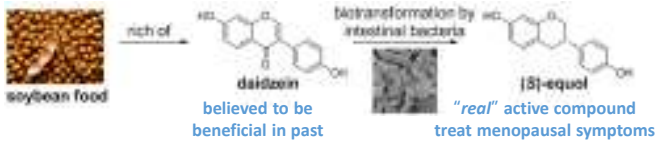


### 1. Introduction

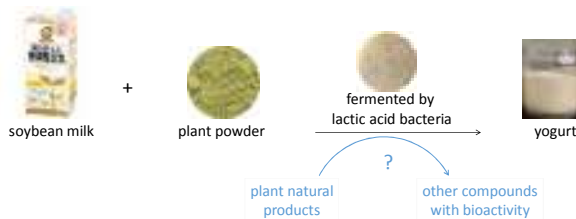
Many natural products are used as drugs. After taken orally, some of natural products may be transformed by intestinal flora into other structures, which have "real" activity. For example, daidzein and (S)-equol:



However, not all people have the ability of transformation according to their different intestinal flora. If this transformation can be accomplished outside human body, the "real" activity can be used by everyone.

### 2. Biotransformation

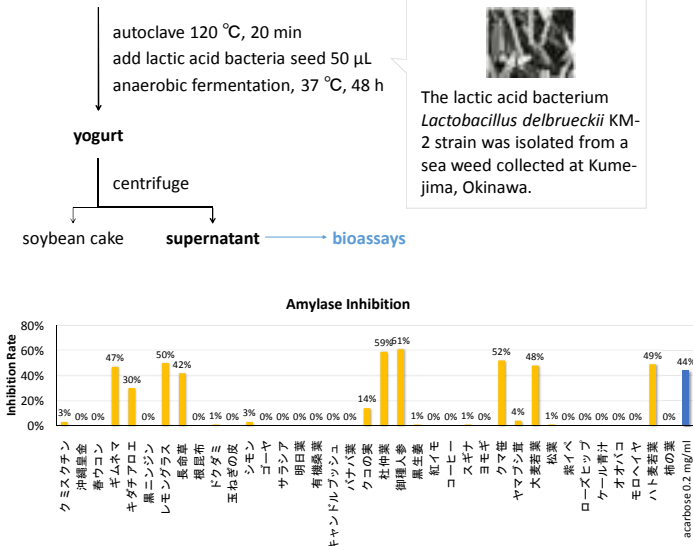
Many medicinal and edible herbs may be convert to other bioactive compounds by intestinal flora after taken. To apply this biotransformation widely and efficiently, a fermentation method was designed:



Investigate this conversion from inactive plant natural products into bioactive compounds by fermentation.

### 3. Initial screening results

soybean milk (100 mL) + plant powder (0.3 g)



\*Water extract of these powders, plane soybean milk yogurt showed no bioactivity in the bioassays.

#### α-amylase Inhibition

Amylase and maltase are the enzymes that catalyze the hydrolysis of starch into glucose. Inhibition of amylase is considered as a strategy for the treatment of disorders in carbohydrate uptake, such as diabetes and obesity.

Other enzymes tested: tyrosinase, xanthine oxidase, hyaluronidase

### 6. Conclusion

- Lactic acid and succinic acid were isolated as an amylase inhibitor from the culture liquid of sterilized Sasa powder, KM-2 and soybean milk.
- α-Glucosidase inhibition was observed in the culture liquid of non-sterilized plat powder, KM-2 and soybean milk.
- Unidentified microbes may be involved in the transformation of soybean isoflavones which are likely responsible for bioactivity.

### 4. Identification of active compounds

soybean milk (1 L) + Sasa powder (3 g)

autoclave 120 °C, 20 min  
add KM-2 seed 500 μl  
fermentation 37 °C, 72 h  
centrifuge

supernatant (600 mL)

freeze drying

yellow solid (14 g)

dissolved in 200ml water  
add in 800ml EtOH  
centrifuge

sediment

supernatant (12 g)

extraction with MeCN (800 mL) / water (200 mL)

H<sub>2</sub>O layer

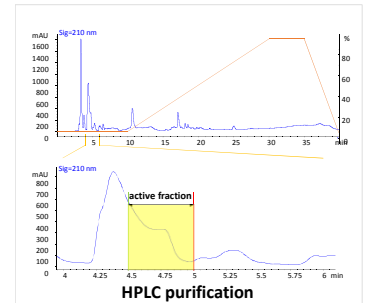
MeCN layer (4.8 g)

Sephadex G25 gel chromatography  
Φ4.2 x 20 cm, water elution  
20 ml x 30 fractions

active fractions (fr. 9-12, 4.0 g)

semi-preparation HPLC  
Cosmosil PBr column

Lactic acid and succinic acid



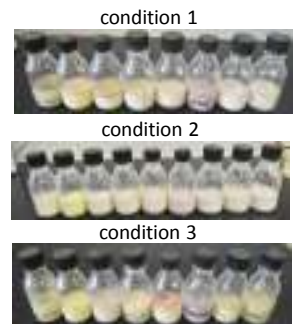
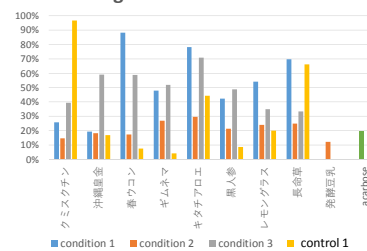
### 5. Reinvestigation : what is the truth?

condition	plant powder	autoclave	KM-2	medium
1	○	-	○	soybean milk
2	○	○	○	soybean milk
3	○	-	-	soybean milk
control 1	○	-	-	water
control 2	-	-	○	soybean milk

Ferment at 37°C for 48 hours.

After centrifuge, the supernatant was filtered and collected for testing.

#### α-glucosidase inhibition



#### HPLC Analysis (254 nm): wild turmeric

