

# EFFECTS OF SPICES BLACK PEPPER, RED PEPPER AND GINGER ON PROVITAMIN-'An' INTAKE BY RAT INTESTINE

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ARTICLE INFO	ABSTRACT

Corresponding Author:	Vitamin "a" deficiency i	n the plant food addiction	population, it is desirabl	e to improve
Corresponding Author: <b>Prof. Rasheed Al Fakri<sup>1</sup></b> <sup>1</sup> Faculty at department of chemistry in university of Lahore, Pakistan rashi.78264@yahoo.com	Vitamin 'a" deficiency i the bioavailability of pro permeability of the intes black pepper, red pepper, for the possible effects of vitro absorption of pro-v animals. Dietary piperine	n the plant food addiction -vitamin A. Certain spices tine. Here, by examining ginger, piperine, and caps pro-vitamin A on intestina itamin A in the intestine and ginger increased pro-	population, it is desirable can alter the ultrafine the intestinal absorption aicin, some common spi l absorption. I looked it has been demonstrated vitamin A intake by 147	e to improve structure and n of rats fed ces are given up. Higher in in all spiced 7% and 98%,
	respectively. Absorption of animals fed black pepper and red pepper increased by 59% and 27%, respectively, while capsaicin from the diet increased by 50%. Therefore, significantly improved intestinal absorption of beta-carotene has been demonstrated as a result of the consumption of hot spices, which may form a food-based strategy that may reduce vitamin A deficiency. <sup>20</sup>			
KEYWORDS	Pro Vitamin-A; Spices; M	Aicronutrient Deficiency; In	ntestinal Uptake;	

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### INTRODUCTION

Vitamin A deficiency is a serious health problem that leads to blindness in children in India (WHO, 1998)<sup>19</sup>. Animal-derived foods (eggs, milk, liver) are an excellent source of preformed vitamin A, but the majority of India's population relies on plant-based foods that provide carotene, especially pro-vitamin A. doing. Several factors, such as dietary composition (fat, fiber, protein) and food processing methods, affect the bioavailability of pro-vitamin A from foods (Rodriguez & Irwin, 1972)<sup>10</sup>. Studies have shown that poorly cooked foods absorb less carotenoids, and mild cooking improves their absorption (Ogulensi and Lee, 1979, Veda et al., 2006)<sup>9, 17</sup>. <sup>20</sup>

The presence of nutrients such as acidulants and antioxidant seasonings in foods affects the retention of pro-vitamin A and bio-accessibility. Previous studies have shown that ingestion of food acidulants (tamarind and citric acid) and antioxidant spices (turmeric and onions) during vegetable heat treatment generally improves pro-vitamin A retention (Gayathri et al., -2004, Veda et al., -2008)<sup>3, 18</sup>. Given that many of India's populations rely on plant-based foods to meet their vitamin A needs, develop nutritional strategies to improve the bioavailability of pro-vitamin A from these sources. Is desirable. Food acidulous (Amchoor and Lime or citric) improved the bioavailability of pro-vitamin A from green leafy and yellow-orange vegetation (Veda et al., 2008)<sup>18</sup>. This improved bioavailability was evident for both raw and cooked vegetables. The presence of turmeric significantly improved the bio-accessibility of pro-vitamin A, especially from these vegetables during heat treatment, while the presence of onions was pressure-cooked carrots and amaranth leaves, and open-cooked pumpkin. Also improved bio-accessibility of provitamin A from pumpkin leaves (Veda et al., 2008)<sup>18</sup>.

Spices are widely used in Indian cuisine. Certain spices can alter the hyperfine structure and permeability of the intestine. Piperine, the main alkaloid of black pepper, is known to enhance bioavailability of drugs and other photochemical by increasing absorption due to changes in membrane lipid dynamics and changes in the conformation of enzymes in the intestine. (Srinivasan, 2007, Srinivasan, 2009)<sup>13, 15</sup>. The lipophilic spice compounds capsaicin (red pepper), gingerol, and zingerone (ginger physiochemical) have considerable structural homology with piperine. It is necessary to demonstrate whether such edible spices, which can alter the hyperfine structure and permeability of the intestinal brush border, have a beneficial effect on the absorption of pro-vitamin A. <sup>20</sup>

Spices are a group of esoteric nutritional supplements that have

been used for thousands of years to improve the sensory properties of foods. The amount and variety of spices consumed in tropical countries is particularly abundant. These spice ingredients give the food its characteristic flavor and attractive color (Srinivasan, 2008)<sup>14</sup>. In addition to these sensory properties, spices also result from a variety of beneficial physiological effects. Among these, the ability to stimulate digestion, its positive effects on lipid metabolism, its effectiveness as an anti-diabetic drug, its antioxidant properties, its anti-inflammatory and cancer-preventing potential are widely documented (Srinivasan, 2005)<sup>12</sup>. <sup>20</sup>

The purpose of this study is to assess the effect of certain spices on pro-vitamin A intake, especially through an inverted pouch of intestinal sections isolated from test rats. In this study, black pepper, red pepper, ginger, and their active ingredients in foods were specifically investigated for their effects on pro-vitamin A absorption due to changes in the ultrafine structure and fluidity of the intestinal brush border. Such basic information about the bioavailability of beta-carotene is needed to improve and improve the diet and streamline the recommended daily intake of vitamin A ( $\beta$ -carotene).<sup>20</sup>

#### MATERIALS AND METHODS

#### **1. MATERIAL**

Fresh carrots (Daucus carota) which were refined and used as a source of pro-vitamin A in this study were purchased from the market in Mysore. All chemicals used were analytical grade and solvents distilled prior to use. The bioactive spice compounds were purchased from M/S Fluka Chemie, Buchs, Switzerland and those were piperine and capsaicin. Standard pro-vitamin A, porcine pancreatic pepsin, pancreatin, and bile extract (porcine) were obtained from Sigma Chemicals Co., St. Purchased from Louis, MO, USA. Casein used in animal feed was purchased from M/S. Nimesh Corporation (Mumbai, India). In the whole study double, distilled water was used. All glassware were acid-washed properly before use. <sup>20</sup>

#### 2. ANIMAL TREATMENT

Animal experiments were performed with appropriate measures to minimize pain or discomfort in accordance with the Guidelines for the Care and Use of Animals for Laboratory Procedures and appropriate approval of the Institutional Animal Ethics Committee (Permit No. IAEC66/05). Young male Wistar rats (8 per group) weighing 80-85 g obtained from the laboratory animal laboratory of the Central Food Research Institute of Mysore were maintained on a special semi synthetic diet for 8 weeks. The basic diet includes: casein - 21%; Sugarcane 10%; Corn Starch 54%; Refined Peanut Butter, 10%; Salt mixture ( Bernhart & Tommarelli, 1966)<sup>2</sup>, 4%, vitamin mixture (National Research Council, 1972)<sup>8</sup>, 1%. Seasonings - Black pepper

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(0.5%), chilli (3.0%), ginger (0.05%), the bioactive spice compounds piperine (0.02%), and capsaicin (0.01%) were included in this basic diet to create a variety of experimental diets. Animals were kept in different stainless-steel cages and for them, in the cages, it was kept accessible to food and water. Dietary intake and weight gain during the experimental regimen in allspice groups were similar to those of the control group.<sup>20</sup>

#### 3. FOOD SOURCES OF PRO-VITAMIN A

Carrots finely chopped and grated. The resultant carrot digesta was used as a source of pro-vitamin A in the study of its intestinal uptake. <sup>20</sup>

#### 4. IN VITRO INTESTINAL ABSORPTION STUDIES

The rats were dozed off with a blow on the head and after laparoto my, the small intestine was quickly excised. Both inside and outside were washed carefully with 0.9% saline, it was transposed and cut into segments of likewise 10 cm long. Uptake of pro-vitamin A in vitro by these segments of intestine isolated from spice pre-treated animals was evaluated (Table 1).<sup>20</sup>

Table 1 - Uptake of #-carotene from carrot homogenate by everted intestinal segments from rats fed spices.							
Rat group	Recovery of $\beta$ -carotene after 3 h of incubation (µg)						
	Mucosal fluid	Serosal fluid	Intestinal epithelium	Percent absorption			
Control	22.09 ± 0.52	0.166 ± 0.022	0.256 ± 0.023	1.87			
Black pepper	21.30 ± 0.51	0.261 ± 0.032	0.392 ± 0.046	2.97			
Red pepper	22.51 ± 0.47	0.169 ± 0.018	0.379 ± 0.052	2.37			
Ginger	22.03 ± 0.40	0.384 ± 0.043	0.465 ± 0.060	3.71			
Piperine	21.27 ± 0.36	0.332 ± 0.047	0.695 ± 0.097	4.61			
Capsaicin	22.36 ± 0.52	0.332 ± 0.036	0.315 ± 0.032	2.81			
Values are mean ± SEM of eight independent determinations.							

\* Denotes significantly higher compared to Control group (p < 0.05).

#### 5. ANALYSIS OF PRO-VITAMIN A

After incubation, pro-vitamin A in aqueous intestinal fluid and mucosal samples was extracted first with acetone/ethanol (1:1, v/v) and then with petroleum ether (Hedren, Mulkozi, & Svanberg, 2002)<sup>5</sup>. This process was repeated several times to completely extract pro-vitamin A. The extracts were combined and the solvent was evaporated to dryness on a rotary evaporator. The residue was re dissolved in petroleum ether and stored for cold hold analysis. Before analysis, petroleum ether was evaporated in a nitrogen atmosphere and the residue was re dissolved in the mobile phase and used for HPLC measurement. pro-vitamin A was extracted from intestinal tissue samples according to the method of Mercado, Holmgren, Fox, and Russell (1989)<sup>7</sup>. Intestinal tissues were homogenized with 10 ml chloroform/methanol (2:1, v/v) in a tissue homogenizer equipped with a Teflon pestle. The homogenate is mixed with 2 ml of 0.9%

saline and then shaken. The mixture was precipitated, separated into two layers, and centrifuged at 2500 g for 10 min. The lower chloroform layer was separated and evaporated to dryness under a stream of nitrogen gas. The residue was re dissolved in the mobile phase used for pro-vitamin a determination by HPLC. The measurement of pro-vitamin A was performed using reversed-phase HPLC (model: Shimadzu LC 10 AVP; Shimadzu Corporation, Kyoto, Japan) equipped with a photodiode array (PDA) detector. Pro-vitamin A was separated on a C18 column (SS Exil, Dandenong, Australia). The mobile phase consisted of mixture (v/v) 65-etonitrile containing 1.3 mmol/L ammonium acetate, 15% methylene chloride, and 20% methanol. pro-vitamin A was monitored at 450 nm using βapo8' carotenal as an internal standard. The identity of the peak and  $\lambda$ max was confirmed by the retention time (14 min 30 sec) and characteristic spectra of the standard chromatogram. During the pro-vitamin A incubation and extraction step, care was taken to prevent oxidative degradation of pro-vitamin A by minimizing exposure of the sample to light and air. Air was replaced with nitrogen at all stages of incubation and storage prior to closing the flask. All work was done under yellow lighting and the glassware was covered with a black cloth to prevent light from entering. 20

## 6. STATISTICAL ANALYSIS

All measurements were performed in 8 replicates and results were expressed as mean  $\pm$  standard error of the mean. Data were analyzed statistically and comparisons between groups were made using unpaired Student's t-test (Snedecor & Cochran, 1976)<sup>11</sup>. The difference is that p &lt; 0.05.<sup>20</sup>

#### **RESULTS AND DISCUSSION**

The amount of digestible carrots to be included in the culture medium as the pro-vitamin A source was optimized in a pilot study using the same intestinal segment length and varying the concentration of the pro-vitamin A source. A digestibility equivalent to 0.08 g of dried carrots present in the culture medium provided the maximum intestinal absorption of pro-vitamin A under the experimental conditions of the incubation period and the length of the intestinal segment of mice (Figure 1). Overall, the uptake of pro-vitamin A from carrot homogenates was significantly increased by intestinal segments of spice-fed animals (Table 1).

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Among the spices tested, edible piperine showed the greatest increase in pro-vitamin A absorption, 247% of the control group. Dietary ginger increased intestinal pro-vitamin A absorption by 98%, whereas dietary black pepper, capsaicin, and red pepper increased by approximately 59%, 50%, and 27%, respectively. Thus, both black pepper and its bioactive ingredient, piperine, have been shown to promote the absorption of  $\beta$ -carotene in the intestine (Figure 2). Chili, another spicy spice, and capsaicin, a spicy ingredient, were also effective in increasing intestinal absorption of pro-vitamin A, but to a lesser extent. Ginger improves intestinal absorption of pro-vitamin A than black or black pepper (Figure 2).



Fig. 2 – In vitro absorption of β-carotene by intestinal segments of spice fed rats. Values are mean ± SEM of eight independent determinations. \*Significantly higher compared to Control group.

In an in vitro study of the effect of piperine on intestinal uptake function using freshly isolated rat small intestinal epithelial cells, piperine (25-100 µM) significantly stimulated γ-glutamyl trans peptidase activity and amino acid uptake. (Johri & Thusu, Khajuria, & Zutshi, 1992)<sup>4</sup> the property of piperine to increase bioavailability may be associated with increased absorption, which is associated with alterations in membrane lipid dynamics and changes in intestinal enzyme conformation. (Khajuria, Thusu and Zutshi, 2002)<sup>6</sup>. The results of membrane fluidity studies using a non-polar fluorescent probe, pyrene (which measures the fluid properties of hydrocarbon cores), showed an increase in long brush boundary membrane fluidity. Micro-structural studies with piperine have shown an increase in the length of microvilli with a significant increase in free ribosomes and ribosomes in the endoplasmic reticulum of enterocytes, suggesting that the synthesis or conversion of cytoskeletal components or membrane proteins may be included in the observed effects. suggest that it is possible (Khajuria et al., 2002)<sup>6</sup>. Therefore, it is suggested that piperine induces changes in membrane dynamics and permeability properties along with inducing protein synthesis related to cytoskeletal function, thereby increasing the absorption surface of the small intestine, thereby promoting efficient infiltration of epithelial cells. barrier. 20

Diet Spices - Pepper, Chili, Ginger, and Bioactive Spice Compounds -Piperine and Capsaicin, Evaluated for Effects on Fluidity, Intestinal Enzyme Activity of the Intestinal Brush Boundary Membrane (BBM) in Rats Interactions with the lipid microenvironment of the membrane and Microstructural changes in the intestinal epithelium showed an increase in BBM fluidity in glutamate-treated animals (unpublished data). These dietary spices have also been shown to stimulate the activities of glycylglycine d ipeptidase, leucine amino peptidase, and  $\gamma$ -glutamyl transpeptidase in the jejunum mucosa, indicating that these hot spices modulate membrane kinetics to alter the conformation of enzymes. Suggests Scanning electron microscopy of intestinal villi of animals fed these spices/spices revealed micro structural changes, such as an increase in the length of the microvilli.<sup>20</sup>

Therefore, the present study of pro-vitamin A uptake by intestinal segments isolated from rats treated with pepper, red pepper, ginger, piperine, and capsaicin showed higher intestinal absorption of pro-vitamin A in the intestines of these seasoned animals. This effect was highest for dietary piperine, followed by ginger and capsaicin. These pungent spices may form a strategy to reduce vitamin A deficiency by increasing the absorbent surface, altering the permeability properties, and thus enhancing the absorption of pro-vitamin A in the intestine. - In-depth study in terms of uptake of trace elements in the body. This promising background information will help develop diet-based strategies to combat vitamin A deficiency diseases.<sup>20</sup>

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