http://www.pjbs.org



ISSN 1028-8880

# Pakistan Journal of Biological Sciences



Asian Network for Scientific Information 308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

ISSN 1028-8880 DOI: 10.3923/pjbs.2021.366.373



## Research Article Functional Cupcake for Preventing Vitamin A Deficiency and Correlated Anemia and Oxidative Stress

<sup>1</sup>Ahmed M.S. Hussein, <sup>2</sup>Rasha S. Mohamed, <sup>2</sup>Karem Aly Fouda, <sup>1</sup>Manal F. Salama and <sup>2</sup>Mona M. Hussein

## **Abstract**

**Background and Objective:** Vitamin A Deficiency (VAD) is a critical public health problem that affects the health of kids worldwide and may induce anemia and oxidative stress. The current study aimed to pre-clinically assess the effect of a cupcake, prepared to be served for primary school children, on vitamin A deficiency and related anemia and oxidative stress in rats. **Materials and Methods:** Flour of flash orange sweet potatoes, as a rich source of pro-vitamin A, was used to prepare the cupcake. The chemical composition, amino acids and sensory evaluation of the cupcake were done. The biological evaluation was carried out using 18 weaning rats in three groups (control group, vitamin A-deficient group and vitamin A-deficient group fed on a diet fortified with 20% of the prepared cupcake for two months). **Results:** The results indicated the high value of vitamin A in the prepared cupcake. Excellent sensory characteristics were noticed. Feeding on the VDA diet fortified with the prepared cupcake suppressed the reduction in Retinol-Binding Protein (RBP), hemoglobin and iron. Total Iron Binding Capacity (TIBC) increased in the VAD group. Also, feeding on the prepared cupcake suppressed the reduction in Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) and the elevation of Malondialdehyde (MDA). **Conclusion:** It can be suggested that the prepared cupcake is promising in preventing of vitamin A deficiency and related anemia and oxidative stress. Thus, the prepared cupcake may be efficient for children to prevent vitamin A deficiency.

Key words: Cupcake, sweet potatoes, vitamin A deficiency, pro-vitamin A, vitamin A and iron, anemia, oxidative stress

Citation: Hussein, A.M.S., R.S. Mohamed, K.A. Fouda, M.F. Salama and M.M. Hussein, 2021. Functional cupcake for preventing vitamin A deficiency and correlated anemia and oxidative stress. Pak. J. Biol. Sci., 24: 366-373.

Corresponding Author: Rasha S. Mohamed, Department of Nutrition and Food Sciences, Research Centre, Dokki, Cairo, Egypt Tel: +201014196767

Copyright: © 2021 Ahmed M.S. Hussein et al. This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

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

<sup>&</sup>lt;sup>1</sup>Department of Food Technology, National Research Centre, Dokki, Cairo, Egypt

<sup>&</sup>lt;sup>2</sup>Department of Nutrition and Food Sciences, Research Centre, Dokki, Cairo, Egypt

## **INTRODUCTION**

Vitamin A (VA) or retinol is an essential fat-soluble vitamin. The term vitamin A refers to a group of retinoid compounds that have characteristics similar to those of retinol, including retinal, retinyl esters and retinoic acid and these are only abundant in animal products while carotenoids, the most abundant in plants, can convert to retinol after oxidative degradation in the animal organism<sup>1</sup>. Vitamin A contributes to normal visual function, synthesis of immunoglobulin normal growth and reproduction process<sup>2,3</sup>. VA Deficiency (VAD) is a critical public health problem that affects the health of about 190 million kids worldwide<sup>4</sup>. According to Galal et al.5, the prevalence of VA deficiency among primary school children in Egypt was about 30% due to the poor quality of diet and micronutrient deficiencies. Vitamin A deficiency induces elevation of lipid peroxidation and oxidative stress<sup>6</sup>. Vitamin A deficiency can moderate iron metabolism and cause iron deficiency and spleen iron holding via the modulation of hepcidin (the hormone that regulates the quantity of iron that is liberated into the circulation from enterocytes and spleen macrophages) expression<sup>7</sup>. It was demonstrated by several studies that supplementation with vitamin A positively improved iron absorption and mobilization8-10.

Orange Flash Sweet Potatoes (OFSPs) are rich in beta-carotene (pro-vitamin A) which converts to vitamin A in the body. Besides, OFSPs act as antioxidants and contain fiber, minerals and vitamins. Nogueira *et al.*<sup>11</sup> used OFSPs in the preparation of bakery products, pasta and foods for the school children to improve their health. Van Jaarsveld *et al.*<sup>12</sup> reported that the 53-day consumption of OFSPs supplied 150% of the Recommended Daily Allowance (RDA) of primary school children who suffering from vitamin A deficiency and declared that the consumption of OFSPS improved the level of vitamin A.

Therefore, the current study aimed to pre-clinically assess the effect of a cupcake prepared, to be served for primary school children, from the flour of flash orange sweet potatoes, as a rich source of pro-vitamin A, on vitamin A Deficiency (VAD) and related oxidative stress and iron deficiency in rats.

## **MATERIALS AND METHODS**

**Study area:** The study was carried out at the Department of Nutrition and Food Sciences and Department of Food

Technology, National Research Centre, Egypt from September, 2019-February, 2020.

**Materials:** Orange sweet potato, wheat flour (72% extraction rate), sugar, butter, eggs, cocoa powder, baking powder and full cream milk were obtained from the local market at Giza Egypt.

**Chemicals:** All chemicals and reagents were of high-quality analytical grade.

**Animals:** Weaned male Wistar rats (21 days old) of 58.05±10.72 g as mean±SD were obtained from the animal house of National Research Centre, Cairo, Egypt. Animals were kept individually in stainless steel cages under standard laboratory conditions (23-25°C, 12 hrs light/dark cycle) and with free access to diet and water. This study has been carried out according to the Medical Research Ethics Committee, National Research Centre, Cairo, Egypt and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Animal's diets: Animal diets were prepared according to AIN-93<sup>13</sup>. Balanced diet contained 12% protein supplemented from casein, 10% corn oil, 10% sucrose, 58.5% maize starch, 5% fiber, 3.5% AIN-93 salt mixture and 1% AIN-93 vitamin mixture. Vitamin A-deficient diet contained 12% protein supplemented from vitamin free-casein, 10% corn oil, 10% sucrose, 58.5% maize starch, 5% fiber, 3.5% AIN-93 salt mixture and 1% AIN-93 vitamin mixture (without vitamin A). Prepared cake containing-diet was the same components of vitamin A-deficient diet with adding 20% of the prepared cupcake. The contents of protein, fat, crude fiber and carbohydrate of the 20% prepared cupcake were reduced from casein, corn oil, cellulose and starch.

## Methods

**Preparation and drying of sweet potatoes:** Sweet potatoes were washed and cut into thin slices at around 1 mm thickness and rinsed in tap water for 1 min then soaked in 0.2% sodium metabisulphite solution for 15 min. The thin slices of sweet potatoes were freeze-dried at 10<sup>-1</sup> m bar and 30°C for 72 hrs, (Edwards Modulyo Freeze Dryer, United Kingdom). Then the dried sweet potatoes were ground into a fine powder and kept at -20°C until further analysis.

**Preparation of the cupcake:** Cupcake ingredients were as follows: Wheat flour (16%), sweet potatoes powder (13%), sugar (14%), egg (16%), cocoa powder (1%), full cream milk (23%), butter (15%) and baking powder (2%). The dried ingredients were combined except sugar. The sugar was added to the butter and beaten for 3 min. Eggs and milk were beaten and added gradually to the mixture and creaming for 2 min. Then cupcake dough was weighed and put in a cupcake container and baked in an oven at 180°C for 7 min.

**Nutritional analysis of the cupcake:** The proximate composition of the prepared cupcake was determined using the standard procedures as described by Hussein *et al.*<sup>14</sup>. Vitamin A was determined using the HPLC technique according to Panfili *et al.*<sup>15</sup>. Fe and Zn were determined by using Atomic Absorption Spectrometry (pyeunicm Model 3300, PyeUnicam Ltd., Cambridge, England) at 422 nm as described by Hussein *et al.*<sup>14</sup>. Amino acids were determined using an LC3000 amino acid analyzer (Eppendorf-Biotronik, Germany). B vitamins (B6, Folic acid and B12) were determined by the HPLC technique according to Vinas *et al.*<sup>16</sup>.

**Sensorial evaluation of the prepared cupcake:** The prepared cupcake was determined for its sensory characteristics (crumb color, crust color, flavor, tenderness, softness, moistness and taste). The evaluation was carried out by trained 15-member panelists from the staff members of the Department of Food Technology, National Research Centre, Dokki, Cairo, Egypt. Each panelist was provided with the sample in an unlabeled transparent cup under white lights. The general acceptability was calculated.

**Physical measurements of the prepared cupcake:** The height (cm) was measured in the center of the baked cupcakes. The volume of baked cupcake was measured by rapeseed displacement. The baked samples were weighted after removed from the pan and the specific volume was also calculated (cupcake volume/cupcake weight).

## Texture profile of the prepared cupcake (crumb firmness):

The crumb texture of the prepared cupcake was evaluated using a Stable Micro System TA-XT2 texture analyzer (Stable Micro Systems, Surrey, England) with a cylindrical aluminum sensor probe.

**Crust and crumb color of the prepared cupcake:** The crust and crumb color of the prepared cupcake was measured using Hunter lab color (model, CIE lab color scale, Scan XE-Reston VA, USA). (L\* lightness a\* redness and b\* yellowness) parameters were measured according to Hunter Apparatus.

**Design of the animal's study:** Eighteen rats were divided after one-week acclimatization into three groups (6 rats in each group) as follows:

- G<sub>1</sub>: Control normal (CN) group where rats received the balanced diet
- G<sub>2</sub>: Vitamin A-deficient (VAD) group where rats received the vitamin A-deficient diet for two months
- G<sub>3</sub>: Vitamin A-deficient+prepared cupcake (VDA+C) group where rats received the vitamin A-deficient diet fortified with 20% of prepared cupcake for two months

During the experiment, food intake was recorded daily. After two months (end of the experiment) total food intake, body weight gain and feed efficiency ratio (Bodyweight gain/total food intake) were calculated. Blood samples were collected under slight anesthesia from all rats after an overnight fast.

Biochemical analysis: The portion of the whole blood was analyzed for hemoglobin (Hb) according to Drabkin<sup>17</sup> using reagent kits obtained from Spinreact company (Spain) and the remaining blood was centrifugated for 10 min at 1000 g and serum of each rat was kept under -20°C until used. Erythrocytes (RBCs) were washed five times with cold saline and kept under -20°C until used. Fasting blood glucose levels were determined according to Trinder<sup>18</sup> using reagent kits obtained from Spinreact company (Spain). Serum iron and Total Iron Binding Capacity (TIBC) were determined according to Stookey<sup>19</sup> and Betts and Stuart<sup>20</sup>, respectively using kits obtained from Spectrum company (Egypt). Serum ferritin and Retinol Binding Protein (RBP) were determined using Eliza kits (Glory Science Co., Ltd. China). The activities of Aspartate Transaminase (AST) and Alanine Transaminase (ALT) were determined according to Reitman and Frankel<sup>21</sup>. The levels of creatinine and urea were determined using reagent kits obtained from Spinreact company (Spain) depending on Larsen<sup>22</sup> and Fawcett and Scott<sup>23</sup> in succession as indicators of kidney functions. RBCs malondialdehyde (MDA) and Glutathione Peroxidase (GPx) were determined according to

Ohkawa *et al.*<sup>24</sup> and Paglia and Valentine<sup>25</sup>, respectively. The activity of RBCs superoxide dismutase (SOD) was determined using the Eliza kit (SinoGeneclon Biotech Co., Ltd). All the colorimetric determinations were carried out using a UPVC spectrophotometer (Jasco V-730, serial No. A 112361798, Japan).

**Statistical analysis:** Statistical analyses were done using SPSS version 16. The results were expressed as mean $\pm$ standard error (SE) and analyzed statistically using one-way analysis of variance (ANOVA) followed by the Duncan test. The statistical significance of difference was taken as p $\leq$ 0.05. The correlation between iron and serum RBP at the 0.01 level was done.

## **RESULTS**

The chemical composition of the prepared cupcake (Table 1) showed that the protein content was 10.3%, the fat content was 14.5% and the carbohydrate content was 44.9%. The supplied calories were 351.2 Kcal. Also, the prepared cupcakes showed moderate light and weight in proportion to the volume. The cupcake recorded high scores for color, taste, moistness and softness. It could be observed that the cupcake samples were low in hardness, gumminess and chewiness (20.30, 7.22 and 4.62, respectively). Table 2 showed that sweet potato cupcakes with cocoa powder showed dark brown crust and crumb (the redness was 9.63 and 8.86 for crust and crumb, respectively). The results (Table 3) indicated that 100 g of cupcake provides the daily requirements of vitamin A, vitamin B6, folic acid and vitamin B12 for school children by 90, 40, 44 and 40%, respectively according to World Health Food (2016). Also, 100 g of cupcake provides 40.37% of iron and 56.25% of zinc from the daily requirement of school children according to World Health Food (2016). Results of the essential amino acids profile (Table 4) showed that the cupcake contained a different amount of essential amino acid, the highest one was tryptophan and the lowest one was leucine. Each gram protein of cupcake can achieve about 72.72 and 10.9% of the daily requirement from tryptophan and leucine as compared with FAO/WHO (1991).

As observed from Table 5, the final body weight (188.67, 189.17 and 191.17 g for control rats, rats fed on vitamin A deficient diet and rats fed on vitamin A deficient diet fortified with the prepared cupcake, respectively), the body weight gain (130.67, 131.17 and 133 g for control rats, rats fed on

Table 1: Proximate composition (%), physical, sensorial and textural properties of the prepared cupcake

Parameters	
Proximate analysis (%)	
Moisture	26.54
Protein	10.30
Ash	2.24
Fiber	1.52
Fat	14.5
Carbohydrates	44.9
Energy (Kcal)	351.2
Physical properties	
Weight (g)	25
Volume (cm³)	61
Specific volume (cm <sup>3</sup> g <sup>-1</sup> )	2.44
Density (g <sup>-1</sup> cm <sup>3</sup> )	0.406
Sensorial properties	
Crust color	8.5
Crumb color	8.1
Moistness	8.4
Taste	8.5
Tenderness	8.2
Softness	8.6
Overall acceptability	8.1
Textural properties	
Hardness (N)	20.30
Adhesiveness (N)	0.35
Springiness (mm)	12.70
Cohesiveness	0.36
Gumminess (N)	7.22
Chewiness (N)	4.62

Table 2: Color properties of the prepared cupcake

Color	L*	a*	b*	Hue
Crust	26.95	9.63	15.22	-13.3
Crumb	24.38	8.86	16.42	15.86

L\*: Lightness, a\*: Redness, b\*: Yellowness

vitamin A deficient diet and rats fed on vitamin A deficient diet fortified with the prepared cupcake, respectively) and the total food intake (753.83, 791.33 and 793.00 g for control rats, rats fed on vitamin A deficient diet and rats fed on vitamin A deficient diet fortified with the prepared cupcake, respectively) were found to be not significantly (p>0.05) different between the studied groups.

As notable from Table 6, Retinol Binding Protein (RBP) was significantly reduced in vitamin A-deficient rats in comparison to normal rats. On the other hand, rats fed on the prepared cupcake recorded RBP value higher than that of vitamin A-deficient rats. Blood glucose was found to be significantly increased in vitamin A-deficient rats in comparison to normal rats. While rats fed on the prepared cupcake showed a significant decrease in the blood glucose level when compared to normal rats. Hb, iron and ferritin were found to be decreased in vitamin A-deficient rats in comparison to

Table 3: Vitamins and minerals content of the prepared cupcake compared to the World Healthiest Food (2016)

Nutrients	World healthiest food (2016)*	Prepared formula	**Percentage
Zinc (mg/100 g)	8	3.23	40.37
Iron (mg/100 g)	8	4.5	56.25
Vitamin A (μg/100 g)	600	540	90
B <sub>6</sub> (μg/100 g)	1.5	0.6	40
Folic acid (µg/100 g)	250	108	43.2
B <sub>12</sub> (μg/100 g)	1	0.4	40

<sup>\*</sup>World healthiest food (2016) for children aged from 4-13 years. \*\*Percentage: Achieved by 100 g cupcake compared to the World healthiest food (2016)

Table 4: Amino acids profile of the prepared cupcake compared with provisional FAO/WHO amino acid (1990)

	FAO/WHO* amino acid		
Essential amino acids	Amino acids (mg $g^{-1}$ protein)	requirement (mg $g^{-1}$ protein)	**Percentage
Threonine	7.2	34	21.2
Valine	5.41	35	15.46
Total sulfur amino acids	6.33	25	25.32
Isoleucine	8.6	28	30.7
Leucine	7.22	66	10.9
Total aromatic amino acids	20.1	63	31.9
Lysine	6.6	58	11.4
Tryptophan	8	11	72.72

<sup>\*</sup>From FAO/WHO expert consultation, \*\*% that achieved by 100 g cupcake compared to the World Healthiest Food (2016)

Table 5: Nutritional parameters of different experimental groups

Parameters (g)	Groups			
	Control normal	Vitamin A deficient	Vitamin A deficient+cupcake	
Initial body weight	58.00±2.42°	58.00±5.93°	58.17±4.91°	
Final body weight	188.67±12.65 <sup>a</sup>	189.17±6.18ª	191.17±5.25ª	
Body weight gain	130.67±12.74°	131.17±6.20 <sup>a</sup>	133.00±3.09°	
Total food intake	753.83±20.46 <sup>a</sup>	791.33±17.37ª	793.00±14.56°	
Food efficiency ratio	$0.17\pm0.02^{a}$	0.17±0.01ª	0.17±0.01ª	

In each row, the same letters mean non-significant differences, different letters mean the significance among the tested groups. The confidence level is 95%. The data are expressed as mean values  $\pm$  standard error

Table 6: Glucose, RBP, Hb, iron, TIBC and ferritin of different experimental groups

	Groups		
Parameters	Control normal	Vitamin A deficient	Vitamin A deficient+cupcake
RBP (μg mL <sup>-1</sup> )	50.97±1.22°	33.96±1.09 <sup>a</sup>	44.85±1.77 <sup>b</sup>
Glucose (mg dL <sup>-1</sup> )	78.32±3.57ab	86.57±2.14 <sup>b</sup>	76.70±3.23ª
Hb (g dL <sup>-1</sup> )	14.60±0.33 <sup>b</sup>	9.57±0.28 <sup>a</sup>	14.02±0.57 <sup>b</sup>
Iron (mg dL <sup>-1</sup> )	235.82±7.76 <sup>b</sup>	196.21±4.38 <sup>a</sup>	228.57±5.53 <sup>b</sup>
TIBC (μg dL <sup>-1</sup> )	71.25±2.41°	86.48±2.44 <sup>b</sup>	77.04±2.07ª
Ferritin (ng mL <sup>-1</sup> )	81.68±3.13 <sup>b</sup>	72.41±1.92°	81.30±2.91 <sup>b</sup>

In each row, the same letters mean non-significant differences, different letters mean the significance among the tested groups. The confidence level is 95%. The data are expressed as mean values  $\pm$  standard error. RBP: Retinol-binding protein, Hb: Hemoglobin, TIBC: Total iron-binding capacity

normal rats while TIBC significantly increased in vitamin Adeficient rats in comparison to normal rats. On the other hand, rats fed on the prepared cupcake recorded Hb, iron and ferritin values higher than those of vitamin A-deficient rats and TIBC values less than that of vitamin A-deficient rats.

The data of Fig. 1 showed a positive correlation between iron and serum RBP at the 0.01 level.

As shown in Table 7, liver functions (AST and ALT) and kidney functions (urea and creatinine) were found

to be not significantly different between the studied groups. The activities of SOD and GPx were found to be decreased in vitamin A-deficient rats in comparison to normal rats while MDA value significantly increased in vitamin A-deficient rats in comparison to normal rats. On the other hand, rats fed on the prepared cupcake recorded SOD and GPx activities higher than those of vitamin A-deficient rats and MDA value less than that of vitamin A-deficient rats.

Table 7: Liver and kidney functions as well as erythrocytes' oxidative markers of different experimental groups

Parameters	Groups			
	Control normal	Vitamin A deficient	Vitamin A deficient+cupcake	
AST (U L <sup>-1</sup> )	33.79±1.23ª	34.80±1.59°	35.18±1.81 <sup>a</sup>	
ALT (U L <sup>-1</sup> )	25.82±0.92ª	26.80±1.20°	27.28±1.44ª	
Urea (mg $dL^{-1}$ )	24.32±0.52ª	25.43±1.32ª	25.08±1.11ª	
Creatinine (mg dL <sup>-1</sup> )	0.49±0.05ª	$0.48\pm0.04^{a}$	$0.46 \pm 0.02^{a}$	
MDA (nmol g <sup>-1</sup> Hb)	6.23±0.30 <sup>a</sup>	9.60±0.42 <sup>b</sup>	6.05±0.45ª	
SOD (U g <sup>-1</sup> Hb)	158.11±3.15 <sup>b</sup>	122.63±4.51ª	152.50±4.23 <sup>b</sup>	
GPx (U g <sup>-1</sup> Hb)	47.55±1.13 <sup>b</sup>	41.42±1.41°	$43.95 \pm 1.43$ ab	

In each row, the same letters mean non-significant differences, different letters mean the significance among the tested groups. The confidence level is 95%. The data are expressed as mean values  $\pm$  standard error. AST: Aspartate transaminase, ALT: Alanine transaminase, MDA: Malondialdehyde, GPx: Glutathione peroxidase, SOD: Superoxide dismutase, Hb: Hemoglobin

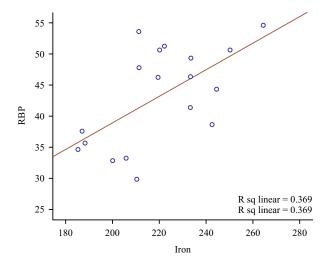


Fig. 1: Correlation between RPB and iron is significant at the 0.01 level (2-tailed)

RPB: Retinol-binding protein

## **DISCUSSION**

In the current study, vitamin A was found to be high in the prepared cupcake may be due to the added butter and egg as Górska-Warsewicz *et al.*<sup>26</sup> and Lima and Souza<sup>27</sup> confirmed that butter and egg are good sources of vitamin A. The orange flash sweet potato was used in the current study as a dietary source of beta-carotene that converts in the body to vitamin A. Drapal and Fraser<sup>28</sup> reported that the beta-carotene (pro-vitamin A) content in the lyophilized orange sweet potato was  $21 \mu g q^{-1}$ .

Regarding the physical properties of the prepared cupcake, the results revealed the moderate weight of the prepared cupcake due to the presence of wheat flour in the prepared cupcake<sup>29</sup>. The prepared cupcake showed dark brown crust owing to Millard's reaction to cocoa powder during the baking process. According to Popov-Raljić *et al.*<sup>30</sup>, cakes that have high L \*values are lighter in color because they

have higher rates of reflectance for light. Maillard reaction products were the most responsible for the color formation of the cupcake. Cupcake samples were low in hardness. The decrease in the crumb hardness can be explained by the increase in the cupcake volume. These results are consistent with those obtained by Yamsaengsung *et al.*<sup>31</sup> who reported that the increased bread volume is related to the decreased hardness values.

As a result of Retinoids insolubility in water, they are usually present bound to specific Retinoid-Binding Protein (RBP) in the aqueous media of the body<sup>32</sup>. Serum RBP determination highly connects to serum retinol level to predict vitamin A status<sup>33</sup>. In the present study, the reduction of RBP in VAD rats indicated the vitamin A deficiency while the increase of RBP in rats fed on the prepared cupcake indicated the efficiency of this cupcake in prevention the vitamin A deficiency and improving vitamin A status. This effect can be attributed to the high content of dietary vitamin A in the prepared cupcake in addition to the high content of beta-carotene in sweet potato.

The blood glucose level was found to be significantly increased in the VAD group when compared to the normal group. Reddy *et al.*<sup>34</sup> reported that chronic vitamin Adeficiency changed the structure and function of the pancreas via decreasing the islet cells and diminished the levels of plasma insulin. On the other hand, the blood glucose level was found to be significantly decreased in rats fed on the prepared cupcake when compared to the VAD group. This result may be attributed not only to the ability of the prepared cupcake to improve the vitamin A status but also to its content of sweet potato' fiber as it was reported before that fiber-rich food able to maintain a normal blood glucose level<sup>35</sup>.

In the present study, a positive correlation between iron and serum RBP was found at the 0.01 level. Also, Hb, iron and ferritin were found to be decreased in VAD rats. It was reported that anemia often correlates with vitamin-A deficiency as iron metabolism, hematopoiesis and increased

susceptibility to infections can be induced by VAD<sup>36</sup>. Severe VAD was found to be linked to anemia in preschool children<sup>37</sup>. On the other hand, Hb, iron and ferritin were found to be increased in rats fed on the prepared cupcake. TIBC was found to be low in rats fed on the prepared cupcake. He *et al.*<sup>38</sup> also found that TIBC increases when serum iron content is low and decreases when the serum iron concentration is high. The efficiency of the prepared cupcake in improving vitamin A status may be contributed to the improvement of iron status.

In the results of the present study, the activities of erythrocytes' SOD and GPx were found to be decreased in VAD rats. While MDA significantly increased in VAD rats when compared to the control group. Sangeetha *et al.*<sup>6</sup> reported the association between vitamin A deficiency and oxidative stress and found that the activities of catalase and glutathione reduced while lipid peroxidation increased in VAD rats.

Rats fed on the prepared cupcake recorded SOD and GPx activities higher and less MDA value than VAD rats. This also may be due to the high content of powerful antioxidant compounds in orange flash sweet potatoes<sup>28</sup>. These antioxidants can prevent oxidative stress and protect against the free radical attack<sup>39</sup>. Sangeetha et al.<sup>6</sup> also found that supplementation with beta-carotene can modulate the activities of catalase and glutathione in addition to the level of lipid peroxidation in VAD rats. It is recommended to incorporate the orange flesh sweet potatoes as a source of pro-vitamin A in food products especially that prepared for children to avoid vitamin A deficiency. The current study suggested that the prepared cupcake may be efficient for children to prevent vitamin A deficiency. However, further investigation on the effect of this cupcake on children is required as the major limitation of the current work was the inability to perform the study directly on children although the prepared cupcake was formulated from edible ingredients the pre-study on rats was required to obtain adequate data about the probable biological effects.

## CONCLUSION

The results indicated that the prepared cupcake contained dietary vitamin A and can provide school children with the daily requirement of vitamin A, protein and fat. The prepared cupcake recorded high scores of the sensory properties. The prepared cupcake is promising in control of vitamin A deficiency and the related oxidative stress and reduction in iron.

## SIGNIFICANCE STATEMENT

The current study as a pre-clinical assay indicated the efficiency of the prepared cupcake from the flour of flash orange sweet potatoes in control of vitamin A Deficiency (VAD) and related oxidative stress and iron deficiency in rats. This study will assist the researcher to expose the critical area of the role of the dietary intervention of dietary vitamin A sources on vitamin A deficiency and correlated anemia as well as oxidative stress that many researchers were not able to explore. Thus, a new theory on this functional sweet potato cupcake and possibly other combinations, may be arrived at.

#### REFERENCES

- Green, A.S. and A.J. Fascetti, 2016. Meeting the vitamin a requirement: The efficacy and importance of β-carotene in animal species. Sci. World J., 2016: 1-22.
- Osanai, M., N. Nishikiori, M. Murata, H. Chiba, T. Kojima and N. Sawada, 2007. Cellular retinoic acid bioavailability determines epithelial integrity: Role of retinoic acid receptor α agonists in colitis. Mol. Pharmacol., 71: 250-258.
- Liu, X., Y. Li, Y. Wang, Q. Wang and X. Li et al., 2014. Gestational vitamin A deficiency reduces the intestinal immune response by decreasing the number of immune cells in rat offspring. Nutrition, 30: 350-357.
- 4. WHO., 2016. Guideline: Daily Iron Supplementation in Infants and Children. World Health Organization, Geneva.
- 5. Galal, O.M., I. Ismail, A.S. Gohar and Z. Foster, 2005. Schoolteacher's awareness about scholastic performance and nutritional status of Egyptian schoolchildren. Food Nutr. Bull., 26: S275-S280.
- Sangheeta, R.K., N. Bhaskar and B. Baskaran, 2009. Comparative effects of β-carotene and fucoxhantin on retinol deficiency induced oxidative stress in rats. Mol. Cell. Biochemis., 331: 59-67.
- 7. Mendes, J.F.R., E.M. de Almeida Siqueira, J.G.M. de Brito e Silva and S.F. Arruda, 2016. Vitamin A deficiency modulates iron metabolism independent of hemojuvelin (*Hfe2*) and bone morphogenetic protein 6 (*Bmp6*) transcript levels. Genes Nutr., Vol. 11. 10.1186/s12263-016-0519-4.
- 8. Al-Mekhlafi, H.M., E.M. Al-Zabedi, M.T. Al-Maktari, W.M. Atroosh and A.K. Al-Delaimy, 2014. Effects of vitamin A supplementation on iron status indices and iron deficiency anaemia: A randomized controlled trial. Nutrients, 6: 190-206.
- Willows, N.D., B.N. Barbarich, L.C.H. Wang, D.L. Olstad and M.T. Clandinin, 2011. Dietary inadequacy is associated with anemia and suboptimal growth among preschool-aged children in Yunnan Province, China. Nutr. Res., 31: 88-96.

- Jiang, S., C.X. Wang, L. Lan and D. Zhao, 2012. Vitamin A deficiency aggravates iron deficiency by upregulating the expression of iron regulatory protein-2. Nutrition, 28: 281-287.
- 11. Nogueira, A.C., G.A. Sehn, A.P. Rebellato, J.P. Coutinho and H.T. Godoy *et al.*, 2018. Yellow sweet potato flour: Use in sweet bread processing to increase β-carotene content and improve quality. An. Acad. Bras. Ciênc., 90: 283-293.
- van Jaarsveld, P.J., M. Faber, S.A. Tanumihardjo, P. Nestel, C.J. Lombard and A.J.S. Benadé, 2005. β-Carotene-rich orange-fleshed sweet potato improves the vitamin A status of primary school children assessed with the modifiedrelative-dose-response test. Am. J. Clin. Nutr., 81: 1080-1087.
- Reeves, P.G., F.H. Nielsen and G.C. Fahey Jr., 1993. AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr., 123: 1939-1951.
- 14. Hussein, A.M.S., M.M. Hussein, M.F. Salama, I.M. Hamed, K.A. Fouda and R.S. Mohamed, 2018. Formulation and evaluation of functional cookies for improving health of primary school children. Pak. J. Biol. Sci., 21: 401-408.
- 15. Panfili, G., P. Manzi and L. Pizzoferrato, 1994. Highperformance liquid chromatographic method for the simultaneous determination of tocopherols, carotenes, and retinol and its geometric isomers in Italian cheeses. Analyst, 119: 1161-1165.
- 16. Viñas, P., C. López-Erroz, N. Balsalobre and M. Hernández-Córdoba, 2003. Reversed-phase liquid chromatography on an amide stationary phase for the determination of the B group vitamins in baby foods. J. Chromatogr. A, 1007: 77-84.
- 17. Drabkin, D.I., 1949. The standardization of heamoglobin measurements. Am. J. Med. Sci., Vol. 21.
- 18. Trinder, P., 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem., 6: 24-27.
- 19. Stookey, L.L., 1970. Ferrozine-a new spectrophotometric reagent for iron. Anal. Chem., 42: 779-781.
- 20. Betts, C.A. and B. Stuart, 1973. Determination of serum total iron-binding capacity. J. Clin. Pathol., Vol. 26. 10.1136/jcp.26.6.457-a.
- 21. Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 56-63.
- 22. Larsen, 1972. Creatinine assay by a reaction-kinetic principle. Clin. Chim. Acta, 41: 209-217.
- 23. Fawcett, J.K. and J.E. Scott, 1960. A rapid and precise method for the determination of urea. J. Clin. Pathol., 13: 156-159.
- 24. Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.
- 25. Paglia, D.E. and W.N. Valentine, 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med., 70: 158-169.

- Górska-Warsewicz, H., K. Rejman, W. Laskowski and M. Czeczotko, 2019. Butter, margarine, vegetable oils and olive oil in the average polish diet. Nutrients, Vol. 11. 10.3390/nu11122935.
- 27. Lima, H.J.D. and L.A.Z. Souza, 2018. Vitamin A in the diet of laying hens: Enrichment of table eggs to prevent nutritional deficiencies in humans. World's Poultry Sci. J., 74: 619-626.
- 28. Drapal, M. and P.D. Fraser, 2019. Determination of carotenoids in sweet potato (*Ipomoea batatas* L., Lam) tubers: implications for accurate provitamin A determination in staple sturdy tuber crops. Phytochemistry, Vol. 167. 10.1016/j.phytochem.2019.112102.
- 29. Tucar, M.T., 2017. Sensory characterization of cupcakes made of sweet potato (*Ipomoea batatas*) flour with turmeric (*Curcuma longa*) powder. Asia Pac. J. Multidiscip. Res., 5: 75-83.
- 30. Popov-Raljić, J.V., J.S. Mastilović, J.G. Laličić-Petronijević and V.S. Popov, 2009. Investigations of bread production with postponed staling applying instrumental measurements of bread crumb color. Sensors, 9: 8613-8623.
- 31. Yamsaengsung, R., R. Schoenlechner and E. Berghofer, 2010. The effects of chickpea on the functional properties of white and whole wheat bread. Int. J. Food Sci. Technol., 45: 610-620.
- 32. O'Byrne, S.M. and W.S. Blaner, 2013. Retinol and retinyl esters: Biochemistry and physiology. J. Lipid Res., 54: 1731-1743.
- 33. Mahmood, K., A.H. Samo, K.L. Jairamani, G. Ali, A. Talib and W. Qazmi, 2008. Serum retinol binding protein as an indicator of vitamin A status in cirrhotic patients with night blindness. Saudi J. Gastroenterol., 14: 7-11.
- 34. Reddy, M.R.G., S.M. Venkata, U.K. Putcha and S.M. Jeyakumar, 2018. Vitamin A deficiency induces endoplasmic reticulum stress and apoptosis in pancreatic islet cells: Implications of stearoyl-CoA desaturase 1-mediated oleic acid synthesis. Exp. Cell Res., 364: 104-112.
- 35. Mohamed R.S., S.S. Abozed, S. El-Damhougy, M.F. Salama and M.M. Hussein, 2020. Efficiency of newly formulated functional instant soup mixtures as dietary supplements for elderly. Heliyon, Vol. 6. 10.1016/j.heliyon.2020.e03197.
- 36. Semba, R.D. and M.W. Bloem, 2002. The anemia of vitamin A deficiency: Epidemiology and pathogenesis. Eur. J. Clin. Nutr., 56: 271-281.
- 37. Gamble, M.V., N.A. Palafox, B. Dancheck, K. Briand and R.D. Semba, 2004. Relationship of vitamin A deficiency, iron deficiency and inflammation to anemia among preschool children in the Republic of the Marshall Islands. Eur. J. Clin. Nutr., 58: 1396-1401.
- 38. He, H., Q. Huang, C. Liu, S. Jia, Y. Wang, F. An and H. Song, 2019. Effectiveness of AOS-iron on iron deficiency anemia in rats. RSC Adv., 9: 5053-5063.
- 39. Marcadenti, A., 2015. Dietary antioxidant and oxidative stress: Interaction between vitamins and genetics. J. Nutr. Health Food Sci., 3: 1-7.