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Comparison of Nutrients Contents in Genetically Modified Herbicide-tolerant Dried Red Pepper and Its Parental Cultivars

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ABSTRACT

This study performed compositional analyses of a genetically modified herbicide-tolerant red pepper (GMHT), developed by the Rural Development Administration, and a parental red pepper cultivar 'Subicho', and compared the nutrient composition of them. Using the Association of Official Analytical Chemists (AOAC) methods, the study measured the concentration of nutrients, including the proximate components (protein, fat, fiber, ash, and carbohydrates) and minerals (Ca, K, Na, Fe, Mg, Zn) of GMHT and 'Subicho'. Nutritional composition of GMHT and 'Subicho' were compared with the nutritional composition of conventional red peppers. The nutrient composition of GMHT and 'Subicho' were found similar, and GMHT's nutrient contents were in the range of those of the conventional red peppers. These results showed that GMHT's nutrient contents were equivalent to those of the parental red pepper and other conventional red peppers.

Key words: dried red pepper, genetically modified herbicide tolerance, compositional analysis, nutrients, substantial equivalence

I. Introduction

Genetically modified organisms (GMOs) are referred to living organisms of vegetable or animal origin in which the artificially introduced foreign genes are able to confer novel characteristics not found in wild variety. There is an increasing need of analytical methods for GMOs detection especially in food due to the increasing use of GMOs or their derivatives in food industry (Meric et al. 2004). Rigorous specifications are necessary to ensure the safety of these products for human health and for the environment. Approaches to the regulation and

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safety assessment of genetically modified crops have been developed in a very proactive manner. The first international and national provisions for the safety assessment and regulation of genetically modified organisms including GM crops and derived foods were drawn up by scientific experts in the mid-1980s (OECD 1986; US OSTP 1986). This was nearly a decade before the first regulatory approval of a genetically modified crop was made in 1995. Since then, the global area of commercial cultivation of such crops has risen to 58.7 million hectares in 2002(James 2002). Commercially cultivated GM crops include soybean, rice, maize, cotton, canola, potatoes, and tomatoes. At present, the most widely grown GM crops contain new genes that confer herbicide tolerance or insect resistance. It is important to continue to proactively assess the current safety assessment approaches in terms of their appropriateness for present and future GM crops and those with more complex traits.

One important aspect of the safety assessment of GM crops destined for the food industry is characterizing their nutrient composition. The safety assessment can be focused on the constituent nutrients in the host plant or in its close relatives and the changes in the levels of consumption that may affect human health and nutrition (König et al 2004; Padgette et al 1996; Taylor et al. 1999). Nutrients are components in a particular food that may have a substantial nutritional impact on the consumer or animal. These may be macronutrients (moisture, proteins, fat, fiber, carbohydrates, and ash) or micronutrients (minerals).

This study was conducted to evaluate the nutrient composition of the herbicide tolerant red pepper (GMHT), developed by genetic modification. The composition study was conducted to find out whether GMHT's macronutrients and micronutrients were comparable with those of the parental red pepper, and whether they were within the nutrient ranges of conventional red peppers. This study was designed to focus on any effect a genetic change may have on seed composition; therefore, the red pepper seeds were analyzed after separating from the pericarp.

II. Materials and Methods

1. Materials

The GMHT developed by scientists at the Rural Development Administration (RDA) in Korea, and 'Subicho' (SC), the parental control of GMHT, were obtained from RDA's National Institute of Agricultural Biotechnology (NIAB). Developing the herbicide resistance of the herbicide-susceptible cultivar 'Subicho' involved modifying it by adding phosphinotricin acetyltransferase, using Agrobacterium mediated transformation.

The nutrients contents were analyzed according to the red peppers' physical status (pericarp, seed and placenta).

2. Analytical Assays

The red peppers were analyzed for their moisture, protein, fat, fiber, carbohydrate, ash, and mineral contents. This study used the Association of Official Analytical Chemists methods to determine the proximate components and minerals (AOAC 2001).

1) Proximate nutrients

Energy. Energy was calculated using the Food and Agriculture Organization/World Health Organization energy conversion factor.

Moisture. Moisture loss was determined at 105℃ in a forced draft oven.

Protein. Total nitrogen was determined by the Kjeldahl method. Protein was calculated from total nitrogen using N×6.25.

Fat. Ether-soluble material (primarily "free" fats and oils) was extracted from the sample with petroleum ether in a Soxhlet extractor. The ether was volatilized and the dried residue was quantified gravimetrically and calculated as the percentage of fat. The method was based on the previously published methods (Lee et al. 2004).

Fiber. The sample was dried to remove excessive moisture, ground to pass through a 1.0 mm screen, and extracted with petroleum ether to remove fat. It was then digested in refluxing 0.22 mol/L H_2SO_4, filtered, digested in refluxing 0.30 mol/L NaOH, and filtered. The residue was washed, dried, weighed, ignited, and reweighed. Crude fiber was calculated from the residue's loss on ignition.

Ash. The sample was charred and ashed to a constant weight at 550℃ in a muffle furnace. The residue was quantified and the percentage of ash was determined

Carbohydrate. Carbohydrate was calculated by difference using the fresh weight-derived data, according to the following equation: g/100g carbohydrate = 100g/100g - (g/100g protein + g/100g fat + g/100g ash + g/100g moisture).

2) Minerals

The red peppers' calcium, phosphorus, iron, sodium, potassium, magnesium, and zinc contents were analyzed. An atomic absorption spectrophotometer was used to measure the mineral contents, except that of phosphorus, which was analyzed by a spectrophotometer.

3) Comparison with the nutrients contents of conventional red peppers

The literature range of conventional red peppers was cited from food composition tables (NRRDI, 2001; Kweon et al. 1998; Son et al. 1995; Ku et a.. 2001; Lee et al. 1995; Jung et al. 1993; Lee et al. 2004). GMHT's moisture, protein, fat, fiber, carbohydrates, ash, and mineral contents were compared with the nutritional composition of conventional red peppers.

3. Statistical Analysis

This study used SPSS 10.0 for Windows (SPSS Institute Inc. 2002) to analyze the compositional results (proximate nutrients and mineral levels) for GMHT and SC. The means and standard error mean (SEM) were calculated, and the difference between GMHT and SC as a control was determined by using t-test. The nutrient levels of GMHT were compared with those of the control using the t-test comparison procedure at the 5% level.

Ⅲ. Results and Discussion

1. Proximate nutrients

The basic characteristics of GMHT and the parental red pepper cultivar 'Subicho' are shown in Table 1. Data for red peppers were reported as means of the measurements from three samples from the different field sites for GMHT and SC, along with the ranges of values determined for each sample (Table 2 and 3).

Energy. The energy contents of GMHT and SC pericarps were 241.2 kcal and 251.4 kcal, respectively, and those of seeds and placentas in GMHT and SC were 281.2 kcal and 290.3 kcal, respectively. There was no significant difference in the energy levels

Table 1. Basic characteristics of GMHT and SC.

Sample Name	Part	Bar Gene	Mediator
GMHT[1)]	Pericarp	phosphinotricin-	Agrobacterium
	Seed+placenta	acetyltransferase	
SC[2)]	Pericarp	-	-
	Seed+placenta		

[1)] GMHT: Genetically modified herbicide tolerant red peppers
[2)] SC: 'Subicho' as a control

Table 2. Proximate nutrients of pericarps in GMHT and SC.

Nutrients	GMHT[1)]			SC[2)]			Literature Range
	Mean	Range	SEM	Mean	Range	SEM	
Energy (Kcal)	241.2	238.7-243.6	2.5	251.4	237.5-265.2	13.9	159.0-221.0
Moisture (%)	11.4	11.2-11.6	0.1	11.4	11.3-11.5	0.0	11.6-22.0
Protein (%)	12.7**	12.6-12.8	0.1	14.3	14.2-14.4	0.1	11.0-16.7
Fat (%)	5.1	5.0-5.2	0.0	5.6	5.0-6.0	0.2	5.9-8.9
Fiber (%)	16.4**	16.1-16.7	0.2	17.9	18.0-18.0	0.0	22.0-37.3
Ash (%)	7.5	7.3-7.7	0.1	7.4	7.3-7.4	0.0	4.4-8.9
Carbohydrate (%)	46.9	46.2-47.6	0.7	46.8	43.0-50.5	3.7	16.4-59.0

[1)] GMHT: Genetically modified herbicide tolerant red peppers

[2)] SC: 'Subicho' as a control

** Means are significantly different at p<0.01 by t-test, respectively

of pericarp between GMHT and SC while the energy level of seeds and placentas in GMHT was lower than that of SC. But the difference was considered biologically unimportant. Thus, the energy levels of GMHT's pericarps, and the seeds and placentas were comparable with those of SC.

Moisture. Both GMHT's and SC's pericarps had moisture contents of 11.4% respectively, and GMHT's and SC's seeds and placentas had 6.8% and 7.0%, respectively. No significant difference was found in the levels of moisture in the pericarp between GMHT and SC. The moisture contents of seeds and placentas in GMHT were significantly lower (p<0.01) than those of SC. But their moisture levels were similar and comparable with those of literature range.

Protein. GMHT's and SC's pericarps had protein levels of 12.7% and 14.3%, respectively, and GMHT's and SC's seeds and placentas had 18.7% and 19.1 %, respectively. The protein contents of GMHT were significantly lower than those of SC. However, their protein levels were in the range of

Table 3. Proximate nutrients of seeds and placentas in GMHT and SC.

Nutrients	GMHT[1)]			SC[2)]			Literature Range
	Mean	Range	SEM	Mean	Range	SEM	
Energy (Kcal)	281.2*	276.6-283.6	2.3	290.3	287.3-294.2	2.0	159.0-173.7
Moisture (%)	6.8**	6.7-6.9	0.1	7.0	7.0-7.1	0.1	6.8-9.3
Protein (%)	18.7*	18.7-18.7	0.0	19.1	19.0-19.2	0.1	17.2-18.2
Fat (%)	18.2	17.5-19.1	0.5	18.8	18.5-19.1	0.2	18.8-23.6
Fiber (%)	26.2*	25.5-26.6	0.4	23.9	23.0-24.4	0.5	13.5-16.7
Ash (%)	3.9	3.8-4.0	0.0	3.8	3.7-3.8	0.0	2.9-3.6
Carbohydrate (%)	23.3	22.0-24.2	0.7	24.1	23.7-24.6	0.3	34.8-47.8[3)]

[1)] GMHT: Genetically modified herbicide tolerant red peppers

[2)] SC: 'Subicho' as a control

[3)] Carbohydrates with fiber

*, ** Means are significantly different at p<0.05 and p<0.01 by t-test, respectively

Table 4. Mineral contents of pericarps in GMHT and SC.

Nutrients	GMHT[1)]			SC[2)]			Literature Range
(mg%)	Mean	Range	SEM	Mean	Range	SEM	
Calcium	40.2	39.4-40.8	0.4	42.0	40.3-43.0	0.8	13.8-58.0
Phosphorus	252.6	245.7-258.9	3.8	238.1	232.8-245.4	3.8	230.0-354.1
Iron	3.3	3.2-3.3	0.1	2.6	2.5-3.1	0.3	3.2-6.8
Sodium	110.0	108.0-112.0	2.0	109.0	101.0-116.0	8.0	56.0-702.4
Potassium	3558.6	3480.4-3673.7	58.8	3457.0	3402.8-3561.0	52.0	293.0-3932.8
Magnesium	151.5**	149.1-153.1	1.2	169.1	165.8-171.4	1.7	96.0-190.0
Zinc	1.7	1.7-1.7	0.0	1.6	1.5-1.7	0.0	1.5-2.0

1) GMHT: Genetically modified herbicide tolerant red peppers
2) SC: 'Subicho' as a control
** Means are significantly different at p<0.01 by t-test

the conventional red peppers. And some reports suggested that allergens must be checked along with the increase of protein content because all food allergens are proteins (Health and Human Services 1992).

Fat. GMHT's and SC's pericarps had fat contents of 5.1% and 5.6 %, respectively, and GMHT's and SC's seeds and placentas had 18.2% and 18.8 %, respectively. There was no significant difference in the fat levels of the pericarps, and the seeds, and placentas of two red peppers.

Fiber. GMHT's and SC's pericarps had crude fiber contents of 16.4% and 17.9%, respectively, and GMHT's and SC's seeds and placentas had 26.2% and 23.9 %, respectively. The crude fiber level of pericarp in GMHT was lower than that in SC, while the crude fiber level of seeds and placentas in GMHT was higher than that in SC. This difference was considered biologically unimportant because of the different culture places of these two kinds of red peppers (Padgette et al. 1996).

Ash. GMHT's and SC's pericarps had ash

Table 5. Mineral contents of seeds and placentas in GMHT and SC.

Nutrients	GMHT[1)]			SC[2)]			Literature Range
(mg%)	Mean	Range	SEM	Mean	Range	SEM	
Calcium	17.8**	17.4-18.1	0.2	24.7	23.8-25.3	0.4	11.5-12.9
Phosphorus	501.0	456.5-535.6	23.3	516.6	497.3-527.0	9.7	377.6-468.0
Iron	4.6	4.3-5.2	0.3	5.5	4.8-6.2	0.4	4.7-5.7
Sodium	49.0*	48.0-51.0	1.0	62.0	60.0-64.0	2.0	56.0-456.1
Potassium	1465.6*	1446.6-1487.5	11.9	1528.5	1526.3-1530.7	2.2	1510-2385
Magnesium	312.3**	308.5-315.9	2.1	282.4	280.1-286.0	1.8	163.0-192.0
Zinc	2.8	2.6-3.0	0.1	3.2	2.9-3.7	0.2	2.1-2.2

1) GMHT: Genetically modified herbicide tolerant red peppers
2) SC: 'Subicho' as a control
*, ** Means are significantly different at p<0.05 and p<0.01 by t-test, respectively

contents of 7.5% and 7.4 %, respectively, and GMHT's and SC's seeds and placentas had 3.9% and 3.8%, respectively. There was no significant difference in ash levels between GMHT and SC. Thus GMHT's pericarps, and the seeds and placentas had ash contents comparable with those of SC's. The ash content of pericarp in GMHT was in the range of ash content in conventional red peppers (Kweon et al. 1998; Son et al. 1995; Ku et a.. 2001; Lee et al. 1995; Jung et al. 1993).

Carbohydrate. GMHT's and SC's pericarps had carbohydrate contents of 46.9% and 46.8%, respectively, and GMHT's and SC's seeds and placentas had 23.3% and 24.1%, respectively. There was no significant difference in the levels of carbohydrates between GMHT and SC.

2. Minerals

Calcium. GMHT's and SC's pericarps had calcium contents of 40.2 mg% and 42.0 mg%, respectively, GMHT's and SC's seeds and placentas had 17.8 mg% and 24.7 mg%, respectively. There was no significant difference in the levels of calcium in pericarps between GMHT and SC. The calcium level of seeds and placentas in GMHT was significantly lower ($p<0.05$) than that of SC. But the calcium level of seeds and placentas in GMHT were higher than the range of ash content in conventional red peppers, and the difference was considered biologically unimportant because the calcium content of the pepper could be variable in different cultural sites (NRRDI, 2001; Kweon et al. 1998; Son et al. 1995; Ku et a.. 2001; Lee et al. 1995; Jung et al. 1993).

Phosphorus. GMHT's and SC's pericarps had phosphorus contents of 252.6 mg% and 238.1 mg%, respectively, and GMHT's and SC's seeds and placentas had 501.0 mg% and 516.6 mg%, respectively. The study found no significant differences between GMHT and SC in their levels of phosphorus. The phosphorus levels of GMHT's pericarp were in the range of those of the conventional red peppers.

Iron. GMHT's and SC's pericarps had iron contents of 3.3 mg% and 2.6 mg%, respectively, and GMHT's and SC's seeds and placentas had 4.6 mg% and 5.5 mg%, respectively. There was no significant difference in iron contents of pericarps, and the seeds and placentas between GMHT and SC. The iron contents of GMHT's pericarps, and the seeds and placentas were comparable with those of conventional red pepper.

Sodium. GMHT's and SC's pericarps had sodium contents of 110.0 mg% and 109.0 mg%, respectively, GMHT's and SC's seeds and placentas had 49.0 mg% and 62.0 mg%, respectively. There was no significant difference in pericarps's sodium content between GMHT and SC. The difference in sodium content of seeds and placentas between GMHT and SC was considered biologically unimportant because of the different culture places of these two kinds of red peppers (Padgette et al. 1996).

Potassium. GMHT's and SC's pericarps had potassium contents of 3558.6 mg% and 3457.0 mg%, respectively, and GMHT's and SC's seeds and placentas had 1465.6 mg% and 1528.5 mg%, respectively. There was no significant difference in pericarps's potassium content between GMHT and SC. But potassium content of seeds and placenta in GMHT was significantly ($p<0.05$) lower than that in SC, and this difference was small due to the genetics of the peppers' varieties, the location where the plants were grown, and the crop year (Padgette et al. 1996).

Magnesium. GMHT's and SC's pericarps had magnesium contents of 151.5 mg% and 169.1 mg%, respectively, and GMHT's and SC's seeds and placentas had 312.3 mg% and 282.4 mg%, respectively. The magnesium level of pericarps in GMHT was significantly lower than that of SC, while the magnesium level of seeds and placentas in GMHT was significantly higher than that of SC. The magnesium level of pericarp in GMHT was in the range of conventional red peppers.

Zinc. GMHT's and SC's pericarps had zinc contents of 1.7 mg% and 1.6 mg%, respectively, and GMHT's and SC's seeds and placentas had 2.8 mg%, and 3.2 mg%. There was no significant difference in zinc content between GMHT and SC. The zinc contents of GMHT's pericarps, and the seeds and placentas were comparable with those of SC.

This study analytically confirmed that the levels of major nutrients and minerals would not change in the GMHT. But many studies with various species of red peppers cultured in several locations need to be conducted. For the data presented above, the literature ranges for most of the compositional variables were provided, where available. Although the information was useful to frame the experimental results obtained in this study, the literature data may not be directly comparable because of the differences in the analytical methodology or sample preparation. In addition, much of the literature data may not completely encompass the compositional variables of modern red pepper varieties and different cultural cites.

Proximate analysis is the standard method of determining the base chemical composition of red peppers. Any meaningful increase in protein content resulting from the potential inhibition and promotion of protein synthesis as a result of the application of herbicide resistance should be detected. Proximate data generated from these results showed similarity of the protein level or any other proximate components of GMHT and the conventional red peppers. Some levels of proximate nutrients in both the herbicide-tolerant GMHT and the control (SC) were out of the ranges of literature, although the mean levels of their proximate nutrients were not statistically different. The reason was that the range of proximate nutrients for red peppers was variable across multiple sites and it was due to the limited research results for red peppers. These results were not unexpected because the level of proximate nutrients, minerals, and some components in plants were dependent upon the genetics of their variety, the location where the plantsare grown, and crop year (Padgette et al. 1996; Govindarajan and Anil 1985; Bosland PW 1994; Estrada et al 1999; Estrada et al 2002). The results of the analyses for GM herbicide-resistant plants, reported previously, demonstrate that the application of herbicides does not affect the levels of protein, fat, fiber, carbohydrates, or ash composition, as compared with conventional plants (Taylor et al 1999). The study found no deleterious effects on the pericarps, and seeds and placentas of red peppers. The data demonstrated the substantial equivalence of GMHT to the parental control and to other conventional red peppers as previously reported (NRRDI, 2001; Kweon et al. 1998; Son et al. 1995; Ku et a.. 2001; Lee et al. 1995; Jung et al. 1993). The only difference between GMHT and conventional red peppers is their tolerance to herbicide.

In summary, the proximate composition and the micronutrients (minerals) of GMHT developed by RDA were found to be substantially equivalent to the composition of SC that was not genetically modified in terms of herbicide resistance.

초 록

본 연구는 농촌진흥청에서 개발한 유전자 변형(제초제 저항성) 고추와 모종인 수비초 고추의 영양성분을 분석하고 유전자 변형된 경우 영양성분에 차이가 있는지 비교하기 위하여 수행하였다. 영양성분은 AOAC법을 이용하여 주요 영양소(단백질, 지방, 섬유소, 회분, 당질)와 무기질(칼슘, 칼륨, 나트륨, 마그네슘, 아연, 철) 함량을 분석하였고, 식품성분표에 제시된 일반 고추의 영양성분과 비교하였다. 그 결과 유전자 변형 고추는 모종 고추와 영양성분 함량이 유사하였고, 일

반적인 고추의 영양성분 함량 범위 내에 있어 실질적인 동등성을 갖는 것으로 나타났다.

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