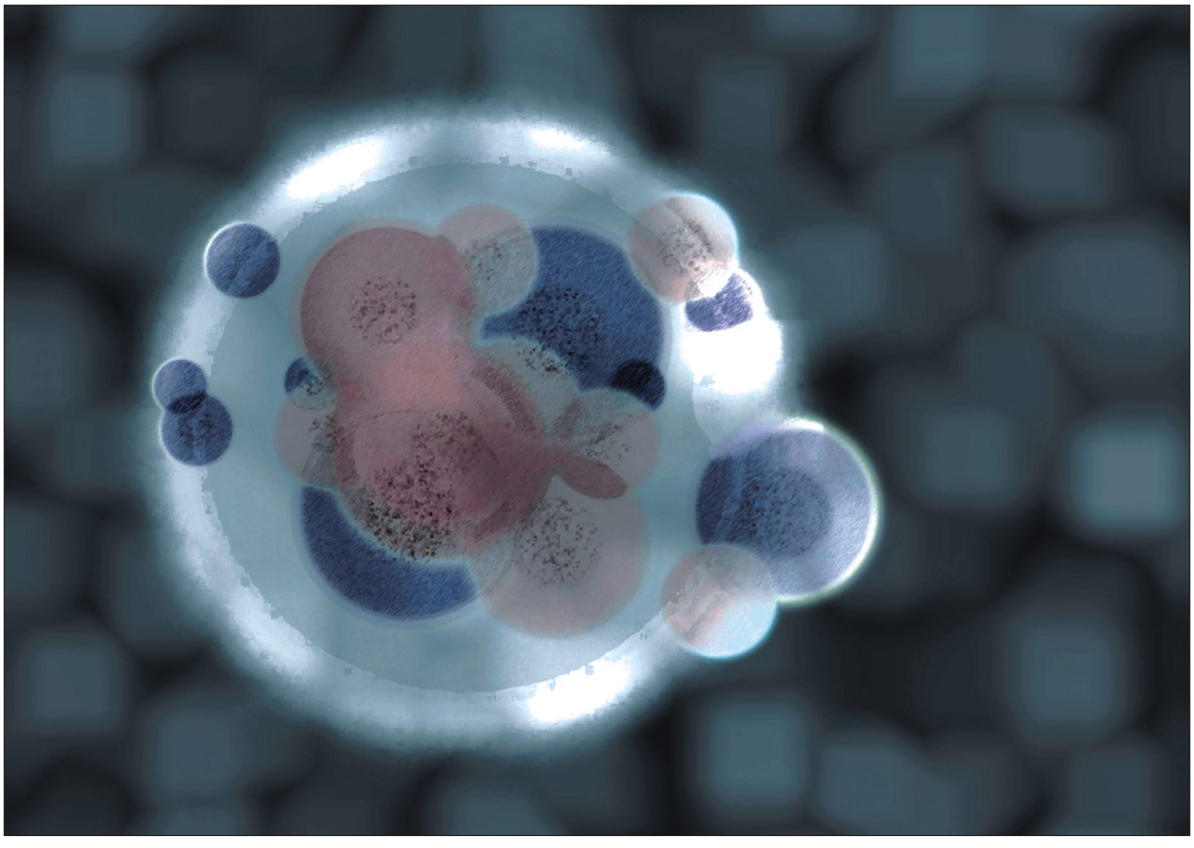

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Research Article
Effect of Natural Tal-enzyme on Functional Foods for Tyrosinemia Treatment in Mice Fed on

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Abstract

Background and Objective: The cornerstone management options for tyrosinemia treatment are tyrosine and phenylalanine low diet, plus nitisinone (2-[2-nitro-4-trifluoromethylbenzoyl]-1,3-cyclohexanedione) (NCTB). Tyrosine ammonia lyase (TAL) converts tyrosine to harmless metabolites, i.e., P-coumaric acid and ammonia. This study aimed to produce functional foods using tyrosine ammonia-lyase enzyme (TAL) for tyrosinemia treatment through feeding of female mice as animal model. **Materials and Methods:** Extracted TAL enzyme was used to treat egg white and mushroom flour to produce functional foods for tyrosinemia treatment. These functional foods were used to examine their effectiveness on food quality by estimation of color characteristics and determination of tyrosine concentrations. Moreover, determination of the levels of tyrosinemia related-genes expression and DNA damage were carried out. **Results:** This study found that treated egg white and mushroom flour with TAL enzyme were stable in color. Tyrosine reduction percentages in female mice fed Tal-enzyme treated egg white and Tal-enzyme treated mushroom flour were 72.3 and 30.96%, respectively, compared with untreated diets using tandem mass spectrometry. In addition, the expression levels of tyrosine aminotransferase (TAT) and 4-hydroxyphenylpyruvate dioxygenase (HPD) genes were remarkably elevated in mice feeding on TAL-egg white than other groups. Also, the DNA damage rate in mice fed on TAL-egg white showed lowest was decreased significantly compared with other groups. **Conclusion:** Addition of TAL enzyme reduced tyrosine to egg white and mushroom flour could be utilized and applied as functional food for tyrosinemia treatment regimes.

Key word: Tyrosinemia, tyrosine ammonia lyase enzyme, egg white, mushroom flour, gene expression, DNA damage

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

An autosomal recessive genetic disorder called Tyrosinemia, which is characterized by an elevation of the amino acid tyrosine in the blood. In this case the tyrosine and its byproduct are accumulating in organs and tissues and lead to dangerous medical problems especially if it left without treatment[1].

Tyrosinemia is observed in three types: Tyrosinemia type I (MIM276700), the severe form, resulting from a lacking of the enzyme fumarylacetoacetate hydrolase (FAH). Its medical signs include failure to thrive, diarrhea, jaundice and lead to kidney and liver failure[1]. Tyrosinemia type II (MIM276600) characterized by a shortage in the tyrosine aminotransferase enzyme activity (TAT) and the tyrosine blood level is higher than types I and III. Its clinical picture includes excessive tearing, eye pain and painful skin lesions on the palms and soles[2]. Tyrosinemia type III (MIM276710) is uncommon disorder, in which the enzyme 4-hydroxyphenylpyruvate dioxygenase is deficient. Its clinical features are intellectual disability, intermittent ataxia, without hepatorenal involvement or skin lesions[3].

A definitive diagnosis of tyrosinemia is based on clinical examination and laboratory test, which requires the confirmation of increased tyrosine by using tandem mass spectrometry. Detection of high levels of succinylacetone and the metabolites 4-OH-phenylacetate, 4-OH-phenyllactate and OH-phenylpyruvate in urinary are considered as primary markers for tyrosinemia disease[1].

Tyrosine is found in food and as well get internally from phenylalanine as a metabolite.

For treatment, it is important to act quickly to prevent the clinical symptoms especially liver dysfunction in tyrosinemia type I. So, the current medical strategy is based on two components: (1) The use of nitisinone to block the formation of fumarylacetoacetate and succinylacetone in tyrosinemia type I, whereas, close to 90% of patients are reported to react with nitisinone as long as the treatment initiated early[4-6], (2) A lifelong low tyrosine and phenylalanine diet to minimize the tyrosine level that needs to be metabolized in three types of tyrosinemia[4-7].

Tyrosine Ammonia Lyase (TAL) enzyme is a way to treat Tyrosinemia' patients by producing of p-coumaric acid through the biotransformation of L-tyrosine[8] with liberate of ammonia, in an irreversible reaction. Young *et al.*[9] and Guruprasad *et al.*[10] found that the TAL enzyme is one of the main enzymes that participate in phenol biosynthesis pathway in plants and it is only found in plants.

In higher plants, Phenylalanine ammonia lyase enzyme (PAL, EC 4.3.1.24) and tyrosine ammonia lyase enzyme (TAL, EC 4.3.1.23) are the essential enzymes in the biosynthesis of phenolic compounds. The PAL is a main enzyme in the pathway of phenylpropanoid compound biosynthesis and deaminates the aromatic amino acids phenylalanine. The TAL is a predominantly occurring enzyme in Gramineae which deaminates L-tyrosine forming coumaric acid. However, a few reports about its occurrence in dicotyledones were published[9,11,12]. Dogbo *et al.*[13-15] estimated that the tyrosine ammonia-lyase (TAL, EC 4.3.1.5) and phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) enzyme activities are participating in the biosynthesis of phenylpropanoids in cassava cells extracted with salicylic acid (SA). These results displayed that PAL enzyme is different from TAL enzyme and the both enzymes are participated in the phenylpropanoids biosynthesis in cassava.

There are few articles that extracted TAL enzyme from plants and discussed its ability to treat tyrosinemia. Bhattacharjee *et al.*[16] reported that TAL enzyme was extracted from *Plectranthus amboinicus* with high enzyme activity levels. Also, they pointed that extracted TAL can be effectively utilized for the treatment of Tyrosinemia II in the future[16].

In the same line, Guruprasad *et al.*[10] extracted TAL enzyme from *Clitoria ternatea* Linn. and discussed the ability of TAL enzyme in the treatment of tyrosinemia II.

Moreover, a United States patent carried out by Huisman *et al.*[17] provided methods for treating and preventing the signs of tyrosinemia by using the compositions comprising the engineered TAL polypeptides as therapeutic purpose.

Few of research papers were carried out in the domain of TAL enzyme extract to decrease tyrosine level in food processing. In this work, crude tyrosine ammonia-lyase enzyme (TAL) was extracted from banana fruit (*Musa cavendishii* L., cv. Enana) and used to reduce tyrosine in food to be utilized as tyrosinemia functional food and in female mice fed on. To perform this goal egg white and mushroom flour food treated with TAL enzyme were used. So, several parameters such as color characteristics, determination of tyrosine concentrations in untreated and TAL-treated egg white and mushroom flour, as well as in female mice fed on were assessed. Moreover, expression alteration of tyrosine metabolism related genes and DNA damage were investigated.

MATERIALS AND METHODS

Place and duration of study: This study was carried out at National Research Centre, Egypt, between December, 2015 and March, 2017.

Drugs and chemicals: Trizol was bought from invitrogen (Carlsbad, CA, USA). The reverse transcription and PCR kits were obtained from fermentas (Glen Burnie, MD, USA). The SYBR Green Mix was purchased from Stratagene (La Jolla, CA, USA). Also, Reagent Kit for the LC-MS/ MS analysis of amino acids and acylcarnitines was purchased from MassChrom (GmbH, Germany). L-tyrosine was obtained from Sigma (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade and obtained from standard commercial suppliers.

Fruit samples: Banana (*Musa cavendishii*, cv. Enana) fruits cultivated in Egypt were obtained from a local market in Cairo. Banana fruits were placed in refrigerator at 3-4°C for a maximum 2 h at the day of use. The collected banana fruits were washed, peeled and pulped using a blender (modelNo.:SHB 3093, made in PRC).

Enzyme preparation

Extractions of TAL enzyme: The TAL-enzyme was extracted using the method of Lister *et al.*[18] and Montero *et al.*[19].

Assay of TAL-enzyme activity: The TAL-enzyme activity was assayed by a little modification on the method of Peixoto *et al.*[20] and Nita-Lazar *et al.*[21]. One unit of the TAL-enzyme activity was explained as the amount of enzyme that proceed a change of 0.001 in absorbance per hour.

Food processing

Egg white processing: Red chicken egg was obtained from a commercial market, Cairo, Egypt. Freshly egg white separated from the egg yolk. Afterwards, egg white was treated by 1% TAL-enzyme and incubated at room temperature for 2 h then 0.05% SO_2 was added to stop of the activity of TAL-enzyme.

Mushroom flour production: Fresh mushroom (*Pleurotus ostreatus*) was obtained from a commercial mushroom farm at Cairo, Egypt. The fresh mushroom obtained was pre-treated with TAL-enzyme (1%) and incubated at room temperature for 2 h then 0.05% SO_2 was added to stop the activity of TAL-enzyme and dried in an oven (SheL, LAb 1370 FX, Shel Don manufacturing, Inc. and Germany) at 60°C for 8 h. Dehydration was continued until the moisture content of mushroom slices reached about 14%, using modified method of Parab *et al.*[22].

Nutrition experiment: Fifty Swiss albino female mice were obtained from the animal house of the National Research Centre, Dokki, Giza, Egypt and allocated in 5 groups of 10 mice each as follows: Group 1: Animals used as a control group with normal feeding (22% protein, 3.48% fat and 3.71% dietary fiber as 60% yellow corn, 34% soy bean, calcium phosphate, sodium chloride, calcium chloride, oil soya, vitamins, antitoxins and molasses). Group 2: Animals fed on raw egg white without any treatment. Group 3: Animals fed on TAL enzyme treated egg white. Group 4: Mice fed on dried mushroom flour without any treatment. Group 5: Mice fed on dried TAL enzyme treated mushroom flour.

All groups were fed with 0.5 mg kg^{-1} b.wt., for one month according to Yang *et al.*[23] and Maeta *et al.*[24]. Animals were housed under temperature and light-controlled conditions with standard laboratory rodent chow and water provided *ad libitum*. Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (publication no. 85-23, revised 1985).

Determination of amino acids in egg white and mushroom flour: All amino acids were analysis for untreated and TAL-enzyme treated egg white and mushroom flour samples according to the method of AOAC[25]. The following formula was used to calculate the tyrosine reduction:

$$\text{Reduction of tyrosine (\%)} = \frac{\text{Tyrosine content in TAL-enzyme treated}}{\text{Tyrosine content in untreated sample}} \times 100$$

Color characteristics determination of egg white and mushroom flour: Color of untreated and TAL-enzyme treated egg white and mushroom flour samples was measured with a color difference using a spectro-colorimeter (Tristimulus Colour Machine) with the CIE lab color scale (Hunter, Lab Scan XE-Reston VA, USA) in the reflection mode (International Commission on Illumination) as mentioned by Hunter[26] and Sapers and Douglas[27]. The color values were expressed as L* (lightness or brightness/ darkness), a* (redness/greenness) and b* (yellowness/blueness). The Hue angle (H)*, Chroma (C)*, E and Browning Index (BI) was calculated according to the method of Palou *et al.*[28]. Where, all values were recorded as the mean of triplicate readings.

Non-enzymatic browning determination: Non-enzymatic browning was measured spectrophotometrically by 4054-UV/Visible spectrophotometer (LKB-Biochrom Comp., London, England) as absorbance at 420 nm using ethanol as a blank according to the method of Stamp and Labuza[29] and Cohen *et al.*[30].

Determination of tyrosine concentration in dried blood spot: Blood samples from mice were spotted on Guthrie cards, left to dry at room temperature for at least 24 h. The procedures of extraction and mass analysis were applied according to instruction of manufacture chromsystems, the concentration of the analyte in the sample was calculated according to: The concentration C analyte (μmol L^{-1}) = (signal intensity of analyte A in the sample spectrum/single intensity of the internal standard IS in the spectrum of sample) × sample related concentration C of the internal standard.

Mass spectrometry (MS): Waters Xevo triple quadrupole mass spectrometer (Milford, USA) equipped with electrospray ionization (ESI) source in positive ion mode was used for analysis. The MassLynx V4.1 and NeoLynx were used for data acquisition and processing of MS analysis. A designed MS/MS program for automatic profiling of acylcarnitines and amino acids was used.

Gene expression analysis

RNA extraction: The total RNA from liver tissues of female mice was extracted using TRIzol® Reagent (Invitrogen, Germany) according to the manufacturer's instructions with minor modifications. Aliquots of isolated RNA were used immediately for Reverse Transcription (RT), otherwise they were stored at -80°C[31].

Reverse transcription reaction: Complete Poly(A)$^+$ RNA isolated from liver tissues was reverse transcribed (RT) into cDNA in a total volume of 20 μL using RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, Germany). The reaction tubes containing RT preparations were flash-cooled in an ice chamber until being used for cDNA amplification through real time polymerase chain reaction (qPCR)[32,33].

Real time-PCR: One step's real-time PCR cycler (Applied Biosystem, USA) was used to determine the liver cDNA copy number. The PCR reactions were set up in 25 mL reaction mixtures containing 12.5 mL 1×SYBR® Premix Ex *Taq*TM (TaKaRa, Biotech. Co. Ltd.). The sequences of specific primer of tested genes were as follows: F: 5'-tgg cca atc ctg gac aga ac-3', R: 5'-tgt tga cca cga gac aag c-3'; for tyrosine aminotransferase; F: 5'-gta ctg cgt agg aga tga gg-3', R: 5'-ggt gag aca ctt gga aaa cc-3' for Maleylacetoacetate isomerase; F: 5'-ggt gga gca aga caa att tg-3', R: 5'-gat tcc tcg tag ttg gtc ac-3' for 4-Hydroxyphenylpyruvate dioxygenase. The tested genes were normalized on the b-actin gene, F: 5'-GGA GAT TAC TGC CCT GGC TCC TA-3', R: 5'-GAC TCA TCG TAC TCC TGC TGC-3'. The relative quantification of the target to the reference was determined by using the $2^{-\Delta\Delta CT}$ method[34,35].

Comet assay: Isolated liver tissues of all groups of rats were subjected to the modified single-cell gel electrophoresis or comet assay[36]. The slides were stained with ethidium bromide and examined using a fluorescence microscope with a green filter at ×40 magnification. For each animal about 100 cells were examined to determine the percentage of cells with DNA damage that appear like comets. The cells were randomly selected and were visually assigned a score on an arbitrary scale of 0-3 based on perceived comet tail length migration and relative proportion of DNA in the nucleus[37].

RESULTS

Activity of tyrosine ammonia lyase (TAL) enzyme: The results in Fig. 1 revealed that the activity of tyrosine ammonia lyase (TAL) enzyme increased in fresh mushroom sample compared with fresh egg white sample. Moreover, the activity of TAL enzyme in mushroom flour showed higher values than in egg white.

Tyrosine concentration "Unit/g/h" in Food: Data in Fig. 2 outlined the tyrosine concentration in untreated, treated egg white and mushroom flour with TAL-enzyme using amino acid analyzer. The content of tyrosine was higher in fresh egg white than in mushroom flour samples. However, the tyrosine content was decreased due to the activity of TAL-enzyme in treated egg white more than in mushroom flour samples.

Color characteristics, parameters and non-enzymatic browning (A420 nm) in food: Color values (L*, a*, b*, C*, E, BI and R420 nm, reflectance) were increased, while H* values were decreased in TAL-treated egg white and mushroom flour samples compared with untreated samples (Table 1). While, the addition of TAL enzyme resulted in an increase in the L*, a*, b*and E -values but decrease in H*-value.

Tyrosine concentration in mice blood spot: The Table 2 summarized the difference in tyrosine concentrations under the effect of TAL enzyme in blood spot of female mice fed on untreated and treated mushroom flour and egg white.

The results clarified that, tyrosine concentration was dropped from 151.02-41.84 μmol L^{-1} in case of egg white, while in mushroom flour it decreased from 109.54-75.63 μmol L^{-1} under the treatment of extracted TAL-enzyme. Which were lower than control groups.

Table 1: Effect of TAL-enzyme on color characteristics, parameters and non-enzymatic browning (A420 nm) in egg white and mushroom flour (n = 3)

Parameters	L*	a*	b*	E	400 nm	420 nm	C*	H*	BI
Egg white	11.72	-1.10	1.49	75.63	1.8	1.43	0.5	53.52	-0.00648
STDEV	0.24	0.07	0.07	0.14	0.12	0.06	0.10	2.82	1.62
Mush flour	70.33	4.16	25.41	31.29	14.55	17.43	25.57	80.71	56.01
STDEV	0.20	0.11	0.27	0.12	0.20	0.07	0.27	0.33	1.62
TAL-egg white	14.23	-2.37	2.32	73.6	1.67	1.86	0.71	44.37	7.24
STDEV-EW	0.07	0.06	0.25	0.28	0.06	0.05	0.61	2.53	1.70
TAL-Mush flour	53.16	5.54	26.38	76.51	14.36	17.50	26.59	78.14	119.4
STDEV	1.24	0.09	0.23	0.09	0.16	0.17	0.23	0.09	1.01

±Standard Error (SE): Standard Deviation (SD)/SQR^2 (n), where, n = 3, L*: Lightness or brightness/darkness), a*: Redness/greenness), b*: Yellowness/blueness), H*: Hue angle), C*: Chroma, E: Difference between color values and BI*: Browning index

Table 2: Effect of TAL-enzyme on tyrosine concentration by tandem mass spectrometry with non- derivatised method in egg white and mushroom flour

Treatments	Mice number	Tyrosine concentration (μmol L^{-1})	Reducing (%)	ST. DEV	St. Error
Control	10	199.58	-	±15.5	±8.95
Egg white untreated	10	151.02	-	±15.54	±8.98
Egg white TAL-treated	10	41.84	72.3	±11.0	±6.36
Mushroom flour untreated	10	109.54	-	±13.5	±7.80
Mushroom flour TAL-treated	10	75.63	30.96	±15.25	±8.82

Standard Deviation (SD): SQRT ($\sum$ (x-x-)^2/n), Standard Error (SE): SD/SQRT(n), n = 3

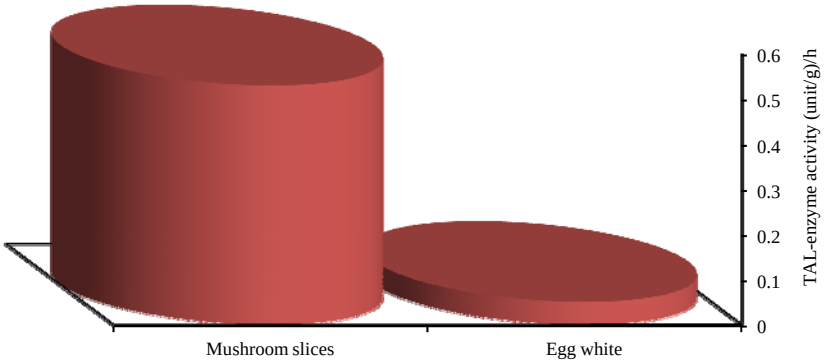

Fig. 1: Tyrosine ammonia lyase activity (Unit/g)/h in fresh egg white and mushroom slices

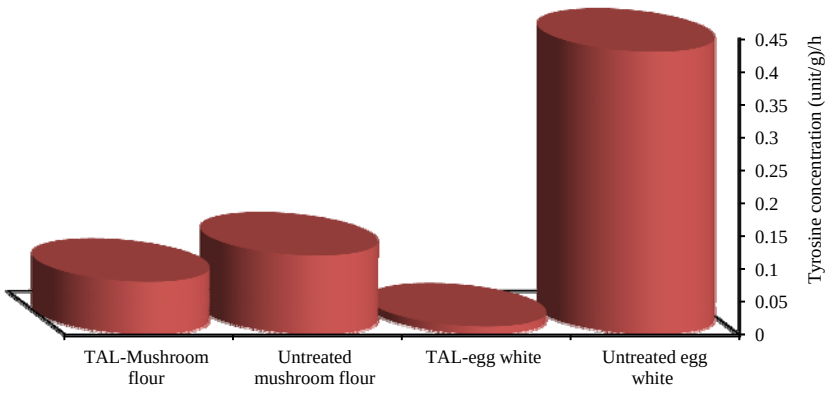

Fig. 2: Effect of TAL-enzyme on tyrosine concentration in egg white and mushroom flour by amino acid analyzer

Table 3: DNA damage in hepatic tissues of female mice fed diet contained untreated or treated egg white and mushroom analyzed by comet assay

	No. of cells		Class**				
Treatments	Analyzed*	Comets	0	1	2	3	DNA damaged cells (%)
Control	500	24	476	17	7	0	4.8±0.2
Egg white untreated	500	33	467	18	14	1	6.6±0.1
Egg white TAL- treated	500	21	479	15	6	0	4.2±0.3
Mushroom flour untreated	500	38	462	27	9	2	7.6±0.4
Mushroom flour TAL-treated	500	36	464	22	13	1	7.2±0.3

*Number of cells examined per a group (n = 5), **Class 0: No tail, 1: Tail length<diameter of nucleus, 2: Tail length between 1X and 2X the diameter of nucleus, 3: Tail length>2X the diameter of nucleus

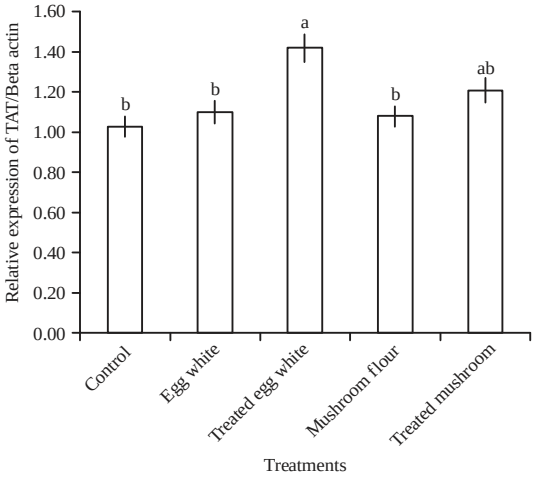

Fig. 3: Alteration of Tyrosine aminotransferase-mRNA (TAT-mRNA) in liver tissues of female mice fed diet contained untreated or treated egg white and mushroom

Data are presented as Mean±SEM, [a,b]Mean values within tissue were insignificantly different (p>0.05)

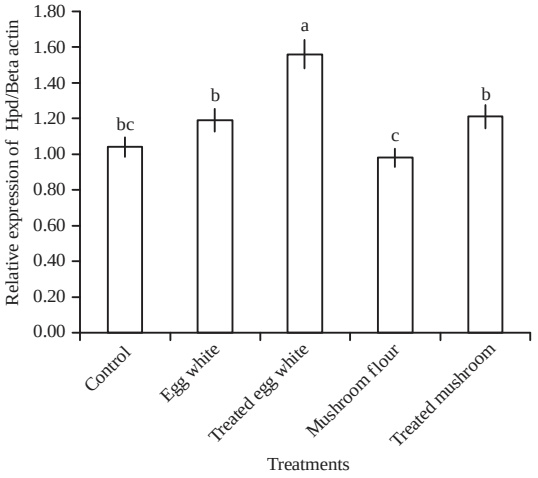

Fig. 4: Alteration of 4-hydroxyphenylpyruvate dioxygenase-mRNA (Hpd-mRNA) in liver tissues of female mice fed diet contained untreated or treated egg white and mushroom

Data are presented as Mean±SEM, [a,b,c]Mean values within tissue with unlike superscript letters were significantly different (p<0.05)

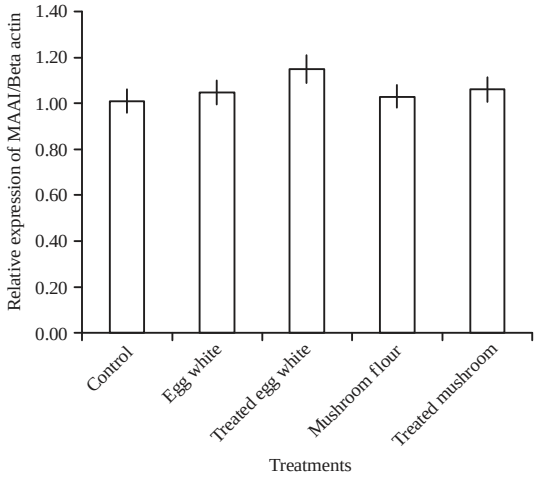

Fig. 5: Alteration of Maleylacetoacetate isomerase-mRNA (MAAI-mRNA) in liver tissues of female mice fed diet contained untreated or treated egg white and mushroom

Data are presented as Mean±SEM

Furthermore the results displayed, the reduction (%) of tyrosine concentration between untreated group and TAL enzyme-treated group was 72.3% in egg white and 30.96% in mushroom flour Table 2.

Expression of tyrosine metabolism related genes: Figure 3-5 summarized the expression levels of tyrosine metabolism related genes [Tyrosine aminotransferase (TAT), Maleylacetoacetate isomerase (MAAI), 4-Hydroxyphenylpyruvate dioxygenase (Hpd)] in liver tissues of female mice fed diet contained untreated or treated egg white and mushroom.

The results exhibited that the levels of TAT and Hpd genes expression were decreased in liver tissues of mice fed standard diet as control group compared with female mice in other groups (Fig. 3, 4). In the same line, expression of TAT and Hpd genes exhibited that there were no significant differences between female mice fed diet contained untreated egg white and those fed untreated mushroom compared with control mice (Fig. 3, 4). However, the levels of TAT and Hpd expression

genes in female mice fed on treated egg white were significantly higher ($p<0.05$) than control and other treated mice (Fig. 3, 4).

However, the expression levels of MAAI gene in liver tissues of mice fed on diet containing treated egg white was increased significantly ($p>0.05$) compared with other groups. In addition, the expression levels of MAAI gene in liver tissues of control mice fed standard diet were relatively similar to all other treated mice without significant differences (Fig. 5).

DNA damage: The results of DNA damage showed that female mice fed on diet containing untreated or treated mushroom and egg white showed insignificant varying of DNA damage (Table 3). Rate of DNA damage in liver tissues of mice fed on diet with treated egg revealed lowest rate of DNA damage in comparison with control and other groups including control mice. While, DNA damage rate was increased slightly in female mice having a diet contained untreated egg white in comparison with those in control mice (Table 3).

In contrary, supplementation of female mice with diet contained untreated mushroom exhibited highest rate of DNA damage but without significant differences compared with other groups (Table 3). In addition, the rate of DNA damage in female mice with treated mushroom was increased insignificantly, compared with those in control mice (Table 3).

DISCUSSION

The present study aimed to use crude tyrosine ammonia-lyase enzyme (TAL) extracted from banana fruit (*Musa cavendishii* L., cv. Enana) to reduce tyrosine in food to produce tyrosinemia functional food.

The activity of TAL enzyme in mushroom flour showed a higher activity than in egg white due to TAL enzymes are in charge of the different changes in chemical compositions, which will influence the quality characteristics and deterioration of fresh or processed mushroom. Also, TAL-enzyme can be used to reduce tyrosine in tyrosinemia disease humans as the tyrosine pathway is similar in production of melanin pigment. Banana (*Musa cavendishii*, cv. Enana) fruits showed good activity of TAL, which has similar function to that of tyrosine ammonia lyase enzyme found in fruits and hence this enzyme can be used to treat tyrosinemia in humans.

The results of this study revealed that addition of TAL enzyme resulted in an increase in the color values compared with other groups. This is perhaps due to release of carotenoids and anthocyanins as a result of TAL enzyme addition. The addition of TAL enzyme resulted in an increase in the a* and b*-values but decrease in L*-value and E. Such a trend is in agreement with previous studies of Ozoglu and Bayindirli[38]. These results exhibited that the increased browning was related to PPO enzyme activity in mushroom flour samples than in fresh egg white samples. According to the current results, the changes of color by increasing of the a* and BI value in mushroom flour were in high relation with browning measurement. Other color parameters, like hue angle and chroma, also cleared that egg white and mushroom flour caused a slight color changes. These results are in consistent with those of Palou *et al.*[28] and Ozoglu and Bayindirli[38], who investigated the effect of anti browning agents on inhibition of enzymatic browning in cloudy apple juice and found similar results. Furthermore, this study found that mushroom flour samples had the higher increased in color as a nonenzymatic browning (A420nm) compared with fresh egg white samples. The increasing in color browning values (as A420 nm) could be resulting from the reaction occurred between active carbonyl groups and amino groups (Maillard reaction) drying process. So, it could be concluded that pretreated drying process did not affect non enzymatic browning with optical density (A420 nm). However, the values of non enzymatic browning (A420 nm) confirmed that the data obtained for drying process showed higher values in mushroom flour compared to fresh egg white.

As mentioned previously, TAL-enzyme is a way to reduce tyrosine concentration through deaminates it and produces harmless metabolites[8], which is clear noticed in the difference of tyrosine reduction percent in blood of mice fed on treated TAL and untreated egg white (72.3%) and mushroom flour (30.96%). Furthermore, this reduction is confirmed with the decreasing of tyrosine concentration on food using amino acid analyzer (egg white and mushroom flour).

The high differences in tyrosine concentration between treated and untreated food especially in egg could be explained due to the presence of TAL and PAL enzyme activities in the extracted solution[39,40]. So, TAL enzyme acts to metabolized tyrosine, which derived from food components. While, PAL tend to metabolize phenylalanine, which is reflecting another source of tyrosine[4] that leads to high decrease in tyrosine concentration. The present study revealed that applying this treatment into different types of food, can give a new route for diet treatment of tyrosinemia patients.

The effect of TAL treated food on the expression of tyrosine metabolism related genes showed that levels of TAT and Hpd gene expression were markedly elevated in mice fed on diet contained treated egg white than control and other treated mice. Additionally, the levels of MAAI gene expression in liver tissues of mice feeding with treated egg white was

higher than other groups, however, the expression values were insignificant. In the same line with the current results, Tanaka *et al.*[41] reported that levels of TAT, MAAI and Hpd genes expression were low in mice strain having high serum tyrosine level. However, they reported that levels of TAT, MAAI and Hpd expression increased with homogentisate (HGA) treatment which is enzyme plays important role in tyrosine catalyze. Additionally, Tanaka *et al.*[41] reported that expression level of Hpd gene was higher than the expression of TAT and MAAI in liver tissues of mice treated with HGA.

The interest in finding novel anti- tyrosinemia factors from natural sources with antioxidant activity is of great interest. Since the key role of these compounds on tyrosine metabolism as tyrosinase promoter have become increasingly important for medicinal and pharmaceutical applications. In this study the impact of treated egg white and mushroom as promising natural products against tyrosinemia was evaluated.

As known that tyrosinemia patients show various clinical symptoms such as liver damage[1,2], the protective effect of treated egg white and mushroom against DNA damage in liver tissues was evaluated in the current study. The present findings showed that the DNA damage rate in liver tissues of female mice on treated egg white diet revealed lowest DNA damage compared with control other groups including control mice. The feedback of present study are in same line with Ishikawa *et al.*[42], who reported that egg compound protected cells from hydroxyl radical formation and consequently protect DNA structure from damage. They found that the protection mechanism against hydroxyl radical formation is attributed to phosvitin which is rich in egg materials. So, phosvitin could be possible to protect liver DNA structure from damage and also might be play an important role in regulation the expression of tyrosine metabolism related genes in liver tissues of female mice fed diet contained treated egg white.

CONCLUSION

In case of implementation of new treatment tool different items should be taken in mind as potential risks, scientific doubts, overheads and sociological outcomes for the kids and their families. Depending on this study, the addition of TAL enzyme in egg white and mushroom flour improved the stability of color characteristics, reduce tyrosine concentration and protect the cells against genetic toxicity that can be utilized and applied in wide range of foods for tyrosinemia patients as a functional food.

SIGNIFICANCE STATEMENT

The current study represents a novel egg white and mushroom flour diet containing tyrosine ammonia-lyase enzyme (TAL) extracted from banana fruit as a functional food for Tyrosinemia which has the potentiality to protect the patients with excess of amino acid tyrosine. This study will help the scientists in pharmacy and in functional food to detect and extract natural products from plants. These natural compounds could be as promising functional foods to save the patients against the side effects of synthesized drugs against tyrosinemia. Thus, incoming active ingredients from plants may be considered at new structure of functional food discovery.

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