

Unpublished data
for reference No.21

Modification of Neohesperidin Dihydrochalcone(NHDC)
by *Bacillus stearothermophilus* Maltogenic Amylase

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ABSTRACT

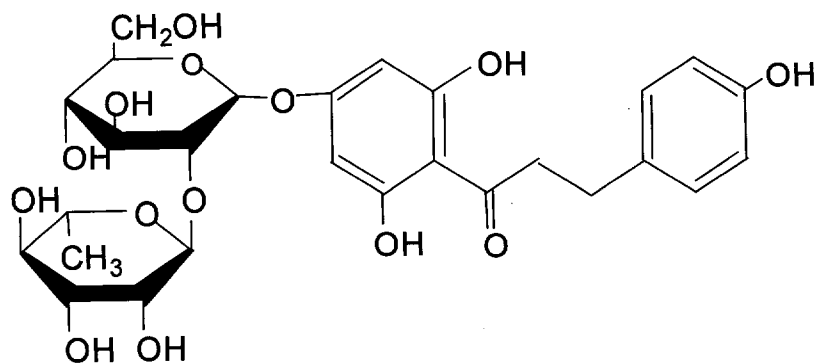
Neohesperidin Dihydrochalcone (NHDC), a sweet compound in grape fruits, was modified to a series of its oligoglycosides by transglycosylation activity of maltogenic amylase from *Bacillus stearothermophilus* (BSMA) with NHDC as an acceptor and maltose as a donor for increasing solubility of NHDC.

Among the several transfer products, maltosyl-NHDC was major transglycosylation product, which was determined by Thin Layer Chromatography (TLC) analysis. Maltosyl-NHDC was purified using sephadex LH-20 lipophylic gel filtration chromatography and TLC. The structure of the purified compound was determined to be maltosyl- α -(1,6)-neohesperidin dihydrochalcone by MALDI-TOF/MS and ^1H - and ^{13}C -NMR. And maltosyl-NHDC showed about 700 times higher solubility and about 7 times less sweetness than that of NHDC.

OBJECTIVES

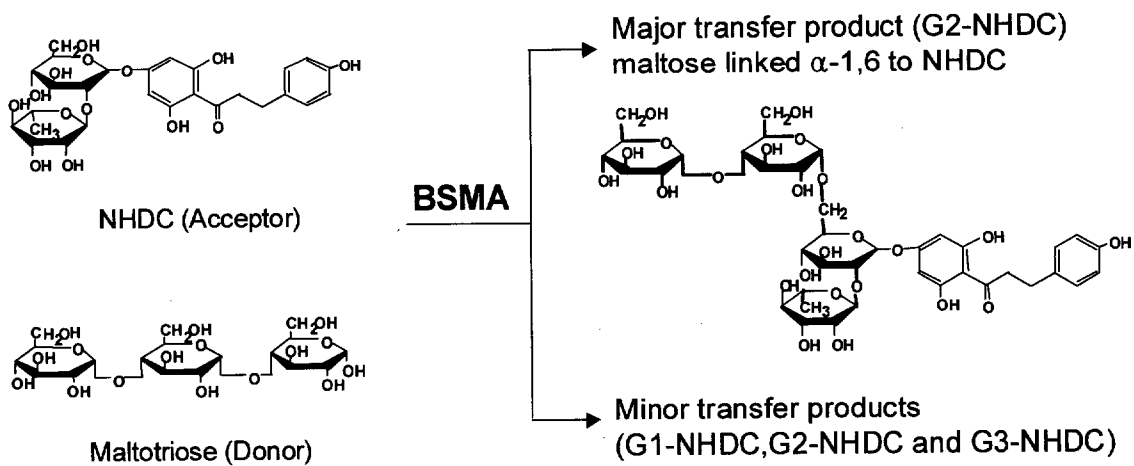
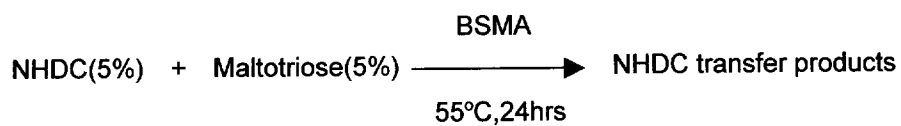
- Modification of Neohesperidin Dihydrochalcone by transglycosylation reaction of maltogenic amylase (BSMA).
- Improvement of physicochemical properties of neohesperidin Dihydrochalcone; increase in solubility.

Neohesperidin Dihydrochalcone(NHDC)

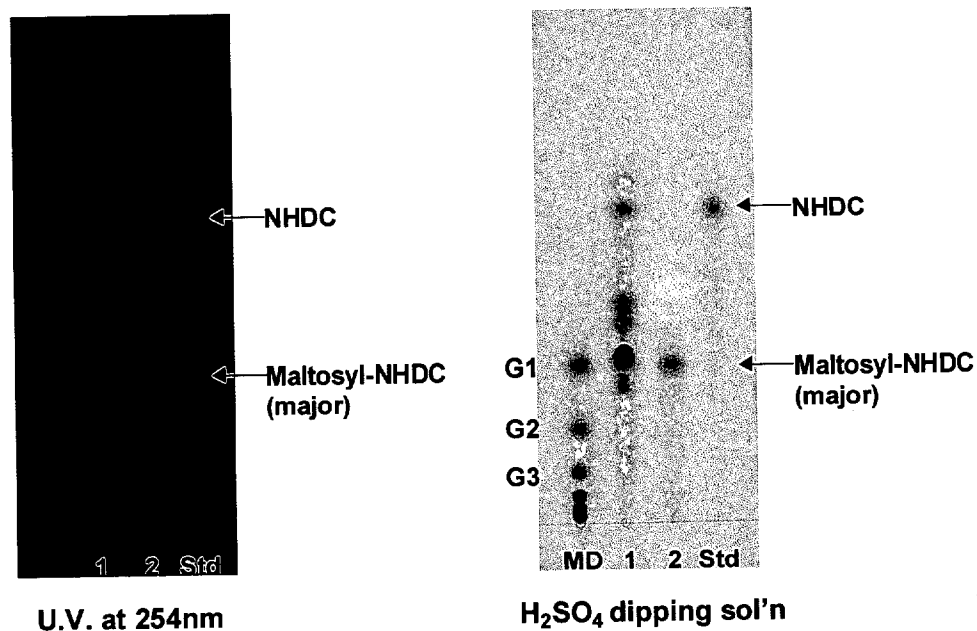


- (1-[4-[[2-O-(6-deoxy- α -L-mannopyranosyl- β -D-glucopyranosyl]oxy]-2,6-dihydroxyphenyl]-3-(3-hydroxy-4-methoxyphenyl)-1-propanone)
- A kind of flavonoid compound in grape fruits
- 1000 to 1500 times sweeter than sucrose
- 2 times sweeter than saccharin
- Low solubility
- Bitterness aftertaste
- Artificial sweetner : chewing gum, dentifrice etc.

Transglycosylation of NHDC by BSMA



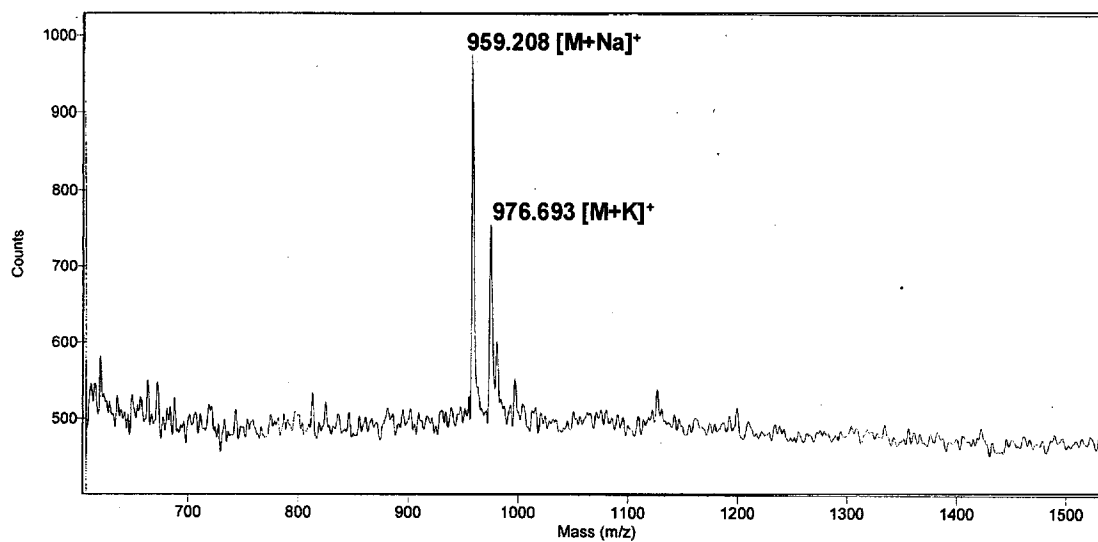
TLC analysis of transfer products



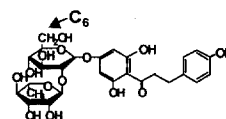
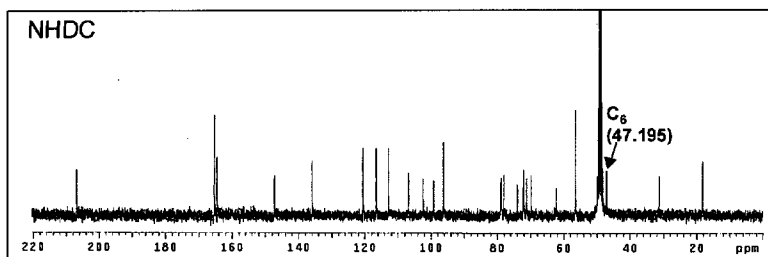
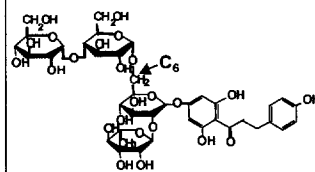
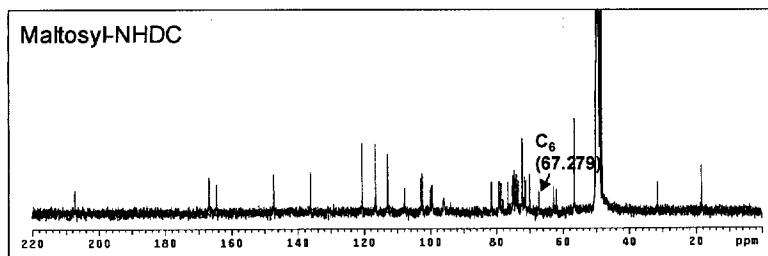
Lane MD : Maltodextrin standard, Lane 1: Transfer products

Lane 2 : Purified maltosyl-NHDC, Lane Std : NHDC

**Molecular weight of purified maltosyl-NHDC
using MALDI-TOF MS**



¹³C-NMR spectra of NHDC and maltosyl-NHDC



Solubility of NHDC and 6-G2-NHDC

Compound	Solubility	Relative solubility
NHDC	1.36mM	1
6-G2-NHDC	977mM	718.4

**Threshold value and relative sweetness
of NHDC and 6-G2-NHDC**

Compound	Taste	Threshold value	Relative solubility
NHDC	sweet	2.04×10^{-3}	100
6-G2-NHDC	less sweet	1.5×10^{-2}	14

CONCLUSIONS

- Several transfer products of NHDC were produced by transglycosylation activity of BSMA.
- Major transfer product was identified as a maltosyl-NHDC by TLC and MALDI-TOF/MS.
- The linkage of maltose to NHDC in the maltosyl-NHDC was determined to be α -1,6 by ^1H - and ^{13}C - NMR.
- Maltosyl-NHDC showed about 700 times higher solubility and about 7 times less sweetness than that of NHDC.

REFERENCES

Horowitz, R. M. and Gentili, B. Dihydrochalcone derivatives and their use as sweetening agents. *J. Agr. Food. Chem.* **17**(4):696-700. 1963

Inglett, G. E., Krbechek, L., Dowling, B. and Wagner, R. Dihydrochalcone sweeteners-sensory and stability evaluation. *J. Food Sci.* **34**:101-103. 1969

Sachiko, E., Kiyotoshi, N., Naoko, S., Ryuta, N., Yoshihiro, T. and Shintaro, K. Preparation and taste of certain glycosides of flavanones and of dihydrochalcone. *Biosci. Biotech. Biochem.* **58**(8):1479-1485. 1994

Kometani, T., Nishimura, T., Nakae, T. and Okada, S. Synthesis of neohesperidin glycosides and naringin glycosides by cyclodextrin glucanotransferase from an alkalophilic *Bacillus* species. *Biosci. Biotech. Biochem.* **60**:645-649. 1996

Lee, S. J. Modification of naringin to naringin glycoside by transglycosylation of maltogenic amylase from *Bacillus stearothermophilus*(BSMA). Master Thesis. Seoul Nat'l Univ. 1999