



ORYZA OIL & FAT CHEMICAL CO., LTD.

Anti-aging, Whitening Effect of Broccoli sprout extract

Broccoli Sprout Extract

**Three Whitening Effects
by Anti-glycation, Anti-carbonylation
and Anti-melanogenesis**



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ver. 1.0MK

1. Components of Broccoli sprout extract

Broccoli has been already known to contain a lot of glucosinolates. We conducted isolation of glucosinolates and the other compounds from broccoli seeds. As a result, we identified following compounds (Fig. 1). A major compound is glucoraphanin which is degraded to sulforaphane (Fig. 2) by myrosinase existing saliva and intestinal bacteria. We standardize sulforaphane content in broccoli sprout extract.

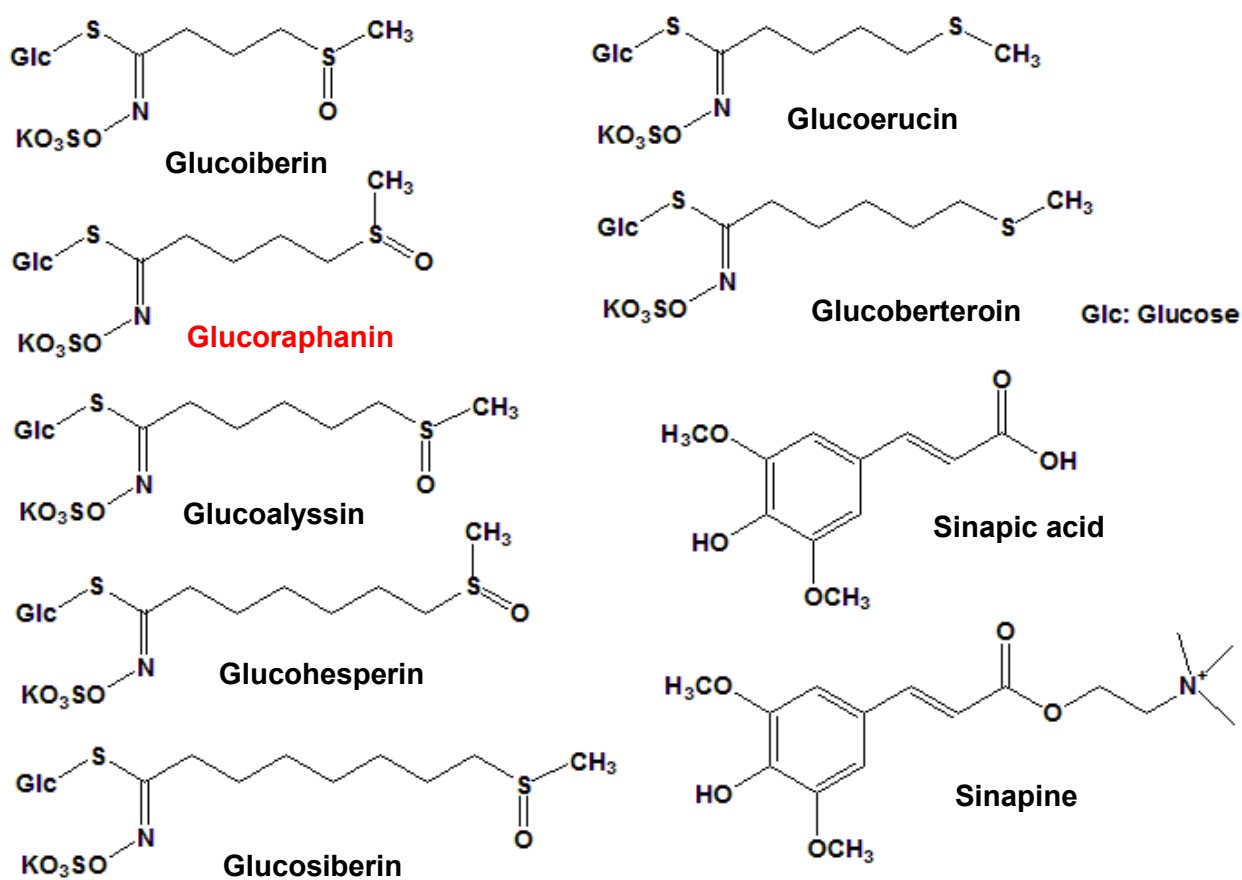


Fig. 1 Isolated compounds from broccoli seeds

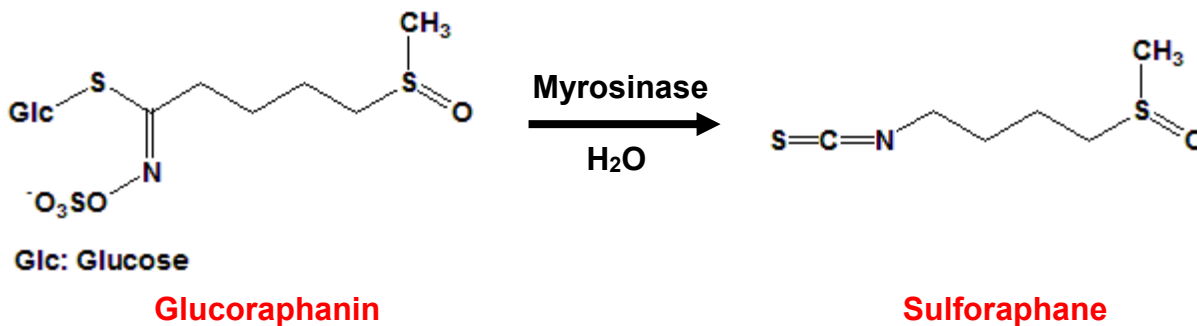


Fig. 2 Production mechanism of sulforaphane from glucoraphanin

2. Anti-glycation effect

2-1. Formation of advanced glycation end products (AGEs)

“Glycation (Maillard reaction)” is a reaction of sugar and amino acid or protein including repeated binding, polymerization and degradation and finally produces melanoidins, browning substances. Maillard reaction also occurs in our body, which leads to the formation of AGEs in collagen and other protein tissues. Formation of AGEs has been shown to contribute to the progression of age-related diseases and diabetes. Glycation of collagen and elastin in skin causes accumulation of AGEs (Fig. 3), which results in intracellular damage and apoptosis in skin cells. So, accumulation of AGEs by aging means deterioration of skin tension and elasticity.

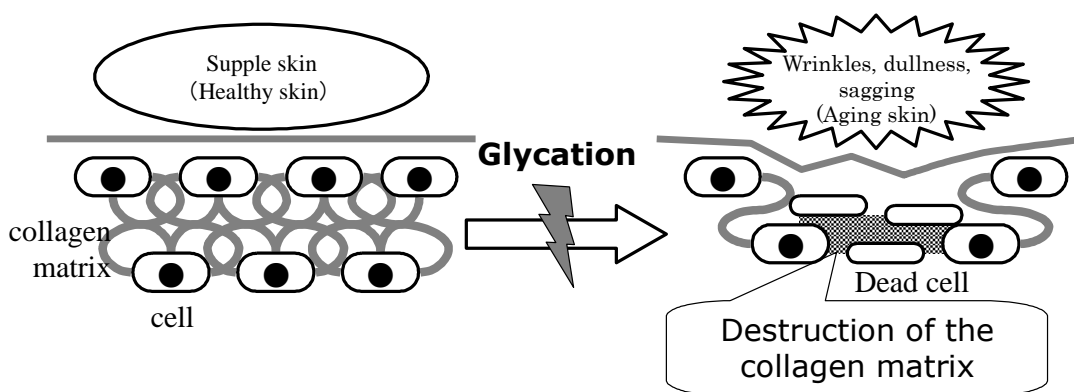


Fig. 3 Skin aging by glycation

2-2. Anti-glycation effect of broccoli sprout extract

After the addition of broccoli sprout extract or sulforaphane to solution of BSA and fructose, the solution was incubated at 37°C. Then we measured fluorescent AGEs in the solution. As a result, broccoli sprout extract and sulforaphane inhibited formation of AGEs (Fig. 4).

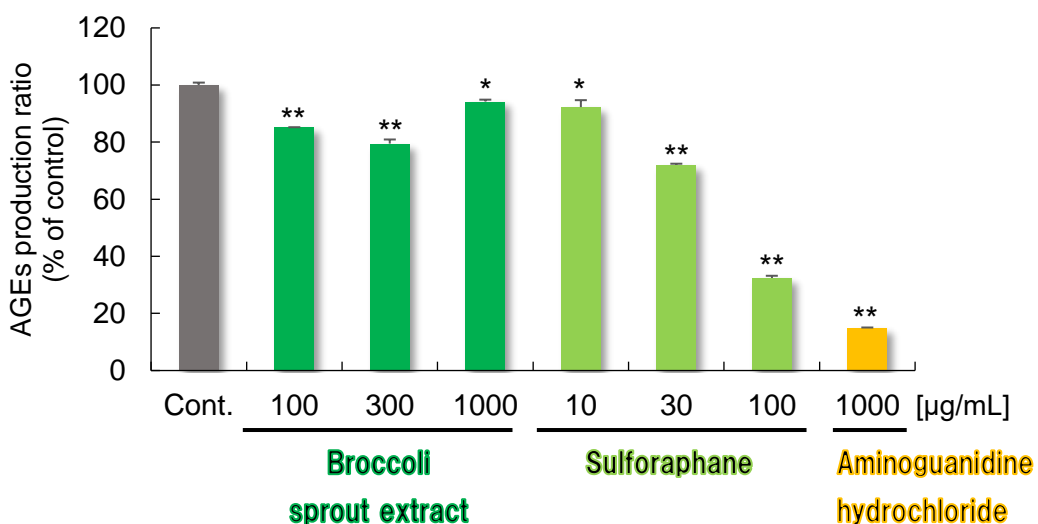


Fig. 4 Anti-glycation effect of broccoli sprout extract and sulforaphane

Mean ± SE (n=3), **: $p < 0.01$, *: $p < 0.05$

3. Anti-carbonyl effect

3-1 Formation of carbonylated proteins

Recently protein carbonylation in skin was shown to cause “yellowish skin discoloration”. “Yellowish skin discoloration” is caused by carbonyl protein formed by the reaction of lipid peroxides and protein[※]).

※) Ogura Y et al., *J Dermatol Sci*, 2011, 64: 45-52.

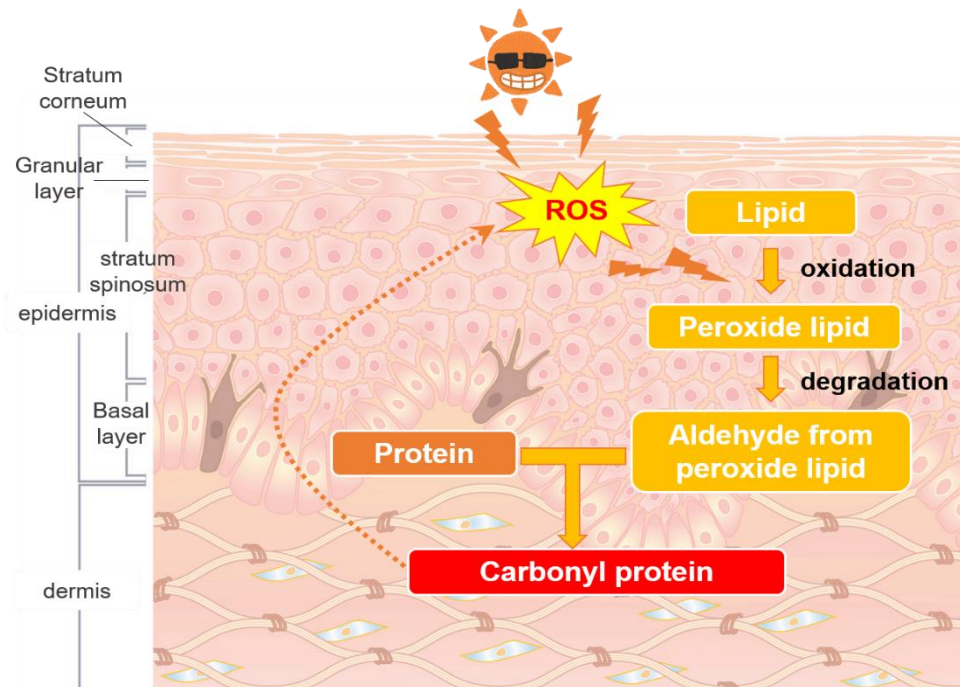


Fig. 5 Formation of carbonylated protein

With the accumulation of carbonyl protein, skin color becomes yellowish and dehydration progresses. As carbonyl protein enhances production of reactive oxygen species (ROS), carbonyl protein causes negative spiral and leads aging skin.

Thus, it is very important to stop the negative spiral. Therefore, we investigated the effect of broccoli sprout extract and the active ingredient on protein carbonylation.



Normal skin



Yellowish skin discoloration

3-2 Anti-carbonyl effect of broccoli sprout extract

After the addition of broccoli sprout extract to the solution of BSA and oxidated linoleic acid, the mixture was reacted for 15 hours at 37°C. Then we determined carbonyl protein by 2, 4-DNP method. As a result, broccoli sprout extract (100 and 1000 µg/mL) as well as aminoguanidine hydrochloride (positive control) inhibited carbonyl protein production. (Fig. 6)

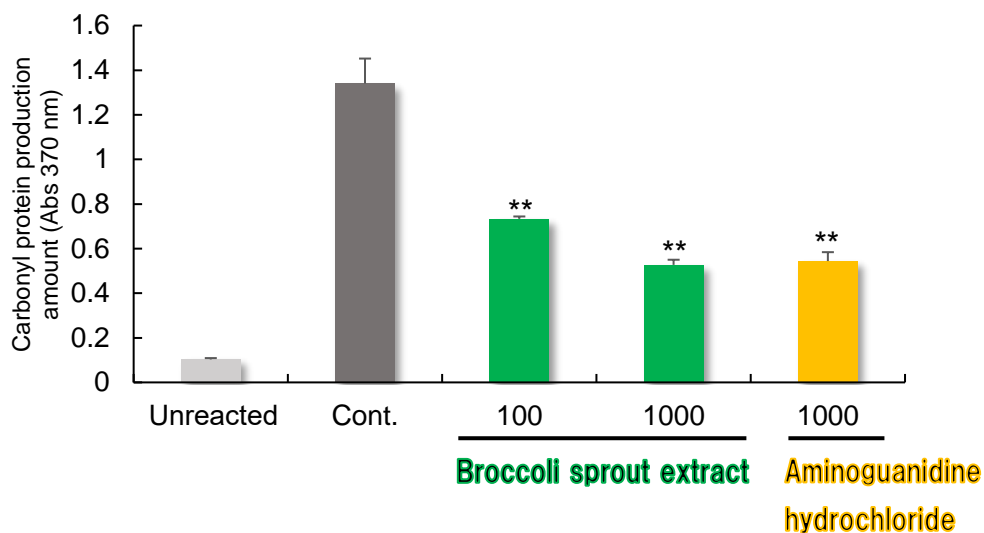


Fig 6. Anti-carbonylation effect of broccoli sprout extract

Mean ± SE (n=4), **: $p < 0.01$

3-3. Anti-carbonyl effect of components in broccoli sprout extract

Next, we examined the effect of the ingredients in broccoli sprout extract on carbonyl protein production. As a result, glucosiberin, glucoerucin and glucoberteroin potently suppressed protein carbonylation. (Table 1)

Table 1. Inhibition ratio of carbonyl protein (CP) production by each active ingredients

Number	Ingredients	Carbone number of the side chain	Inhibition ratio of CP production at each concentration [$\mu\text{g/mL}$] (%)		
			0	100	1000
1	Glucosiberin	3	0.00 \pm 0.02	-1.1 \pm 0.0	33.0 \pm 1.0**
2	Glucoraphanin	4	0.00 \pm 0.04	17.2 \pm 1.0	31.7 \pm 1.8
3	Glucosylsin	5	0.00 \pm 0.02	23.1 \pm 0.5	41.1 \pm 2.4**
4	Glucosesperin	6	0.00 \pm 0.03	12.1 \pm 0.1	34.2 \pm 1.6**
5	Glucosiberin	7	0.00 \pm 0.01	25.0 \pm 0.9**	66.0 \pm 1.2**
6	Glucoerucin	4	0.00 \pm 0.03		79.7 \pm 0.6**
7	Glucoberteroin	5	0.00 \pm 0.04	36.9 \pm 1.3**	103.0 \pm 3.0*
8	Sinapic acid		0.00 \pm 0.01	1.4 \pm 0.1	42.0 \pm 0.9
9	Sinapin		0.00 \pm 0.04	-88.0 \pm 2.2**	-108.0 \pm 2.3**
10	Sulforaphane		0.00 \pm 0.04	11.6 \pm 0.2	35.8 \pm 1.1

Mean \pm SD, Significantly different from the group not treated with sample (0 $\mu\text{g/mL}$) by Dunnett's test (*: $p < 0.05$, **: $p < 0.01$)

4. Whitening

4-1. Inhibition of tyrosinase

Melanin is a major cause of dullness and pigmentation of skin. It is produced by tyrosinase which is a key enzyme in melanogenesis. Thus, melanin production is suppressed by inhibiting tyrosinase activity.

We studied inhibitory effect of broccoli sprout extract on tyrosinase activity. As a result, broccoli sprout extract inhibited tyrosinase in a concentration-dependent manner.

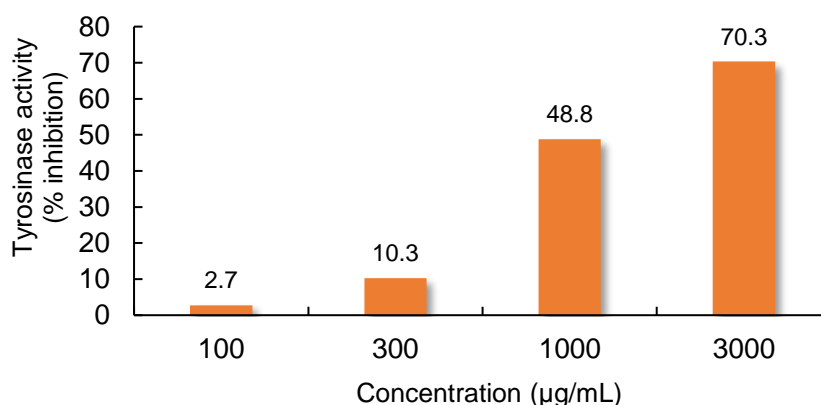


Fig. 7 Inhibition of tyrosinase by broccoli sprout extract

4-2. Inhibition of melanin

Further experiment was prompted using B16 melanoma cells to evaluate the skin lightening effect of broccoli sprout extract. As shown in Fig. 8, broccoli sprout extract demonstrated a dose-dependent inhibitory effect against melanin production in B16 melanoma cells. Therefore, broccoli sprout extract is preventive against skin hyperpigmentation.

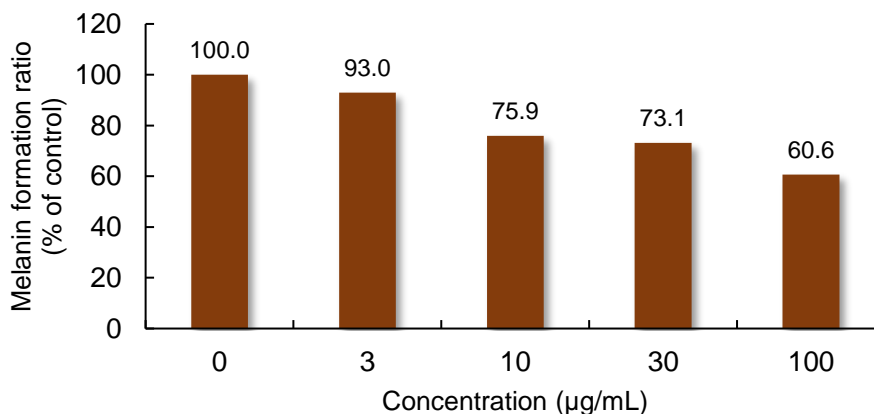


Fig. 8 Inhibition of melanin formation by broccoli sprout extract

4-3. Clinical test

Prior to the study, we measured the color inside of upper arm in volunteers (5 subjects) using spectrophotometer (Nippon Denshoku Industries CO., LTD.). After that, the volunteers took broccoli sprout extract (1 g) after each meal (3 times in total / day) for 1 month. On the next day of final intake, we measured the color including brightness (L* value), hue [reddish (a* value) and yellowish (b* value)]. The result showed increase in brightness and decrease in reddish and yellowish in more than half subjects (Table 2). Therefore, whitening effect of broccoli sprout extract was proved.

Table 2. Change in skin color before and after intake of broccoli sprout extract

Subject		Brightness (L* value)	Reddish (a* value)	Yellowish (b* value)
A	Before	60.39	12.52	21.84
	After	54.06	16.73	24.64
	Change	-6.33	4.21	2.8
B	Before	58.51	11.39	24.29
	After	60.42	10.45	22.5
	Change	1.91	-0.94	-1.79
C	Before	63.54	9.69	18.18
	After	69.11	7.3	17.9
	Change	5.57	-2.39	-0.28
D	Before	64.16	10.25	23.5
	After	69.65	4.96	22
	Change	5.49	-5.29	-1.5
E	Before	63.59	12.47	21.55
	After	66.25	10.51	23.88
	Change	2.66	-1.96	2.33