Sensory Analysis of Calcium-biofortified Lettuce

Sunghun Park and Mark P. Elless

Although vegetables provide some calcium, the dietary quantities of vegetables, fortified foods, and supplements required to realistically replace the calcium contained in dairy products are impractical. In the future, however, vegetables may be able to provide increased calcium in the diet, which could lead to improved bone health. We report here the generation and sensory qualities of novel varieties of lettuce containing enhanced calcium levels.

The majority of Americans do not consume enough calcium to reduce the risk of osteoporosis. Osteoporosis, a condition of reduced bone density, is an underlying cause of bone fragility and a major health concern in the United States. Ample dietary calcium may help alleviate this disease. Consumption of adequate dietary calcium can be accomplished through a variety of diets. Diets rich in dairy products are one straightforward approach; however, intake of carbonated soft drinks now exceeds intake of fluid milk among the majority of Americans. Furthermore, some individuals and ethnic groups limit their consumption of dairy products due to lactose intolerance. After dairy products, vegetables and fruits make the next largest contribution to calcium intake and have the potential to be a significant source of dietary calcium. However, widely consumed fruits and vegetables contain little available calcium and therefore provide only minor contributions to the total calcium intake[1].

The protein sCAX1 can pump calcium into plant cells. One way to alter the calcium content in fruits and vegetables is to directly engineer these foods with calcium transporters. Simplistically, this strategy can be thought of as nutrient mining (biofortification)—where the nutrient is transported from soil into the edible portions of the plant. Specifically, a potential model for increasing the calcium content in edible foods would be to engineer plant endomembrane transporters to transport more calcium. The Ca^{2+}/H^{+} antiporters, termed CAX (for cation exchangers), located on the vacuolar membrane, are important for calcium sequestration. This discovery led to an engineered version of a plant Ca^{2+}/H^{+} antiporter, sCAX1, which could be used for calcium biofortification.

A new generation of calcium-biofortified lettuce is possible. Lettuce is an attractive choice for calcium biofortification. It is rich in vitamin K, and its daily consumption significantly reduces the risk of hip fractures in women compared to women who consumed lettuce at a lower rate[2]. Therefore, the enhancement of calcium in lettuce could provide synergistic benefits to osteoporosis prevention. A large portion of women in the United States eat lettuce daily, suggesting calcium-biofortified lettuce for the prevention of osteoporosis would be consumed by a large target audience.

We have genetically engineered several lettuce varieties to express increased levels of sCAX1. The resulting biofortified lettuce plants contain 25 – 32% more calcium in the edible portion than control lettuce, are fertile, and demonstrate robust growth under greenhouse conditions. In a previous study, genetically engineered carrots expressing high levels of s*CAX1* accumulated almost two-fold more calcium in the edible root compared to control plants, without perturbing growth, development, or fertility, under controlled conditions. In comparison, feeding trials using radiolabeled, biofortified carrots demonstrated that the total amount of calcium absorbed is significantly increased in both mice and humans[3]; however, the taste and tactile components of the carrots need to be measured.

A key component in consumer acceptance requires sensory analysis of genetically engineered high-calcium lettuce. While biofortification efforts attempt to enhance the nutritional quality of numerous foods, few studies have assessed how genetically engineered foods alter the taste qualities and consumer perceptions of these foods. Calcium in the form of a water-soluble salt or complex can be added to food and/or beverages, but may cause a bitter taste[4]. Therefore, it is important to evaluate how genetically engineered high-calcium plants taste.

Sensory analysis applies principles of experimental design and statistical analysis to the use of human senses for product evaluation. This discipline requires recruiting panels of trained human assessors on whom the products are tested and recording their responses. By applying sensory analysis to genetically engineered foods, we can begin to determine if these products are commercially viable from a taste and appearance standpoint.

In this study, we demonstrated increased calcium levels in the edible portion of s*CAX1*-expressing lettuce with no negative impact on lettuce yields. Using a panel of five highly trained descriptive panelists, we also evaluated the flavor, bitterness, and crispness of the biofortified lettuce (**Fig. 1**). No significant differences were observed between the two groups (control or biofortified), indicating that no differences in flavor, bitterness, or crispness were detected between the s*CAX1*-expressing biofortified lettuce and the controls. Therefore, consumer acceptance of the s*CAX1*-expressing lettuce should not be affected based on a change in flavor, bitterness, or crispness.

Additional Benefits and Future Work

In addition to nutritional benefits, the use of genetic engineering to increase calcium levels could improve lettuce productivity and extend product shelf life. Calcium is associated with maintaining the cell wall structure of vegetables by interacting with pectin to form calcium pectate and is reported to maintain firmness by cross-linking with non-esterified pectins in the primary cell wall and middle lamella. Thus, fruit and vegetables treated with calcium are generally firmer than controls during storage due to increased calcium levels in the hypodermal mesocarp tissue.

Calcium has long been used as a firming agent for produce such as cantaloupes, strawberries, and carrots to combat many post-harvest issues. Apples are also immersed in a calcium solution to maintain firmness in shipping and prolong shelf life. Recently *sCAX1* expression was shown to increase calcium levels in tomatoes, increase fruit firmness, and prolong shelf life[4]. Tests for assessing shelf life qualities of s*CAX1*-expressing lettuce are also in progress.

Conclusions

We established unequivocally that modifying a single plant calcium transporter improves calcium content without negatively impacting lettuce quality. While this work focused on the commercialization of s*CAX1*-expressing lettuce, this biofortification approach should be applicable to numerous other crops.

References

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Sunghun Park
Department of Horticulture, Forestry & Recreation Resources
Kansas State University
Manhattan, KS 66506, USA
shpark@ksu.edu

Mark P. Elless
Edenspace Systems Corporation
3810 Concorde Parkway
Chantilly, VA 20151, USA
elless@edenspace.com