

Article

## Inhibitory Action on Aldose Reductase by Soybean Flavonoids

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Os flavonóides kaempherol, genisteína, naringenina, quercetina, morina, rutina, e quercitrina isolados do cultivar de soja UFV-5' foram testados como inibidores de aldose redutase. Os melhores resultados foram obtidos usando morina e quercitrina.

The flavonoids kaempherol, genistein, naringenin, quercetin, morin, rutin and quercitrin isolated from UFV-5' soybean's cultivars were tested as inhibitors of aldose reductase. The best results were obtained by using morin and quercitrin.

**Keywords:** *flavonoids, soybean, aldose reductase*

### Introduction

Flavonoids that are present in higher plants, mainly leguminous, have aroused interest because of their inhibitory action on aldose reductase<sup>1,2,3</sup>. Aldose reductase catalyses the reduction of glucose and galactose, producing sorbitol and dulcitol respectively, using NADH as a co-factor<sup>4</sup>. This enzyme has been found in many animal tissues, such as the retina, blood, the cornea, the sciatic nerve, the liver, kidneys and sperm.

The excessive presence of sorbitol in diabetic rats and humans causes the formation of cataracts<sup>2,5,6</sup>. A similar result was also observed by the presence of dulcitol in the lenses of rats maintained on a diet rich in galactose<sup>7</sup>. In the lenses of diabetic rats the content of sorbitol may be as high as 50  $\mu\text{mol/g}$ , while in the lenses of galactosemic rats, the level of dulcitol can rise to approximately 80  $\mu\text{mol/g}$  wet weight tissue<sup>4</sup>. It has been demonstrated that cataract for-

mation ceases under the systemic administration of aldose reductase inhibitors<sup>7,8</sup>. In order to solve this problem several compounds have been tested<sup>6,8,9,10</sup>, besides flavonoids<sup>1,3</sup>. The purpose of the present study is to isolate, purify and identify flavonoidic compounds of the UFV-5' soybean's cultivar, as well as to test their inhibitory action in aldose reductase *in vitro*.

### Material and Methods

The flavonoids kaempherol (I), genistein (II), quercetin (III), naringenin (IV), morin (V), rutin (VI) and quercitrin (VIII) were extracted from the UFV-5' soybean's cultivar, following Hardin's technique<sup>11</sup>. The sample was heated in a bath-water with acetic acid for 10 min, and then filtered. The filtrate was treated with HCl 1 N for one hour, and then extracted with anhydrous ethyl ether which was evaporated giving a residue containing flavonoidic components isolated by chromatography.

Identification of the flavonoids was made by UV, NMR and IR spectroscopy techniques and comparison with authentic samples available in our laboratory. The verification of the inhibitory action of aldose reductase involved Shimizu's technique<sup>1</sup> at room temperature, by using a phosphate buffer solution (0.01 M; pH 6.2; 0.014 mM of NADPH, 10 mM of glucose or galactose and 25  $\mu$ L of aldose reductase with the addition of H<sub>2</sub>O to a volume of 1.5 mL). The control test contained all the compounds, excepting the glucose or galactose.

The reaction was initiated by the addition of aldose reductase, and the rate of oxidation from NADPH to NADP was observed by the decrease in the absorbancy at 340 nm<sup>1</sup>.

The flavonoids were prepared in concentrations of 10<sup>-4</sup> M, 10<sup>-5</sup> M, 10<sup>-6</sup> M, and 10<sup>-7</sup> M and were dissolved in water with the addition of NaOH, the pH was adjusted to 7.0 and then added to the test mixture.

## Results and Discussion

The inhibitory action of flavonoids on aldose reductase (D-mannitol-1-phosphate-dehydrogenase of *E. coli*) in different concentrations of flavonoids (Table 1), demonstrates that a concentration of 10<sup>-4</sup> M is the best promoter of the inhibitory action of the enzyme. On the other hand, it also confirms that the flavonoids morin and quercitrin presented higher inhibitory action relative to the enzyme. Data on the Table 4 shows that at the same concentration of 10<sup>-5</sup> M, the best results were obtained from the flavonoids rutin, quercitrin, and naringenin. These results are in agreement with those obtained by Varma<sup>12</sup> for quercitrin, acetate of 2-quercitrin and quercetin.

It is known that enzymes as aldose reductase reacts with substrate as glucose or galactose with the formation of a complex. According to these results it can be seen the most preference of the enzyme for reacting with flavonoids

**Table 1.** Inhibitory effect of flavonoids at different concentrations on aldose reductase.

Compounds	Concentrations			
	10 <sup>-4</sup> M	10 <sup>-5</sup> M	10 <sup>-6</sup> M	10 <sup>-7</sup> M
Kaempferol	80.33 <sup>D</sup> *#	60.33 <sup>D</sup>	40.33 <sup>E</sup>	7.00 <sup>C</sup>
Genistein	48.66 <sup>F</sup>	28.66 <sup>E</sup>	18.66 <sup>F</sup>	0.00 <sup>D</sup>
Quercetin	83.66 <sup>C</sup>	63.66 <sup>C</sup>	44.33 <sup>D</sup>	30.66 <sup>A</sup>
Naringenin	75.66 <sup>E</sup>	74.66 <sup>A</sup>	54.66 <sup>A</sup>	21.66 <sup>B</sup>
Morin	96.66 <sup>A</sup>	67.66 <sup>B</sup>	47.66 <sup>C</sup>	0.00 <sup>D</sup>
Rutin	91.66 <sup>A</sup>	71.66 <sup>A</sup>	51.66 <sup>AB</sup>	0.00 <sup>D</sup>
Quercitrin	97.66 <sup>A</sup>	71.66 <sup>A</sup>	51.66 <sup>A</sup>	20.66 <sup>AB</sup>

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

#-Average values of % inhibition of flavonoids on aldose reductase.

**Table 2.** Analysis of Variance<sup>13</sup>.

	free grade	mean square
Flavonoid	6	1777.43*
Concentration	3	18966.14*
Flav x Concentration	18	251.87*
Residue	56	1.6093

\*Significant at level of 5% of probability.

containing sugar unit in its structure. The highest percentage of inhibition obtained for quercitrin 97,66% is in agreement with that possibility.

From the analysis of variance (Table 2) a significant interaction between flavonoids and concentration can be seen.

## Conclusions

From the UFV-5' cultivar of *Glycine max* (soya) it was possible to isolate and identify the flavonoids kaempferol, genistein, quercetin, naringenin, morin, rutin and quercitrin. The identification was achieved by UV, IR and NMR spectroscopic studies. At a concentration of 10<sup>-4</sup> M, the flavonoids morin and quercitrin were those which demonstrated higher inhibitory action. On the other hand, at a concentration of 10<sup>-5</sup> M, the best results were achieved by the flavonoids naringenin, rutin, and quercetin.

These results showed the great possibility to use, in the future, the soybean as prevention of cataract in foods or by using these compounds associated to the medicines, in order to avoid the cataract formation.

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