Total Genistein, Daidzein & Glycitein Content of Soyfoods

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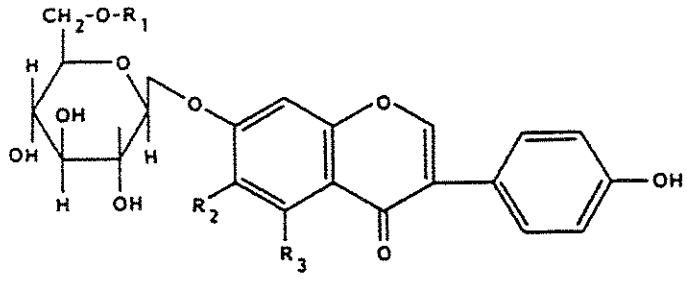
Introduction

The major soybean isoflavones, genistein and daidzein (figure 1), have been identified for a considerable period (Walter, 1941). Because these compounds appear to act as anticarcinogens by exerting a biological antioxidant effect, their content and bioavailability in foods has been a topic of recent interest (Messina and Barnes, 1991). However, in order to evaluate the potential of the isoflavones as a dietary anticarcinogen, the amounts available in typical soy foods and in soybeans must be quantified.

There have been preliminary reports on genistein and daidzein and their glycosides in a few soybean varieties and in soyfoods (Murphy, 1982; Farmakalidis and Murphy, 1985) as well as discussion of the effects of processing on these chemicals. More recently, the variations in glucoside substitution has been recognized (Farmakalidis and Murphy, 1985, Kudou et al., 1991). Glycitein, a 5-methoxy form, has been reported by some researchers (Eldridge, 1982; Naim et al., 1973) but not all soybeans seem to contain this form. The isoflavones appear to be concentrated in the soybean hypocotyl with low to moderate amount in the cotyledon. Since traditional processing of soybeans into food products will not separate these seed parts, we have evaluated soybean seeds without fractionation.

We have estimated the amount of soyfoods that humans would need to consume to provide an anticarcinogenic dose at 0.6 to 18 mg/kg body weight/day. Some soyfoods have isoflavone contents that will easily supply this amount.



ISOFLAVONE	R_1	R_2	R_3
DAIDZIN	H	H	H
GENISTIN	H	H	OH
GLYCITIN	H	OCH_3	H
6"-O-ACETYLDAIDZIN	$COCH_3$	H	H
6"-O-ACETYLGENISTIN	$COCH_3$	H	OH
6"-O-ACETYLGLYCITIN	$COCH_3$	OCH_3	H
6"-O-MALONYLDAIDZIN	$COCH_2COOH$	H	H
6"-O-MALONYLGENISTIN	$COCH_2COOH$	H	OH
6"-O-MALONYLGLYCITIN	$COCH_2COOH$	OCH_3	H

Figure 1. Chemical structures of soybean isoflavone glycones. Soy aglycone isoflavones, genistein, daidzein and glycitein, are free phenols without a glucose moiety.

Materials and Methods

Soybeans were obtained from our collection of food (tofu) soybeans. Commercial soyfoods were purchased locally. Soy

ingredients were purchased locally and made into "homecooked" soyfoods in departmental test kitchens.

Isoflavone standards for HPLC analysis were isolated by methods of Farmakalidis and Murphy (1985) and Kudou et al. (1991). Only genistein and daidzein can be purchased commercially (ICN; CalBiochem, Inc.).

Isoflavones in "homecooked" soyfoods were measured as free isoflavones after acid hydrolysis in 1N HCl (Wang et al. 1990). All other soy products were evaluated as acetonitrile/0.1N HCl extracts (Murphy, 1981). Isoflavones were separated by gradient (A: H_2O with 0.1% acetic acid; B: acetonitrile with 0.1% acetic acid) on a YMC-PACK ODS-AM-303 C_{18} column (Kudou et al., 1991). The gradient is developed for 15 to 35% B over 50 min followed by a 10 min hold at 35% B. The peaks were evaluated with a Waters 990 photodiode array detector between 200-350 nm.

Statistical analysis of differences between means was performed by ANOVA with the SAS package of the ISU computation system.

Results and Discussion

The analysis protocol used can evaluate the 12 isoflavones found in soybeans and soyfoods. Figure 1 presents the chemical structures of the 3 isoflavonoids, genistein, daidzein and glycitein. Additionally, these isoflavonoids occur as the glycosides, genistin, daidzin and glycitin, as the 6"-O-acetylglucosides, and as the 6"-O-malonylglucosides.

Figure 2 represents typical chromatographic profiles for two soy samples. Vinton 81 soybeans show the typical distribution for whole soybeans. Most of the isoflavones were present as glucosides. There were little 6"-O-acetyl forms reflecting minimal heat treatment. The distribution of genistein and daidzein forms was roughly equivalent, however, this ratio varies with crop year and growth environment within a variety. Glycitein and its glucosides were 5% of total isoflavone content. Soybeans from the north-central region of the U.S. appear to have much lower levels of this methoxylated isoflavone than those grown in more southern regions of the U.S. Tofu isoflavones yielded a different chromatographic profile typical of food products were the soy was fully hydrated. The glucosides are reduced while the aglycones and the 6"-O-acetyl forms increased. This reflected the action of the native glycosidases and the effect of heat processing, respectively.

Prior to isolation of glycone isoflavone standards, soyfoods were evaluated for their total isoflavone content by assaying the acid hydrolysates. These data are presented in figure 3. The graph shows that as soy products or soybeans were diluted into foods, the effective dose decreases rapidly compared to whole soybeans alone. Soymilk, fried tempeh, tempeh pizza and soybean casserole were judged to contain isoflavones at levels high enough to provide an isoflavonoid dose in the range required.

Food-use soybeans were evaluated for all isoflavonoid moieties but contained almost no acetyl forms. Total isoflavonoid, total genistein and total daidzein are presented in Table I. The distribution of the isoforms are presented in figure 4 for Vinton 81, Strayer 2233 and Prize varieties, all U.S. tofu beans, and for Keburi, Kurodiazu and Raiden, Japanese

varieties, from several crop years. The contents of isoflavones show considerable variation by variety, crop year and location. The malonyl forms make up a considerable proportion of total isoflavonoid contents of intact soybeans. Almost no 6"-O-acetyl forms appear in intact beans. The total glycitin content was relatively constant for all varieties evaluated at 135 μg/g.

Commercial soy product isoflavone contents are presented in Table II and in figure 5. As total soy protein content was diluted in soy product formulation, the total isoflavone content was reduced, concomitantly. The effects and extent of heat processing is reflected in the appearance and concentration of the 6"-O-acetyl forms in commercial products. The more extensive the heat treatment, the higher the 6"-O-acetyl isoflavonoid contents. Textured soy protein (TVP) was processed by extrusion, a high intensity heat treatment, and yielded the highest levels of the acetyl derivatives. Processing, by heat or with water addition, decreased the 6"-O-malonyl forms significantly while increasing the respective aglycones and unmodified glycones. The commercial soyfoods evaluated were produced from unknown soybean varieties. We have not performed a mass-balance on specific soybean varieties, thus, we cannot calculate the distribution during processing.

Conclusion

Total isoflavone content of food soybeans ranged from 713 to 2772 ppm total, 311 to 1311 ppm daidzein, 402 to 1461 ppm genistein and 82 to 203 ppm glycitein. Isoflavone content of commercial soyfoods ranged from 7 to 2892 ppm total, 5 to 1539 ppm genistein, 2 to 1537 ppm daidzein and 12 to 202 ppm glycitein. The glycoside variation was effected by heat processing and water content. Careful selection of soy products can yield a desired anticarcinogenic dose of 700 to 2000 ppm.

References

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TOTAL ISOFLAVONE CONTENT OF FOOD SOYBEANS

		μg/g			
VARIETY	YEAR	TOTAL	GENISTEIN	DAIDZEIN	GLYCITEIN
PRIZE	1989	2,772^{A}	1,461^{ABCD}	1,311^{A}	110^{GHI}
LS301	1989S	2,747^{A}	1,509^{AB}	1,238^{A}	129^{DEF}
P9111	1989	2,682^{AB}	1,636^{A}	1,046^{BC}	116^{FGHI}
LS301	1990	2,479^{BC}	1,368^{BCDE}	1,111^{BC}	135DE
PRIZE	1990	2,402^{CD}	1,280^{DEFG}	1,122^{B}	122^{EFGHI}
P9202	1989	2,375^{CDE}	1,472^{ABCD}	903DE	131^{DEF}
VINTON 81	1989	2,225^{CDEF}	1,163^{FGH}	1,062^{BC}	122^{EFGHI}
LS301	1989A	2,170^{DEF}	1,300^{CDEF}	870DE	131^{DEF}
P9202	1991	2,132^{FE}	1,481^{ABC}	651^{F}	135DE
P9111	1991	2,065^{F}	1,253^{EFG}	812^{E}	82^{K}
VINTON 81	1990H	2,047^{F}	1,083^{GH}	964^{CD}	122^{EFGHI}
STRAYER 2233	1989	1,443^{G}	837^{JK}	605^{FG}	130^{DEF}
HP204	1989S	1,361^{GH}	854^{JK}	507^{GHI}	132^{DEF}
STRAYER 2233	1991	1,333^{GH}	788^{JK}	545^{FGH}	126^{EFG}
HP204	1990	1,318^{GHI}	840^{JK}	479^{HIJ}	123^{EFGH}
KEBURI	1991	1,278^{GHI}	859^{JK}	419^{IJK}	156^{BC}
XL72	1989	1,242^{GHI}	972^{HIJ}	270^{L}	167^{B}
RAIDEN	1991	1,221^{GHI}	875^{JK}	345^{KL}	203^{A}
HP204	1989A	1,181^{GHIJ}	786^{JK}	394^{IJK}	131^{DEF}
XL72	1990	1,178^{HIJ}	904^{JK}	274^{L}	170^{B}
KURODIAZU	1991	1,138^{HIJ}	820^{JK}	318^{KL}	123^{EFGHI}
VINTON 81	1991I	1,059^{IJ}	732^{KL}	327^{KL}	107^{HIJ}
VINTON 81	1991S	933^{JK}	553^{LM}	380^{JKL}	117^{FGHI}
VINTON 81	1991W	713^{K}	402^{M}	311^{KL}	109^{GHI}

Table 1. Isoflavone values in columns with different letters were significantly different (a=0.05). Isoflavone contents were normalized with respect to glucoside moiety. Crop year with letter code were grown in different locations in Iowa.

Table 2. The total equivalent amounts (μg/g) of daidzein, genistein, and glycitein in soy food products.

Products	Daidzein	Genistein	Glycitein	Total
Soy Ingredients				
Green soybean	546 [a]	729 [c]	79 [de]	1354 [c]
Soy granule	549 [a]	748 [c]	167 [b]	1464 [b]
T.V.P.	473 [b]	707 [cd]	202 [a]	1382 [bc]
Soy flour	226 [f]	810 [b]	88 [d]	1124 [de]
Traditional soy foods				
Soynuts	563 [a]	869 [a]	193 [a]	1625 [a]
Soy beverage[A]	311 [de]	617 [ef]	109 [c]	1037 [ef]
Soy beverage[B]	295 [de]	607 [fg]	111 [c]	1014 [f]
Soy beverage[C]	336 [d]	560 [g]	105 [c]	1001 [f]
Soy beverage[D]	407 [c]	665 [de]	111 [c]	1183 [d]
Organic tofu	146 [g]	162 [k]	29 [ghij]	337 [hi]
Tempeh	273 [e]	320 [h]	32 [fghij]	625 [g]
Bean paste	272 [ef]	245 [i]	77 [e]	593 [g]
Fermented beancurd	143 [g]	224 [ij]	23 [j]	390 [h]
Honzukuri miso	79 [h]	177 [jk]	38 [fg]	294 [i]
2nd-generation soy foods				
Hot dog	34 [hijk]	82 [lm]	34 [fghi]	150 [jk]
Bacon	28 [ijk]	69 [lm]	24 [ij]	122 [jkl]
Tempeh burger	64 [hi]	196 [ijk]	30 [ghij]	289 [i]
Tofu yogurt	57 [hij]	94 [l]	12 [k]	164 [j]
Soy parmesan	15 [jk]	8 [n]	41 [f]	65 [kl]
Cheddar cheese[A]	2 [k]	5 [n]	27 [hij]	34 [l]
Cheddar cheese[B]	34 [hijk]	40 [mn]	35 [fgh]	109 [jkl]
Mozzarella cheese	11 [k]	36 [mn]	30 [ghij]	76 [jkl]
Flat noodle	9 [k]	37 [mn]	39 [fg]	85 [jkl]

List of figures

Figure 2A VINTON 81 SOYBEANS

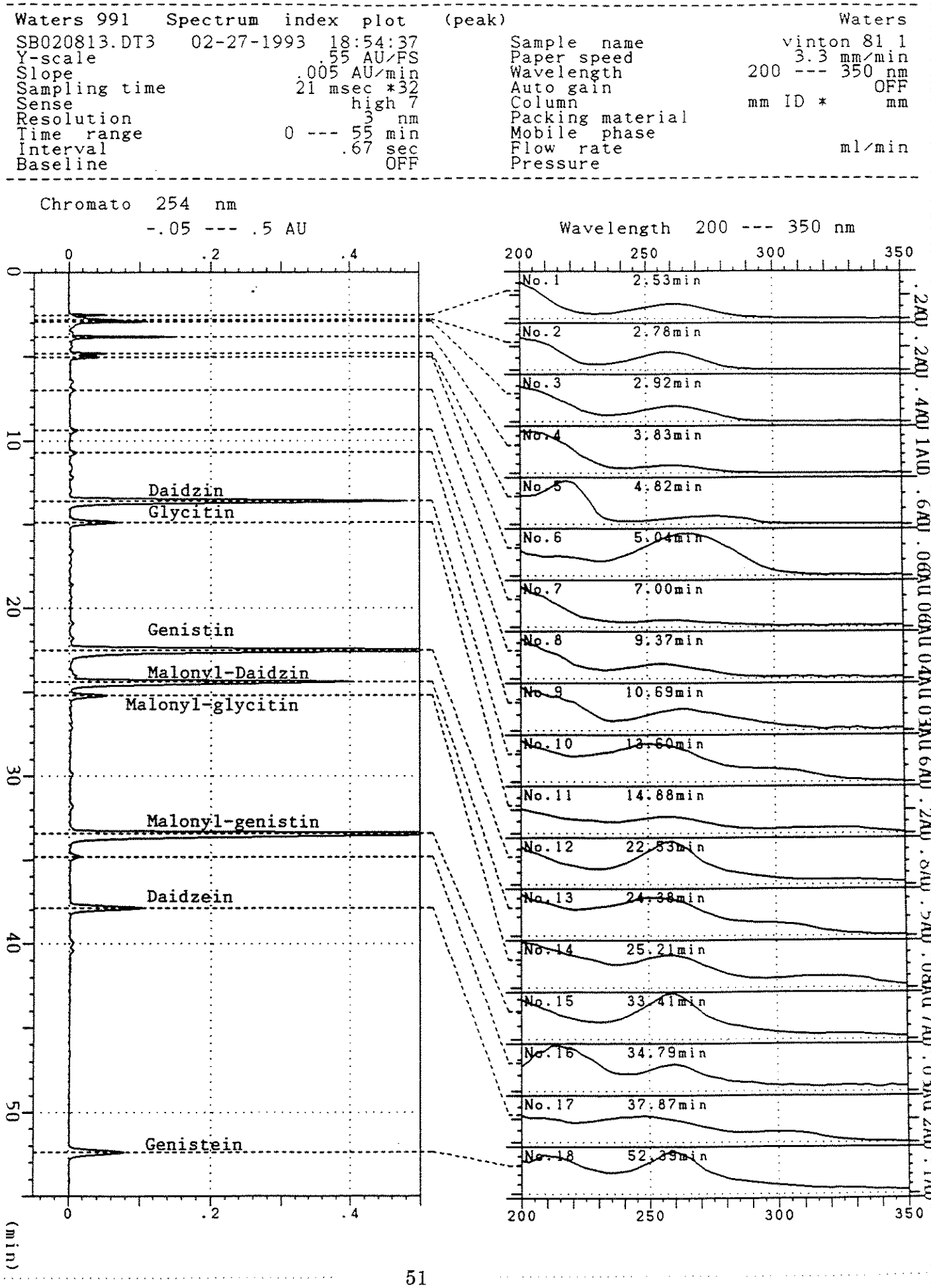


Figure 2B ORGANIC TOFU

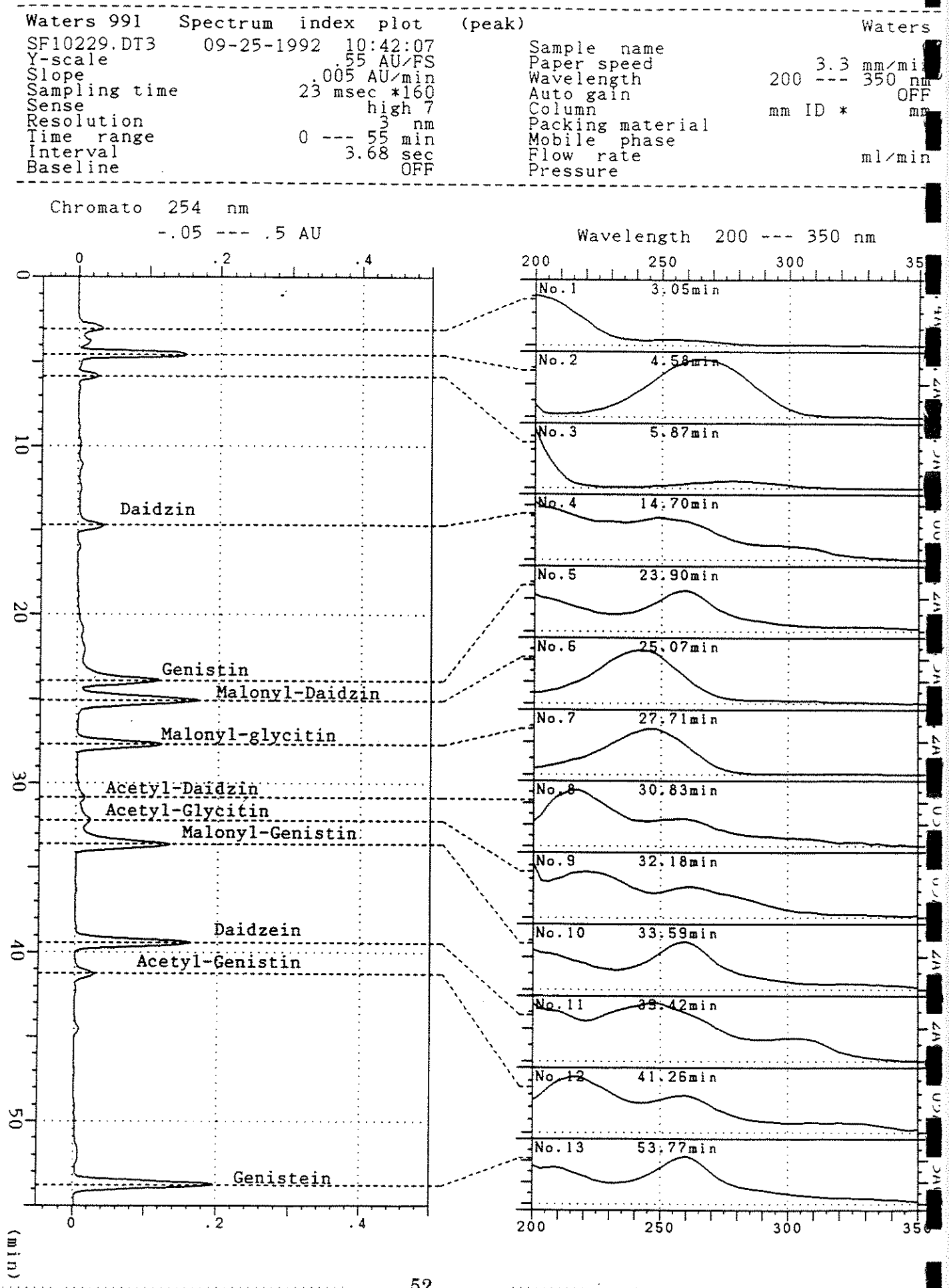
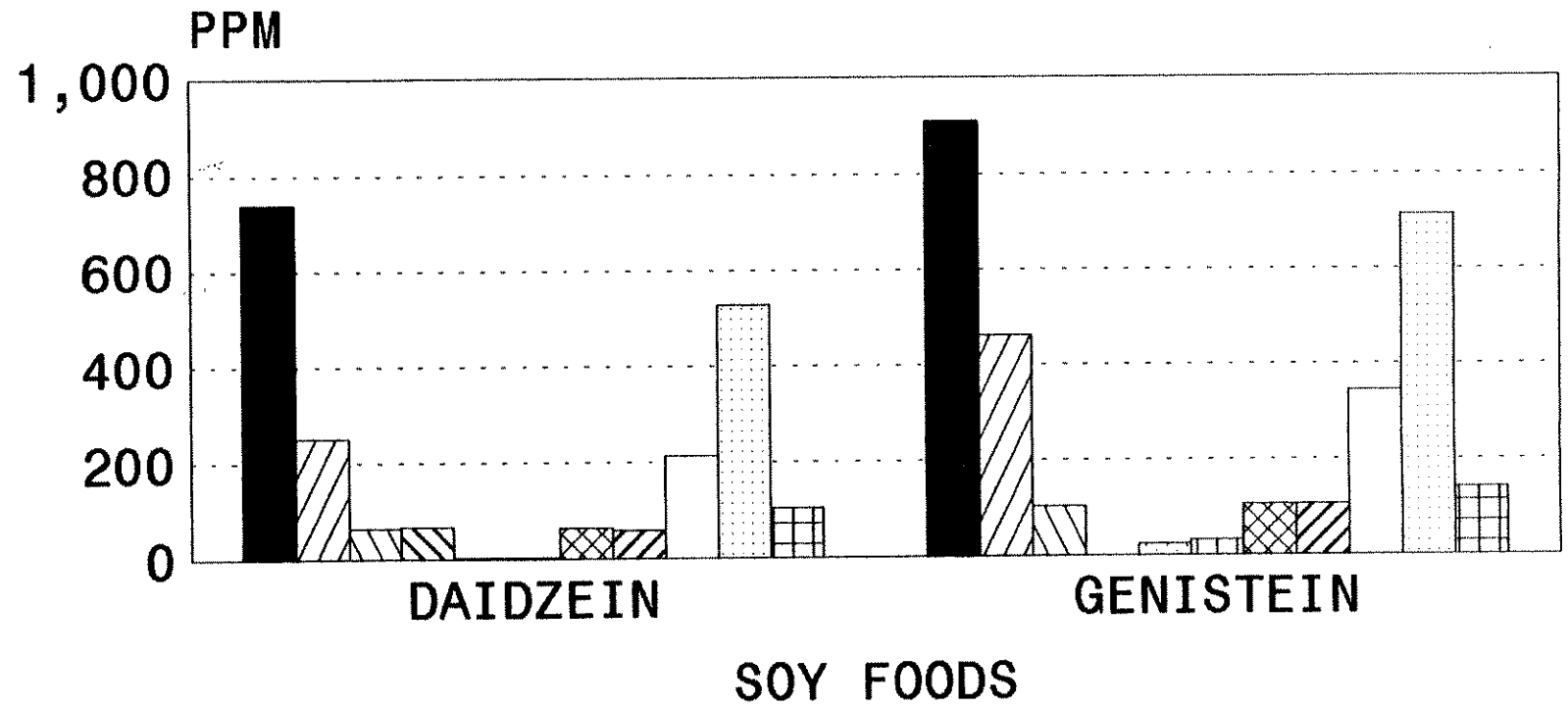


Figure 3

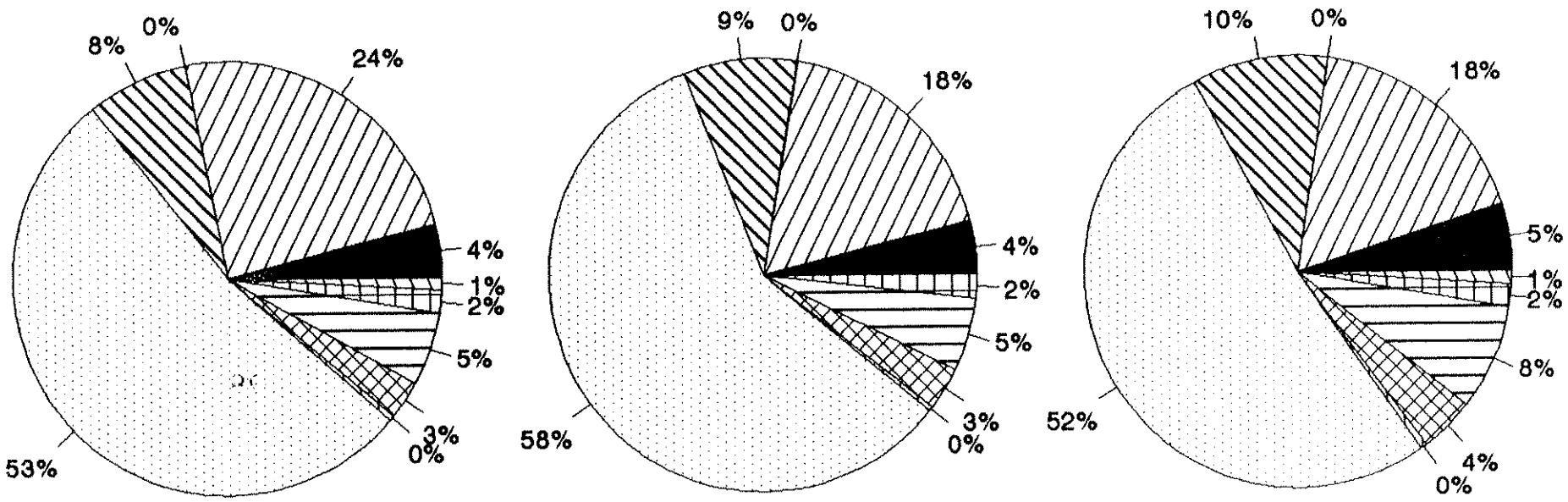
SOY ISOFLAVONES
HOME-MADE FOODS



HYDROLYZED TOTALS

figure 4A

ISOFLAVONES IN FOOD SOYBEANS



Keburi
D=347 G=764 GI=156

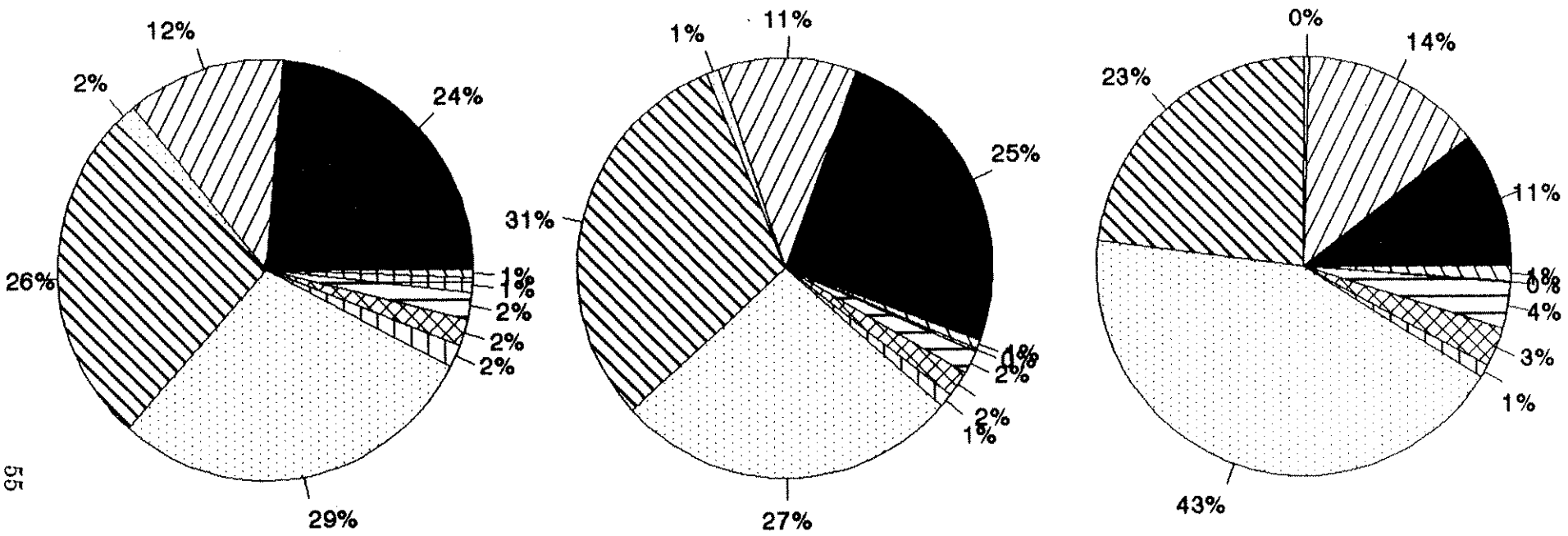
Kurodiazu
D=241 G=735 GI=123

Raiden
D=278 G=781 GI=203 ug/g

DAIDZIN | MAL-DIN | DAIDZEIN | GENISTIN | MAL-GIN
GENISTEIN | GLYCITIN | MAL-GLY | AC-GLY | GLYCITEIN

1991

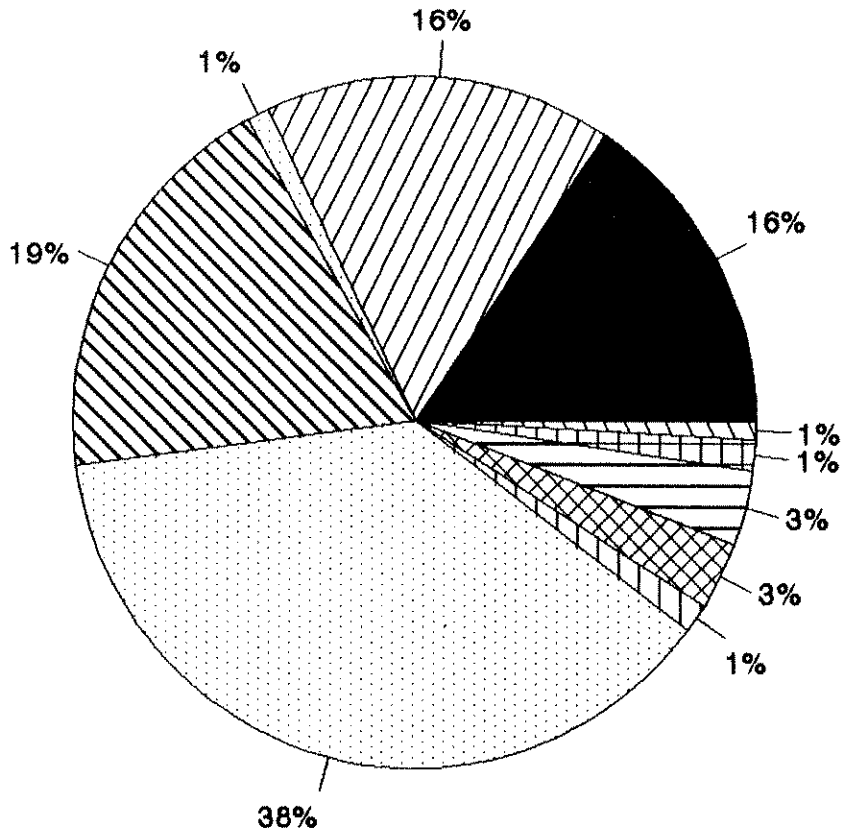
Figure 4B



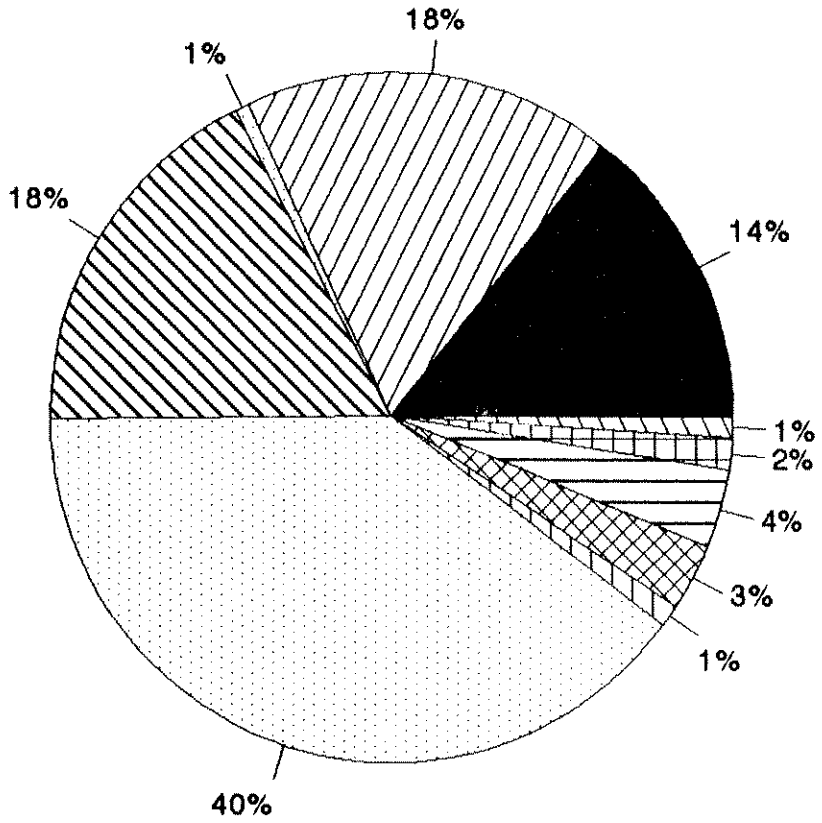
DAIDZIN | MAL-DIN | DAIDZEIN | GENISTIN | MAL-GIN
GENISTEIN | GLYCITIN | MAL-GLY | AC-GLY | GLYCITEIN

VINTON 81

Figure 4C



1989
D=444 G=768 GI=130

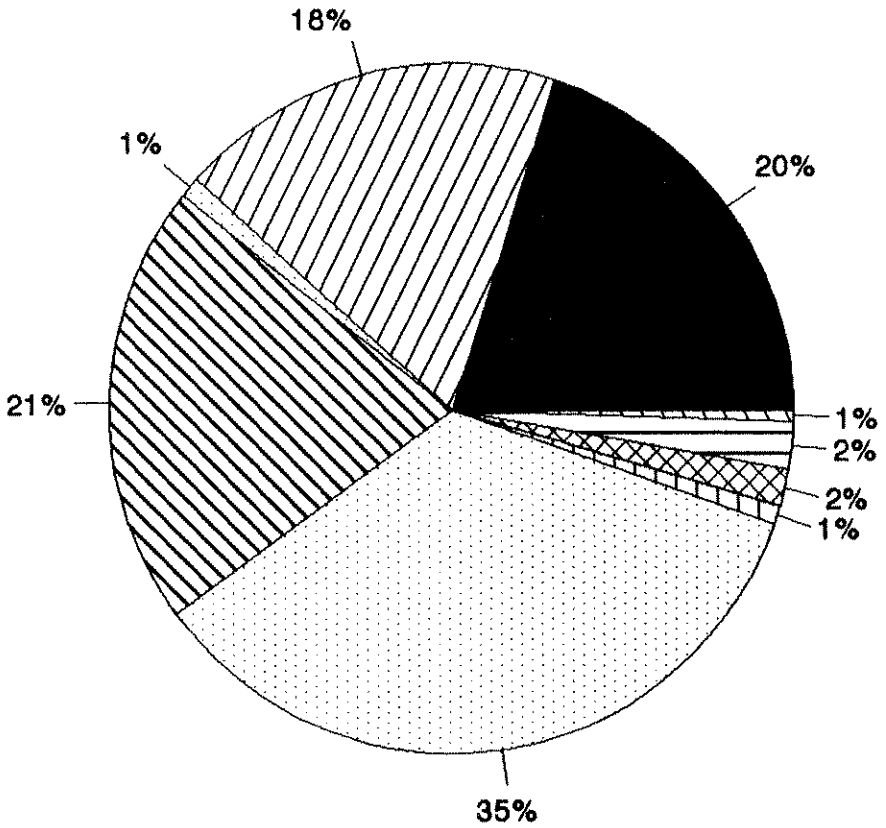


1991
D=403 G=719 GI=126 ug/g

DAIDZIN | MAL-DIN | DAIDZEIN | GENISTIN | MAL-GIN
GENISTEIN | GLYCITIN | MAL-GLY | GLYCITEIN

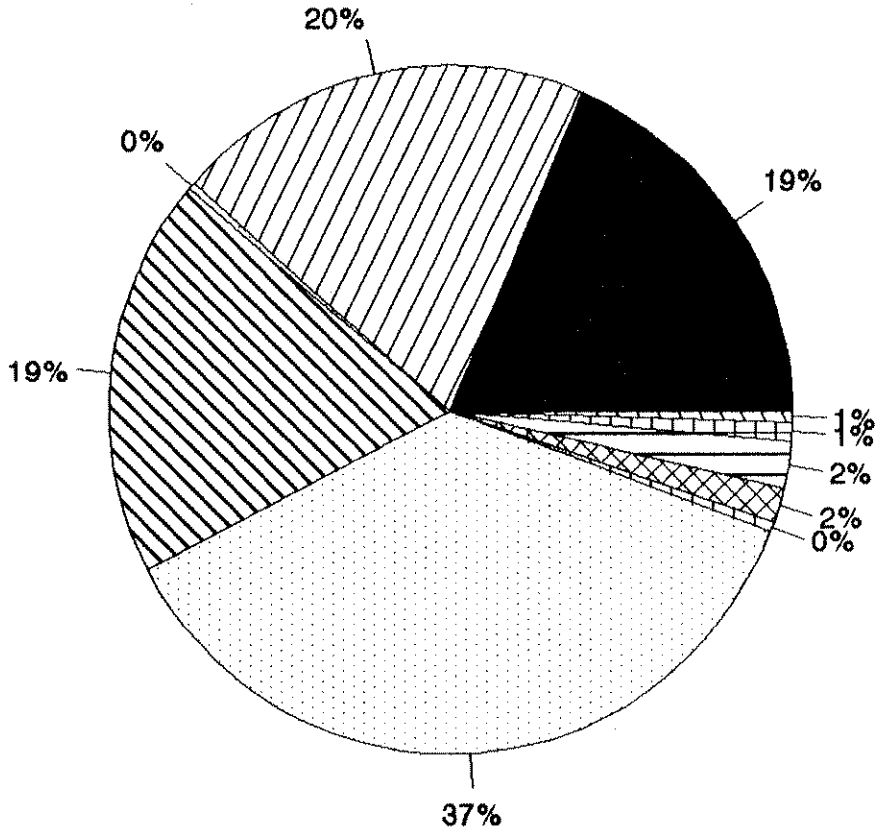
STRAYER 2233

Figure 4D



1989

D=874 G=1237 GI=129



1990

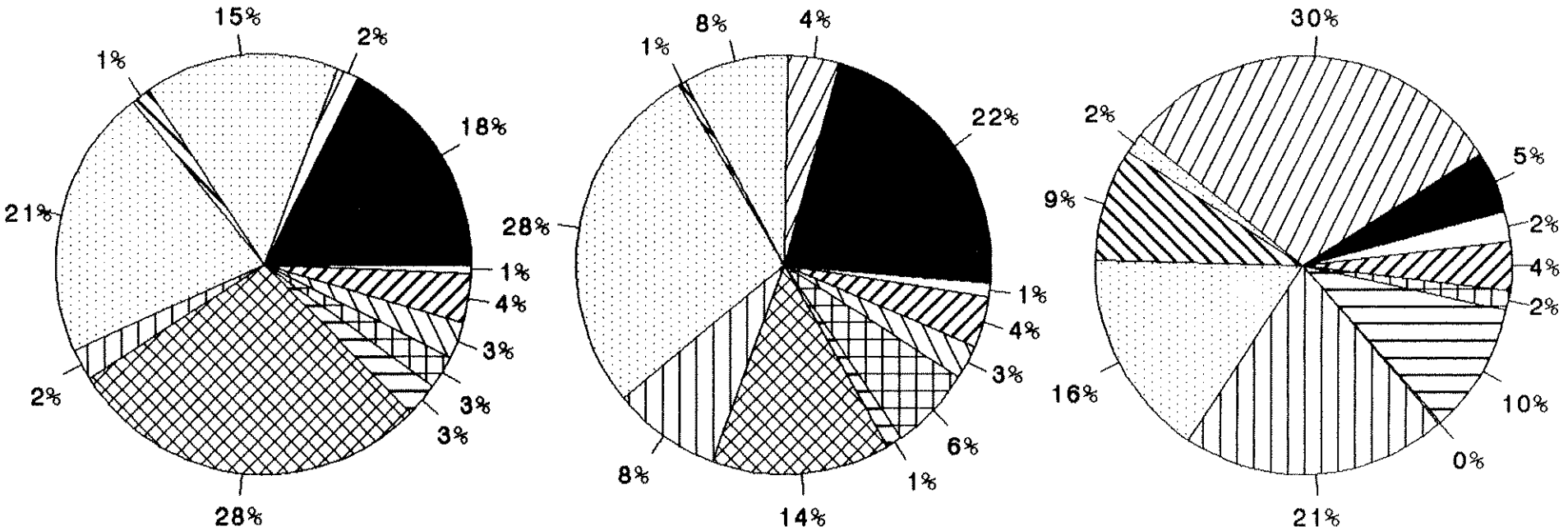
D=817 G=1175 GI=122 ug/g

DAIDZIN | MAL-DIN | DAIDZEIN | GENISTIN | MAL-GIN

GENISTEIN | GLYCITIN | MAL-GLY | GLYCITEIN

PRIZE

Figure 5A



SOYNUTS TEXTURED SOY PROTEIN TOFU

D=563 G=869 GI=193 D=473 G=707 GI=202 D=146 G=162 GI=29 ug/g

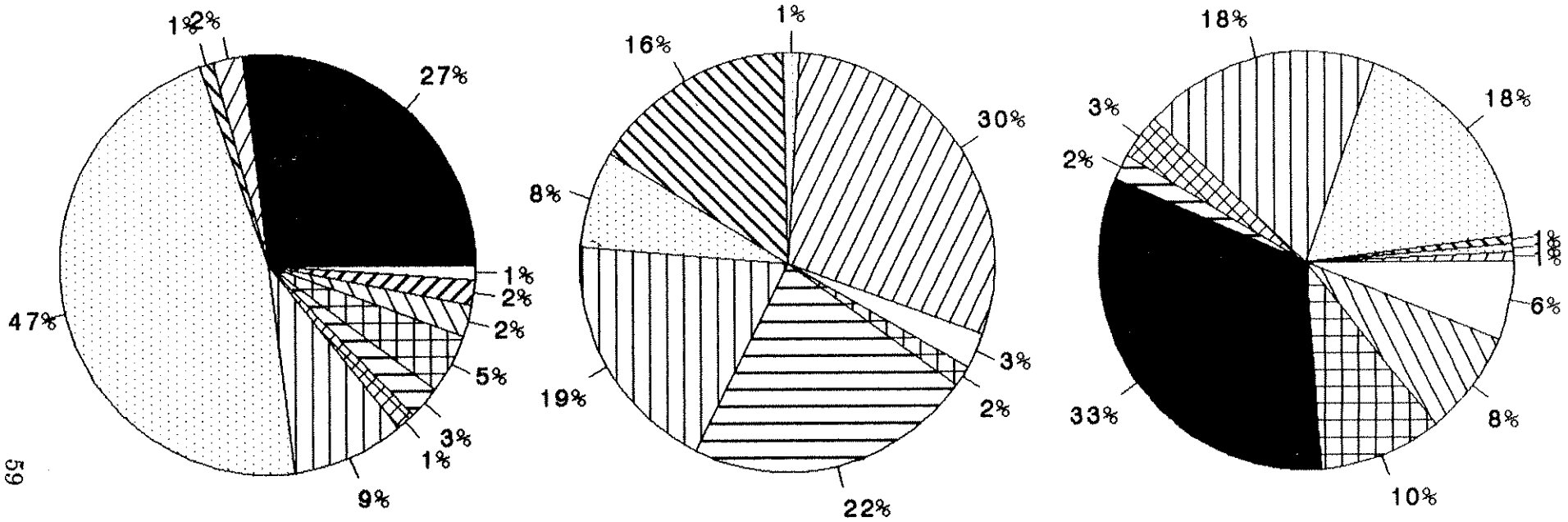
DAIDZIN MAL-DIN AC-DIN DAIZEIN

GENISTIN MAL-GIN AC-GIN GENISTEIN

GLYCITIN MAL-GLY AC-GLY GLYCITEIN

COMMERCIAL SOYFOODS

Figure 5B


1%
2%
27%
47%
1%
2%
2%
5%
3%
1%
9%
SOYMILK
D=311 G=617 GI=109
1%
16%
30%
8%
3%
19%
2%
22%
TEMPEH
D=273 G=320 GI=32
18%
18%
3%
2%
1%
1%
6%
33%
8%
10%
FAKIN' BACON
D=28 G=69 GI=24 ug/g
DAIDZIN
MAL-DIN
AC-DIN
DAIZEIN
GENISTIN
MAL-GIN
AC-GIN
GENISTEIN
GLYCITIN
MAL-GLY
AC-GLY
GLYCITEIN
COMMERCIAL SOYFOODS