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An in vivo study on the use of local phaseolus shoots in food

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Abstract: Legumes grown and consumed all over the world except for the polar regions are used in human nutrition as the main source of plant protein for thousands of years. Although the legumes are rich in minerals, dietary fiber and B vitamins. However, it is known that the activity of phenolic, bioactive compounds, ascorbic acid and antioxidants increases after germination and sprouting in bean seeds. It was aimed to determining nutritional usege of shoots obtained by germination of commercial (Uzbekistan, Mersin) and regional (Malatya) bean varieties (fresh and uncooked). In this direction oxidation quantity and antioxidant activity are defined providing that *Drosophila melanogaster* as model organism is fed on bean shooted during the larval phase. Despite the fact that larval malondialdehit (MDA) quantities seem similar as statistical after nutrition, Glutatyon-S-Trasferoz (GST) activity of larvae that are fed on regional beans is defined higher. Either this result is originated from storage and hiding condition which commercial products getting rich according to vitamin or gene source of the regional bean is thought. Findings obtained; even if bean sprouts show that they can be used as an antioxidant source in nutrition, they are needed extensive studies on safely usage in terms of creatures.

Keywords: Mung bean, Sprouted, Drosophila, Nutrition, Antioxidant.

Introduction

Due to increased nutritional awareness in worldwide, demand for the natural/regional products and functional food has increased. Legumes and cereals, which have a variety of bioactive compounds, have been important in human nutrition for many years as edible seeds (Gan et al., 2016). Although consuming the food that has been germinated in the past, consumption of legumes by germination has become a new culinary trend (Öztürk, 2008). Recent germination studies have shown that the nutritional and medical values of edible seeds can be improved, and they can be used as functional foods (Gan et al., 2016; Ganesan and Xu, 2017; Liyanage et al., 2017). Botanical natural bioactive compounds have increased significant extent in antioxidant capacity by sprouting (Yeap et al., 2015; Gan et al., 2016). Generally, various seeds such as soy, mung bean and adzuki bean, peas, lentils, wheat, barley, oats, rye are being consumed directly by sprouting/germination and cooking (López-Amorós et al., 2006; Sivritepe, 2010). Depending on the plant species and germination conditions, bioactive components (such as plant flavonoids and phenolic compounds) and antioxidant activity are being increased in seed (López-Amorós et al., 2006; Pająk et al., 2014). Mung beans are a protective grain against the heart attack, reducing serum lipids and cholesterol, removing free radicals (Tang et al., 2014; Bai et al., 2016; Liyanage et al., 2017). For example mung beans are being contained two flavonoids, vitexin and isovitexin (Tachibana et al., 2013; Bai et al., 2016; Liyanage et al., 2017). There are many studies on bean germination or sprouting and antioxidant activity (Guajardo-Flores et al., 2014; Pająk et al., 2014; Aguilera et al., 2015; Dueñas et al., 2015; Dueñas et al., 2016).

Drosophila melanogaster Meigen (Drosophilidae), used as an experimental animal in genetic studies, is used to study the ingestion and digestion of many functional foods (Lushchak, 2011; Güneş, 2016). Despite the fact that there is nutritional studies with sprouted beans in the literature (Liyanage et al., 2017), antioxidant activity in local and commercial crops has not been determined *in vivo*. The main purpose of this study are (1) since they will

Figure 1. Mung bean seeds (A. Malatya, B. Mersin, C. Uzbekistan origin) and germinated sprouts (D. Malatya, E. Mersin, F. Uzbekistan origin), X 1000μ.

change or regulate biochemical parameters in the processing of food (Nergiz and Gökgöz, 2007), determination of the antioxidant effect of sprouted mung beans grown regional (Malatya) and commercial (Mersin and Uzbekistan) and (2) the determination of the use of these shoots for vitality in food. For this purpose, it has been tried to determine the oxidant and antioxidant properties formed by feeding of the larval model organism.

Materials and Methods

Commercial and regional mung bean sprouts (Malatya, Mersin and Uzbekistan origin, Fig. 1) germinated for about 48 hours (up to 25-75 mm; Sivritepe, 2010) were added (wet weight 0.2 gr/100 ml) to the potato artificial diet of the *D. melanogaster* by 30% to feed the living beings. The amount used in the feeding study was determined by taking into account previous studies and the insect body weight (Liyanage et al., 2017). Positive control purpose, powder of mung seeds were used by crushing in liquid nitrogen. In order to obtain constant weight of germinated and powder mung beans, there were dried for 24 hours at 60°C (Liyanage et al., 2017). Water was used as a negative control. The first-stage larvae emerging from the egg were inoculated into experimental flasks and observed up to the third stage after three metamorphoses (25±1°C, 60±5 % relative humidity and 12 hours light/12 hours dark photoperiod). The larvae from the third stage were collected without waiting and biochemical analysis were made from total tissues.

For each sample, a total of 10 larval tissues were homogenized (10 s, 30 W) with buffer (0.5 M pH 7.2 potassium phosphate; Güneş and Büyükgüzel, 2017). The amount of oxidation (Malondialdehyde-MDA) in larval stage was determined by Habig et al. (1974), and antioxidant activity (Glutathion-S-transferase-GST-EC.2.5.1.18) according to Jain and Levine method (1995).

The "LSD test" (ANOVA) was used o determine the changes in the biochemical values of insects used as commercial and regional of mung beans, and the "one way variance analysis" (SPSS, 1997) was used to determine the significance of differences between the averages. The averages were evaluated at a probability of 0.05 ($P<0.05$).

Results and Discussion

The amount of MDA that is indicative of lipid peroxidation in living beings is a biochemical parameter that proves the oxidation in vivo. Many studies have indicated that mung bean has antioxidant activity by increasing the activity of superoxide dismutase (SOD) enzyme, reducing the amount of MDA in animals such as mice and rabbits (Wu et al., 2001; Tang et al., 2014; Tiwari et al., 2013; Yeap et al., 2014, 2015; Bai et al., 2016; Liyanage et al., 2017). However in our study, it was

Table 1. MDA amount and GST activity of *D. melanogaster* larvae fed with germinated mung beans and raw powders

gr/100ml	MDA (Mean ± S.E.)†	GST (Mean ± S.E.)†
0.0§	3.71 ± 0.03c	67.98 ± 0.03a
MOP	2.71 ± 0.04c	16.24 ± 0.02c
MeOP	29.49 ± 0.01b	62.50 ± 0.02a
UOP	62.82 ± 0.02a	26.04 ± 0.01bc
MOS	75.38 ± 0.01a	33.33 ± 0.01b
MeOS	37.73 ± 0.01b	5.95 ± 0.02d
UOS	9.33 ± 0.02c	4.43 ± 0.01d

* Average of four repetitions, 10 larvae were used for each repeat, † Values with the same letter in the same column are not different from each other ($P<0.05$; LSD Test), § Control nutrient (without mung beans), MOP: Malatya origin mung bean powder, MeOP: Mersin origin mung bean powder, UOP: Uzbekistan origin mung bean powder, MOS: Malatya origin mung bean shoots, MeOS: Mersin origin mung bean shoots, UOS: Özbekistan origin mung bean shoots

determined that lipid peroxidation was increased 31-fold by changing of MDA amount of insect with use of regional mung beans powder (Control<Malatya<Mersin <Uzbekistan; $P<0.05$). It can be seen that the use of germinated mung bean sprouts is the opposite of this situation, Table 1 (Control<Uzbekistan<Mersin<Malatya; $P<0.05$). It is known that food processing methods increase bioavailability by reducing non-digestible components in raw legumes, because the proteins are hydrolyzed to absorbable polypeptides and essential amino acids (Subuola et al., 2012; Satya et al., 2013; Chandrasiri et al., 2016). In the same way, it is expressed in some studies that feeding with powder and sprouted mung bean will reduce the blood lipid level perfectly (Mohd et al., 2013; Kapravelou et al., 2015; Benil et al., 2017). It was determined that insect lipid peroxidation was increased fed with germinated mung bean, despite increased bioavailability, except for the origin of Uzbekistan. Although Drosophila has a similar digestive system to highly organized organisms (Watson, 1982; Duttlinger et al., 2002; Sharma et al., 2007; Apidianakis and Rahme, 2011; Ren et al., 2014), increasing Kaempferol in germinated mung beans were thought to cause lipid peroxidation leading to deterioration of intestinal homeostasis (Ganesan and Xu, 2017). Because the production of reactive oxygen, which is the main element in the deterioration of resistance in the insect, cannot be controlled by the peritrophic membrane and antioxidant enzymes in the stomach, so that they can adversely affected the insect (Apidianakis and Rahme, 2011).

The amount of GST, which acts as antioxidant against nutrition and insecticidal oxidation, was similar to the control statistically by using MeOP. In insects fed with MOP and UOP, a one third reduction in the amount of GST was detected. It was observed that the amount of GST in insects fed with sprouted mung beans has considerably decreased (Table 1). Except for Malatya origin bean, the antioxidant activity seen in post-treatment products have been similar to previous studies (Liyanage et al., 2017). It was also known that this ratio will increase with application of boiling (Ademiluyi et al., 2015).

It was observed that the GST activity increased from approximately 16 to 33, depending on the amount of MDA being increased in the insect fed with MOP and MOS. Although the amount of MDA was increased from approximately 29 to 37 in the use of Mersin origin beans, GST activity decreased statistically with sprouting. Uzbekistan origin bean showed a similar activity to Malatya origin bean, and the antioxidant activity was decreased depend on the amount of MDA with germination. Germination changes the quantitative and qualitative phenolic composition depending on the type of legumes and germination conditions (López-Amorós et al., 2006). From these results could be shown that MeOS should not be used because oxidation cannot be responded in larval stages, but MOP, UOP, MOS and UOS can be used for feeding.

It was thought that the excess oxidation and lower antioxidant enzyme activity observed in the MOS-fed larvae, due to the vitamin C which is increased during germination (Ganesan and Xu, 2017). Because vitamin C added to diet has increased insect toxicity (Bahadorani and Hilliker, 2008). MeOS consumption of insects was found to significantly reduce amount of GST while increasing oxidation. Antioxidant balance was observed in the same origin powder. It has also known that the antioxidant activity is increased most in the germination of bean sprouts on the fourth day (López-Amorós et al., 2006). A two-day germination was applied instead of four days due to the blackening of the root after 48th hour of germination in our study. This may also be the reason for the inadequate availability of antioxidant activity in grassed legumes. Increase in the amount of MDA; the phytic acid, improves digestibility during germination (Bilgiçli, 2002), may be due to the task of protecting to seed against insects and pests. However, to prevent damage to the content by insects, secondary metabolites can also be synthesized depending on the origin in legumes (Amin et al., 2000; Shahjahan and Amin, 2000; Roy et al., 2010; Ahad et al., 2016). Thus, legumes have been protected by preventing feeding behavior of the insect (Ahad et al., 2016). As a result, the amount of MDA has increased in insects such as *Callosobruchus chinensis* L. (Rahman and Talukder, 2006; Ahad et al., 2016), which are fed with herbal secondary metabolites or not feeding.

Conclusion

The increase in the amount of MDA and GST activity in larvae fed with UOP and the absence of oxidation in those fed with UOS has indicated that commercial seeds of Uzbekistan origin are suitable for commercial use. As a result, although commercial mung bean sprouts can be used as an antioxidant source, detailed studies on biochemically reliable use of local and commercial legumes are needed to be demonstrated by using model organisms.

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