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NUTRITIONAL AND BIOLOGICAL STUDIES ON GREEN BARLEY LEAVES AS A SOURCE OF BETA CAROTENE

Bessar, Badiaa A.
Food Sciences and Technology Dept., Fac. of Agric.,Kafr El-Sheikh, Tanta Univ., Egypt.

ABSTRACT

This study was carried out to evaluate green barley leaves (Giza 117) as a rich source of Beta carotene through feeding study to compare the bioavailability of barley leaves β-carotene with synthetic β-carotene. Also in the present study green barley leaves juice was used for enrichment barely drink and guava juice with β-carotene. The data showed that using the barley leaves diet as a source for β-carotene had the advantage of adding extra nutrients to experimental rats comparing with other diets. These nutrients helped the rats to be healthier, and grow more rapidly. Bioavailability of barley leaves β-carotene was higher than that of synthetic β-carotene. Levels of Vitamin A were higher in plasma and liver of rats fed on barley leaves diet than that of synthetic β-carotene diet. Using barley leaves juice for fortification showed a clear effect in increasing β-carotene levels in barley drink and guava juice.

INTRODUCTION

Recently, natural plants have received much attention as a source of biologically active substances including antioxidants, anti-mutagens, and anti-carcinogens (Osawa et.al. 1992). Synthetic antioxidants such as butylated hyroxyanisole and butylated hydroxytoleuene have been used as antioxidants for foods since the beginning of this century.The use of these synthetic antioxidants, however, has begun to be restrictive because of their toxicity (Bronen 1975 and Ito *et. al.* 1983). Therefore the importance of natural antioxidants has increased greatly. There is a pressing need to find safe economic antioxidants to replace these synthetic chemicals.

Barley has been grown in temperate climates around the world as a fed grains, it competes with several commodities including, corn, sorghum, wheat and Oats. The young barley leaves are green cereal grass that contains the greatest and most perfectly balanced concentration of nutrients found in nature (Anderson, 1999).

These leaves are powerfull source of antioxidants and rich in β-carotene, great source of chlorophyll and other anti-oxidants. Also they are rich with many nutrients which could be useful in the prevention of cardiovascular diseases, prostatic cancer and help arthritics (Michael, 1995 and Yu, *et. al.*, 2002 a).

β-carotene acts as powerful antioxidant to protect against degenerative diseases such as cancer. β-carotene is the non-toxic precursor of vitamin A. It is largely found in orange and yellow coloured vegetables and in greater abundance in leafy greens. The carotene in leafy greens is converted to vitamin-A about twice as efficiently as the carotene in carrots and other root vegetables. β-carotene has never been known to be toxic in any amount, even thcugh consuming excessive amount, it can only cause an orange pigmentation to the skin (Yu, *et. al.*, 2002 b).

In this study, β-carotene was determined in green barley leaves, nutritional and biological studies on green leaves β-carotene were done. Juice of Young green barley leaves was used for enrichment barley drink (fayrouz) and guava juice with bioactive β-carotene.

MATERIALS AND METHODS

Materials:

Barley grain (Hordeum vulgare), Giza 117, was obtained from Agricultural Research Center at Sakha, Kafer El-Sheikh Governorate, Egypt. Synthetic beta-carotene was obtained from Sigma Chemical Company in crystalline form. Barley drink (Fayrouz) and guava juice were obtained from Egyptian local markets.

Methods:

Green barley leaves were grown in containers containing natural soils at room temperature and no pesticides, herbicides or chemical fertilizers are never used. leaves were harvested two weeks after germination for barley grains.

Determination of beta-carotene in green barley leaves.Green barley leaves were dried in an air oven at 60^0 under vacuum, the dried leaves were subsequently ground and silted in 100 mesh metal screen sieve, and mesh size 2-mm sieve was used to form a fine and uniform powder and kept in polyethylene packages for extraction. β-carotene was extracted from dried green barley leaves according to methods of A.O.A.C (1995). Determined β-carotene concentration in the green barley leaves powder was 46.27 mg/100 gm.

Feeding Experiments:

The recommended level of vitamin-A in rats is 200,000 I.U. Vitamin-A activity/kg diet (De-lumen *et. al.* 1982). This is equivalent to 0.4 gm β-carotene, since one retinol equivalent is equal to 3.33 I.U. Vitamin-A activity and to 6 µg. β-carotene.

For evaluating and comparing the bioavailability of barley leaves and synthetic β-carotenes, 15 albino white rats (male and female) with an average weight at 50 ± 5 gm were used. Rats were divided into three groups (5 rats per each). Each animal was individually housed in a wire bottom stainless steel. Two groups of them were fed on diets containing β-carotene from young green barley leaves and synthetic β-carotene as their sole source. While the third group was fed on casein diet and serve as a control (zero β-carotene content). For each diet a 20% protein level was chosen because it provided the marginal amounts of protein necessary for efficient muscle deposition. This experiment was continued for 6 weeks. Animals were weighted twice a week. Feces were collected daily in polyethylene packages and stored in a freezer at (-20 c°) for β-carotene determination. At the end of experiment, rats were weighted and sacrificed with guillotine, immediately their blood were collected and transported to the laboratory for vitamin-A analysis. Liver and fatty tissues were collected in polyethylene packages and stored in a freezer at (–20 c°) for β-carotene determination as described by (Chan, 1981).

Preparation of Diets:

A vitamin premix was first prepared by weighing out individual vitamins except vitamin-A (vitamin A was added only in control diet). The vitamin was slowly blended into the mixture using a mortar and pestle. The minerals mixture, chlorine bitartrate and D-L methionine were then successively added to the vitamin premix and blended in a Hobart mixer for 20 minutes at low speed. This mixture served as a basal premix to which were added appropriate amounts of test materials, corn oil, protein and sucrose for each treatment. This final mixture was further mixed for 30 minutes. The β-carotene content of the two diets (except the control diet) was determined in order to ensure that the experimental diets in the two treated groups were equivalent in the percentage of β-carotene. Stock basal of diets under investigation was prepared according to (DeLumen, *et. al.* 1982). Glass jars were used as food containers and water was dispensed with an inverted bottle with a stainless steel nozzle.

β-carotene determination in barley leaves, feces and fatty tissues of experimental rats was carried out according to the method of A.O.A.C. (1995), while Vitamin-A in plasma and liver of rats was estimated according to the method of Olson (1979).

Fortification of barley drink (Fayrouz) and guava juice:

Fortification of barley drink (Fayrouz) and guava juice with juice of barley leaves as a rich source of β-carotene was run as follow:

500 gm From green barley leaves were blended first using Moulinex machine, blended leaves were transferred to 1 liter flask, water was added to complete the total volume to be 500 ml, the content of the flask was mixed for 15 minutes and filtered . The obtained juice (20.35 mg β-carotene / 100 ml) was used for fortification of both barley drink (Fayrouz) and guava juice with percentages of 10%, 20% and 30% respectively.

β-carotene was determined in previous samples before and after fortification according to the methods of A.O.A.C. (1995).

Sensory Evaluation of Fortified Drinks:

Sensory evaluation was carried out according to the method described by Watts *et. al.* (1989). The panel was composed of 20 judges, using a fully structured g-point rating scale to evaluate colour, taste, flavour and overall acceptability of the experimental drinks.

RESULTS AND DISCUSSION

Bilogical Evaluation of Experimental β-carotene:

Young green barley leaves contains certain materials with anti-inflammatory activity and considers a power source of safe, economic antioxidants which can be replaced synthetic chemicals such as β-carotene, chlorophyll, 2-0 glyco- sylisovitexin (Osawa, *et. al.*, 1992). In addition it contains, satisfied amount from many vitamins and minerals. The effect of feeding rats on diets containing different sources of β-carotene (barley leaves β-carotene, synthetic β-carotene and recommended level of vitamin-A) on body weight gain, percent liver of body weight and food conversion efficiency

(FCE) were studied and the obtained data are given in Table (1). It could be noted from data that weight gain of rats fed on barley leaves was the highest (122.07 gm.) followed by casein diet group (110.70 gm.) and synthetic group (95.52 gm.). This may be due to the additional nutrients that found in barley leaves and added to the diet unavoidably, where all other diets contained equal amount of the basic ingredients. These results are in agreement with Nagasawa *et. al.* (1989), and Ghazi, (1994). In addition, Olson (1979) reported that vitamin-A and β-carotene are considered the main substances that play an active role in promoting the growth action in rats. From these results it was apparent that liver percent of the body weight indicated that weight gain of rats fed on barley leaves as a source of β-carotene was real where liver percent of body weight indicated normal growth. As well as food conversion efficiency (FCE) in tested groups were 3.06, 3.02 and 3.11 in rats fed on barley leaves, synthetic β-carotene and control (casein), respectively, where differences were very little between them.

Table (1): Biological Evaluation of the Experimental β-carotene.

Group	Initial Weight (gm) Mean ±SD	Final Weight (gm) Mean ±SD	Body Weight (gm) Mean ±SD	% Liver of Bodyweight (gm) Mean ±SD	Food Intake (gm) Mean ±SD	β-carotene Intake (gm) Mean ±SD	F.C.E. Mean ±SD
A	52.41±1.21	174.48±2.65	122.07±1.13	4.07± 0.64	373.46±3.42	0.149±0.11	3.06±0.03
B	50.73±1.26	145.25±2.05	95.52±2.41	3.03±0.49	289.37±3.86	0.124±0.09	3.02±0.06
C	51.35±1.38	162.13±3.16	110.70±1.87	3.33±0.32	344.46±4.21	-	3.11±0.07

A: Rats fed on diet containing barley leaves β-carotene.
B: Rats fed on diet containing synthetic β-carotene.
C: Rats fed on diet containing the recommended level of vitamin-A (free of β-carotene.).
F.C.E. = Food Intake / Body weight gain.

These results are in close with those of Ben-Amotz *et. al.* (1988), who found that lack of cell wall in Dunalietta strains was advantageous for making use of β-carotene within the cell for rendering it easily digestibility by most animal species.

Bioavailability of Different Sources of β-Carotene

The effect of using different sources of β-carotene on bioavailability of β-carotene and on β-carotene levels in feces and fatty tissues of tested rats are presented in Table (2).

Data showed that bioavailability of barley leaves β-carotene and synthetic β-carotene were 66.8 and 53.3,respectively, where the experimental β-carotene has higher bioavailability than synthetic β-carotene. This could be related to the presence of extra oil in the barley leaves diet, since the presence of oil helps to increase β-carotene absorption. These results are in close with those of Jalal *et. al.* (1998) and Van het Hof *et. al.* (2000) who reported that, the absorption of β-carotene is markedly reduced when fat intake was low, because the activity of β-carotene dioxygenase depends on fat. In addition the obtained results are in agreement with the findings of Hu *et. al.* (2000) and Ben Amotz, *et. al.* (1988).

Table (2): Effect of Feeding Rats on Diets Containing Different Sources of β-carotene.

Group	Total β-carotene Intake (mg./100gm)	β-carotene in feces (mg./100gm) Mean ±SD	β-carotene Bioavailability Mean ±SD	β-carotene in Fatty Tissues(mg./100gm) Mean±SD
A	40	13.3±0.1	66.8±1.33	1.5±0.06
B	40	18.7±0.2	53.3±1.88	1.3±0.03

$$\text{Bioavailability} = \frac{\text{Intake β-carotene} - \text{Fecal β-carotene}}{\text{Intake β-carotene}} \times 100$$

From Table (2) also the presented data revealed that β-carotene in the feces of synthetic β-carotene group (18.7 gm.) was higher than that of green barley leaves β-carotene (13.3 gm.). This finding reflex the bioavailability of examined and synthetic β-carotene.

It could be observed also from the same table that, β-carotene in the fatty tissues of green barley leaves group was higher than synthetic β-carotene group, this may be due to addition of green barley leaves in sufficient amount to reach the desired levels of β-carotene, added extra lipids to the diet unavoidably Ghazi, (1994) supports our findings.

Effect of Feeding Rats on Diets Containing Different Sources of β-Carotene on Vitamin-A Level in Plasma and Liver:

Data presented in Table (3) show that the highest level of vitamin-A was observed in plasma of rats fed on control casein diet (86 mg./100 ml.) followed by that of rats fed on tested barley leaves diet (23 mg./100 ml.), and finally the one fed on synthetic β-carotene (14 mg./100 ml.). The same trend of vitamin-A level in liver was observed where the concentrations of vitamin-A were (91 mg./100 gm., 82 mg./100 gm. and 56 mg./100 gm.) in liver of rats fed on control diet, barley leaves diet and synthetic β-carotene diet respectively. The conversion of β-carotene to vitamin-A in the barley leaves diet was high, this may be due to the presence of oils in this diet. These results were supported by those of Olsons (1987), Ben Motz (1988) and Gazi (1994). In addition the carotene in leafy greens is converted to vitamin-A about twice as efficiently as the carotene in carrots and other root vegetables (Anderson, 1999).

Table (3): Vitamin-A Level in Plasma, and Liver of Rats Fed on Different Sources of β-carotene.

Group	Vitamin-A Level in Plasma (mg/100 ml) Mean ±SD	Vitamin-A Level In Liver (mg/100gm) Mean ±SD
A	23±2.20	82±3.42
B	40±2.07	56±2.85
C	86±2.80	91±3.15

Effect of Fortification with Green Barley Leaves Juice on β-Carotene Level in Barley Drink and Guava Juice:

The effect of using different percents of green barley leaves juice as a rich source of β-carotene for fortification of barley drink and guava juice are shown in Table (4), where the level of β-carotene increased from 1.06 mg /100 ml to 2.78 mg /100 ml, 4.62 mg /100 ml. and 6.5 mg./100 ml. in barley drink when replaced with 10%, 20% and 30% from green barley leaves respectively, as well as, β-carotene in guava juice of content 0.042% β-carotene) increased to be 1.87, 3.66 and 5.54 when fortified with 10%, 20% and 30% of green barley leaves juice respectively in addition to other nutrients found in green barley leaves which could be useful in the prevention of many danger diseases like cardiovascular disease. These results are in agreement with Michael (1995) and Yu, *et. al.* (2002).

Table (4): Effect of Fortification with Green Barley Leaves Juice on β-carotene Level in Barley Drink and Guava Juice.

Fortified Samples	β-carotene (mg/100 ml)			
	Initial (Control) Mean ±SD	10% Mean ±SD	20% Mean ±SD	30% Mean ±SD
Barley Drink (Fayrouz)	1.06±0.11	2.78±0.22	4.62±0.32	6.50±1.05
Guava Juice	0.042±0.03	1.87±0.07	3.66±0.36	5.54±0.87

Organoleptic Evaluation of Barley Drink and Guava Juice:

The effect of adding different level of green barley leaves β-carotene on organoleptic compound of barley drink and guava juice was studied and received data were illustrated in Table (5).

Table (5): Sensorly Evaluation of Barley Drink and Guava Juice Fortified with Green Barley Leaves Juice.

% of Barley Juice	Colour	Flavour	Taste	Odor	Acceptability
Barley Drink (Fayrouz)					
Control	8.43+1.20	8.22+1.46	7.65+1.01	7.73+1.25	8.21+0.63
10	8.22+1.43	7.76+1.54	7.83+1.63	7.52+1.42	8.32+0.77
20	8.56+1.05	8.41+1.23	8.46+1.09	7.88+0.88	9.11+1.03
30	7.81+1.11	8.12+1.14	7.94+1.13	7.69+0.87	7.84+1.06
% of Barley Juice	Colour	Flavour	Taste	Odor	Acceptability
Guava Juice					
Control	7.86+0.43	8.31+0.24	8.54+0.25	8.13+1.02	8.56+0.52
10	7.73+0.76	7.43+0.36	8.15+0.33	7.81+1.24	8.11+1.14
20	7.81+1.05	8.64+0.41	8.11+0.76	7.94+1.07	8.42+1.03
30	6.84+0.83	8.24+0.61	7.56+0.91	7.44+0.82	7.66+0.73

The obtained data revealed that barley drink which fortified with 20% green barley leaves juice had the highest values of colour, flavour, taste, odor and overall acceptability in comparing with that fortified with 10%, 30% and control. As for guava juice, using 20% of barley juice showed high score especially for colour and flavour followed by using 10% in comparison with control.

Conclusion

Finally, it could be concluded that juice of young green barley leaves (Giza 117) could be considered as a powerful source of antioxidant (β-carotene) in addition to many other nutritive substances which could be increased biological activity to convert β-carotene to vitamin-A to be useful for human nutrition. This juice could be used as a new natural product and to fortify other common juices and drinks with active β-carotene.

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دراسات عن الخواص الغذائية والبيولوجية لأوراق بادرات نبات الشعير كمصدر للبيتاكاروتين

بديعة عبد الرحمن بيصار

قسم علوم وتكنولوجيا الأغذية – كلية الزراعة بكفر الشيخ – جامعة طنطا

- في هذه الدراسة تم إجراء دراسات عن الخواص الغذائية والبيولوجية لأوراق بادرات نبات الشعير (جيزة ١١٧) كمصدر للبيتاكاروتين وذلك بتغذية فئران التجارب على علائق مختلفة لمقارنة التمثيل الحيوي لبيتاكاروتين أوراق بادرات نبات الشعير مع البيتاكاروتين الصناعي.
- كما تم دراسة استخدام عصير أوراق بادرات نبات الشعير لتدعيم مشروب الشعير وعصير الجوافة بالبيتاكاروتين الطبيعي.

وأوضحت النتائج مايلي:

- تغذية فئران التجارب على عليقة تحتوي مسحوق أوراق بادرات نبات الشعير كان له ميزة واضحة في إضافة مواد غذائية أخرى للفئران المغذاه عليها مقارنه بالعلائق التجريبية الأخرى.
- ساعدت المواد الغذائية التي تحتويها عليقة مسحوق أوراق بادرات الشعير في تحسين صحة فئران التجارب كما إزدادت سرعة نموها مقارنه بالفئران التي غذيت على العلائق الأخرى.
- التمثيل الحيوي لبيتاكاروتين أوراق بادرات الشعير كان مرتفعا في فئران التجارب عنه في البيتاكاروتين الصناعي.
- مستويات فيتامين (أ) كانت مرتفعة في بلازما الدم وكبد الفئران المغذاه على عليقة مسحوق أوراق بادرات الشعير عنه في الفئران المغذاه على عليقة البيتاكاروتين الصناعي.
- استخدام عصير أوراق بادرات الشعير لتدعيم المشروبات والعصائر كان له أثر واضح على زيادة مستويات البيتاكاروتين في مشروب الشعير وعصير الجوافة.