

Isoflavone composition and biological activity of extracts from soy bread crumb and crust

Yu Chu Zhang, Daniel Albrecht, Steven J. Schwartz, Joshua Bomser, and Yael Vodovotz

ABSTRACT

Increased dietary intake of soy isoflavones and soy protein have been associated with reduced risk of chronic diseases. A soy bread that meets the FDA guidelines for heart health claim was developed. Research has shown that thermal processing may alter the composition of soy isoflavones and potentially modulate biological activity.

The objectives of our study were to investigate:

- 1) structural conversion of isoflavones during baking, and
- 2) biological activity of extracts from soy bread crumb and crust.

Bread containing 30% soy was formulated. Isoflavones were extracted separately from soy bread crumb and crust and analyzed by reversed-phase HPLC. Biological activity was assessed by inhibition of cellular proliferation of PC-3 human cancer cell lines. PC-3 cells were treated with growth medium containing varying concentration of extracts from soy bread and its two fractions, crumb and crust (0-45µM of genistein and 0-33µM of daidzein per ml of growth medium). After 48 hours cellular proliferation was measured using a MTT assay. Wheat bread was used as a control.

The total isoflavone content in soy bread crumb and crust were 1.35mg/g and 1.27mg/g, respectively. Soy bread crumb contained a mixture of β-glucosides (32.6%), malonylglucoside (41.7%), acetylglucosides (7.9%) and aglycones (17.8%). Soy bread crust contained similar amount of aglycones with a significant increase in acetylglucosides and β-glucosides than those in the crumb, corresponding to a much lower level of malonylglucosides of isoflavones. At genistein level of 45µM, soy bread extract decreased proliferation by 22% compared to wheat bread extract. Soy bread crumb showed weaker inhibitory effect (-18%) than crust.

Heat treatment and yeast fermentation may result in the structural conversion of isoflavones during baking. These chemical alterations influence not only the compositional distribution of isoflavones in soy crumb and crust but also the biological activity of extracts from these bread fractions.

INTRODUCTION

SOY

- Associated with heart health and cancer prevention
- Health benefits may be related to isoflavones and proteins in soy
- Purified isoflavones suppress a wide variety of cancer cell lines in vitro.

Soy Isoflavones

- Occur in four possible forms (aglycones and conjugates)
- Structurally similar to mammalian estrogen
- Biological activity depends on chemical forms and food matrix



A bread with 30 % soy ingredients was developed at OSU.

A serving size of 50gram provides:

- Isoflavones: 28 mg
- Meets FDA soy heart healthy claim



"Diets low in saturated fat and cholesterol that includes 25 grams of soy protein a day may reduce the risk of heart disease."

- FDA soy health claim approved in 1999

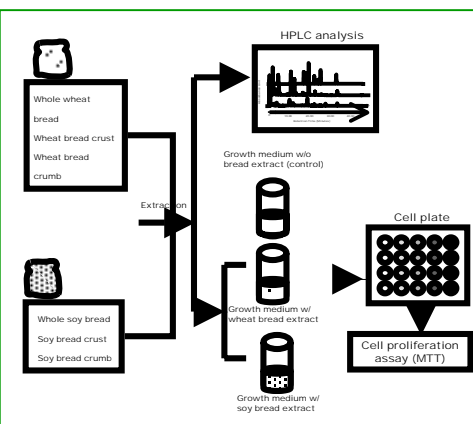
HOWEVER...

- Processing may alter soy components, esp. isoflavones
- Possible interactions of isoflavones with other dietary components
- Different food matrix may affect type and amount of isoflavones
- The biological activity of isoflavones delivered via soy bread is unknown.

PROJECT OBJECTIVES

1. To characterize the isoflavone content and composition after bread baking
2. To investigate the effects of isoflavone extract from soy bread on the proliferation of PC-3 human cancer cells

EXPERIMENTAL PLAN



OBJECTIVE 1

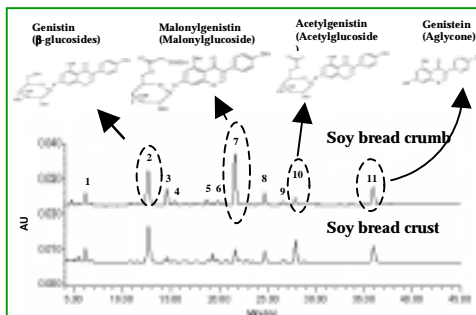


Figure. Gradient HPLC separation of isoflavones in soy bread. Elution: 1. Daidzin, 2. Genistein, 3. Malonyldaidzin, 4. Malonylgenistein, 5. Acetyldaidzin, 6. Acetylgenistein, 7. Malonylgenistein, 8. Daidzein, 9. Glycitein, 10. Acetylgenistein, 11. Genistein

RESULTS

Isoflavones in soy bread:

- Isoflavone content in soy bread crumb and crust (0.8mg/g t.b.) was similar but their HPLC profiles were altered.

- The difference in exposure temperature (crumb -100°C, crust -165°C) and moisture content (crumb -44%, crust -18%) may be among the factors contributing to the different isoflavone profiles

Table. Isoflavone content (nmol/g dry basis) and composition of soy bread (%)

Isoflavone	Soy bread crumb	%	Soy bread crust	%
Daidzin	253.2±20.3		381.9±22.9	
Genistein	782.6±7.8		1000.0±10.0	
β-glucosides	1036.3	32.7	1381.9	43.6
Malonyldaidzin	315.0±1.0		71.2±0.7	
Malonylgenistein	789.6±7.9		100.7±0.0	
Malonylgenistein	35.2±0.4		36.7±0.7	
Malonylglucosides	1139.8	36.0	278.6	8.6
Acetyldaidzin	57.9±1.2		178.7±1.8	
Acetylgenistein	95.2±2.9		488.1±9.8	
Acetylgenistein	70.4±4.2		68.3±3.4	
Acetylglucosides	223.5	7.0	735.1	23.2
Daidzein	306.7±3.1		315.0±6.3	
Genistein	448.9±9.0		447.0±0.0	
Glycitein	14.3±0.9		15.6±0.3	
Aglycones	769.9	24.3	777.6	24.4
Total	3169.5	100	3164.3	100

* Values are mean ± SD of three independent determinations.

OBJECTIVE 2

WHEAT BREAD VS. SOY BREAD

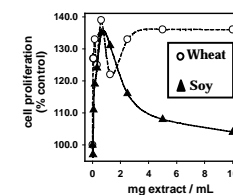


Figure. The effects of the control (wheat) bread extract and soy bread extract on the proliferation of PC-3 prostate cancer cells.

RESULTS

- Wheat bread extract increased PC-3 cell proliferation at all concentrations tested.
- Soy bread extract showed a modest stimulated increase in PC-3 cell proliferation at low concentrations.

RESULTS

SOY BREAD CRUST VS. SOY BREAD CRUMB

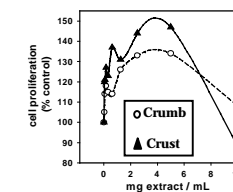


Figure. The effects of soy bread extract (crust and crumb) on the proliferation of PC-3 prostate cancer cells.

- Inhibitory effect on PC-3 cell growth was only observed with extract of soy bread crust.
- This stimulating effect on cell growth was reduced at high concentrations of soy bread extracts.

CONCLUSION

- The mechanism of action of soy bread extract on cancer cell proliferation may be different from that of pure isoflavone compounds.
- The biological activity of soy bread to reduce PC-3 cell proliferation may largely exist in its crust fraction
- Additional studies, such as on antioxidant activity of soy bread, are needed to further explore the observed biological activity.

