7,50	6,85	-	6,85	7,50	H-Glucosil 5,0/3,85-3,20 (6H)
Naringenin	δ CCl_4	5,20	2,50-2,90	-	5,85	-	6,0	7,25	6,75	6,75	7,25	-	
Kaempherol	δ CCl_4	-	-	-	6,15	-	6,45	8,0	6,85	-	6,85	8,0	
Formonometin	δ CCl_4	7,70	-	8,0	6,50	-	6,75	7,35	6,65	6,85	7,35	3,90	
Biochanin A	δ CCl_4	7,65	-	-	6,15	-	6,40	7,35	6,85	6,85	7,35	3,8	



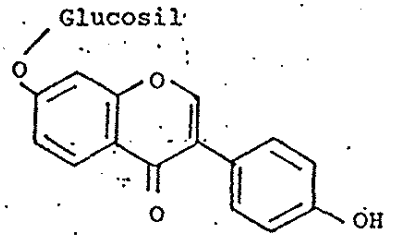
[I] Genisteín



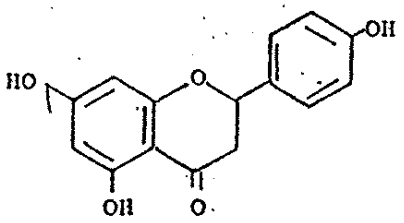
[II] Genistin



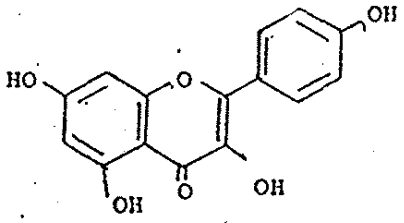
[III] Daidzeín



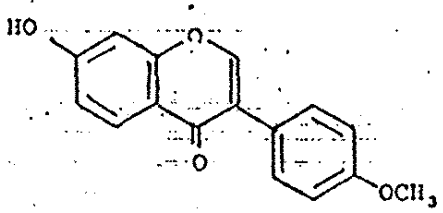
[IV] Daidzin



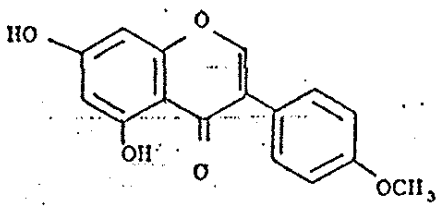
[V] Naringenin



[VI] Kaempferol



[VII] Formonometin



[VIII] Biochanin A

FIGURE 1. CHEMICAL STRUCTURES OF THE FLAVONOID COMPOUNDS STUDIED.

TABLE V. AVERAGE VALUES OBTAINED FOR THE CONTENTS OF FLAVONOID COMPOUNDS IN NINE CULTIVARS OF SOYBEAN.

Cultivars of Soya	Protein (%)	Oil (%)	Genisteín (mg/100g)	Genistin (mg/100g)	Daidzeín (mg/100g)	Daidzin (mg/100g)	Formonometin (mg/100g)	BiochaninA (mg/100g)
Doko	34,76 G	20,93 CD	28,16 F	112,00 D	34,16 F	38,16 F	34,26 G	66,26 G
IAC-8	37,86 F	23,76 A	32,16 E	114,00 CD	39,16 B	50,26 E	35,23 F	68,26 F
FT-Cristalina	38,10 EF	23,10 AB	32,00 E	113,33 CD	39,00 D	50,20 E	35,20 F	68,06 F
UFV-1'	40,13 D	20,36 D	33,16 D	116,66 C	41,50 C	52,23 D	39,40 D	71,33 CD
UFV-5'	45,20 A	23,80 A	38,23 A	123,00 AB	48,16 A	60,13 A	43,23 A	77,86 A
UFV-7'	39,36 DE	21,40 C	33,23 D	120,33 B	36,50 F	52,16 D	38,00 E	70,40 E
UFV-9'	42,20 B	21,40 C	36,00 B	124,00 A	45,23 B	57,30 B	41,16 B	75,30 B
UFV-10'	40,50 CD	22,40 B	33,23 D	122,00 AB	41,13 C	52,40 D	39,26 D	71,16 DE
UFV-15'	41,66 BC	22,40 B	34,33 C	124,00 A	44,33 B	55,16 C	40,23 C	72,20 C

* The values followed by at least one equal letter do not differ statistically by Tuckey's Test (5% probability level).

TABLE VI. ANTIOXIDANT ACTION OF FLAVONOID COMPOUNDS. AVERAGE EXPERIMENTAL VALUES OF ACIDITY, IODINE AND PEROXIDE INDEXES.

Mixture of Soybean Oil with	Acidity Index % Oleic Acid	Iodine Index (mg/100g)	Peroxide Index (mEq/kg)
Genistein	0,42 CD	187,66 E	20,33 D
Genistin	0,41 CD	187,66 E	20,33 D
Daidzein	0,40 CD	186,00 F	18,33 E
Daidzin	0,40 CD	186,00 F	18,33 F
Naringenin	0,43 C	192,33 D	23,00 BC
Kaempherol	0,40 CD	186,00 F	18,33 E
Formonometin	0,45 C	193,00 A	24,00 B
Biochanin A	0,38 D	190,66 C	22,00 C
Soybean Oil without Antioxidant	3,55 A	54,00 G	36,33 A
Refined Soybean Oil with Antioxidant	0,54 B	188,33 D	20,00 D

* The values followed by at least one equal letter do not differ statistically by Tuckey's Test (5% probability level).

workers studying other flavonoids in various plants[1-3,22].

In conclusion we can firmly state that the analysis of nuclear magnetic resonance, ultraviolet and infrared spectra led to the identification of flavonoid compounds in nine cultivars of soya. The quantification results permit us to conclude that the leguminosa (soybean) is rich in flavonoid compounds and also rich in proteins, the highest values being obtained for Cultivar UFV-5'.

The efficiency of flavonoid compounds as antioxidants was verified by acidity, iodine and peroxide indexes. The best results were obtained for genistein (I) and genistin (II).

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