한국농화학회지 Vol. 25, No. 4, Dec. 1982
J. Korean Agricultural Chemical Society

Relationship Between Ginsenoside Content and Stem Color Intensity of *Panax ginseng*

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(Received October, 25 1982)

莖色別人蔘根의 Ginsenoside 含量

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抄　錄

莖色程度가 다른 紫莖種人蔘根胴體의 表皮一周皮(外部)와 導管一隨(內部)의 진세노사이드를 조사하였다. 진세노사이드의 樣相, 다이올계와 트리올계의 比(PT/PD) 및 總 진세노사이드 함량은 두 部位間에만 有意性 있는 差異를 보였으며 莖色度와는 關係를 보이지 아니하였다. 紫色度 감소에 따른 總 진세노사이드 含量의 增加傾向은 試料數를 增大하여 調査할 必要가 있다. 진세노사이드의 含量 순서는 外部에서 $R_{b1}>R_{g1}>R_e>R_c>R_{g2}>R_{b2}>R_f>R_d$였고 內部에서는 $R_{g1}>R_{b1}>R_{g2}>R_e>R_{b2}>R_c>R_f>R_d$였다. PT/PD는 外部에서 1.08 內部에서 1.95였다. 總 진세노사이드 含量은 外部가 內部보다 3倍 높고 두 部位의 무게는 비슷하므로 胴體의 진세노사이드 함량은 外部의 함량에 의존된다.

Introduction

Korea ginseng(Panax ginseng) has been so famous as it had already been praised with in the old Korea(BC 3-AD 668)[1]. Korea ginseng root is still holding the crown of medicinal herb kingdom by the highest frequency in the Oriental medicinal prescriptions[2]. Not only medicine but also old stile ginseng tea that is made by boiling with jujube, and ginseng-chicken soup are favorit ones. It is principally due to the adaptogenic efficacy of Korea ginseng root to correct human physiological imbalances[2]. The adaptogen theory derived from various modern experiments using ginseng seems to be well accordance with the oriental concept of the best(upper class) medicine that it has good efficacy and is not poisonous regardless of the quantity and duration of administration[2]. Korea Ginseng is likely placed on the borderline of health food and medicine. Ginseng saponin has been a target component so that most ginseng researches have been done in relation with saponins[3,4] even though nucleic acids and peptides were reported at effective components very recently[5,6]. Most researches on ginsenosides(Ginseng saponin) were concerned to analytical methods, moleculer structure, and

pharmacological or clinical efficacy[3,4]. Studies on ginsenosides in relation to biological characteristics of ginseng plant and environmental factors are very rare[7,8,9]. Many variants in commonly grown purple stem variety are easily recognized by purple colored area of stem, petiole and peduncle[7]. Similarity of ginsenoside pattern is greater between the same part of two different roots than between different part of the same root[10]. We investigated the relationship among total or each ginsenoside content, part of root and stem color.

Materials and Methods

Root sample: Two healthy roots in each group different in percentage(10, 50 and 100%) of purple color region of stem were randomly chosen from the same field at harvest(6 years old). Main body(Tap root) was separated into the central portion(xylem and pith) and the outside portion(epidermis and cortex), dried at 70°C in a forced draft oven and ground with mortar.

Ginsenoside extraction: According to previous *method*[7] *based on others*[11,12] two grams of dry root powder were extracted 3 times for 3 hours each with 20m*l* of HPLC(High pressure liquid chromatography) grade methanol at 60°C water bath. The extracts were pooled and evaporated in vacuo at 40°C to dryness. The residue was dissolved with 5m*l* HPLC grade water 5 times, transfered to separation funnel. Pooled water fraction was washed once with 50m*l* ethylether and 3 times with 50m*l* HPLC grade chloroform to eliminate nonpolar components. Ginsenoside in water layer was extracted 3 times with 50m*l* of H_2O saturated HPLC grade n-butanol. n-Butanol layer was pooled and evaporated in vacuo to dryness until no n-butanol smell was detectable. The residue was dissolved with 0.5 to 1.0m*l* of HPLC grade methanol for injection.

Ginsenoside analysis[7,11,12]: Methanol solution (20 ul) was injected to HPLC(Waters Associate Model 244) and analytical conditiong were as follow. 1 u-Bondapak Carbohydrate Analysis Column, RI detector, $CH_3CN/H_2O/BuOH=80:20:15$ solvent system, 1.5m*l*/min. flow rate, 1.0cm/min. chart speed, attenuator 8x. Each ginsenoside peak of chromatogram was identified by standard sample and standard ginsenosides. Each ginsenoside content was calculated as Rg_1 equivalent from the peak area by using Rg_1 calibration curve(Fig. 1) and then divided by appropriate factor to each ginsenoside[10]. Reading of each peak area was done by multiplication of height by half width. This reading showed two straight lines(15.5cm²/mg and 12.5cm²/mg) for calibration curve of standard ginsenoside Rg_1 (Fig. 1) and appears to lessen reading error than previous base-height method[10]. At higher concentration range the former likely results in positive error while the latter in negative error as shown in Fig. 1.

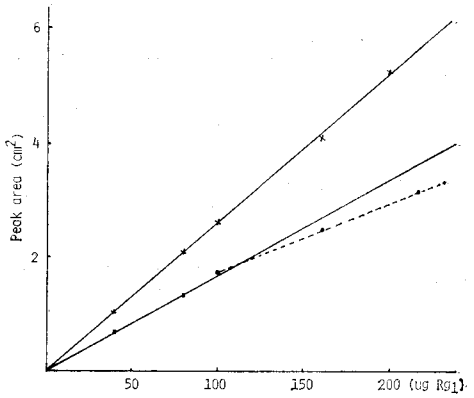

Fig. 1. Calibration curve of gninsenoside Rg_1.
-×-×-; Base line-height method
-·—·—; Half width-height method (Dotted line is for high concentration range).

Statistical Analysis: Stem color, part of root and each ginsenoside were considered main, sub, and sub-subplot, respectively and then analysis of variance was calculated as the case of split-split plot design. Analysis of variance on the ratio of protopanaxadiol(PD) and protopanaxatriol(PT) ginsenosides(PT/PD) was done in relation to stem color and part.

Results and discusson

The content of ginsenosides in epidermis plus cortex and central part of main body of ginseng root different in purple colored area on stem was shown in Table 1 and 2. The content of ginsenoside was in the order of $Rb_1>Rg_1>Re>Rc>Rg_2>Rb_2>Rf>Rd$ in epidermis-cortex while $Rg_1>Rb_1>Rg_2>Re>Rb_2>Rc>Rf>Rd$ in xylem-pith. In epidermis-cortex first three ginsenosides are 62% of total and Rb_1 alone 27%. In xylem-pith first three are 68% and Rg_1 alone 32%. Report on ginsenoside composition of these two parts are hardly found.

The variance of ginsenoside content for stem color, part and their interactions are shown in Table 3. Highly significant difference was held between ginsenoside contents as mentioned above and as shown Table 1 and 2. Significant inter-

Table 1. Ginsenoside content of epidermis-cortex of *P. ginseng* root in relation to stem color.

Dark purple (%)	Root No.	Ginsenoside (mg/100g dw)							
		Rg_2	Rg_1	Rf	Re	Rd	Rc	Rb_2	Rb_1
10	1	247	412	155	296	84	228	141	431
	2	190	545	107	332	87	228	171	889
	Mean	219	479	131	314	86	228	156	660
50	1	144	463	T	353	74	237	216	713
	2	133	461	226	331	81	277	194	481
	Mean	138	462	113	342	77	257	205	597
100	1	122	339	121	248	50	250	155	413
	2	301	335	135	183	32	146	108	338
	Mean	212	336	128	216	41	198	132	376
Grand	Mean	190	426	124	291	68	228	164	544

*Percentage of dark purple color on stem. T: Trace.

Table 2. Ginsenoside content of central part of *P. ginseng* root in relation to stem color.

Dark purple (%)	Root No.	Ginsenoside (mg/100g dw)							
		Rg_2	Rg_1	Rf	Re	Rd	Rc	Rb_2	Rb_1
10	1	65	199	T	92	19	39	32	123
	2	97	185	63	95	30	57	66	152
	Mean	81	192	31	94	25	48	49	138
50	1	148	288	T	79	23	62	33	207
	2	134	217	88	79	24	37	36	105
	Mean	141	253	44	79	24	49	35	156
100	1	68	167	T	39	16	32	14	68
	2	143	184	T	80	T	19	87	68
	Mean	106	176	T	59	8	26	50	68
Grand	Mean	109	207	25	77	19	41	45	121

*Percentage of dark purple color on stem. T: Trace.

Table 3. ANOVA for ginsenoside content in two parts of *P. ginseng* in relation to stem color.

Source	df	SS	MS	F
Total	95	2364116		
Replication	1	4401	4401	0.68NS
Color(C)	2	56018	28009	4.33NS
Error(a)	2	12952	6476	
Part(P)	1	724538	724538	285***
C×P	2	13897	6949	2.73NS
Error(b)	3	7626	2542	
Ginsenoside(G)	7	947142	135306	28.9***
C×G	14	33888	2421	0.52NS
P×G	7	257360	42480	9.09***
C×P×G	14	70007	5001	1.07NS
Error(c)	42	196287	4674	

***: Significant at P=0.001, NS: Nonsignificant.
Color: Percentage of dark purple area of stem.
Part: Central(Xylem-pith) part and Epidermis-cortex.
Gin.: Ginsenosides.

action was shown only between part and ginsenosides but between color and ginsenosides indicating that ginsenoside pattern is similar among the roots regardless of stem color but significantly different between epidermis-cortex and central part. This fact reaffirmed the previous results on pattern similarity of ginsenosides in relation to stem color[10].

Dry weight of main body, weight ratio of two parts and total ginsenosides in each part are shown in Table 4. Ratio of two parts seemed no relation to root weight but average of 6 roots was 1.1 suggesting that weight of two parts are likely equal. The content of ginsenosides was about 3 times higher in epidermis-cortex than in xylem-pith. It is much different from those of others. According to Sakamoto et al.[13] the content was 5.3% in epidermis-cortex and 0.12 in the central, that is, 44 times higher in epidermis-cortex. Furthermore Tani et al. did not find any ginsenoside peak in two wave length chromatoscanner profile of thin layer chromatography of xylem-pith extract[14].

Such extreme difference might be attributed to sampling. If separation of two parts are done after drying the root as when white ginseng was perchased at market, ginsenosides in the central part could move to the outside part with water flow during drying.

Such difference in ginsenoside content is highly significant only between parts and not between same parts of different roots with stem different in purple colored area(Table 3). Average ginsenoside content of main body calculated from Table 1 and 2 with the ratio of two part in Table 4 was in the order of $Rb_1>Rg_1>Re>Rg_2>Rc>Rb_2>Rf>Rd$. Rg_1 was, however, higher than Rb_1 in 4 of 6 roots. In spite of such difference ginsenoside pattern of main body was similar regardless of stem color(Table 3). Rb_1 and Rb_2 were 25 and 23% of total ginsenosides, respectively. Rb_1 was highest not only in the main body of fresh(33%), white(39%) and even red(63%) ginseng[12] but also in the whole root with the range of 34 to 51%[15]. Since these were all pooled samples the variation between individual roots were unknown.

Total ginsenoside content in main body ranged from 1.0% to 1.66%(Table4). This difference was not significant for the stem color as shown in Table 3. The mean of total ginsenoisdes

Table 4. Ginsenoside content of *P. ginseng* root in relation to stem color.

Dark purple* (%)	Root No.	Main body		Ginsenosides(% dw)			
		Weight (g.dw)	E/C	Epidermis-Cortex	Xylem-Pith	E/C	Total(main body)
10	1	17.19	1.59	1.994	0.570	3.50	1.44
	2	23.98	1.03	2.549	0.745	3.42	1.66
	mean	20.59	1.31	2.272	0.658	3.46	1.55
50	1	10.16	1.12	2.201	0.841	2.62	1.56
	2	11.49	0.83	2.184	0.720	3.03	1.38
	mean	10.83	0.98	2.193	0.781	2.83	1.47
100	1	11.69	0.87	1.698	0.405	4.19	1.00
	2	18.11	1.16	1.578	0.583	2.71	1.12
	mean	14.95	1.02	1.638	0.494	3.45	1.06
Total	average	15.46	1.10	2.034	0.644	3.22	1.36

*: Percentage of dark purple area on stem.
E/C: Ratio of Epidermis-Cortex and Xylem-Pith(Central part).

tended to decrease with the increase of purple colored area. F value increased when total content in Table 4 was used though it was not significant. F value for color in Table 3 was based on the ratio as one. Thus it might be significant with greater sample size.

Total ginsenosides in this case was very low comparing with other's report that it was 2.64% in main body of white ginseng and 2.67 in red ginseng[12]. The reason is not clear in these case. But seasonal variation of total ginsenosides in the root was considerably high[9]. Total ginsenosides as the sum of each ginsenosides as in this case is still far below of the crude saponin by weighing method. The former was 54, 53 and 52% of the latter in the main body of fresh, white and red ginseng[12] and similar result was obtained for whole root[15,16].

Protopanaxadiol ginsenosides(PD, Rb_1+Rb_2+Rc+Rd), protopanaxatriol ginsenosides(PT, Rg_1+Rg_2+Re+Rf) and their ratio in two parts and main body were shown in Table 5. The ratio of PT to PD(PT/PD) was about 2 in xylem-pith but about 1 in epidermis-cortex. Since total ginsenosides in 3 times higher in epidermis-cortex PT/PD of main body depends much on that of epidermis-cortex resulting in 1.2 PT/PD was only significantly different between epidermis-cortex and the central. No significant difference was found between stem color(Table 6). It was also true when PT and PD, or PT/PD of main body in Table 5 were used for analysis of variance. PT/PD was a

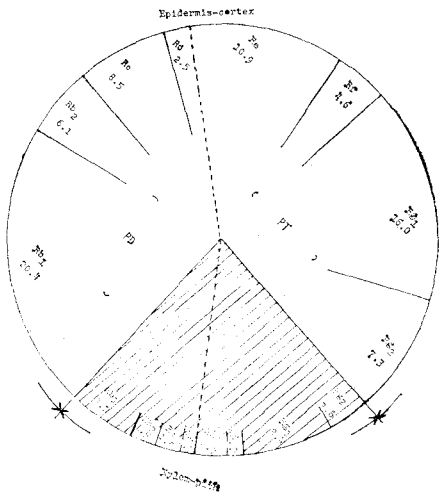

Fig. 2. Composition of ginsenosides in main body of Panax ginseng root.
PT: Protopanaxatriol
PD: Protopanaxadiol
Numerals indicate percentage contribution to total ginsenosides. Shaded part indicates Xylem-Pith.

Table 5. Content of protopanaxatriol(PT) and protopanaxadiol(PD) ginsenosides in *P. ginseng* root.

(mg/100g dw)

Dark purple* (%)	Root No.	Xylem-Pith(Central part)			Epidermis-Cortex			Total(main body)		
		PT	PD	PT/PD	PT	PD	PT/PD	PT	PD	PT/PD
	1	356	213	1.66	1110	884	1.26	819	625	1.31
10	2	440	305	1.44	1174	1375	0.85	812	848	0.96
	mean	398	259	1.55	1142	1130	1.06	816	737	1.14
	1	516	325	1.59	961	1240	0.78	751	808	0.93
50	2	518	202	2.56	1151	1033	1.11	805	579	1.39
	mean	517	264	2.08	1056	1137	0.93	778	694	1.12
	1	275	130	2.12	830	868	0.96	533	473	1.13
100	2	408	175	2.33	954	624	1.53	701	416	1.69
	mean	342	153	2.23	892	746	1.25	617	445	1.41
Total	average	419	225	1.95	1030	1004	1.08	737	625	1.22

*: Percentage of dark purple area of stem.
PT: $Re+Rf+Rg_1+Rg_2$, PD: $Rb_1+Rb_2+Rc+Rd$

Table 6. ANOVA for the ratio of PT/PD in main body of *P. ginseng* root in relation to stem color.

Source	df	SS	MS	F
Total	11	3.68		
Replication	1	0.18	0.18	0.72NS
Color(C)	2	0.38	0.19	0.76NS
Error(a)	2	0.49	0.25	
Part(P)	1	2.27	2.27	45.4***
C×P	2	0.21	0.11	2.2NS
Error(b)	3	0.15	0.05	

***: Significant at p=0.001, NS: Nonsignificant.
Color: Percentage of dark purple area of stem.
Part: Central(Xylem-Pith) part and Epidermis-cortex.

little higher in this case comparing with 0.70, 0.74 and 0.33 in main body of fresh, white and red ginseng, respectively[12]. In physiological efficacy PT was known as stimulative while PD sedative and thus unity of PT/PD was suggested as ideal[2]. *Panax quinquefolius*(American sheng) was known to have higher PT/PD than Korean ginseng[2]. However data on PT/PD seems not enough for comparison. PT/PD was lower in lateral root[12] and fiber root(unpublished data of authers) because of higher ratio of epidermis-cortex. From the above results ginsenoside composition of main body could be schematically shown as Fig. 2. Total ginsenoside content and PT/PD for white or red ginseng will depend not only on ginsenoside content and composition but also on the weight ratio of main body and lateral root because fine roots are eliminated. Ginsenoside content in lateral roots was about twice that of main body[12] but weight ratio of lateral root to main body was not known. The contribution rate of each part such as central, epidermis, cortex, lateral and fiber root for the weight, total ginsenosides, and PT/PD of whole root should be further studied in relation to species, variety, strain and environmental factors.

Abstract

Ginsenosides in epidermis-cortex(EC) and xylem-pith(XP) of main body of *Panax ginseng* (var. atropurpureacaulo) root were investigated in relation to dark purple area on stem. Pattern of ginsenosides, ratio of protopanaxatriol(PT) to diol(PD) and total ginsenoside content were

significantly different between EC and XP, and not related with stem color. The increasing trend of total ginsenosides with decreasing in purple area on stem needs to be tested with greater sample size. The order of ginsenoside content was $Rb_1>Rg_1>Re>Rc>Rg_2>Rb_2>Rf>Rd$ for EC, $Rg_1>Rb_1>Rg_2>Re>Rb_2>Rc>Rf>Rd$ for XP. PT/PD was 1.08 for EC, 1.95 for XP. Since total ginsenoside content was 3 times higher in EC than in XP and weight of two parts was almost same, the content of ginsenosides of main body mostly depends on those of EC.

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