

Analysis of green immature soybeans for their content in sucrose and glutamate

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Introduction

Food soybeans can be used to make soymilk and tofu but also consumed as a vegetable when they are harvested in their immature state; these green soybeans are also known as Edamame, from their name in Japanese. The grain quality group at AAFC works closely with the soybean breeder to analyze their genetic lines and provide him with screening tools to select the most appropriate lines for each end-use. Edamame soybeans will be steamed before consumption. The green soybeans must taste delicious on their own and have within them components that contribute to the taste as humans perceive it. Besides the oil and protein content which are analysed routinely for all food soybeans, two components of the grain that will influence taste are the amount of sugar, as well as the content in free glutamate, the amino acid known to bind to our "savoury" taste receptor.

Megazyme International, a company based in Ireland, supplies diagnostic technology for measuring components in a variety of food matrices and its technology was chosen for this project. Breeding for edamame has just started at AAFC /Ottawa so it was the first time that green immature soybeans were analyzed for these components.

Material method

Three different soybeans were analyzed : They were all evaluated as green soybeans and as mature dry soybeans. Enzyme-based protocols from Megazyme were used to measure free sucrose and free glutamate. Their content is obtained using a colorimetric measurement of absorbance with a spectrophotometer at a specific wavelength for the reaction product and appropriate calculations based on the absorbance of a standard.

L-Glutamic acid is oxidized by nicotinamide-adenine dinucleotide in the presence of glutamate dehydrogenase, leading to the formation of 2-oxoglutarate, reduced nicotinamide-adenine dinucleotide (NADH) and ammonium ions. However, as the equilibrium of this deamination reaction lies markedly in favor of the reactants, a further reaction catalyzed by diaphorase is required, in which NADH reduces iodinitrotetrazolium chloride to an INT-formazan product. The amount of INT-formazan formed in this reaction is stoichiometric with the amount of L-glutamic acid. It is the INT-formazan's absorbance that is measured by a spectrophotometer at 492 nm.

This protocol is specific to L-glutamic acid.

Free sucrose is hydrolyzed to glucose and fructose by the enzyme invertase. Glucose is then measured with a reagent (GOPOD reagent) made up of glucose oxidase with a peroxidase paired with 4-aminoantipyrine which will allow a red color to develop in the solution as the enzyme reactions proceed in the presence of glucose. Absorbance is measured at 510 nm. Free glucose is subtracted by incubating a duplicate sample with the same GOPOD reagent only without the invertase step.

Results and discussion

An oilseed type was included to measure the sucrose and glutamate present in commercial soybeans, already known to not be adequate as Edamame soybeans. The Japanese variety is our objective in terms of taste. We can see in table 1 and as expected, the oilseed variety is under, for both sucrose and glutamate, the Japanese Edamame. It is also clear that for both traits, the genetic line does not have enough of both sucrose and glutamate since its contents are even below oilseed soybean. The difference in sucrose between the three soybeans was maintained in the dry mature soybeans increasing by approximately 1% for all of them.

Table 1: Comparison of the content (%dw) in sucrose and glutamate of three green soybeans : a Japanese Edamame variety , an oilseed soybean and a genetic line from the AAFC Edamame breeding program

| Variety | Glutamate (±0.05) | Sucrose (±0.3) |
|--------------|----------------------|-------------------|
| Edamame | 0.46 | 6.3 |
| Oilseed | 0.43 | 5.3 |
| Genetic line | 0.36 | 4.9 |

Conclusion

The objectives of the project were achieved: we established the Megazyme methods for green soybeans and determined the sucrose and glutamate content were for the Edamame AAFC genetic line in relation to Japanese Edamame. The breeding program has the screening tools it needs and the breeder is also knows that he needs to improve his genetic line for both traits as the content are below to the Japanese Edamame soybean.



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