

## MARUKYOU BIO FOODS Co.

# Melanin Production Inhibitor Using Chondroitin Sulfate Oligosaccharides

Patent pending



### Inhibits melanin production

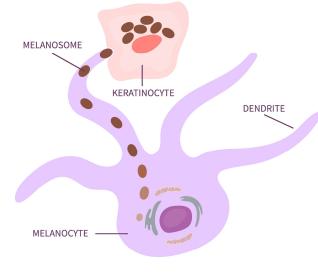
Melanin-producing cells (melanocytes) increase the production of tyrosinase and related proteins when exposed to ultraviolet radiation, activating melanin synthesis from tyrosine.

The synthesized melanin is transported outside the cell, forming a protective melanin cap around the cell nucleus within skin cells.

However, in modern society, there are times when it is desired to suppress melanin production and maintain fair

skin from an aesthetic perspective.

<u>Chondroitin sulfate oligosaccharides</u> <u>are expected to help maintain fair and</u>





healthy skin through a combination of effects, including suppression of melanin production, antioxidant and anti-inflammatory effects via Nrf2 activation, and moisturizing functions.

#### 1. Cytotoxicity Evaluation

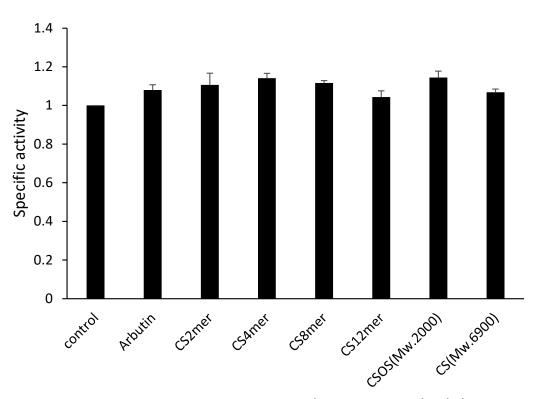
The test substance was added to B16F10 melanoma cells and cultured, and cytotoxicity was evaluated using WST8.

Proliferation activity ratio in each test substance-added culture relative to control set at 1

Item	Average (n=3)	Standard deviation
Control	1.00	0.000
Arbutin	1.08	0.047
CS2mer	1.11	0.106
CS4mer	1.14	0.044
CS8mer	1.12	0.022
CS12mer	1.04	0.057
CSOS(Mw.2000)	1.14	0.058
CS(Mw.6900)	1.07	0.029

<sup>•</sup> The test substance was added at a concentration of 1 mM.

<sup>•</sup>The test was performed three times, and the average was evaluated.



Error bars are standard deviations (SD).

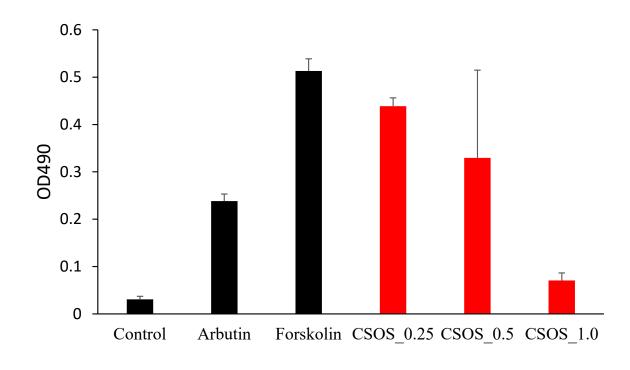
Chondroitin sulfate oligosaccharides did not inhibit cell proliferation and showed no cytotoxicity.

#### 2. Evaluation of Melanin Production Suppression in Melanoma Cells (1)

The melanin production inhibitory effect was evaluated by culturing melanoma cells (B16F10) with the test substance.

Forskolin was added as a melanin production inducer, and the test substance was added. The blackness of the culture medium was measured over time at OD490.

Arbutin was used as a comparative control.



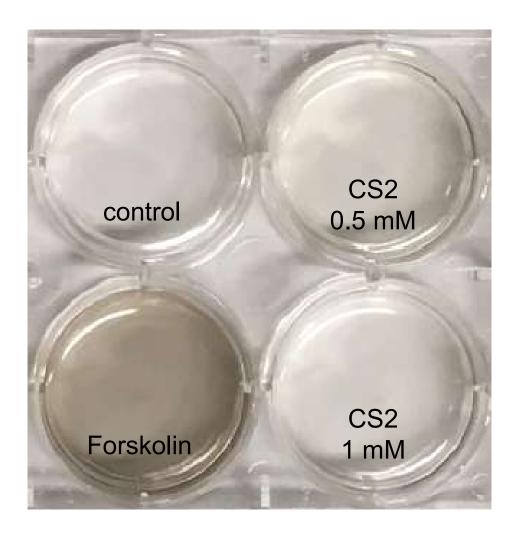
Melanin Production Inhibition Test in Melanoma Cells (B16F10)

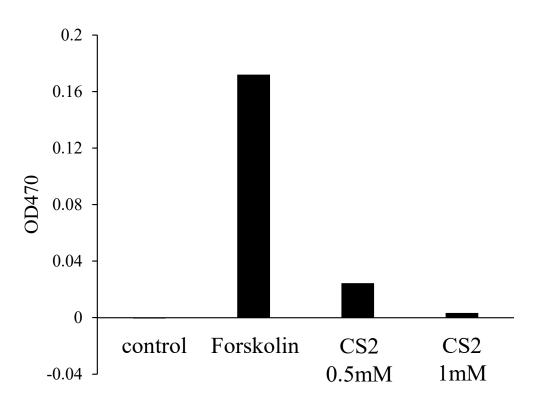
The number at the end of CSOS denotes the final concentration (mM).

Error bars are standard deviations (SD)

The graph shows results 11 hours after the onset of darkening in the Forskolin-added group. While darkening of the medium progressed in the Forskolin-only group, it was suppressed in the CSOS (chondroitin sulfate oligosaccharide)-co-added group. CSOS suppressed melanin production in a concentration-dependent manner, with a strong inhibitory effect observed in the 1 mM-added group.

#### 3. Evaluation of Melanin Production Suppression in Melanoma Cells (2)





**Melanin Production Inhibition Test in Melanoma Cells** (B16F10)

The photograph shows the appearance of melanoma cells (B16F10) cultured with the test substance on day 5. The graph shows the results of measuring the blackness of the culture medium at OD470. Chondroitin sulfate oligosaccharide (disaccharide) strongly inhibited melanin production induced by Forskolin.

# Conclusion

Chondroitin sulfate oligosaccharides safely suppress melanin production

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