Table of Contents

Editorial
1. Antimicrobial-Resistance Bacteria in Food Products  e1-e2  
   – Malik A. Hussain

Review
2. Food as Medicine: The New Concept of "Medical Rice"  38-50  
   – Shaw Watanabe, Azusa Hirakawa, Chizuru Nishijima, Ken'ichi Ohtsubo, Kozo Nakamura, Shigeru Beppu, Patcharee Tungtrakul, Sun Jian Quin, E-Siong Tee, Takuo Tsuno and Hajime Ohigashi

Literature Review
3. Non-Alcoholic Fatty Liver Disease and Nutrition: A Literature Review  51-63  
   – Sahar Jafari, Esmail Hajinasrollah and Mehdi Foroughi

Research
4. Pesticide Residue Dissipation Upon Storage and Processing in Chickpea Legume for Food Safety  64-72  
   – Geetanjali Kaushik, Santosh Satya and S. N. Naik

Review
5. Nutraceuticals for Athletes  73-82  
   – Charu Gupta, Dhan Prakash and Sneh Gupta

Research
6. An In Vitro Estimation of Glycemic Index of White Bread and Improvement of the Dietary Fiber  83-87  
   – Anteneh Taye, Ephrem Engidawork and Kelbessa Urga
Antimicrobial-Resistance Bacteria in Food Products

Malik A. Hussain, PhD

Department of Wine, Food and Molecular Biosciences, Lincoln University, Springs Road, Lincoln, New Zealand

Antimicrobial resistance (AMR) is one of the emerging issues that can seriously impact human health directly and food supply indirectly. Antimicrobial agents are commonly used in food production environments to control animal diseases. Repeated exposure to different concentrations of antimicrobial agents may result in the foodborne bacteria to become resistant through specific genetic resistance mechanisms or the selective pressure of antimicrobials. Antimicrobial-resistant foodborne pathogens are dangerous in many ways i.e., infection with a resistant microorganism may have prolonged illness due to limited treatment options or have acquired more virulent characteristics. AMR among some foodborne pathogens has been increasing during 15 to 25 years.

Foods contamination with antimicrobial-resistant bacteria may occur at primary production stage or at other stages in the supply chain. The antimicrobial-resistant bacteria can be passed to food products through several routes. Animal origin foods may have the highest risk of carrying antimicrobial-resistant bacteria. Contamination of such foods is more likely because of the animals that have received antimicrobial treatments. Animal products such as meat, poultry and eggs could have AMR microbes transferred into them during slaughtering process due to faecal contamination. It is also to remember that antimicrobial-resistant bacteria can spread to other animals in a herd/flock.

Results of some studies have found the colistin resistant \textit{E. coli} and other bacterial isolates from food animals. The resistance was reported due to the presence of a gene (\textit{mcr-1}) on a plasmid. Colistin belongs to a group of antibiotics called polymyxins that are the last option to treat multiple-resistant infections in human. This sort of bad consequences happen when clinically important drugs are given to animals. The \textit{mcr-1} gene could be detected in sequenced genomes of bacteria isolated from imported foods and from the blood a hospitalised patient in Denmark.

Water can serve a source of AMR microbes in certain food products. For example, vegetables and fruits can become contaminated with antimicrobial-resistant bacteria if the irrigation water has AMR microbes. Moreover, washing of fresh produce using such water can also pass resistant microorganisms. Fishery products may be contaminated with antimicrobial-resistant bacteria if they live in water that has AMR microbes or given feed that contains antibiotics.

Antimicrobial-resistant bacteria may also enter into food products through environmental sources. A variety of antimicrobial agents such as antibiotics, antifungals, sanitizers, and food preservatives are used during food production, processing, storage and distribution. These agents are primarily applied to improve the efficiency of the food system and increase the safety and quality of food products. Soil, manure and dust particles in the air generally carry AMR microbes. Preparation and handling of food in an unhygienic manner can spread antimicrobial-resistant bacteria from one type of food or environmental source to another food through cross-contamination.

Above-mentioned information present a glimpse of the actual threatening situation
that world will be facing due to AMR. In fact, AMR microbes are around us all the times in our bodies to our foods and in our environment to food production systems. These all factors are strongly inter-connected, just adding to the complexity of this problem.

It would be self-deceiving to believe that AMR problem is easy to address and can be tackled. The global facts and predictions are indicating AMR as a serious threat to global public health and economy. Scientific and technical reports estimate that world could possibly see the global impact of AMR spread in the form of 10 million deaths annually and total economic cost up to US $100 trillion by 2050. Recent high-level UN meeting on AMR demonstrated that world has realised an urgent need to take practical measures and develop a strong global political commitment to address the challenge. This could be regarded as a very positive global move to increase and improve awareness of AMR.

REFERENCES


Food as Medicine: The New Concept of “Medical Rice”

Shaw Watanabe, MD, PhD1; Azusa Hirakawa, BS1; Chizuru Nishijima, BS1; Ken’ichi Ohtsubo, PhD2; Kozo Nakamura, PhD3; Shigeru Beppu, PhD4; Patcharee Tungtrakul, PhD5; Sun Jian Quin, MD7; E-Siong Tee, MD8; Takuo Tsuno, PhD9; Hajime Ohigashi, PhD10

1Lifescience Promoting Association, Tokyo, Japan
2Kagawa Nutrition University, Sakado, Japan
3Niigata University, Niigata, Japan
4Department of Agriculture, Shinshu University, Matsumoto, Japan
5Forica Foods Co. Ltd., Niigata, Japan
6Institute of Food Research and Product Development, Kasetsart University, Bangkok, Thailand
7Clinical Nutrition Center, Huadong Hospital, Fudan University, Shanghai, China
8Nutrition Society of Malaysia, Petaling Jaya, Malaysia
9Tsuno Rice Fine Chemical Co., Ltd., Wakayama, Japan
10Kyoto University and Human Health Foundation, Kyoto, Japan

ABSTRACT

In many countries, rice contributes to health by supplying dietary energy, proteins and fat. Many different species of rice have been developed in Japan and other rice producing countries. Some varieties are expected to prevent various diseases, or to be used for dietary therapy. The health effects of brown rice are empirically well known, and accumulating evidence about the physiological and pharmacological activity of rice bran strongly supports the use of brown rice in the dietary therapy. These could be categorized in the new concept, “medical rice”. For example: medical rice for diabetes (glycemic index<55), medical rice for chronic kidney disease (CKD) (protein<1/20), medical rice for mental health (high gamma-aminobutylic acid or γ-aminobutylic acid (GABA), gamma oryzanol (γ-oryzanol) and/or ferulic acid), and medical rice for cancer prevention (high antioxidant capacity). Organic cultivation is necessary to avoid toxic substances from fertilizers and insecticides. In response to the enormous increase of medical costs in many countries, encouragement of healthy longevity by changes of dietary habits is mandatory. Functional food labeling has started in 2015 in Japan, so the proper food labeling of medical rice could help people who want to control and/or improve their health status.

KEYWORDS: Brown rice; Rice bran; Rice ingredients; Glycemic index; Low protein rice; Gamma-aminobutylic acid or γ-aminobutylic acid (GABA); γ-oryzanol; Ferulic acid; Phytate.

ABBREVIATIONS: CKD: Chronic Kidney Disease; GABA: Gamma-aminobutylic acid or γ-aminobutylic acid; γ-oryzanol: Gamma Oryzanol; LDL: Low-density lipoprotein; HbA1c: Glycated hemoglobin; BMI: Body Mass Index; DRI: Dietary Reference Intake; MHLW: Ministry of Health, Labour and Welfare; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid; FTLD: Frontotemporal lobar degeneration; RCT: Randomized Clinical Trials; HDL: High-density lipoproteins; HPLC-UV: High-performance liquid chromatography-ultraviolet; TD2: Type-2 diabetes; BRAVO: Branch Retinal Vein Occlusion; MDRD: Modification of Diet in Renal Disease; WHO: World Health Organization; IGF-1: Insulin Like Growth Factor 1; POMS: Profile of Mood States; TMD: Total Mood Disorders.

INTRODUCTION

Rice is the main staple food for approximately 70 percent of the world’s population, principally living in ten areas of the Asia-Pacific region. In many countries, rice contributes to health
by supplying dietary energy, proteins and fat. It accounts for more than 50% of the diet in Bangladesh, Myanmar, Lao PDR, VietNam and Indonesia. In this regards, the nutritional aspects of rice should be re-evaluated, especially the integrated composition of functional ingredients.

BROWN RICE AND HEALTH

Until the late 19th century, Japanese traditional meals were composed of unpolished brown rice and barley as staple food, miso (fermented soy) soup and side dishes cooked with vegetables, soybean products, and various varieties of roots. In the Meiji era (1868-1905), polished rice became popular, and beri-beri increased to epidemic proportions until vitamin B1 was found in rice bran. After the World War II, polished rice, meat, eggs, and dairy products became the major food items composing main and side dishes. Consequently, new dietary habits largely account for the high prevalence of the metabolic syndrome and other lifestyle related chronic diseases.

On the other hand, there is a traditional way of eating in Japan. Macrobiotic is one of the school of dietary therapy founded by Sagen Ishizaka, Kenzo Futaki, and Yukikazu Sakurazawa (George Ohsawa). Whole grains and whole foods have been emphasized as central to macrobiotic diet. Locally-produced and organically grown, and minimally processed foods are also recommended. Macrobiotic meals are practically plant-based: seasonal vegetables, beans, and sea vegetables with brown rice as staple food. Recently a variety of rice species have been harvested, and they are expected to contain various ingredients, in addition to the ordinary nutrients.

NUTRITIONAL ASPECTS OF BROWN RICE

According to our research, macrobiotic practitioners consume more magnesium, iron, vitamin E, vitamins B and dietary fibers, although their energy intake is less than that for average Japanese. Their body mass index (BMI), blood pressure and low-density lipoprotein (LDL) cholesterol levels are often found to be low, while glycated hemoglobin (HbA1c) remained within normal levels. Even when analyzed in comparison with other vegetarian dietary data, daily nutritional values were higher in those who ate rice more frequently than noodles, and even higher in brown rice than white rice. The macrobiotic dietary habit of eating brown rice seemed to contribute to their healthy state. The consumption of small fish, in the shape of whole food, for macrobiotic practitioners supplemented vitamin B12, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

Using sample meals, we investigated whether or not macrobiotic meals (we say genmai-shoku) could fulfill nutritional requirement. In the radar charts displayed in Figure 1, central circle (in blue) represent the dose of Dietary Reference Intake (DRI) 2010 recommended by the Ministry of Health, Labour and Welfare, (MHLW). The outer green lines represent the relative intake doses from genmai-shoku. Sample meals of genmai-shoku provided enough energy, fat and protein, and several times more minerals and vitamins than required.

In addition to the functional effects of ingredients in brown rice, the frequency of mastication influences the brain function. In Japan, fast foods with soft texture have recently become popular for younger generation. The mastication frequency has been decreasing in proportion. The brown rice increases the chewing number of times than a meat or fish dishes. National Health and Nutrition Survey, Labour and Welfare (2010) showed it was only 800 times per American meal compared to the 30,000 times by genmai meal. Longer eating time acts to prevent fast eating, which would be lead to obesity, and relaxes stress.

So, brown rice could be called the “medical rice for...
The effects of eating brown rice have been gaining attention for preventing and treating not only beri-beri and constipation, but also other chronic diseases. Organic rice can remove arsenic and other toxic chemicals ingested from fertilizers and/or insecticides.

FUNCTIONAL INGREDIENTS IN RICE BRAN

Compared with white rice, whole brown rice, is rich in vitamins, minerals, dietary fibers and various functional chemicals (Tables 1A, 1B and 1C). About 8.52 million metric tons of brown rice are produced every year in Japan. Rice bran makes about 10% of unprocessed rice by weight, and contains 18-22% oil, of which up to 5% of unsaponifiable dark oil (Figure 2). Rice bran can be used in a variety of applications such as food, animal feed and fertilizer, but most of the rice bran is discarded at present.

Recently, much attention has been paid to rice bran, because of various pharmacological properties of its ingredients, like anti-oxidation. A current study further clarified the properties of many functional ingredients in rice bran. A current study has further clarified the properties of many functional ingredients in rice bran. It is separated to gum, wax, dark oil and scum by different boiling temperature for further extraction of a number of chemicals (Figure 2). The biological activities of each factor have been clarified by many in vivo and in vitro experiments. Human data by randomized clinical trials (RCT) are also accumulating.

(1) Lipophilic Ingredients

The nutritional benefits of rice bran oil are well known. γ-oryzanol and tocotrienol are considered to be the active ingredients in the oil. The pharmacological effects are: a decrease in total and LDL cholesterol, an increase in high-density lipoproteins (HDL) cholesterol, a decrease in triacylglycerol and ApoB, and the inhibition of platelet aggregation. γ-oryzanol is contained in the non-saponifiable fraction of rice bran. γ-oryzanol is

![Figure 2: Ingredients in rice bran.](image)

Brown rice is produced in amounts of about 8.52 million metric tons (MT) a year in Japan. This means that 0.8 million MT of rice bran are produced annually, because rice bran makes 8 to 10% of the brown rice. Rice bran is used in a variety of applications such as food, animal feed and fertilizer, but most of the rice bran is discarded at present. Recently much attention has been paid to rice bran, because it has been reported that the ingredients of rice bran show various interesting properties, such as anti-oxidation and lowering of serum lipid levels.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Whole</th>
<th>Polished</th>
<th>Bran</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy content (J)</td>
<td>1520-1610</td>
<td>1460-1560</td>
<td>1670-1990</td>
</tr>
<tr>
<td>Energy content (kcal)</td>
<td>363-385</td>
<td>349-373</td>
<td>399-476</td>
</tr>
<tr>
<td>Crude protein (g)</td>
<td>7.1-8.3</td>
<td>6.3-7.1</td>
<td>11.3-14.9</td>
</tr>
<tr>
<td>Crude fat (g)</td>
<td>1.6-2.8</td>
<td>0.3</td>
<td>15.0-19.7</td>
</tr>
<tr>
<td>Available carbohydrate (g)</td>
<td>73-87</td>
<td>77-89</td>
<td>34-62</td>
</tr>
<tr>
<td>Total dietary fiber (g)</td>
<td>2.9-4.0</td>
<td>0.9-2.3</td>
<td>17-29</td>
</tr>
<tr>
<td>Water-insoluble fiber (g)</td>
<td>2.0</td>
<td>0.5</td>
<td>15-27</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>1.4</td>
<td>0.2-0.5</td>
<td>0.8-0.5</td>
</tr>
<tr>
<td>Phytic acid (g)</td>
<td>0.04-0.9</td>
<td>0.1-0.2</td>
<td>3.0-7.4</td>
</tr>
<tr>
<td>Phenolic (g catechin)</td>
<td>0.01-0.02</td>
<td>0.01-0.02</td>
<td>0.01-0.02</td>
</tr>
</tbody>
</table>

Table 1A: Nutrient composition of brown rice, milled rice and rice bran at 14% moisture content.
### Table 1C: Various nutrients and ingredients in rice per 100 g.

<table>
<thead>
<tr>
<th>Major nutrients</th>
<th>Whole brown rice</th>
<th>Brown rice</th>
<th>Polished rice</th>
<th>Pre-germinated rice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Water (g)</strong></td>
<td>14-15</td>
<td>60-64</td>
<td>61-64</td>
<td>60-62</td>
</tr>
<tr>
<td><strong>Energy (kcal)</strong></td>
<td>353-357</td>
<td>150-204</td>
<td>145-154</td>
<td>156-160</td>
</tr>
<tr>
<td><strong>Protein (g)</strong></td>
<td>5.7-6.8</td>
<td>2.8-3.7</td>
<td>1.8-2.4</td>
<td>2.4-2.6</td>
</tr>
<tr>
<td><strong>Fat (g)</strong></td>
<td>3.2-3.3</td>
<td>1.2-1.7</td>
<td>0.2-0.3</td>
<td>0.8-1.0</td>
</tr>
<tr>
<td><strong>FFA (g)</strong></td>
<td>2.26-2.81</td>
<td>0.72-1.21</td>
<td>0.23-0.29</td>
<td>0.5-0.57</td>
</tr>
<tr>
<td><strong>SFA (g)</strong></td>
<td>0.58-0.71</td>
<td>0.2-0.3</td>
<td>0.09-0.11</td>
<td>0.16-0.17</td>
</tr>
<tr>
<td><strong>UFA (g)</strong></td>
<td>1.68-2.1</td>
<td>0.52-0.9</td>
<td>0.14-0.2</td>
<td>0.35-0.4</td>
</tr>
<tr>
<td><strong>Carbohydrate (g)</strong></td>
<td>74-76</td>
<td>32-35</td>
<td>34-35</td>
<td>34-35</td>
</tr>
<tr>
<td><strong>maltose (g)</strong></td>
<td>0.3</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>glucose (g)</strong></td>
<td>0.2-0.3</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Dietary fiber (g)</strong></td>
<td>2.2-3.1</td>
<td>2.1-2.5</td>
<td>0.3-0.6</td>
<td>1</td>
</tr>
<tr>
<td><strong>Ash (g)</strong></td>
<td>1.1-1.3</td>
<td>0.5-0.7</td>
<td>0.1-0.2</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>Ca mg</strong></td>
<td>8-9</td>
<td>1.2-1.7</td>
<td>3-4</td>
<td>4-5</td>
</tr>
<tr>
<td><strong>P mg</strong></td>
<td>290-300</td>
<td>120-150</td>
<td>22-24</td>
<td>63-69</td>
</tr>
<tr>
<td><strong>Fe mg</strong></td>
<td>0.9-1</td>
<td>0.5-0.6</td>
<td>0.1</td>
<td>0.2-0.3</td>
</tr>
<tr>
<td><strong>K mg</strong></td>
<td>220-250</td>
<td>100-140</td>
<td>16-20</td>
<td>53-63</td>
</tr>
<tr>
<td><strong>Mg mg</strong></td>
<td>110-120</td>
<td>51-68</td>
<td>2-3</td>
<td>20-23</td>
</tr>
<tr>
<td><strong>Zn mg</strong></td>
<td>1.9-2.2</td>
<td>0.8-1.3</td>
<td>0.6-0.7</td>
<td>0.8-0.9</td>
</tr>
<tr>
<td><strong>Cu mg</strong></td>
<td>0.12-0.27</td>
<td>0.1-0.16</td>
<td>0.04-0.08</td>
<td>0.11-0.14</td>
</tr>
<tr>
<td><strong>Mn mg</strong></td>
<td>2.0-2.5</td>
<td>1.0-1.5</td>
<td>0.17-0.25</td>
<td>0.8-0.9</td>
</tr>
<tr>
<td><strong>Se mg</strong></td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td><strong>vitamin B1 mg</strong></td>
<td>0.32-0.46</td>
<td>0.11-0.22</td>
<td>0</td>
<td>0.11-0.12</td>
</tr>
<tr>
<td><strong>vitamin B2 mg</strong></td>
<td>0.02-0.03</td>
<td>0.005-0.01</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>vitamin B6 mg</strong></td>
<td>0.36-0.41</td>
<td>0.17-0.32</td>
<td>0.007-0.008</td>
<td>0.054-0.094</td>
</tr>
<tr>
<td><strong>niacin mg</strong></td>
<td>5.3-5.9</td>
<td>2.1-3.4</td>
<td>0.008-0.1</td>
<td>0.4-0.8</td>
</tr>
<tr>
<td><strong>αtocopherol mg</strong></td>
<td>1.3-1.5</td>
<td>0.6-0.8</td>
<td>0</td>
<td>0.5-0.7</td>
</tr>
<tr>
<td><strong>βtocopherol mg</strong></td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>γtocopherol mg</strong></td>
<td>0.1-0.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>δtocopherol mg</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Protein (g)</strong></td>
<td>5.7-6.8</td>
<td>2.8-3.7</td>
<td>1.8-2.4</td>
<td>2.4-2.6</td>
</tr>
<tr>
<td><strong>Arg mg</strong></td>
<td>445-534</td>
<td>201-285</td>
<td>149-194</td>
<td>185-208</td>
</tr>
<tr>
<td><strong>Lys</strong></td>
<td>228-253</td>
<td>100-137</td>
<td>65-77</td>
<td>85-93</td>
</tr>
<tr>
<td><strong>His</strong></td>
<td>139-164</td>
<td>92-137</td>
<td>41-53</td>
<td>53-59</td>
</tr>
<tr>
<td><strong>Phe</strong></td>
<td>269-329</td>
<td>130-175</td>
<td>95-124</td>
<td>111-125</td>
</tr>
<tr>
<td><strong>Tyr</strong></td>
<td>164-196</td>
<td>87-111</td>
<td>67-86</td>
<td>78-88</td>
</tr>
<tr>
<td><strong>Ala</strong></td>
<td>325-383</td>
<td>144-213</td>
<td>105-136</td>
<td>134-146</td>
</tr>
<tr>
<td><strong>Gly</strong></td>
<td>274-316</td>
<td>121-176</td>
<td>86-111</td>
<td>111-121</td>
</tr>
<tr>
<td><strong>Pro</strong></td>
<td>233-284</td>
<td>107-158</td>
<td>80-105</td>
<td>99-116</td>
</tr>
<tr>
<td><strong>Glu</strong></td>
<td>922-1130</td>
<td>413-603</td>
<td>321-423</td>
<td>400-443</td>
</tr>
<tr>
<td><strong>Ser</strong></td>
<td>297-354</td>
<td>137-198</td>
<td>104-135</td>
<td>129-141</td>
</tr>
<tr>
<td><strong>Thr</strong></td>
<td>220-240</td>
<td>100-148</td>
<td>171-219</td>
<td>85-93</td>
</tr>
<tr>
<td><strong>Asp</strong></td>
<td>518-611</td>
<td>236-335</td>
<td>171-219</td>
<td>213-233</td>
</tr>
<tr>
<td><strong>Trp</strong></td>
<td>53-75</td>
<td>19-38</td>
<td>17-20</td>
<td>22-23</td>
</tr>
<tr>
<td><strong>Cys</strong></td>
<td>141-177</td>
<td>54-85</td>
<td>50-55</td>
<td>58-63</td>
</tr>
<tr>
<td><strong>GABA</strong></td>
<td>3-7</td>
<td>4-6</td>
<td>&lt;0.5</td>
<td>3-5</td>
</tr>
<tr>
<td><strong>Phytic acid</strong></td>
<td>1.18-1.21</td>
<td>1.3-2.0</td>
<td>0.8-1.3</td>
<td>0.4-0.5</td>
</tr>
</tbody>
</table>

Table 1C: Various nutrients and ingredients in rice per 100 g.
bound to ferulic acid, and thus belongs to the family of ferulated sterols. γ-oryzanol exists in 4 chemical forms with similar functional activities: two are triterpene alcohol esters and the other two are sterol esters (Figure 3).\textsuperscript{19,20} The solubility of γ-oryzanol is only 0.06% in water, and 0.2% in 20% ethanol. The absorption of γ-oryzanol may not be optimal after oral intake of brown rice. The proportion of γ-oryzanol is 0.1% in rice bran, but it is possible to take 300 mg of γ-oryzanol by oral intake of brown rice.

Phenolic compounds are major antioxidant and radical scavenging ingredients in rice. Nakamura et al.\textsuperscript{19,20} developed a method for the simultaneous determination of phenolic compounds in rice by high-performance liquid chromatography-ultraviolet (HPLC-UV). Eleven kinds of phenolic compounds were identified in rice: ferulic acid, caffeic acid, sinapinic acid, p-coumaric acid, vanillic acid, protocatechuic acid, syringic acid, hydroxybenzoic acid, chlorogenic acid, 6′-O-feruloylsucrose and 6′-O-sinapoylsucrose (Table 2). In unpolished rice, the three most abundant ones are: 6′-O-feruloylsucrose, 6′-O-sinapoylsucrose, and ferulic acid.\textsuperscript{20} With their representative concentrations of 1.09, 0.42 and 0.33 mg/100 g rice flour, they represent 84.0% by weight of the total amount of soluble phenolic compounds (2.19 mg/100 g brown rice flour). Polished rice contains only 0.28 mg of phenolic compounds/100 g rice flour.

Tocotrienol and tocopherol are lipid-soluble antioxidants, which prevent cardiovascular diseases and cancer.\textsuperscript{21} Squalene, an isoprenoid compound structurally similar to beta-carotene, is an intermediate metabolite in the synthesis of cholesterol. In humans, about 60 percent of dietary squalene is absorbed.

(2) Water-Soluble Ingredients

Inositol and phytic acid are water-soluble ingredients like GABA.\textsuperscript{22-28} Magnesium, calcium and other trace elements are

<table>
<thead>
<tr>
<th>Phenolic Compounds</th>
<th>Unpolished</th>
<th>Polished</th>
</tr>
</thead>
<tbody>
<tr>
<td>ferulic acid</td>
<td>0.33 0.07</td>
<td></td>
</tr>
<tr>
<td>caffeic acid</td>
<td>0.02 0.025</td>
<td></td>
</tr>
<tr>
<td>sinapinic acid</td>
<td>0.02 0.005</td>
<td></td>
</tr>
<tr>
<td>p-coumaric acid</td>
<td>0.098 0.02</td>
<td></td>
</tr>
<tr>
<td>vanillic acid</td>
<td>0.072 0.032</td>
<td></td>
</tr>
<tr>
<td>protocatechuic acid</td>
<td>0.037 0.013</td>
<td></td>
</tr>
<tr>
<td>syringic acid</td>
<td>0.03 0.01</td>
<td></td>
</tr>
<tr>
<td>hydroxybenzoic acid</td>
<td>0.04 0.021</td>
<td></td>
</tr>
<tr>
<td>chlorogenic acid</td>
<td>0.033 0.028</td>
<td></td>
</tr>
<tr>
<td>6′-O-feruloylsucrose</td>
<td>1.089 0.026</td>
<td></td>
</tr>
<tr>
<td>6′-O-sinapoylsucrose</td>
<td>0.417 0.032</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2.186 0.282</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Contents of soluble phenolic compounds in rice (mg/100 g).
also included in this fraction.

In 2008, Maeba et al. reported an interesting clinical observation about the preventive effect of inositol on metabolic syndrome. Seventeen subjects with metabolic syndrome were given inositol per os for 2 weeks (5 g a day for one week and 10 g a day thereafter). The authors observed a significant decrease in total cholesterol, LDL cholesterol, small dense LDL cholesterol and apolipoprotein B (a marker of post-prandial hyperlipidemia). Waist circumference, high-sensitivity CRP and fasting blood glucose level also improved. Interestingly, a significant decrease in blood glucose level was only observed among subjects with metabolic syndrome. This may reflect higher concentrations of serum plasminogen, which is a protective factor against oxidative stress. The finding suggests that plasmalogen is a key factor mediating the beneficial effect of inositol on the metabolic syndrome.

Myo-inositol is a ring-shaped polyalcohol (Figure 4). It has half the sweetness of sucrose. It is an element of the vitamin B complex, although it is not a real vitamin. It is present in human colostrum, and considered to be essential for babies’ growth. It is also effective for the prevention of metabolic syndrome. It has shown anti-fatty liver effect, anti-diabetic effect, improvement of metabolic syndrome, effectiveness against panic disorders and obsessive-compulsive disorders, and inhibitory effect on lung cancer in animal experiment.

Phytic acid is a phosphatized inositol, and has a strong chelating effect, pH adjustment effect, and antioxidant action. It is used for the prevention of discoloration and as a deodorant. In vivo, it is expected to have various effects, for example: detoxification, anti-fatty liver effect, immuno-stimulatory action, and anti-cancer effect by inhibition of the phosphoinositide (PI) 3-kinase system.

CONCEPT OF MEDICAL RICE

Many different kinds of rice have been developed in Japan and other rice producing countries. Some varieties are expected to prevent various diseases, or to be used for dietary therapy. For example, ‘super-hard’ high-amylose rice could be used for diabetic patients, low-protein or low-gluterin rice for patients with renal failure, GABA-rich large germ rice is expected to improve mental health, and rice with high antioxidant properties would be effective for the prevention of cancer and other diseases. Human data are accumulating, so we believe it is time to introduce the concept of medical rice for disease prevention and treatment (Table 3).

(1) Medical Rice for Diabetes

In 2012, a meta-analysis reported an association between white rice intake and increased risk of type-2 diabetes (T2D), suggesting the need to replace white rice by brown rice in the Japanese diet. The effects of brown rice on visceral obesity and endothelial function were shown in the Okinawa branch retinal
vein occlusion (BRAVO) study. Participants were between 30 and 60-years-old males with metabolic syndrome. Brown rice reduced their post-prandial blood glucose level and insulin level. A decrease in body weight and an improvement of various biochemical abnormalities were also observed. The benefit of brown rice and brown rice with legumes for glycemic and insulinemic control were also shown by Mulan et al.

Recently, Ohtsubo et al. succeeded in harvesting special super-hard rice, which contained a high concentration of resistant starch, due to long amylopectin chains. It showed good effect on postprandial glucose level and insulin secretion (Figure 5). However, the taste is different from ordinary Japonica rice. So, they next developed super-hard-rice powder after boiling. Now, the powder of super-hard rice is available for a number of new food items. For example, medical “Tomato Bread” is made of super-hard rice powder, containing resistant starch, GABA rich pre-germinated brown rice, tomato as a source of lycopene, and gelatinized rice flour for durable palatability. The size and taste is comparable to wheat bread. Tasty rice noodle is also made from this powder.

(2) Medical Rice for Chronic Kidney Diseases (CKD) and Renal Dysfunction.

One of the benefits of a low-protein diet is the preservation of the kidney function. Distinct mechanisms could be identified: (1) improvement of hyperphosphatemia and hyperkalemia, (2) decrease in urinary protein, (3) improvement of subjective symptoms, (4) prevention of complication, (5) good control even after indication of hemodialysis for better survival.

The protein in rice is stored in two different types of compartment. The major proteins are prolamin and glutenin. Prolamin is the alcohol soluble protein fraction remaining after salt extraction of globulin. Glutelin is the dilute-acid or dilute-alkaline soluble protein fraction after prolamin extraction. Most of the prolamin was present at the periphery in whole rice grains, implying that prolamin is removed by enzymatic digestion on polished white rice.

Low-protein rice is available in Japan in five different packages, depending on their different amount of protein. The rice content varies between 150 g and 180 g to reach a total content of 160 kcal (2 E-unit). The lowest protein concentration is 0.1 g/pack, which is 1/25 of normal rice (Figures 6A and 6B). The palatability period is usually 7 months, but some packages have an extended storage period of 3.5 years for disaster situations.

Ideura confirmed the effects of a low-protein diet on patients with chronic kidney diseases. At the threshold of renal failure of 6 mg serum creatinine/dl, low protein diet had started. With a content of 0.4-0.5 g/kg body weight, the median survival

![Figure 5: Postprandial blood glucose and insulin level. Super hard rice showed suppression of postprandial glucose level and insulin secretion. Super hard rice or purple rice, and new technology, such as co-extrusion with red onion germination, make it possible to fortify bio-active rice bread.](image-url)

![Figure 6: (a) Aseptic Package of low protein rice and (b) nutritional components of low protein rice.](image-url)
was 4 years.

With a content of 0.6 and 0.7 g/kg, no beneficial effect was observed compared with a control group (>0.8 g/kg body weight). The optimal low-protein content was 0.3 g/kg body weight. Low potassium and phosphate concentrations are additional benefits for CKD patients.

Sun et al^4^ performed a preliminary study in Huadong Hospital (Shanghai, China), examining the effect of 12-weeks of low-protein rice as dietary therapy (0.6 g/kg body weight) for CKD patients. Cooked rice was provided 3 packs/day containing 1.35 g proteins and 900 kcal energy. The meal plan was checked by trained research dietitians, and dietary intake and compliance were monitored through diet diaries.

Compared with baseline levels, the total dietary energy increased from 1606 kcal/d (27.9 kcal/kg bwt) to 1748 kcal (30.8 kcal/kg bwt). Dietary daily vitamin B1 intake increased from 0.34 mg to 0.78 mg, and vitamin B2 intake from 0.42 mg to 1.08 mg. Serum albumin slightly increased from 44 g/L to 46 g/L. The total serum protein concentration increased from 74 g/L to 77 g/L. Meanwhile, changes in body weight, BMI, and hemoglobin were not significant. After 12 weeks, urinary protein levels decreased from 0.4 g/d to 0.1 g/d. Urine albumin decreased from 130.8 mg/24 h to 60.8 mg/day. Twenty-four urinary protein, albumin excretion, and urinary albumin/creatinine ratio decreased by 63.7%, 55.0% and 52.0%, respectively.

Low-protein rice was well accepted by Chinese CKD patients. It is an important tool for CKD dietary therapy as it increases energy and micronutrients intake and improves the nutritional status. A long-term and large sample size RCT study is planned in Thailand to confirm the protective effects of low-protein rice on CKD progression.

The average Japanese citizen consumes 60 g protein a day, and half comes from rice. By using low-protein rice, we can reduce the protein intake by half. The amount of fish or chicken on side dishes does not need to be strictly restricted. However, if the main dish contains a large portion of beef like in the American diet, meat is the source of both protein and energy. In the well-designed multicenter Modification of Diet in Renal Disease (MDRD) study,^4^ the benefit of a very low protein diet could not be shown. We analyzed their data and found the reason why MDRD study was failed. The energy intake was less than 70% of the protocol, probably due to the cut of meat from main dish. So, energy deficiency could plausibly have worsened the disease.\(^4^9\)

Medical rice for CKD should contain enough energy source and low protein, as well as low potassium and phosphate.

(3) Medical Rice for Mental Health

As the society is aging, the number of people with impaired cognitive function becomes serious problem in the world. In Japan the number of people with dementia is estimated to be 2 million, and World Health Organization (WHO) estimates that 47.5 million people have dementia, with 7.7 million new cases every worldwide.\(^5^0\)

Large-germ brown rice and pre-germinated brown rice contain functional ingredients to prevent dementia, such as GABA, γ-oryzanol, in addition to nutritional elements such as vitamins, minerals, and dietary fibers.\(^5^1,5^2\) GABA and γ-oryzanol are involved in the metabolism of hypothalamic catecholamines.

γ-Oryzanol is known to have anti-stress effects, to palliate menopausal disorders and dysautonomia. Other effects have recently been reported, for example: improvement of hypotension, curative effect of Alzheimer’s disease, amelioration in muscular fatigue.\(^5^3,5^4\) Antioxidant effect, radical eliminating action, ultraviolet absorptive action, anti-inflammatory effect, antidiabetic effect, anti-allergic effect, increase of insulin like growth factor 1 (IGF-1) and antibacterial action are also reported, but the main hope is an improvement of cognitive function.\(^5^2\)

GABA is also a candidate for mental health. Large-germ rice and pre-germinated brown rice (GBR) contain a high amount of GABA.\(^5^4\) Pre-germinated brown rice was developed for easy cooking, keeping the many nutritional and functional ingredients, such as dietary fiber, vitamins and minerals, GABA, γ-oryzanol, acyl-sterol glycoside, etc. GBR contained not only GABA, but also ferulic acid.

The effect of ferulic acid mixed with Angelica archangelica extract on cognitive functions and behavioral and psychological symptoms of dementia have been examined by Kimura et al^5^2 (Figure 7) and many symptoms were shown to improve (Table 4).

![Figure 7: Mental health: Control of behavioral and psychological symptoms of dementia (BPSD). Treatment with ferulic acid extract led to reduced NPI scores in 19 (95.0%) out of 20 patients and to significantly decreased NPI scores overall (p<0.001).](image)

Pregnant women often become unstable in mood. In an intervention study, 41 pregnant women were randomized to take germinated rice or white rice for 14 days.\(^5^4\) A psychological test profile of mood states (POMS) was done before and after the study, and salivary amylase was measured as a stress marker. POMS test measures 6 dimensions of mood, and depression,
anger-hostility, fatigue score significantly improved by brown rice eating, and total mood disorders (TMD) was nearly half of that of white rice (Figure 8).

Mothers who in took pre-germinated rice is shown by black column and those who ate white rice is shown by white column. Depression, anger-hostility, fatigure score significantly improved by brown rice eating, and TMD was nearly half of that of white rice.

In addition to their mental effects, the giant-germ rice and GBR are also useful for diabetic and hypertensive patients. In a randomized-controlled trial comparing two packs of GBR rice with white rice, 24 healthy volunteers (10 males and 14 fe-
males, aged from 27 to 47) were studied for the effect on blood pressure. The GBR group displayed a significant reduction in systolic blood pressure after 12 h of GBR intake, particularly marked after 6 minutes of physical load with an ergometer. It was also shown that the blood glucose levels and the incremental area under the curve (IAUC) were lower after taking GBR. The IAUC was 1448 mg/min/dl in GABA rich group, whereas it was 1601 mg/min/dl in white rice group.

These results demonstrate that this special pre-germinated brown rice (GABA+ferulic acid) may be effective in sub-
jects with mild hypertension and diabetes mellitus, in addition to the mental health.

Other components of rice bran (steryl glucosides [PSG] for example) were found to be effective for coping against stress. So, medical rice for mental health is at least defined to contain high GABA and γ-Oryzanol or ferulic acid.

(4) Medical Rice for Cancer Prevention

In various animal experiments, Muto et al showed that fer-
mented rice bran (FBRA) strongly prevented the incidence of colon, breast, head and neck, esophageal, and pancreatic cancers almost half. The antioxidant activity of rice bran could be con-
sidered to be a major factor.

The effects of many phytonutrients are expected to work well beyond free radical protection. Of late, an antioxidant test known as Oxidation Radical Absorbance Capacity (ORAC) has become popular. Other similar assays, such as DPPH, TRAP, TEAC etc. are available to specify the antioxidant capac-
ity of food ingredients.

This is why consumers are often confused by different values as there is no readily available comparison method among the values obtained by different assay systems. It is proposed that the antioxidant capacity of complex supplements should be expressed in terms of standardized antioxidant units (AOU), pondering the antioxidant values obtained in aforementioned assay system. Japanese intake of AOU per day is estimated more than 10000 AOU unit throughout a year.

Beyond the standard antioxidant vitamins, such as vita-
mion C and E, we should consider antioxidants found in brightly pigmented whole fruits and vegetables, mostly due to anthocya-
nins and proanthocyanins. We measured antioxidant activities

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Follow-up</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPI total score</td>
<td>28.3±9.6</td>
<td>17.7±9.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Delusions</td>
<td>2.2±2.7</td>
<td>1.3±2.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Hallucinations</td>
<td>2.8±3.4</td>
<td>1.1±2.1</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Agitation/aggression</td>
<td>4.6±3.2</td>
<td>2.5±1.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Depression/dysphoria</td>
<td>1.7±2.9</td>
<td>1.2±2.0</td>
<td>NS</td>
</tr>
<tr>
<td>Anxiety</td>
<td>1.9±2.3</td>
<td>1.5±2.0</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>Euphoria</td>
<td>0.2±0.9</td>
<td>0.2±0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Apathy/indifference</td>
<td>5.9±2.4</td>
<td>3.3±1.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Disinhibition</td>
<td>1.9±3.1</td>
<td>1.8±2.9</td>
<td>NS</td>
</tr>
<tr>
<td>Irritability/habitility</td>
<td>4.0±3.2</td>
<td>2.3±2.0</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Apathy/indifference</td>
<td>3.4</td>
<td>2.6</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

4 weeks after Feru-guard® treatment in 20 patients with frontotemporal lobar degener-
ation or dementia with Lewy bodies.

Table 4: Changes in neuropsychiatric inventory score.

Figure 8: Mother’s stress measured by POMS: Changes in POMS scores before and after dietary intervention by GABA rich rice.
of various rice varieties, and found that only brown rice showed antioxidant activity (Table 5). Black rice showed the highest anti-oxidant activity. Both brown rice and black rice retained the high anti-oxidant activity even after cooking.66 Polished rice did not show antioxidant activity at all. The presence of antioxidant activity in daily meals should prevent carcinogenesis and diseases caused by free radicals.

CONCLUSION

The health effects of brown rice are empirically well known, and accumulating evidence about the physiological and pharmacological activity of rice bran strongly supports the use of brown rice in meals, although this is not popular in Japan and other countries. However, in response to the enormous increase of medical costs, the Japanese government starts to encourage healthy longevity measures by changes of dietary habits. Functional food labeling has started in 2015, so the proper food labeling of medical rice could help people who want to control and/ or improve their health status.44 An example of food label for ‘Medical Rice for Health’ is shown in the figure (Figure 9).

A word in the lower part of the mark could be changed according to the purpose.

ACKNOWLEDGEMENTS

The authors deeply appreciate the participants in the East Asia Conference of Standardization of Rice Function, which was held in Kyoto from December 10 to 12, 2014. All authors attended the conference and contributed to make the entity of medical rice, providing their original data.

<table>
<thead>
<tr>
<th></th>
<th>ORAC_W (AOU P)</th>
<th>ORAC_L (AOU C)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw brown rice</td>
<td>13</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td>Raw brown rice</td>
<td>15</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>Raw brown rice</td>
<td>11</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Cooked brown rice by pressure pan</td>
<td>5</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Cooked brown rice by pressure pan</td>
<td>6</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Cooked brown rice by pressure pan</td>
<td>6</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Cooked brown rice without pressure</td>
<td>6</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Cooked brown rice without pressure</td>
<td>5</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Cooked brown rice without pressure</td>
<td>7</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Pregenerated rice</td>
<td>1</td>
<td>&lt;0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Pregenerated rice</td>
<td>2</td>
<td>&lt;0.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Cooked polished rice “kinme”</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Cooked polished rice “kinme”</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Cooked polished rice “kinme”</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>cooked polished white rice</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>cooked polished white rice</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>cooked polished white rice</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
</tr>
</tbody>
</table>

AOU-F: Antioxidant unit by flavonoids; AOU-C: Antioxidant unit like carotenoids.

Table 5: Antioxidant activity of various rice and cooked rice. These rice with high antioxidant activity could be categorized in the medical rice for cancer prevention.

Figure 9: Food labeling of medical rice and licensed medical rice for health. This rice is organic brown rice, containing enough vitamins and minerals with high antioxidant ability, without any detectable herbicide or toxic heavy metals.
The authors also thank the financial support of Human Health Foundation, Toyo Rice Cooperation, Tsuno Rice Fine Chemicals Co. Ltd. Fancl Cooperation, Genmai-koso Co. Ltd., and Forica Food Co. Ltd. to open this conference. A part of this work was presented at the 9th Asia Pacific Conference on Clinical Nutrition, which was held in Kuala Lumpur, Malaysia in 2015. They also thank to Dr. Philippe Calain for his contribution to complete this manuscript.

CONFLICTS OF INTEREST

The authors do not have any conflicts of interest regarding this paper to any company.

REFERENCES

23. Dilworth L, Omoruyi F, Asemota H. Effects of IP6 on carbohydrate and lipid metabolism in rat model. In: Tsukuba Agri-


42. Kopple JD, Massry SG. Nutritional Management of Renal Disease. Baltimore, Maryland, USA: Williams & Wilkins; 1997.


Non-Alcoholic Fatty Liver Disease and Nutrition: A Literature Review

Sahar Jafari, MSc1; Esmail Hajinasrollah, MD2; Mehdi Foroughi, MSc3*

1Department of Nutrition Sciences, College of Medicine, Urmia University of Medical Sciences, Urmia, Iran
2Loghman Medical Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3Department of Nutrition Science, College of Nutrition Sciences, Isfahan University of Medical Science, Isfahan, Iran

KEYWORDS: Non-Alcoholic Fatty Liver; Obesity; Patients; Weight loss.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is considered as a well-known disease which is determined by without using alcohol. There is high prevalence of non-alcoholic fatty liver in the community and this condition potentially can progress to hepatic cirrhosis and hepatic defect.1-3 In developed country, it is estimated that 20% to 30% of adult populations have non-alcoholic fatty liver.4-8 50% of diabetic people and approximately 80% of obese people with morbidity obesity are having non-alcoholic fatty liver. 6,9-10 Prevalence of non-alcoholic fatty liver in Western countries is likely concurrent with epidemic obesity and also it is associated with metabolic disorders.5,11-14 Patients with non-alcoholic fatty liver usually have insulin resistance.14-19 The prevalence of non-alcoholic fatty liver has been increased in individuals who have normal body mass index (BMI). However, these people have central obesity and latent insulin resistance.17,18,20,21 In epidemiologic studies, these patients with normal weight have unhealthy diet.22-24

The efficacy and immunity of medication therapy is unclear towards treatment of non-alcoholic fatty liver.25 Obesity has the most correlation with non-alcoholic fatty liver.26 Therefore, to modify the lifestyle is the first line treatment for non-alcoholic fatty liver. Managing the non-alcoholic fatty liver includes gradually decreasing the weight and increasing the physical activity.27-33

Studies did not determine which material or micronutrient in the diet potentially increases the risk of developing to non-alcoholic fatty liver; however, it is obvious that keeping low weight will be difficult in long term.34 To change the diet composition without decreasing the rate of calorie will be a practical and realistic variation for treatment of non-alcoholic fatty liver. Therefore, it is very important to evaluate the relationship between particular micronutrients and compounds of diet to non-alcoholic fatty liver.

This review study has been performed on epidemiological studies which evaluated the relationship between non-alcoholic fatty liver to diet compounds, weight loss, and physical activity.
CARBOHYDRATE AND FAT CONTENT OF DIET

Three main sources are available for increasing the triglyceride (TG) concentration in liver. These sources include increased inflow of free fatty acids from internal tissues to liver, increased intra-hepatic lipogenesis and increased receiving of fatty diet. The recent human studies suggested that high amount of fat in diet is removed by liver. Therefore, post-prandial high changes in the rate of fat metabolism may be observed in patients with non-alcoholic fatty liver. In a study consisting of 15 patients with non-alcoholic fatty liver and 15 healthy people, the total rate of TG and very low density lipoprotein (VLDL) was higher in individuals with non-alcoholic fatty liver after receiving the single oral fat. The rate of ApoB48 and ApoB100 did not change after receiving fat by patients with non-alcoholic fatty liver. These findings suggested that increased removing of TG in the post-prandial period was associated with decreased secretion of VLDL which in turn increases the rate of hepatic steatosis. The relationship between total fat of diet and the content of liver fat has been studied directly in the human studies. In a cross-sectional study, 10 obese women received two types of diets with equal rate of calories during two weeks. The rate of fat in one diet was 16% and 56% in the other one. The rate of fatty liver was measured by spectrophotometer. The rate of liver content was decreased up to 20% by using ow fat diet, however, it is increased up to 35% by using high fat diet. The rate of changes in fatty liver was concurrent to changes in the rate of fasting plasma insulin. These changes are considered very important because people did not change their weight during the study. In the other study, the bariatric surgery was performed on 74 patients with morbidity obesity (90% developed to non-alcoholic fatty liver). The diet of these patients was evaluated through a 24-hour recall. The rate of consuming the meals was compared to liver histological data. There was not any meaningful correlation between the rates of received calorie or proteins to steatosis, fibrosis and hepatitis. Nevertheless, taking the high carbohydrate (approximately 56%) had significantly correlation to the rate of inflammation. However, the rate of taking the fat had reverse correlation to the rate of inflammation. The findings of this study determine neither the correlation between each kind of dietary fats to non-alcoholic fatty liver nor the correlation between each kind of simple or complex carbohydrate to the rate of inflammation and histological findings in the patients with non-alcoholic fatty liver. The correlation between carbohydrate and non-alcoholic fatty liver was evaluated in one study. The diet of 28 patients with non-alcoholic steatohepatitis was compared to 18 patients with simple steatosis. This study determined that receiving carbohydrate in group with non-alcoholic steatohepatitis was much more than the other group. Especially, the rate of taking simple carbohydrate was higher in the group with non-alcoholic steatohepatitis.

It is reasonable that fat and carbohydrate should be consumed according to recommendable rates (according to the recommendations of the American Heart Institute). Therefore, it is emphasized that the components of fat and the kind of carbohydrates (simple and complex) should be changed in the diet of patients with non-alcoholic fatty liver.

Types of Fat in Diet and Other Micronutrients

Contrary to metabolic and cardiovascular diseases, there are sparse epidemiologic studies that evaluate the correlation between the types of fat in diet to the rate of fatty liver. One study was performed with low number of samples but the diet was evaluated accurately (based on 7 days of recording the food intake). 25 patients with non-alcoholic steatohepatitis were compared to the control group in terms of their diets. The diet of patients with non-alcoholic fatty liver was rich of saturated fat and cholesterol, but the content of polyunsaturated fatty acid (PUFA), fiber, ascorbic acid and tocopherol were very low in their diet. The results of this study were supported by the other study and ratio of the receiving the unsaturated fatty acid to saturated fatty acid in the both groups of non-alcoholic steatohepatitis and fatty liver were lower than control group. The relationship between the kind of fat in the diet to the oxidative stress markers was evaluated in patients with non-alcoholic fatty liver. Diet analysis has been done by using of a Food frequency Questionnaire in 43 people with non-alcoholic steatohepatitis and 33 healthy people. There was negative relationship between saturated fat and total received fat to the ratio of glutathione with oxidized glutathione of plasma to fiber, carbohydrate, monounsaturated fatty acids (MUFA) and PUFA. Several kinds of fats can have protective effect on non-alcoholic fatty liver. It is proved that omega 3 improves non-alcoholic fatty liver. The lab studies demonstrated that the diet rich of omega 3 increased the insulin sensitivity in rats. In addition, it decreases the rate of intra hepatic TG and also it improves steatohepatitis.

Two observational studies determined that the rate of omega 3 consumption is low in patients with non-alcoholic fatty liver. In the first study which was performed as case-control study, 45 patients with non-alcoholic fatty liver were adjusted to 856 people in control group in the terms of age and sex. The history of diet was evaluated through food frequency questionnaire (FFQ). The results of study demonstrated that intake of omega 6 and the ratio of omega 6 to omega 3 was higher in the patients with non-alcoholic fatty liver. These results suggested that the quality and quantity of the received fat is more likely higher than the rate of calorie in patients with non-alcoholic fatty liver.

The second cross-sectional study was performed on 349 volunteers. The history of diet was assessed by FFQ. The results of the study indicated that the patients with non-alcoholic fatty liver consumed more red meat and less fish (rich of omega 3). The red meat is rich in omega 6 in terms of fatty acid. Therefore, data suggested that consuming higher red meat increases the ratio of omega 6 to omega 3 in patients with non-alcoholic fatty liver. Two clinical trials suggested the protective role of omega 3 in patients with non-alcoholic fatty liver. The first study was a non randomized controlled study which evaluated the ef-
fect of receiving one year complementary dose of 1000 mg/day omega 3 (EPA, DHA) by 42 patients with non-alcoholic fatty liver compared to 14 people as control group. PUFA supplement significantly improved the serum enzymes (alanine aminotransaminase (ALT), aspartate transaminase (AST), and Gamma Glutamyl Transferase (GGT)) and it decreased the rate of fat development in liver.\textsuperscript{48} The second non-control clinical trial was performed on 23 patients with non-alcoholic fatty liver. These patients received 2700 mg EPA/per day for one year. The level of serum ALT has been improved meaningfully. 7 subjects were under hepatic biopsy after treatment. The sampling demonstrated that the rate of inflammation, steatosis and fibrosis has been improved.\textsuperscript{49} In the both clinical trials, the body weight did not change.

There are sparse studies about the relationship between trans-fatty acids and MUFA to non-alcoholic fatty liver. So, further investigations should be performed on fatty liver and trans-fatty acids as well as MUFA.

**Trans-Fatty Acids**

Quantitative studies were performed to determine the role of trans-fatty acids in the development of non-alcoholic fatty liver. Consuming trans-fatty acids increases the risk of developing insulin resistance and cardiovascular diseases.\textsuperscript{50,51}

In a study which was performed on Syria rats, one group was given PUFA and the other group was given trans-fatty acids. A group who received trans-fatty acids developed impaired glucose tolerance. In addition, the rate of insulin resistance had increased in this rats.\textsuperscript{52} In one study, the effect of Western lifestyle was tested on Syria rats. The rate of liver steatosis significantly increased in rats which received trans-fatty acids associated with beverages rich of fructose.\textsuperscript{53} Therefore, the role of trans-fatty acids should be evaluated in progress of the non-alcoholic fatty liver.

**MUFA**

Oleic acid is consumed as the main source of MUFA in the diet. Olive oil is the most important source of oleic acid (other sources are avocado and seeds). MUFA decreases the blood lipid indices, and it reduces the ratio of low-density lipoprotein (LDL) and total cholesterol to high-density lipoprotein (HDL) as well. In one meta-analysis study, the effect of various diets on lipid and glycemic indices has been evaluated. The result of this meta-analysis indicated that diets rich of monounsaturated fatty acids decreases TG concentration and blood cholesterol 19\% and 22\%, respectively. Also, it increases the rate of HDL but it does not affect LDL.\textsuperscript{54,55}

In one study, rats received methionine and choline deficient (MCD) diet (lack of colin and methionine) associated with monounsaturated fatty acids. Olive oil decreased the rate of TG concentration in liver up to 30\% compared to the other rats that received only MCD diet. Olive oil improves the rate of insulin resistance, increases the rate of hepatic secretion of TG, and decreases the TG flow from the peripheral organs to liver.\textsuperscript{56} The rate of hepatosteatosis was improved in the rats that received olive oil associated with balanced diet.\textsuperscript{57} Contrary to polyunsaturated oil, olive oil prevents progression of hepatic fibrosis.\textsuperscript{58} Nevertheless, it is unclear whether the patients with non-alcoholic fatty liver have received olive oil or MUFA less than healthy people. The role of MUFA in development or improvement of non-alcoholic fatty liver is not fully understood.

**CHOLESTEROL**

The observational studies for cholesterol indicated controversial results. Some studies concluded that there is not any difference between the rate of intake cholesterol in patients with non-alcoholic fatty liver and control group.\textsuperscript{59-61} Nevertheless, Musso et al\textsuperscript{18} in a study showed that people with non-alcoholic fatty liver intake higher cholesterol. Also, a recent study advocated the role of cholesterol of diet in the development of non-alcoholic fatty liver. In this study, 12 subjects with normal weight who developed to non-alcoholic fatty liver were compared to 44 obese subjects with non-alcoholic fatty liver. It should be considered that the rate of intake cholesterol in first group was very higher than second group; however, the rate of intake of unsaturated fatty acids was very low in first group. Therefore, this difference in consuming cholesterol and PUFA likely is associated with progress of non-alcoholic fatty liver in people with normal weight.\textsuperscript{24} In one study on the non-obese animal models, it was shown that diet rich of cholesterol causes non-alcoholic fatty liver.\textsuperscript{60} Increased cholesterol in diet results in increasing the synthesis of fatty acids in hepatic cells.\textsuperscript{24} Eventually, further investigations are required to determine the effect of various diets with different fats and the effect of these fats on progression or improvement of non-alcoholic fatty liver.

**The Relationship between Sweetened Beverages and Non-alcoholic Fatty Liver**

The sweetened beverages have increased the sugar consumption throughout the world.\textsuperscript{62} In the recent decades, consumption of sweetened beverages has elevated in the world.\textsuperscript{62} The recent studies (2005-2006) demonstrated that children and adults intake 172 and 175 Kcal/day, respectively due to drinking sweetened beverages.\textsuperscript{63} Consumption of sweetened beverages is associated with the risk of developing obesity, diabetes, metabolic syndrome, fatty liver, and related cardiac diseases which result from increased intake of calorie as well as very rapid absorption of the available sugar in these beverages.\textsuperscript{59,64-69}

The diets rich of sucrose increase the TG synthesis in the liver. It is evident that the rats and human who intake diet rich of fructose and sucrose are developed to fatty liver.\textsuperscript{70} Therefore, it is reasonable that patients with non-alcoholic fatty liver should lower fructose intake.\textsuperscript{71} In addition, the sweetened beverages such as cola have caramel dye and they are rich of advanced glycation end products (AGEs). These compounds increase the insulin and inflammation resistance.\textsuperscript{61} In the recent years, some
studies confirmed the relationship between non-alcoholic fatty liver and consumption of sweetened beverages. In a study, 31 people with normal weight that developed to non-alcoholic fatty liver were compared to 30 healthy subjects. The patients with non-alcoholic fatty liver significantly consumed sweetened beverages (43% more) and juices (8% more). In the other study, patients with non-alcoholic fatty liver were compared to control group. The patients with fatty liver received sweetened beverages twofold than control group. In one study after adjusting for age, sex and calorie intake on 427 participants with non-alcoholic fatty liver disease, it was demonstrated that daily fructose intake meaningfully was associated with hepatic fibrosis directly. Generally, it should be considered that sweetened beverages play a significant role in developing to non-alcoholic fatty liver. The physician should ask the patient questions about the history of drinking sweetened beverages.

THE PATTERN OF WESTERN DIET AND FAST FOOD

Meals have different kind of food materials and the compound of food materials can have synergistic effect on each other or they can interfere. The investigators consider Western diet as diet with higher fructose, sweetened beverages, red meat, cholesterol, saturated fatty acids, lower fiber, vegetables and fruits. This diet has direct correlation to development of non-alcoholic fatty liver.

In human, fast food consumption has a direct correlation to increased insulin resistance. In coronary artery risk development in young adults (CARDIA) study, the results of the prospective study during 15 years on 3031 participants with non-alcoholic fatty liver demonstrated that people who eat more fast food (more than 2 times in week) have insulin resistance twofold than people who eat fast food less than once a week. In an animal trial, a diet similar to fast food results in impaired hepatic cells. In one study on 18 healthy students, they had received fast food 2 times a day for 4 weeks. The rate of taking energy and weight of these people increased and the rate of insulin resistance became twofold. Also, the rate of serum TG and ALT increased in this people. These foods are rich of energy, saturated fatty acids and trans-fatty acids, simple carbohydrates, and fructose, but they have little fiber. As a result, they increase the fatty acids in liver and they produce local inflammation.

WEIGHT LOSS

In the past decades, during the clinical trials three types of diet have been prescribed for reducing the weight of the patient with non-alcoholic fatty liver. The first diet is very low calorie diet (VLCD) that significantly decreases the weight. The second diet in clinical trials is the balanced diet associated with physical activity and behavior therapy. The third diet is associated with the lifestyle modification.

The examples from first diet includes the number of clinical trials in decades of 1970 and 1960, a low calorie diet (1500 Kcal) and/or fasting that has significantly decreased weight. Steatosis had been decreased in all patients, however, it damaged liver, increased the hepatic necrosis and fibrosis in people with abruptly decreased weight.

In one study which was performed by Anderson et al in 1991, 41 subjects with mortality obesity received a diet associated with a formula generating 400 Kcal. The rate of steatosis was improved but it increased 24% inflammation in hepatic duct and increased hepatic fibrosis. In the other study, VLCD diet with more low weight or equal to 10% normalized hepatic enzymes. Two studies with small sample evaluated the effect of a standard diet associated with gradual decreasing of weight on histology of liver. In a study, after three months treatment there was significantly improvement in the rate of steatosis as well as hepatic inflammation and fibrosis.

In a study on 15 participants with fatty liver, these persons received a standard diet along with behavior therapy. The weight of 9 subjects among 15 reduced up to 7% and as a result it improved the condition non-alcoholic fatty liver; however, in 6 subjects the weight and also of fat concentration in liver did not change.

In the other study, 32 patients with non-alcoholic fatty liver divided to two groups randomly. One group only received education about lifestyle modification (control group) and the other group received a weight loss diet and physical activity (case group). The rate of steatosis in the case group significantly improved than control group. The participants with more than 7% weight loss demonstrated meaningfully improvement in the rate of steatosis and hepatic duct inflammation.

In a clinical trial, ERlistat was used for decreasing the weight in patients with non-alcoholic fatty liver, and 9% weight loss improved steatosis in these patients. In the other study, the lifestyle of participants with non-alcoholic fatty liver and diabetes type II were modified. These patients were given a weight loss diet associated with increased physical activity for 12 months. The control group received some recommendations about improved nutrition and physical activity. After 12 months the case group had more weight loss (approximately 8.5%) than control group and the rate of hepatic steatosis was improved.

In a clinical trial by Suzuki et al, 348 participants with increased ALT received an instructional brochure for weight loss diet. After three months, these persons were evaluated. The rate of serum ALT improved in subjects with more than 5% weight loss, and also the rate of serum ALT in 136 subjects reached to normal rate. In a clinical trial, 152 participants with increased liver enzymes were divided into two groups randomly. One group had more lifestyle modification but the other group had...
less (nutrition and physical activity). At the end of the study, the rate of reduction in the hepatic enzymes was higher in the group with more modification in the lifestyle than other group.67 67 individuals with fatty liver participated in a study. Once a month, they were visited by practitioner and also once every three months they were consulted by nutritionist. In addition, they had equal lifestyle modification (increased physical activity and they received a weight loss diet). At the end of six months, the interference of hepatic enzymes and the ratio of liver to spleen improved in these people.90 Determining the therapeutic effect of weight loss in the clinical trial has two restrictions as follow: the first restriction is due to the little number of samples in the study and the second one is to determine the effect of weight loss on liver histology because liver biopsy is performed in the studies due to ethical considerations. The liver biopsy is essential for evaluating the effect of weight loss on hepatic steatosis. This is important because some diets apparently decrease the hepatic enzymes, but they cause damage of liver. The noninvasive methods should be used for evaluating the histological features of liver and they determine the real effect of weight loss on liver. Nevertheless, in the reviewed studies the weight loss improved the liver function. Weight loss has been confirmed as therapeutic method.

PHYSICAL ACTIVITY

Higher physical activity is beneficial for people. It decreases the risk of developing diabetes type II, insulin resistance, blood pressure, dyslipidemia, impaired glucose tolerance and metabolic syndrome.90-95 The studies demonstrated that physical activity play key role in treatment of patients with non-alcoholic fatty liver. Several observational studies showed that there is reverse correlation between prevalence of fatty liver to the time of physical activity. In one study with more samples, 349 individuals with fatty liver spent less time for physical activity (aerobic and resistance exercises).96 In the other study with 218 participants, there was reverse correlation between physical fitness and developing fatty liver.97 In one study on 37 persons with non-alcoholic fatty liver, hepatic biopsy demonstrated that patients with lower physical activity had higher steatosis in liver.98 The useful effects of physical activity have been supported by recent clinical trials. In a clinical trial on 141 participants with non-alcoholic fatty liver, the subjects were given instruction for physical activity during 3 months. After three months, the weight of persons with physical activity >60 minutes/week significantly decreased (mean 2.4 kg), and also their insulin resistance and hepatic enzymes reduced.99 In other clinical trial, the aerobic physical activity along with a low energy diet helped to normalizing the level of hepatic enzymes.100 Therefore, it appeared that increased physical activity improves the level of hepatic enzymes. In a clinical trial on 19 subjects with obesity, the short time effect of aerobic physical activity on liver, blood, visceral fats and muscular lipid was evaluated by using magnetic resonance imaging (MRI). Cycling for four weeks (three sessions per week for 30 to 45 minutes) meaningfully decreased the rate of plasma TG up to 4%, the rate of visceral fats up to 12%, and the rate of hepatic TG up to 21%. It should be considered that these changes were produced without weight loss.101 For three months, a study has been done on 12 obese subjects with fatty liver. They received the resistance exercise program including two one-hour sessions per week. The physical activity increased the strength and muscularity in participants. Although, the rate of fatty liver did not change, but the rate of insulin resistance was increased without weight loss.102,103 Physical activity causes weight loss and it likely results in increased insulin resistance and glucose homeostasis.104 Physical activity increases insulin receptors in muscles. As a result, the rate of glucose inflow into the muscles is elevated.105 Also, physical activity has beneficial effect on fatty acids oxidation by increasing the rate of oxidation.106 TG concentration will be decreased by higher physical activity.107 The rate of removal of free fatty acids in plasma is decreased in athletes than nonathletes people.108 The similar findings in monozygote twins defined that increased physical activity in one of them (due to the lack of genetic impairment effect) decreases the fatty acids removal by liver.109 In the recent years, there is great attention to resistance exercises for increasing the physical activity.109-111 A study demonstrated that resistance exercises significantly reduce the rate of visceral fat and also it increases the lipid indices.112 A randomized clinical trial showed the effect of aerobic and resistance exercises on cardiovascular diseases. Resistance exercises not only increase the rate of lean body mass but also decrease the rate of total fat of body.113 In one meta-analysis study, the aerobic and resistance exercises were compared and the resistance exercise increased the lean body mass than aerobic one.114 Increased the volume of muscle through increasing the area of reserving glucose reduces the required insulin for normalizing the level of glucose.115 The US Centers of Prevention and Control of Diseases (CDC) recommend that healthy people do more than 30 minutes moderate to severe activity, all days per week, and also they should do resistance exercises more than 3 times a week for >20 minutes in each session. However, these instructions extensively have been recommended, only 27.7% American adults do the moderate to vigorous physical activity and 29.2% do not have any regular activity.116,117 In addition, the prevalence of physical activity in adults with diabetes is very less than non diabetic people.118 People with diabetes less likely perform the recommendations related to physical activity.119,120 In one study, the time of sedentary life of persons was measured. The sedentary time had direct correlation to the rate of fasting insulin.121 Environmental factors such as driving by car instead of walking, sedentary activity and watching TV reduce physical activity.122

DIETARY SUPPLEMENTS

Vitamin E

Vitamin therapy with high dose vitamin E supplement as 1000-300 IU/day (recommended as approximately 30 IU/daily) has been associated with conflict results. In the uncontrolled clinical trials, receiving the vitamin E was associated with reduced hepatic enzymes123; however, simultaneously using of vitamin E, lifestyle modification, diet and physical activity in controlled
trials did not show the therapeutic effects of vitamin E. In randomized clinical trial, 247 patients with non-alcoholic fatty liver randomly divided to three groups: the first group was given 30 mg/day pigolitazone, the second group was given 800 IU/day vitamin E, and the third group received placebo. The duration of this study was 2 years. There was significantly improvement in non-alcoholic fatty liver by vitamin E therapy compare to placebo. Vitamin E and pigolitazone reduced ALT, AST and the rate of steatosis than placebo, but the rate of hepatic fibrosis did not change. Nevertheless, some clinical trials demonstrated that using high dose vitamin E causes stroke and death due to different reasons.

**Vitamin D**

Many studies have suggested that vitamin D potentially play key role in decreasing the development of diabetes type 2, hypertension, and cardiovascular diseases. The level of vitamin D of serum independently is related to the beta cells functions and insulin sensitivity in patients with diabetes type II. In one study, the level of serum vitamin D was low in patients with non-alcoholic fatty liver. In a study, Targer et al. compared 60 patients with non-alcoholic fatty liver 60 healthy individuals in terms of the level of serum vitamin D. the level of serum vitamin D in the group with non-alcoholic fatty liver was lower than healthy individuals. In patients with non-alcoholic fatty liver, the level of serum vitamin D was related to the level of steatosis, inflammation and hepatic fibrosis. In one study, the relationship between vitamin D of serum, fatty liver and cardiac diseases was evaluated. In this study, 670 patients with non-alcoholic fatty liver were compared to 30 healthy individuals. Patients with non-alcoholic fatty liver had very lower level of serum vitamin D than control group. Eventually, further investigations are required for evaluating the correlation between the level of serum vitamin D, non-alcoholic fatty liver and the therapeutic effects of serum vitamin D on non-alcoholic fatty liver.

**CONCLUSION**

Non-alcoholic fatty liver not only is a chronic hepatic disease but also it predisposed to development of diabetes type II. Also, in some studies the non-alcoholic fatty liver is related to cardiovascular diseases. It is important to know the risk factors which result in non-alcoholic fatty liver because prevention of these risk factors can reduce the risk of non-alcoholic fatty liver. The relationship between nutrition to non-alcoholic fatty liver and risk factors for hepatic steatosis in Northern Italy. The treatment team for patients with this disease should be consisted of dietician, psychology, and expert of physical exercise so as to achieve the therapeutic purposes.

**REFERENCES**

8. Bedogni G, Bellentani S. Fatty liver: How frequent is it and


97. Church TS, Kuk JL, Ross R, Priest EL, Biltoft E, Blair SN. Association of cardiorespiratory fitness, body mass index, and waist circumference to non-alcoholic fatty liver disease. *Gastroenterology.* 2006; 130: 2023-2030. doi: 10.1053/j.gastro.2006.03.019


120. Newton JL, Jones DE, Henderson E, et al. Fatigue in non-alcoholic fatty liver disease (NAFLD) is significant and associates with inactivity and excessive daytime sleepiness but not with liver disease severity or insulin resistance. Gut. 2008; 57: 807-813. doi: 10.1136/gut.2007.139303


144. Kessler A, Levy Y, Roth A, et al. Increased prevalence of


148. Anderson AS. How to implement dietary changes to prevent the development of metabolic syndrome. *Br J Nutr*. 2000; 83(Suppl 1): S165-S168. doi: [10.1017/S0007114500001112](http://dx.doi.org/10.1017/S0007114500001112)


Pesticide Residue Dissipation Upon Storage and Processing in Chickpea Legume for Food Safety

Geetanjali Kaushik, PhD; Santosh Satya, PhD; S. N. Naik, PhD
Centre for Rural Development and Technology, Indian Institute of Technology (IIT), New Delhi, Delhi 110016, India

ABSTRACT

Aim: Pesticide residue dissipation in chickpea legume under simulated storage conditions as well as effect of processing techniques was investigated. Further, the impact of pesticide residues was studied on micronutrient bioavailability.

Background: Food quality and safety have assumed an important dimension in current scenario. It is important to investigate the pesticide residues present in the grains from storage in warehouses and their dissipation through processing.

Methods: Pesticide residues in chickpea grains were analyzed through gas chromatography (GC) while simulated gastric digestion was undertaken for bioavailability.

Results: The dissipation pattern of chlorpyrifos and its metabolites under grain storage conditions for 5 months revealed that the residues exceeded the maximum residue limit (MRL) values right from the beginning of the storage. The effect of processing techniques showed that soaking and germination eliminated almost all the pesticide residues. However, the build-up of toxic metabolite oxon during pressure cooking and microwave (MW) cooking is a matter of great concern because of its greater toxicity than parent pesticide molecule. The impact of pesticide residues on the bioavailability of micronutrients showed that chlorpyrifos did not impact the bioavailability of Fe and Zn but significantly reduced the bioavailability of Cu and Mn at the highest spiking level (25 ppm) of chlorpyrifos.

Conclusion: Storage of grains leads to accumulation of residues which are eliminated by household processing techniques. Pesticide residues impact micronutrient bioavailability in grains.

KEYWORDS: Chlorpyrifos; Chickpea; Storage; Processing.

ABBREVIATIONS: GC: Gas Chromatography; MRL: Maximum Residue Limit; NCR: National Capital Region; PCB: Polychlorinated biphenyls; AchE: Anti-cholinesterase; TCP: 3,5,6-trichloro-2-pyridinol; DON: Deoxynivalenol; ANOVA: Analysis of variance; DDT: Dichlorodiphenyl-trichloro-ethane.

INTRODUCTION

Food is the basic necessity of life and food contaminated with toxic pesticides is associated with severe ill effects on the human health. Food legumes are an important part of the human diet, as these are good sources of proteins, carbohydrates and dietary fibres with satvik characteristics. On account of their high nutritive value they would play an important role in ensuring nutritional security especially for the developing countries. Keeping in view the fact that legumes are more susceptible to pest infestation, these are likely to be contaminated with certain chemical pesticides right from the crop growth to grain storage which may affect the food safety. Bengal gram or chickpea (Cicer arietinum) commonly called chana has been one of the widely consumed pulses in India. It is used in preparing a variety of snacks, sweets and condiments. Fresh green seeds are also consumed as green vegetable. Chickpea grains are usually stored for long periods in warehouses where various pesticides are intensively and successively applied many times resulting in their bioaccumulation. Many studies have shown...
that pesticide residues penetrate the grains and accumulate over time, thus indirectly exceeding the recommended doses. Therefore, studies in pesticide contaminated stored grain matrix under tropical conditions need to be undertaken to study pesticide behavior and dissipation. In this regard, pesticide chlorpyrifos was selected for the present study as it is widely used pesticide for various applications including grain storage system.

In the initial phase of green revolution, chemical pesticides have contributed to the increase of yields in agriculture by successfully controlling pests and diseases. Inappropriate application of pesticides affects the whole ecosystem by entering the residues in food chain and polluting the soil, air, ground and surface water. As a result increasing incidences of cancer, chronic kidney diseases (CKDs), suppression of the immune system, etc. have been reported.

Major pesticides used in crop production include organophosphates (such as malathion, chlorpyrifos), organochlorines (endosulfan, lindane, aldrin, dieldrin), synthetic pyrethroids (deltamethrin, cypermethrin, bifenthrin) and carbamates (carbaryl, bendiocarb) while in storage mainly pyrethroids (deltamethrin, cypermethrin, bioresmethrin) and organophosphates (malathion, chlorpyrifos) are employed. Chlorpyrifos is one of the world’s most widely used organophosphorus pesticides in agriculture. The use of chlorpyrifos has been restricted in US and some European countries but it is still in use in developing countries like India, where in the year 2000, it was the fourth highest consumed pesticide. Also during a survey in National Capital Region (NCR) in 2009, it was found that chlorpyrifos is the most consumed pesticides. Chlorpyrifos is bioactivated in the liver to chlorpyrifos-oxon, which then rapidly hydrolyzed to 3,5,6-trichloro-2-pyridinol (TCP). TCP has insignificant anti-cholinesterase (AChE) activity and is not regarded as toxicologically important, whereas chlorpyrifos-oxon is a potent cholinesterase inhibitor. Studies have indicated that the insecticide chlorpyrifos and its oxon metabolite inhibit acetylcholinesterase activity (AChE).

Some studies on pesticide residue dissipation in grains established that during storage the residues of pesticides were able to penetrate into the grains and accumulated with time. Stored grains being treated with chemical pesticides show presence of bound residues even after fairly long periods of storage contributing to dietary intake of pesticides. Food processing at domestic and industrial level would be effective in the dissipation of pesticide residues in food. Metabolic and biochemical pathways lead to biotransformation of pesticides and their dissipation through domestic processing techniques. Further, in case of pesticide residue dissipation in food system, most of the research work overlooked the metabolites of pesticides which in some cases are even more toxic than the main pesticide. Also, an important aspect related to the bioavailability of micronutrients in the pesticide contaminated food matrix has not even been considered. Therefore, this research investigates the pesticide residues present in the grains under simulated storage (warehouse) conditions and their dissipation through processing.

MATERIALS AND METHODS

Reagents and Chemicals

Chlorpyrifos (20 EC) was purchased from Agrochemical dealer Dev Udyog, Nehru Place, New Delhi, India. The analytical standard of metabolite—TCP, PESTANAL (99.3% purity, 100 mg) was procured from Sigma-Aldrich and Chlorpyrifos-Oxon (94.0% purity, 50 mg) was of Dr. Ehrenstorfer GmbH make. All the reagents used in the experiments were procured from Qualigens Fine Chemicals, Mumbai, Maharashtra, India.

Sample Collection and Processing

Organic chickpea seeds were procured from organic farms of Navdanya, situated in Dehradun, India. The seeds were cleaned, separated from broken seeds and the dust and other foreign materials were removed.

Simulated Storage Design

The dissipation of chlorpyrifos in chickpea under simulated storage conditions was studied for which chlorpyrifos was applied according to the specifications given by Central Warehousing Corporation, 2007 according to which 20 EC chlorpyrifos diluted with water (1:19) is sprayed at a dose of 3 L/100 m². Gunny bags of 5 kg capacity were taken and filled with chickpea grains (2 kg) and placed on separate tables with plastic sheets spread over them. Then chlorpyrifos was sprayed at the recommended (6 g a.i./m²) and double recommended doses (12 g a.i./m²). The experiment was setup for 5 months (May-September 2009) in field laboratory (Micromodel Complex), IIT Delhi. The samples...
at various time intervals during storage were collected and analyzed for chlorpyrifos and its metabolites (oxon and TCP) by GC method. Three replications were taken for each treatment including control.

**Recovery Studies**

The levels selected for spiking with the pesticide standard after literature review were 1, 5 and 10 ppm. One kilogram of chickpea was finely ground in a domestic electric grinder out of which 50 g of ground chickpea sample was taken in conical flasks. The samples were spiked with 1.0 mL of 1, 5 and 10 ppm standard solutions to each conical flask. The samples were then kept at room temperature and homogenized by stirring with a glass rod until the solvent is evaporated.

**Sample Preparation**

Twenty-five grams of sample was extracted with 200 mL acetone—water (8:2). The extracts were filtered through Buchner funnel using Whatman no.1 under suction. After rinsing twice with 25 mL of acetone the sample was concentrated in rotary vacuum evaporator over a water bath at 50 °C to about 50 mL. The concentrated extract was taken in 500 mL separatory funnel and diluted with 250 mL of 5% aqueous sodium chloride and added hexane (100 mL thrice) for partitioning. Passed the combined layers of hexane containing pesticide residue through anhydrous sodium sulphate and concentrated to near dryness. The concentrate was taken in about 10 mL hexane for adsorption chromatography. In a glass column wet packed 5 g anhydrous sodium sulphate, 20 g of silica gel and 10 g anhydrous sodium sulphate bottom upward and pre-washed with 50 mL hexane. Just as the extract drained into the sodium sulphate, added 150 mL of 5% ethyl acetate in hexane and eluted chlorpyrifos. After concentrating as before, it was taken in a suitable volume of hexane for estimation by GC.

**Pesticide Treatment**

Twenty emulsifiable concentrate (EC) of chlorpyrifos was diluted with water to form a stock solution of 1000 ppm which was further diluted to contain 10, 15 and 25 ppm solutions. One kilogram of chickpea thinly spread in a tray lined with aluminium foil was sprayed with the pesticide of above concentration. The samples were spiked with 1.0 mL of 1, 5 and 10 ppm standard solutions to each conical flask. The samples were then kept at room temperature and homogenized by stirring with a glass rod until the solvent is evaporated.

**Determination of Bioavailability of Minerals**

For determination of mineral bioavailability, chlorpyrifos was diluted to 1000 ppm and further diluted to contain 1, 5, 10, 15 and 25 ppm solutions which were applied to chickpea as mentioned in the “Pesticide treatment” section. Bioavailability of minerals from chickpea samples were determined by an in vitro method. All grain samples were finely ground in a stainless steel blender. The ground samples were subjected to simulated gastric digestion by incubation with pepsin (pH 2.0) at 37 °C for 2 h. Titratable acidity was measured in an aliquot of the gastric digest by adjusting the pH to 7.5 with 0.2 M sodium hydroxide in the presence of pancreatin—bile extract mixture (1 L of 0.1 M sodium bicarbonate containing 4 g pancreatin+25 g of bile extract). To simulate intestinal digestion, segments of dialysis tubing (molecular mass cut-off 10 kDa) containing 25-ml aliquots of sodium bicarbonate solution, being equivalent in moles to the sodium hydroxide needed to neutralize the gastric digest (titratable acidity) determined as above, were placed in Erlenmeyer flasks containing the gastric digest and incubated at 37 °C with shaking for 30 min or longer until the pH of the digest reached 5.0. Five milliliters of the pancreatin—bile extract mixture were then added and incubation was continued for 2 h or longer until the pH of the digest reached 7.0. At the end of the simulated gastrointestinal digestion, the minerals present in the dialysate were analyzed by atomic absorption spectrometry. The dialyzable portion of the total minerals present in the sample (expressed as percent) represented the bioaccessible minerals.

**Processing Techniques**

The effect of simple domestic processing techniques like soaking and germination, ordinary cooking, pressure cooking and microwave cooking on chlorpyrifos and its metabolites was studied in chickpea seeds. Processed seeds were ground and analyzed for residues. The spiked unprocessed seeds had residue levels of 8.22, 11.95 and 22.41 ppm, respectively, for spiking at levels of 10, 15 and 25 ppm, respectively.

Soaking and germination soaked seeds were germinated for 24, 48 and 72 hours at 25 °C in sterile petri-dishes lined with damp filter papers in incubator.

Ordinary cooking seeds were cooked in distilled water (1:5, w/v) on a hot plate for 120 min till they became tender.

Pressure cooking seeds were autoclaved at 15 lbs/inch² for 20 min in beakers covered with aluminium foil containing distilled water (1:5, w/v).

Microwave cooking Seeds containing distilled water (1:5, w/v) were cooked in a domestic Samsung Bioceramic microwave oven (model CE283DN-850 W cooking power) for 15 min.

After each type of processing, excess water was drained and the samples were dried in a hot air oven at 55 °C for 24 h. The samples were then ground in a domestic electric grinder to pass through a 100 mesh sieve and then stored in resealable plastic bags at room temperature for further analysis.

**Analysis of Chlorpyrifos and its Metabolites**

Perkin Elmer Gas Chromatograph model Clarus-500 with NPD detector was used for analysis of samples. A sample of the reduced eluate (2.0 IL) was injected into the GC. Temperature pro-
Programming for the above instrument was as follows:

Temperature: 120 °C for 3.00 min then 5 °C/min to 270 °C, hold for 10.00 min, detector: NPD at 300 °C, injector temperature 250 °C; carrier gas: helium at 1.5 mL/min. The retention times of TCP, chlorpyrifos and chlorpyrifos oxon were 5.29, 17.35 and 17.96 min, respectively.

Statistical Analysis

Mean and standard deviations were calculated from the results of the analysis performed. The data were subjected to analysis of variance (ANOVA). Means comparison was performed using Duncan’s Multiple Range Test (MRT). Significance was defined as \( p < 0.05 \).

RESULTS

Effect of Simulated Storage Conditions on the Fate of Chlorpyrifos and its Metabolites

It is evident from the results that chlorpyrifos is able to penetrate the grains even in gunny bags over which a plastic sheet was spread. It is very peculiar to note that initially (0-20 days) the concentration of chlorpyrifos in the grains is less than that of its metabolite oxon (0.39 ppm and 1.37 ppm) which might be due to the rapid conversion of penetrating chlorpyrifos to oxon facilitated by high temperature and intense sunlight on roof of lab room during peak summer season of the study.

With time, initially the accumulation of pesticide in chickpea grain increases and becomes maximum (4.61 ppm, recommended dose and 5.59 ppm, double recommended dose) after 30 days of storage (Figures 1 and 2). Even the concentration of its toxic metabolite oxon (2.66 ppm and 2.69 ppm at the two doses) is highest at this storage period. Further, the concentrations of chlorpyrifos and oxon decrease continuously during the remaining storage period. TCP which is not present initially appears after 30 days and then builds up in concentration and reaches maxima on 75th day and subsequently follows a continuous decline. It is probably as a result of conversion of oxon into TCP. The concentration of TCP becomes maximum after 75 days (3.56 and 4.35 ppm at two doses respectively).

The point that needs to be stressed here is that the maximum toxicity generated due to the metabolites is at 30th day.
(oxon concentration is maximum, toxicologically significant); though the calculated equivalent chlorpyrifos residues come out to be highest in chickpea grain samples after 75 days viz. 10.05 ppm and 11.55 ppm respectively. These two samples drawn at 75th day appear less toxic as compared to the grain samples taken in the initial storage phase (1 to 30 days) as the concentration of oxon is highest in this duration. As mentioned earlier that oxon is 3000 times more neurotoxic than the parent compound chlorpyrifos so the initial samples are highly unsuitable for consumption on account of very high toxic content. It is also clear that conversion of residues of metabolites into equivalent chlorpyrifos residues for ease of simplicity does not give correct picture of degree of hazards as it masks the very important fact of manifold toxicity of the metabolites.

The dissipation pattern of chlorpyrifos was almost similar for both the treatments (i.e. recommended and double recommended dose), but slightly faster dissipation was noticed in the case of higher rate of application. This could be attributed to the fact that at higher dose of pesticide application, the quantity of chlorpyrifos available per unit area is more as compared to the lower dose of chlorpyrifos. Figures 1 and 2 give the dissipation pattern of chlorpyrifos and its metabolites under simulated storage condition.

If the MRL value reported for chlorpyrifos in case of legumes (0.05 ppm) set by the European Union is extended to chickpea, the present data reveals that the residues of chlorpyrifos and its metabolites present in grains even after 5 months of storage are not at a safe level and may pose hazards if offered for consumption without decontamination.

### Effect of Domestic Processing on Dissipation of Chlorpyrifos and its Metabolites

The chlorpyrifos treatment on chickpea grains was found to be persistent for protection from storage pests. Significant amount of residues (including metabolites) were observed even after 5 months of storage which indicates the concern regarding food safety. Hence, the effect of some simple, feasible domestic processing techniques on pesticide residues in chickpea was studied to see whether the grains could be made acceptable and safe for consumption. The processing methods include soaking coupled with germination, ordinary open cooking, pressure cooking and microwave cooking. Data are presented in Table 1.

It is evident from Table 1 that germination (24 h) of chickpea seeds leads to almost complete dissipation of chlorpyrifos residues without the generation of its metabolites. Data shows that various cooking techniques used lead to high chlorpyrifos dissipation in chickpea but pressure cooking and microwave cooking were associated with generation of toxic metabolites oxon and TCP. Oxon is believed to be around 3000 times more neurotoxic than chlorpyrifos, the research findings reveal a very important concern regarding use of pressure cooker and microwave for cooking of legumes, as in ordinary open cooking, toxic metabolites are not generated at same concentration of chlorpyrifos treatment. At 15 ppm level of chlorpyrifos contami-

### Table 1: Effect of processing techniques on chlorpyrifos and its metabolites in chickpea.

<table>
<thead>
<tr>
<th>Spiking level</th>
<th>Chlorpyrifos and metabolites</th>
<th>Processing Techniques</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soaking &amp; Germination</td>
<td>Ordinary cooking</td>
</tr>
<tr>
<td>10 ppm (8.22 ppm)</td>
<td>Chlorpyrifos</td>
<td>0.15±0.03</td>
</tr>
<tr>
<td></td>
<td>Oxon</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>TCP</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Equivalent Chlorpyrifos residues</td>
<td>0.15a</td>
</tr>
<tr>
<td>15 ppm (11.95 ppm)</td>
<td>Chlorpyrifos</td>
<td>0.18±0.04</td>
</tr>
<tr>
<td></td>
<td>Oxon</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>TCP</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Equivalent Chlorpyrifos residues</td>
<td>0.18a</td>
</tr>
<tr>
<td>25 ppm (22.41 ppm)</td>
<td>Chlorpyrifos</td>
<td>0.29±0.09</td>
</tr>
<tr>
<td></td>
<td>Oxon</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>TCP</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Equivalent Chlorpyrifos residues</td>
<td>0.29a</td>
</tr>
</tbody>
</table>

Note: Values in parentheses of first column (Spiking level) denote actual level of initial residues. Results are means of triplicate±standard deviation. Different alphabets (a,b,c) in same row denote significant differences (p<0.05).
nation, MW cooking generates almost 3 times more oxon than pressure cooking while in ordinary cooking oxon is not detected. With the increase in contamination to 25 ppm, the generation of oxon during MW becomes almost 5 times to that generated in pressure cooking.

**Impact of Chlorpyrifos Residues on Micronutrient Bioavailability in Chickpea**

In nutritional science, the bioavailability of an essential metal is determined by its metabolic utilization. For this purpose, the concept of “total utilization” defines the fraction of a nutrient that is ultimately used in metabolism after its digestion, absorption, and distribution.\(^{21}\)

The data given in Table 2 clearly indicates that the bioavailability of micronutrients varied depending on the type of mineral and the concentration of the spiked pesticide. Bioavailability of iron and zinc did not change in pesticide contaminated chickpea (1-25 ppm). In case of manganese, the pattern was very different. An increase in bioavailability was noted with the increase in chlorpyrifos concentration up to 15 ppm but at higher spiked level (25 ppm) substantial decrease (54%) in bioavailability was observed. In case of copper there was slight but insignificant increase in bioavailability in chickpea grains spiked with chlorpyrifos concentrations ranging from 1 ppm to 10 ppm, however the bioavailability decreased significantly (26.66%) at the highest spiking level of 25 ppm of chlorpyrifos.

### Table 2: Variation of mineral bioavailability (percent) with chlorpyrifos spiking.

<table>
<thead>
<tr>
<th>Spiking Level</th>
<th>Fe</th>
<th>Zn</th>
<th>Mn</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.88±0.21(^a)</td>
<td>1.56±0.16(^b)</td>
<td>0.65±0.07(^b)</td>
<td>0.15±0.04(^c)</td>
</tr>
<tr>
<td>1 ppm</td>
<td>0.85±0.09(^a)</td>
<td>1.59±0.13(^b)</td>
<td>0.86±0.05(^c)</td>
<td>0.17±0.01(^b)</td>
</tr>
<tr>
<td>5 ppm</td>
<td>0.91±0.03(^a)</td>
<td>1.49±0.04(^a)</td>
<td>0.79±0.04(^b)</td>
<td>0.16±0.02(^b)</td>
</tr>
<tr>
<td>10 ppm</td>
<td>0.86±0.06(^a)</td>
<td>1.57±0.11(^a)</td>
<td>0.73±0.08(^a)</td>
<td>0.16±0.05(^b)</td>
</tr>
<tr>
<td>15 ppm</td>
<td>0.97±0.09(^a)</td>
<td>1.58±0.15(^a)</td>
<td>0.75±0.11(^b)</td>
<td>0.15±0.01(^a)</td>
</tr>
<tr>
<td>25 ppm</td>
<td>0.94±0.08(^b)</td>
<td>1.41±0.07(^a)</td>
<td>0.30±0.05(^a)</td>
<td>0.11±0.03(^a)</td>
</tr>
</tbody>
</table>

**Note:** Results are means of triplicate±standard deviation. Different alphabets in same column denote significant differences (\(p<0.05\)).

In another study, steeping was found to be the most important processing step in the removal of fenitrothion and nu-arimol residues (52%) followed by germination (25%) during the malting of barley.\(^{37}\) Recently, it was reported that cooking in a microwave oven causes a decrease in the residue levels of pesticides trifluralin, chlorpyrifos, decamethrin, cypermethrin and dichlorvos in rice and beans (pesticides spiked at levels of 1.0 ppm) when they were cooked in a microwave at 500 W and 800 W (power) for 15-45 min, respectively. After cooking, 92% to 99% of pesticides were eliminated.\(^{38}\) In a study on the effect of cooking on dichloro-diphenyl-trichloro-ethane (DDT) and its derivatives in green beans it was concluded that pressure cooking for 3 min at 15 psi resulted in a greater decrease in total amounts of DDT and its derivatives than microwave cooking for 6 min.\(^{39}\) A few studies for pesticide contaminated vegetables have been reported. For example, processing of spinach for 66 min at 122 °C reduced the pesticide residues and 100% dissipation of azinphos-methyl, 96% of malathion and 100% of methyl parathion was noted.\(^{40}\) Cooking of tomatoes at 100 °C for 30 min lead to 71.0-81.6% reduction in organophosphorous pesticides.\(^{17}\) In a recent study, it was found that boiling resulted in 100%, 92% and 75% organophosphate reduction respectively in brinjal, cauliflower and okra.\(^{41}\) It is inferred that processes involving heat treatment can increase volatilization, hydrolysis or other chemical degradation and thus reduce residue levels in cooked food.\(^{14,42}\)

An important aspect of micronutrient availability in
peptide contaminated food has not been given attention, so data of the present study could not be corroborated. However, a few clinical studies report the impact of pesticide residues on mineral bioavailability. Pesticides did not affect the retention of zinc in the mucosa and small intestines of rats. Certain carbohydrates chelate with essential metal copper and reduce its availability in animals. Both lindane and linuron affect calcium metabolism. Dietary polychlorinated biphenyls (PCB), DDT and butylated hydroxyanisole (BHA) raised the level of copper in liver, kidney, or serum. The incubation of hepatocytes for 60 min with 2, 4-D pesticide resulted in a significant increase in calcium to massive levels which was accompanied with the loss of cell viability.

CONCLUSION

The dissipation pattern of chlorpyrifos and its metabolites in chickpea grains under simulated storage conditions revealed that chlorpyrifos and its metabolites were present in stored grains. Also, the residues exceeded the MRL values right from the beginning of the storage duration and even a 5 month period could not bring the residues below MRL. The chlorpyrifos residues were found 4 times (at the recommended dose) above the MRL highlighting the concern regarding safety of such stored grains for human consumption. The issue which further complicates the matter is the presence of metabolites viz. oxon (along with TCP) generated during grain storage which is even more toxic than the parent compound, chlorpyrifos. Here, it is important to note that existing MR values (by International Authorities) do not reflect any information about the harmful metabolites generated in grain matrix. Therefore, concerted efforts by considering toxic metabolites for evolving dietary guidelines for ensuring food safety have to be made. Fortunately, domestic processing techniques can thus reduce the residues of chlorpyrifos thus decontaminating the grains for human consumption. It was found that soaking and germination eliminated almost all the residues in stored chickpea while cooking processes also resulted in high chlorpyrifos dissipation but the build-up of toxic metabolite oxon especially during pressure and microwave cooking is a matter of great concern regarding food safety.

In fact, overall such studies reported in literature are scanty and scattered. Present systematic study focusing on metabolites of pesticide (chlorpyrifos) applied revealed very important findings related to food safety and nutritional security. Hence, fast cooking by microwave has to be recommended carefully and significance of traditional cooking system (through require more time) should be reinvestigated from the view point of food safety. Further, extensive and intensive investigation considering successive spray of various pesticides used in the warehouse for controlling storage pests needs to be carried out in the context of food safety.

ACKNOWLEDGEMENTS

Authors are grateful to Dr. K. Srinivasan for laboratory facilities at CFTRI, Mysore and Ms. Poonam Singhal (CRDT, IIT Delhi) for scientific discussion.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES


4. Agnihotri NP. Pesticide safety and monitoring. All India coordinated research project on pesticides residues. Pusa, New Delhi, India: Indian Council of Agricultural Research; 1999.


Nutraceuticals for Athletes

Charu Gupta, PhD1*; Dhan Prakash, PhD1; Sneh Gupta, MSc2

1Amity Institute for Herbal Research and Studies, Amity University, Noida, UP 201313, India
2Department of Zoology, RGPG College, Chippi Tank, Meerut, UP 250001, India

*Corresponding author
Charu Gupta, PhD
Amity Institute for Herbal Research and Studies
Amity University
Noida, UP 201313, India
E-mail: charumicro@gmail.com

ABSTRACT

It is widely known that athletes used to rely on training and good nutrition to provide maximum results. But now to maintain a competitive edge, athletes are switching to nutraceuticals. They are dietary supplements that provide health benefits. Nutraceuticals and dietary supplements are now commonly used to enhance and improve the health and performance of athletes. This has triggered the sale of nutraceuticals in the market and stores and gyms are selling hundreds of different supplements. These nutritional supplements usually claim to improve strength, performance and stamina, help build lean muscle, and help to burn excess fat. There are many health and nutritional supplements available in the market for the athletes. It is a difficult choice to choose the suitable and appropriate nutraceutical for oneself. The present article will introduce the athlete to a variety of different nutritional health supplements available in the market including fat burners, conjugated linoleic acid (CLA), numerous essential fatty acids (EFAs), creatine, whey proteins, glutamine, Tribulusterrestris, beta-hydroxy beta-methylbutyrate (HMB), methoxyisoflavone, and Cordyceps.

KEYWORDS: Nutraceuticals; Athletes; Nutrition; Health; Whey protein; Creatinine; Cordyceps; Tribulus.

ABBREVIATIONS: CLA: Conjugated Linoleic Acid; EFAs: Essential Fatty Acids; HMB: Beta-hydroxy beta-methylbutyrate; NHIS: National Health Interview Survey; FDA: Food and Drug Administration; IOC: International Olympic Committee; RDA: Recommended Dietary Allowance; BCAAs: Branched Chain Amino Acids; GMP: Glycomacropeptide; TGF-β: Transforming Growth Factor-Beta; PUFAs: Polyunsaturated fatty acids; CLA: Conjugated Linoleic Acid; DHA: Docosahexaenoic acid; EPA: Eicosapentaenoic acid; ATP: Adenosine triphosphate.

INTRODUCTION

The demand for sports nutraceuticals is increasing day-by-day due to the greater consumer awareness and increasing acceptance of nutraceutical supplements in the market. This increasing consumer demand for supplements geared towards sports nutrition will continue to see strong sales in coming years. According to BCC Research, the global market for sports nutrition products is expected to grow at a rate of 24.1%, and is projected to reach $91.18 billion this year.1

The major reasons for explosive growth of such types of athlete performance supplements are due to changing product market focus. Traditionally, sports endurance supplements mainly target towards body-building and other professional athletes. However, sales of sports nutrition products have now been promoted and have been classified into multiple fitness categories. The sports industry has expanded to include the individuals that also participate in recreational workouts and other physical activity. Although athletes performance products have been geared towards male consumers, now-a-days the sports performance nutritional supplements are popular in both the genders. There has also been growth within the youth segment for athletes performance nutritional supplements. A survey performed by the National Health Interview Survey (NHIS) shows that 1.6% of kids, are now users of sports performance formulations. These changing demographics of the typical sports endurance supplement user have
been responsible for the growth of the base market for sports supplements and nutraceuticals.1

The term nutraceutical is a hybrid of the words ‘nutrient’ and ‘pharmaceutical’. Nutraceuticals are generally dietary supplements derived from food sources with added health benefits. They are defined as any extract (derived from plant or animal origin) that has health-related benefits beyond those obtained by normal nutritional means. Some common examples include elk velvet antler, Tribulus while caffeine and androstenedione does not belong to the nutraceutical group. They are not Food and Drug Administration (FDA)-regulated and their therapeutic value has been debated for years, including their potential in the prevention of several chronic diseases like diabetes, slowing the ageing process, mitigate addiction tendencies, relieve anxiety, improve mental acuity and rally antioxidant defenses. As quoted by Greek physician Hippocrates, a proponent of using food as medicine, probably recognizes their potential.

Nutraceuticals can be developed for the benefit of the athletic community. They can be used to influence the athlete’s performance either directly or indirectly since nutraceuticals are not drugs; they are natural and healthy and they compromise the health of the athletes. Moreover, nutraceuticals use is also not banned by the International Olympic Committee (IOC) athletes commission. There is no ethical and legal dilemma. So such form of nutritional therapy cannot be denied to the athletes. However, there might be certain problems with the use of nutraceuticals by the athletes. As nutraceuticals can induce positive drug tests, it can result in disqualification.

Gurley et al2 reported in a study that 11 out of 20 ephe-dra supplements that claimed to contain the particular amount of ingredient (active phytochemical) as labeled on their container, they either failed the quality test or had more than 20% difference from the actual amount. It was also reported in another meet that Tribulus is often contaminated with testosterone, nor-testosterone and sometimes androstenedione also; around 25% of Gingko biloba preparations failed to qualify the quality tests. Thus to make nutraceuticals acceptable for the athletes, the issue should be addressed with valid scientific investigations.

The scientific issues can be resolved by scientific validation of the nutraceuticals with respect to quality controls. Controlled clinical trials should be conducted in order to determine if nutraceuticals can influence athletic performance or drug tests. In the present paper, the role and functions of nutraceuticals in enhancing athletic performance is studied.

NUTRACEUTICAL TYPES FOR ATHLETES

**Organic Fat burners**

These are one of the largest sold supplements to athletes. Fat burners serve a variety of functions that includes enhancing basal metabolic rate and energy levels of the body, suppresses appetite, and reduces excess water levels. Athletes typically use fat burners to improve their energy levels to enhance their training and performance. There are numerous ingredients and their combinations present in fat burners. Some of the common and popular ingredients present in organic fat burners are guarana and/or kola nut (natural sourced caffeine), Citrus aurantium (bitter orange), and cayenne pepper. These are mostly used to increase metabolism and energy. Chromium and Garcinia cambogia are amongst some most frequently used appetite suppressants, while Dandelion root is a common diuretic.1

Fat burners, while providing benefit for many people, are also the most abused supplements in the market. Exceeding the recommended dosage is a common practice by many people in the hope of obtaining quicker and more profound results. This practice poses a danger to many people and they can have serious consequences on their health. The recent ban of the herb Ephedra by Health Canada is a prime example. Ephedra was commonly used by athletes as a fat burner for many years. The abuse of this supplement in recent years has caused serious side effects in many people, which prompted Health Canada to ban this product from the market. Fat burners that are used correctly can be an excellent addition to a work out regime.1

The natural fat burners most commonly used are Garcinia cambogia, green coffee bean and raspberry ketone. Raspberry ketone is a primary aroma compound present in red raspberries. It regulates the release of a protein called adiponectin (used by body to regulate the metabolism). The raspberry ketone compound causes the breakdown of fat cells more effectively, which in turn helps to burn the fats in the body. The raspberry ketone is very effective when combined with regular work out along with a nutritious diet. The recommended intake is 100 mg per day.1

Another natural fat burner is a plant Garcinia cambogia whose fruits are utilized in making weight loss products. This small pumpkin shaped fruit prevents the storage of fat while controlling the appetite. Unlike other supplements, garcinia does not require any special diet but in order to have better results, a sensible balanced diet along with regular exercise is preferred.

Green coffee beans are another group of natural fat burners. These include the coffee beans before they are roasted. This is because the amount of active phytochemical called chlorogenic acid gets reduced after roasting. Chlorogenic acid has various health benefits like it aids in weight loss, reduces heart disease risk, diabetes and boosts the immune system. The green coffee beans regulate blood sugar and metabolism that helps in weight reduction.3

Thus, organic fat burners are more preferable than the artificial fat burners as they are natural and have no side effects. This should be accompanied with balanced diet and regular exercise. Just to cite an example, one of the commercially available fat burners is T5 fat burner marketed by Biogen. This nutraceutical...
In addition to promoting healthy fat burning capabilities, it also increases the body’s natural production of metabolic hormones T4 (thyroxine) and T3 (tri-iodothyronine). Both these hormones are produced by the thyroid gland and are primarily responsible for the regulation of metabolic rate and increase the levels of focus and mental alertness. It contains more thermo-genic ingredients that aids in increased fat cell lipolysis, super-charged metabolism, extreme fat loss, enhanced energy levels, greater concentration and maximum thermogenesis.1

**Muscle Building Supplements**

These supplements are the important component of an athlete’s diet. There are many essential nutrients that are required to stimulate growth hormone, testosterone and maintain metabolic rate. Some of the important muscle building supplements are:

**Whey proteins (WP):** As per recommended dietary allowance (RDA), the daily protein intake of a normal person should be 1-1.5 g of protein per kg body weight.1 This amount cannot be recovered from the diets alone. Protein is essential for athletes to build and repair muscle that is broken down during exercise. Protein supplements in the form of whey proteins are widely recommended. Whey proteins are considered to have the highest nutritional values of all food proteins as they contain all the essential and important amino acids required by humans, in the right proportions. Whey proteins are rich in branched chain amino acids (BCAAs), a major component that provide energy for people undergoing intense or prolonged periods of exercise and help prevent loss of body mass and muscle. They are also readily digestible and completely bio-available. Whey proteins supply additional nutritional benefits, for example, α-lactalbumin, the second most abundant whey protein, has a high content of the amino acid tryptophan, a precursor of the vitamin niacin.3

Whey protein is a complete protein containing all the essential amino acids with a high biological value. Biological value is actually a measure of protein quality, measuring the amount of protein that is retained from the absorbed protein in the body for maintenance and growth. This means that the absorbed whey protein is retained in the body and not quickly excreted. This will help the athlete by allowing them to use the components of whey protein to aid muscle recovery after a workout and would also promote their skeletal muscle growth. Whey protein is an excellent choice to promote muscle growth and used as a high protein meal replacement. It enhances the workout of athletes and also improves the effectiveness of many other supplements, like creatine.1

Several researches have also focused on soy and whey protein in athletes diets. For example, Kalman et al4 studied the effects of soy- and milk-based proteins on circulating androgens and changes associated with exercise-induced body composition. For 12 weeks, 20 subjects took 50 g of one of four protein sources (soy isolate; soy concentrate; soy isolate and whey blend; or whey blend only)/day during their participation in a resistance training program.4 Protein supplementation resulted in a significant increase in lean body mass independent of the protein source.4

There are several categories of whey protein ingredients that are commonly used in food and nutrition products, including whey powder, whey protein concentrate and whey protein isolate.3 The variation in their composition is due to the difference in extent and method of processing.

*Whey powder* is prepared from whey, a by-product of cheese manufacture. Whey is then clarified, pasteurized and dried to provide a fine white powder known as whey powder.

*Whey concentrate* typically uses ultra-filtration membrane technology to filter or concentrate various whey components on the basis of the membrane pore size and/or molecular weight. The fluid whey is allowed to pass through a semi-permeable membrane thereby removing lactose and ash and concentrating the protein content.3

*Whey protein isolate* have a protein content of 90% or more and is produced through a variety of processes like microfiltration and ion exchange. Microfiltration removes additional lactose and fat to increase protein concentration up to 90% or higher. The advantage of this process is that it maintains the various important naturally occurring bioactive components in whey. Advanced chromatography technology can be used to further separate the individual protein components such as lactoferrin and lactoperoxidase from the main whey proteins (alpha-lactalbumin, beta-lactoglobulin and bovine serum albumin). Advanced membrane technology is used for the enrichment of whey concentrate with whey bioactive components such as sphingolipids, lactoferrin, immunoglobulins, glycomacropeptide (GMP) and transforming growth factor-beta (TGF-β).1 The whey solids are used as concentrated whey for human and animal food, as rich source of proteins.3

**BRANCHED CHAIN AMINO ACIDS (BCAA)**

Branched chain amino acids (BCAAs) account for 14-18% of amino acids in skeletal muscle proteins and are the most widely used supplements among natural bodybuilders.5 It helps to stimulate protein synthesis and promote muscle building and a faster recovery from exercise. The examples of BCAAs are amino acids such as leucine, isoleucine and valine but out of all BCAAs, leucine is of particular interest because it stimulates protein synthesis to an equal extent as a mixture of all amino acids. However, it has been observed that ingestion of leucine only can decrease the level of plasma valine and isoleucine; therefore, all 3 amino acids need to be consumed to prevent plasma depletion of any 1 of the BCAAs.7 The presence of leucine in the muscles has a direct effect on protein synthesis pathway. Studies have shown that BCAAs are metabolized in the muscle to manufacture glutamine, a precursor to GSH and another important component of the immune system.8
The safe upper limit of leucine is 550 mg/kg body-weight/day in adult men; however, future studies are needed to determine the safe upper limit for other populations. Intake of BCAAs before and after the workout reduces the amount of muscle damage and muscle soreness post-workout. It has been suggested a daily intake of 10-15 g of 2:1:1 ratio of BCAAs before and after the workouts for best results.

ESSENTIAL FATS

The body can synthesize some of the required fats from the diet. However, the 2 essential fatty acids, linolenic and linoleic acid as they cannot be synthesized in the body, must be obtained from food. These basic fats, found in plant foods, are used to produce specialized fats in the body called omega-3 and omega-6 fatty acids.

Research has shown that essential fats help in speedy recovery, support the immune system, promote the production of testosterone, improve muscle function and helps to maintain lean muscle mass. Another example of essential fats is fish liver oil. Fish oil is high in omega-3 polyunsaturated fatty acids (PUFAs). Omega-3 fatty acids are popular amongst athletes and non-athletes alike for their ability to improve blood vessel (endothelial) function, anti-inflammatory, and increase provision of energy from fat. Fish oil supplementation decreases the oxidative stress in response to strenuous exercise. This is positive in that it reduces exercise-induced inflammation, reduces the risk for infection due to immuno-deficiency, decreases delayed-onset muscle soreness, and increases the rate of recovery. Fish oil can reduce heart rate during rest and exercise implying a more efficient heart. This effect is due to direct effect on electrophysiological function of the heart.

Approximately consumption of 1-2 g of fish oil in a ratio of 2:1 eicosapentaenoic acid: docosahexaenoic acid or EPA:DHA per day improves cardiovascular function and exercise performance. Most studies have shown that this regimen reduces exercise-induced muscle soreness and many have shown a lowering of blood pressure and improved blood flow during exercise.

VITAMINS AND MINERALS

Micronutrients play a vital role in energy production, hemoglobin synthesis, and maintenance of bone health, adequate immune function, and protect body against oxidative damage. They also assist in synthesis and repair of muscle-tissues during recovery from exercise and injury. During exercise, body is stressed and requires various micronutrients in their metabolic pathways, and training during exercise may result in muscle biochemical adaptations that increase micronutrient needs. The routine exercise may also increase the turnover and loss of these micronutrients from the body. As a result, greater intakes of micronutrients may be required to cover increased needs for building, repair, and maintenance of lean body mass in athletes.

Most athletes are deficient in essential micronutrients. Training alone can deplete minerals and vitamins, due to sweating and fuels muscle contraction. Thus it is essential to fortify the food with vital micronutrients to unleash the energy from the food consumed.

The most common vitamins and minerals found to be of concern in athletes diets are calcium and vitamin D, the B vitamins, iron, zinc, magnesium, as well as some antioxidants such as vitamins C and E, β-carotene, and selenium. Iron is critical for athletes because it helps the body use and carry oxygen to active muscles. Iron from plant sources such as beans, lentils, seeds, soy, whole grain or fortified cereals, breads and pastas is not well absorbed, so a source of vitamin C like citrus fruit and juices, strawberries, bell peppers or broccoli should be included in the diet to help absorb the iron.

B vitamins are needed for releasing energy in the body, building and repairing tissues and for healthy red blood cells. There is scientific evidence that athletes may need higher amounts of B-complex vitamins, alternatively, eating foods from all 4 food groups and taking enough calories will ensure to meet these needs.

Besides, there are a variety of antioxidants that help protect the body’s cells from damage. The antioxidants from nutrient-rich foods, especially plant foods like vegetables, fruit, nuts, whole grains and legumes. The antioxidant rich nutrients are beta-carotene, found in brightly coloured vegetables and fruit like sweet potatoes, carrots, pumpkin and apricots; vitamin E is present in vegetable oils, avocado, wheat germ, nuts and seeds; vitamin C is found in many vegetables and fruits such as citrus, strawberries, bell peppers, tomatoes and broccoli.Selenium is found in meat, fish and poultry, milk and milk alternatives such as cheese and yogurt, whole grains, mushrooms, nuts, seeds and legumes.

Therefore, reviewer suggest that athletes should not take supplements to get antioxidants but they should be essentially obtained from the diet.

SPECIFIC NUTRACEUTICALS SUPPLEMENTS FOR ATHLETES

Conjugated Linoleic Acid (CLA)

Conjugated Linoleic Acid (CLA) is another supplement often labeled as a fat burner and is a potent antioxidant. It is a mixture of different types of isomers of linoleic acid, primarily position and geometric isomers, which is found preferentially in dairy products and meat. Current research findings suggest that CLA has many advantages both for bodybuilders and any type of athlete. CLA is also unique because it is present in foods obtained from both animal and dairy sources, besides it also possess anti-cancer activity and is expressed at concentrations close to human consumption levels. CLA appears to reduce the body’s ability to store fat and increase the body’s use of fat for energy. Another
advantage of CLA is that once it is soaked into muscle cells, it can trigger an increase in muscle mass. It is also a potent anti-oxidant, anti-carcinogen, and anti-catabolite, as well as a powerful immune system enhancer. Some of the common accolades of CLA are assistance in fat burning, building and retention of lean muscle, and is anti-cancer. Some of the most common benefits of CLA supplementation are that it increases metabolic rate, lowers cholesterol and triglyceride level, enhances muscle growth, lowers insulin resistance, reduces food-induced allergic reactions, and enhances immune system. This would obviously be a positive benefit for any type of athlete that is trying to lose weight and improve body composition. CLA supplementation was also shown to improve the lean mass to body fat ratio, decreases fat deposition, especially on the abdomen, and enhances muscle growth. CLA achieves this reaction by reducing body fat and by enhancing insulin sensitivity so that fatty acids and glucose can pass through muscle cell membranes and away from fat tissue. This results in an improved muscle to fat ratio. Compelling evidence also indicates that CLA also promotes youthful metabolic function and reduces body fat. The unique mechanism by which this fatty acid protects against disease makes it an important addition to any supplement program (Table 1).

**Essential Fatty Acids (EFAs)**

Omega-3’s are the essential fatty acids, because they are necessary for health and must be included in diet (because the human body cannot manufacture them on its own). There are mainly 3 types of fatty acids that are collectively referred to as omega-3: alpha-linolenic acid (ALA), eicosapentaenoic (EPA), and docosahexaenoic acid (DHA).

ALα is found in foods of plant origin. The richest source of ALα is flaxseed, but it is also found in hempseed, canola oil, soybeans, soybean oil, pumpkin seeds, pumpkin seed oil, linseeds, walnuts, and walnut oil.

Docosahexaenoic acid (DHA) is found in seafood, algae, and cold water fish such as salmon, sardines and albacore tuna.

Eicosapentaenoic (EPA) is found in many of the same foods as DHA, including cold-water fish such as salmon, and sardines, including cod liver, anchovies, halibut, herring and mackerel.

EFAs include both omega 3 and 6, which play an important role in the functioning of the human body. A balanced intake of these 2 EFAs is essential for healthy cellular function and optimal athletic performance. Studies have proved that EFAs can improve stamina and endurance, decrease recovery time and inflammation after exercise, and improves amino acid utilization to help build and maintain lean muscle mass. Many athletes include EFAs as a staple in their diet to receive these benefits.

**Creatine**

Creatine is another supplement that is extremely popular with athletes. It is naturally synthesized in the human body from amino acids primarily in the kidney and liver and transported in the blood for use by muscles. Approximately 95% of the body’s total creatine content is located and concentrated in skeletal muscle. It is required for the production of adenosine triphosphate (ATP), the cellular fuel that runs the body. ATP that is stored in the muscle is the only fuel available for energy and the only fuel source capable of generating 100% muscle contraction. Muscle contraction by stored ATP is most effective for building strength and once it has been depleted, the other fuel sources will dominate and muscle contraction will decline. Vigorous exercise will deplete muscles from their routine creatine supply. Creatine supplementation re-energizes tired muscles, allowing the athlete to work out harder for a longer duration, and also increases muscle and strength. Taking 30 to 40 grams of mixed sugars with each dose will aid and enhance in muscle uptake of creatine. The sugars stimulate insulin, which is essential to push creatine through the cell membranes. Creatine should be cycled and not taken on a continuous basis. It has maintained a consistent popularity amongst body builders and also remains a top choice for athletes looking to build lean muscle.

The benefits of creatine include an increased strength, enhanced performance, increased testosterone levels, improved brain activity, and also helps in regeneration of muscle cells. Washington State University (WSU) study concluded that creatine supplements can increase muscle mass and strength to the participants and also provides an opportunity to power through intense workouts. Another study conducted by Australian researchers determined that intake of 5 g of creatine daily over a period of 6 weeks, the neurological performance of the participants improved significantly including the speed in which information was processed as well as it also increased the memory. However, creatine may not benefit to those participating in lower-intensity exercises, such as aerobics. So supplement distributors should focus on other formulations and ingredients to target average consumers.

**Glutamine**

It is the most abundant free amino acid found in the muscles of the body and is readily available for the synthesis of skeletal...
muscle proteins. This amino acid helps to build and maintain muscle, and therefore nutritional supplements containing glutamine is useful for athletes. L-glutamine is the natural form of the amino acid glutamine and it is therefore one of the top sports supplements sold on the market. L-glutamine can naturally boost the level of human growth hormone and has gained a lot of recognition as a supplement used to enhance muscle recovery.21 Major dietary sources of glutamine are plant and majority animal proteins such as beef, pork and poultry, milk, yogurt, ricotta cheese, cottage cheese, raw spinach, parsley and cabbage. Glutamine is found in many foods high in protein, such as beans, fish, meat and dairy products. Free L-glutamine is also present in vegetable juices and fermented foods such as yogurt.

Glutamine is a non-essential amino acid and the major part of the de novo synthesis in the human body. However, during strenuous physical exercise, critical illness, injury or heavy stress loads, the need for glutamine can increase beyond the body’s known ability to synthesize it, and therefore it becomes ‘conditionally essential’.

The other key benefits of glutamine are:

- Increasing glutamine in muscle tissue to produce an anabolic effect for body builders and other athletes.
- Glutamine prevents muscle wasting in post-surgical patients and for those with trauma and with conditions causing muscle catabolism.
- Glutamine stimulates and supports the immune system.
- It increases mental alertness and supports neurological health.
- It helps to promote proper glucogenic function necessary for balancing low blood sugar levels.
- It also affects the appetite center to help reduce cravings for sugar and alcohol.
- It also supports the proper intestine function.

Athletes who undergo intense, prolonged exercise have an increased risk of infections, apparently as a result of immunosuppression. Glutamine plays an important role in immune function, and plasma glutamine levels decline after intense, prolonged exercise.23

In a double-blind trial, supplementation with 10 g of glutamine after a marathon or ultra-marathon run significantly decreased the incidence of infections over the next 7 days. In a double-blind fashion study, 151 marathon and ultra-marathon runners received 5 g of glutamine or placebo in 330 ml of water immediately after completing the run and again 2 hours later (total glutamine dose, 10 g). In the 7 days following the event, infections occurred in significantly fewer individuals in the glutamine group than in the placebo group (19.2% vs. 51.2%; p<0.001).28

Arginine

L-Arginine is a semi-essential amino acid that is though body has the ability to manufacture this amino acid, sometimes additional supplementation may be necessary. This is to increase the natural benefits of this amino acid. L-Arginine is a precursor to nitric oxide and necessary for the synthesis of creatine.1

“NO supplements” containing arginine are widely known as a vasodilator and are consumed by bodybuilders to increase blood flow to the muscle during exercise, increase protein synthesis, and improve exercise performance. These supplements are very popular among professional athletes and the body building sportspersons as it increases the blood flow and oxygen throughout the entire body.

However, there is little scientific studies to support these claims. In a research, healthy young men were supplemented with 7 g arginine or a placebo prior to exercise and were observed with no significant change in blood flow following exercise.25

However, the effects of arginine supplementation on performance are still controversial. Approximately one-half of acute and chronic studies on arginine and exercise performance have found significant benefits with arginine supplementation, while the other one-half has found no significant benefits.26

In another study, it was found that arginine supplementation significantly reduced muscular endurance by 2-4 repetitions on chin up and pushes up endurance tests.27 Based on these results, the authors of a review concluded that arginine supplementation had little impact on exercise performance in healthy individuals.28

Tribulus Terrestris (Puncture Vine)

Tribulus Terrestris is a nutritional supplement that has been growing in popularity over the last few years. Traditionally, it was used by men to improve libido but recently has gained recognition as a muscle builder. Tribulus Terrestris functions by enhancing testosterone levels by increasing luteinizing hormone levels. Increasing testosterone levels also promote protein synthesis and positive nitrogen balance. The benefits for the athlete are muscle cell growth, increase in body strength, and faster recovery from injury. Tribulus Terrestris is an extremely powerful medicinal plant with a strong effect on testosterone along with muscle building, restorative sleep, protection of cardiovascular system and pain reduction. It is famous amongst athletes because it significantly increases endurance and is anabolic. The plant fruit has been well documented and used in both traditional Chinese Medicine and in Indian Ayurvedic medicine for more than 3000 years.

Tribulus Terrestris is composed of large number of active elements including steroidal saponins such as dioscin, di-
Research has shown that HMB is particularly effective in catabolic populations such as the elderly and patients with chronic disease. However, studies on the effectiveness of HMB in trained, non-calorically restricted populations have been mixed.

The reasons for discrepancies in the results of HMB supplementation studies in healthy populations may be due to many factors including clustering of data in this meta-analysis to include many studies from similar groups, poorly designed, non-periodized training protocols, small sample sizes, and lack of specificity between training and testing conditions.

However, overall HMB appears to be effective in a majority of studies with longer-duration, more intense training protocols and may be beneficial to bodybuilders, particularly during planned over-reaching phases of training.

Beta-hydroxy Beta-methylbutyrate (HMB)

The leucine metabolite beta-hydroxy-beta-methylbutyrate (HMB) has been extensively used as an ergogenic aid; particularly among bodybuilders and strength/power athletes to promote exercise performance and skeletal muscle hypertrophy. It decreases muscle protein catabolism and increases muscle protein synthesis. HMB may also decrease blood pressure, total and low density lipoprotein (LDL) cholesterol, especially in hypercholesterolemic individuals. HMB is particularly effective in catabolic populations such as the elderly and patients with chronic disease. While numerous studies have supported the efficacy of HMB in exercise and clinical conditions, there have been a number of conflicting results.

In a recent study, the effect of 12 weeks of HMB supplementation on skeletal muscle hypertrophy, body composition, strength, and power in trained individuals were studied. The effects of hydroxyl methyl butyrate (HMB) on muscle damage and performance during an overreaching cycle were also determined and it was concluded that HMB enhances hypertrophy, strength, and power following chronic resistance training, and prevents decrements in performance following overreaching.

β-hydroxy β-methylbutyrate (HMB) is a supplement that was once quite popular with athletes but has now faded into obscurity. HMB is a metabolite of the normal breakdown product of the amino acid leucine and enhances the effects of vigorous exercise by building muscle and reducing body fat. Studies have shown a pronounced effect of HMB on decreasing protein breakdown while increasing nitrogen retention by the body. This results in an increase in lean muscle mass and strength. However, its results are visible along with regular exercise.

In case an athlete is not on a regular routine of strenuous exercise, it is unlikely that there would be not many noticeable results. However, the safety of HMB supplementation has also been studied with no adverse effects on liver enzymes, kidney function, cholesterol, white blood cells, hemoglobin, or blood glucose have been observed. In another study, 2 meta-analysis on HMB supplementation have concluded that HMB is safe and does not result in any major side effects. HMB is actually advantageous by decreasing blood pressure, total and LDL cholesterol, especially in hyper-cholesterolemic individuals.

Research has shown that HMB is particularly effective in catabolic populations such as the elderly and patients with chronic disease. However, studies on the effectiveness of HMB in trained, non-calorically restricted populations have been mixed.

The reasons for discrepancies in the results of HMB supplementation studies in healthy populations may be due to many factors including clustering of data in this meta-analysis to include many studies from similar groups, poorly designed, non-periodized training protocols, small sample sizes, and lack of specificity between training and testing conditions.

However, overall HMB appears to be effective in a majority of studies with longer-duration, more intense training protocols and may be beneficial to bodybuilders, particularly during planned over-reaching phases of training.

Methodology

Methodology

Methoxy Isoflavone

Methoxy-isoavone (M), another supplement, is a non-hormonal member of the isoflavone family. It is highly anabolic (increases protein synthesis) with no androgenic effects. Methoxy-isoavone partially suppresses the catabolic hormone, cortisone, while improving nitrogen retention by the body. Thus, it is suitable for athletes looking to gain more lean muscle. The long term effects of its higher dosing are unknown, with no short-term effects.

In a study, the effect of M, along with other supplements such as 20-hydroxyecdysone (E), and sulfo-polysaccharide (CSP3) were analyzed on the athletes as dietary supplements that improve strength and muscle mass during resistance training. However, little is known about their potential ergogenic value. The purpose of this study was to determine whether these supplements affect training adaptations and/or markers of muscle anabolism/catabolism in resistance-trained athletes. The results indicated that M, E, and CSP3 supplementation do not affect body composition or training adaptations nor do they influence the anabolic/catabolic hormone status or general markers of catabolism in resistance-trained males.

Smilax Officinalis

Smilax officinalis (SO) is a plant that contains plant sterols purported to enhance immunity as well as provide an androgenic effect on muscle growth. Some data supports the potential immune enhancing effects of SO. However, there is no data available to show that SO supplementation increases muscle mass during training.

Growth Hormone Releasing Peptides (GHRP) and Secretagogues

Research has indicated that growth hormone releasing peptides (GHRP) and other non-peptide compounds (secretagogues) help to regulate growth hormone (GH) release. These observations
have served as the basis for development of nutritionally-based GH stimulators (amino acids, pituitary peptides, pituitary substances, *Mucunapruriens*, broad bean, alpha-GPC). Although there is clinical evidence that pharmaceutical grade GHRP’s and some non-peptide secretagogues can increase GH and IGF-1 levels at rest and in response to exercise, it has not been demonstrated that such increases lead to an increase in skeletal muscle mass.\(^{38}\)

**Cordyceps Sinensis (Caterpillar Fungus)**

The caterpillar fungus is a traditional medicine that was widely used as a tonic and/or medicine by the Chinese for hundreds of years. *Cordyceps* was traditionally used to combat fatigue and promote vitality. It improves athletic performance by opening up the breathing passages, thereby allowing more oxygen flow into the body. Oxygen is essential for energy production, thereby enhancing the athlete’s training and performance.

The use of this fungus was relatively unknown in this country until it was known and credited for the success of a Chinese women athlete at the National Games in Beijing, in 1993. Three Chinese track runners set new world records during that Games at 3 different distances, 10,000 m, 15,000 m and 30,000 m. Their coach, Mr. Ma Zunren, attributed the athlete’s success to intensive training as well as a stress-relieving tonic prepared from the caterpillar fungus. Since then *Cordyceps* on an upward trend and as athletes see more positive results; it will become a mainstream supplement.

The various mode of action of *Cordyceps* are firstly it increases ATP level as one of the active ingredient of cordyceps is adenosine, a nucleic acid that is a crucial component of ATP; secondly it boosts testosterone levels thereby increasing the ability to add muscle mass; thirdly it improves oxygen utilization by up to 50% and finally cordyceps also boosts perceived energy levels.

A number of studies have also shown that supplementing cordyceps can lower the heart-rate, which explains why people report being able to train harder for longer duration of time when using this supplement. Another study published in 1999 also pointed to long-term cardiovascular health benefits from cordyceps, indicating that it can lower total cholesterol by 10-20% and increase HDL by a quarter.\(^{37}\)

*Cordyceps* creates the effect similar to caffeine, but without the side effects of jitters or an inability to sleep several hours later. This is because cordyceps attaches to the same receptors as caffeine, providing a noticeable feeling of enhanced energy and thus it is used as a popular anti-aging supplement in China.\(^{37}\)

**Ornithine-α-ketoglutarate (OKG)**

OKG is commonly administered through enteral feeding and has been shown to significantly shorten wound healing time and improve nitrogen balance in severe burn patients.\(^{39}\) OKG has the ability to improve nitrogen balance, thus it is helpful for athletes engaged in intense training.

A scientific study reported that OKG supplementation (10 g/day) during 6-weeks of resistance training promoted greater gains in bench press. However, no significant differences were observed in squat strength, training volume, gains in muscle mass, or fasting insulin and growth hormone. Therefore, additional research is needed before conclusions can be drawn.\(^{39}\)

**CONCLUSION**

Thus, from the above discussion it can be safely concluded that the contribution of nutraceuticals towards athlete’s health is appreciable. Nutraceutical market is therefore consistently growing and has turned into a billion dollar industry. But it is advisable that an athlete should research a supplement before using to ensure the most appropriate effect on their training and performance. However, the banned nutritional supplements should be avoided by the athletes. The athlete should always choose the product based on its quality and effectiveness, not necessarily on what an advertisement states. The use of nutraceuticals/dietary/functional supplements to improve athletic performance will likely to remain in the forefront of the nutritional and dietary supplement industry in the coming years.

**ACKNOWLEDGEMENTS**

The authors are grateful to Dr. Ashok K. Chauhan, Founder President and Mr. Atul Chauhan, Chancellor, Amity University, Noida, UP, India for their encouragement, research facilities and financial support.

**DECLARATION BY THE AUTHORS**

This article is intended for general reading only and is based on the data available from the scientific literature and as reported in blogs, posts and some unofficial sources. The authors do not claim any efficacy of the above cited products. In addition, the authors do not promote or advertise any commercial products/supplements mentioned in the article.

**ETHICAL ISSUES**

There is none to be declared.

**CONFLICTS OF INTEREST**

The authors confirm that this article content has no conflicts of interest.

**REFERENCES**

1. Kreider RB, Wilborn CD, Taylor L, et al. ISSN exercise &


An In Vitro Estimation of Glycemic Index of White Bread and Improvement of the Dietary Fiber

Anteneh Taye, MSc1; Ephrem Engidawork, PhD2; Kelbessa Urga, MSc3

1Department of Food Science and Technology, Hope College of Business, Science and Technology, Addis Ababa, Ethiopia
2Department of Pharmacology and Clinical Pharmacy, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia
3Ethiopian Public Health Institute, Addis Ababa, Ethiopia

ABSTRACT

Consumers of products preferably demand minimally processed products that do not contain chemical preservatives and maintain health benefits. These encourage the use of dietary fiber (DF) to combat some metabolic disorders like diabetes and other immunological compromisings. This study indicates the potential of DF to satisfy the lowering of estimated glycemic index from improved white bread. The sources were selected from crude fiber composition of different cereals and fruits. Fruits of ripen avocado mesocarps and baobab pulps composed of total pectin composition 13%, 30% respectively and selected as good sources. Analysis of total dietary fiber (TDF), insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) were carried out according to the manufacturer megazyme total dietary fiber kit protocol and methods described in Association of Official Analytical Chemists (AOAC). Improvement of DF was observed by enhancing on white breads (WB) based on the daily requirement of soluble fibers (pectin) composition, i.e. 6 g/day. Samples of avocado improved bread (A VB) and baobab improved bread (ADB) were made by mixing in proportion of wheat flour to avocado/baobab pulps (97:3); (94:6); (88:12). Analysis of proximate composition and in vitro estimation of hydrolysis index (HI) and glycemic index (GI) were done for products to evaluate the quality and impact on blood glucose level. Significant differences were observed for predicted GI between improved bread and controls, 82.84±0.37 (WB), 80.63±0.21 (ADB), 78.50±0.30 (A VB). As a result, the reductions in GI for the improved white breads indicate the delay in release of glucose from absorption by the cell due to the viscous nature of dietary fiber component. Thus, the improved white breads with appropriate soluble dietary fiber (pectin) content, reveals the potential for the reduction of blood glucose level and can be considered as functional foods (neutraceuticals) along with its moderate level degree of likeness or sensory acceptability. In general, the study has indicated that the soluble fibres can have a potential to reduce the post-prandial glucose by delaying absorption in cells.

KEYWORDS: Dietary fiber; In vitro; In vivo; Hydrolysis index; Glycemic index; Neutraceuticals.


INTRODUCTION

Food industries are mainly challenged by consumer’s demand in quality, safety and minimally processed products that do not contain chemical preservatives and maintain health benefits.
These encourage the use of dietary fiber (DF) as functional food (neutaceuticals). Functional foods play a special role to satisfy hunger, provide the necessary nutrients, and prevent nutrition related diseases.

DF compounds composed of non-digestible carbohydrate by endogenous enzymes and a mixture of plant carbohydrate polymers. The physical properties of DF in food determine the physiologic effects and potential health benefits. Such as, influence on glucose and fat absorption and bowel function.

The difference in pattern of diabetes varies in countries due to economic status and forecasted to be present in 2030 according to International Diabetes Federation (IDF). Hence, this paper has shown the significant advantage of improving white breads with soluble DF (pectin) through providing an important information on a) using of agricultural sources for therapeutic and to alleviate nutrient related health problems, b) aware the value of waste disposal of agricultural or fiber sources, such as bran or peel of fruits; c) increasing the advantage of using soluble dietary fiber SDF (pectin) as raw materials in food industries.

METHODS

Potential crude fiber sources were screened from cereals and fruits. Analysis of TDF, IDF and SDF were done using methods no. of AOAC 985.29 and 993.19. Pectin, SDF, extracted from sources was based on the method of Sadasivam and Manickam. Quantitative determination of pectin content was done using ultraviolet-visible (UV-V) spectrophotometer (λ=520 nm) for the reaction of galacturonic acid with carbazole in presence of sulfuric acid.

Samples of improved products, baked bread, were prepared at 3rd level by proportionate wheat flour to baobab (adansonia) and/or avocado pulps accordingly [ADB1/AVB1 (97:3); ADB2/AVB2 (94:6); ADB3/AVB3 (88:12); Control (100:0)]. The proximate values were analyzed for the above samples using methods for determination of content for crude fat (100:0), ADB2/A VB2 (94:6); ADB3/A VB3 (88:12); Control (100:0)]. The proximate values were analyzed for the above samples using methods for determination of content for crude fat (100:0), ADB2/A VB2 (94:6); ADB3/A VB3 (88:12); Control (100:0)].

Estimation of in vitro kinetic of starch digestion was done using the method of Goni. The rate of starch digestion was expressed as the percentage of total starch hydrolyzed at difference of 30 minutes in time intervals. The areas under curves (AUC) for hydrolysis of all products were calculated for the release of glucose concentration against time. The HI was calculated as the relation between the AUC for a food and the AUC for a reference food, white bread, expressed as a percentage.

Sensory evaluation was performed in 12-18 hours after baking in evaluating color, texture, taste, odor and overall acceptability of the bread samples.

RESULTS AND DISCUSSION

Compositions of crude fibre were screened from cereals (teff white (TW), barely without bran white (BWB), wheat whole white (WWW), wheat whole black (WWB) barely whole white (BWW)) and fruits (avocado pulp (AVP), baobab pulp (ADP)) as a source (Table 1). However, baobab pulp (Adansonia Digitata) was found higher in crude fiber content than avocado and cereals, avocado was selected among others in consideration to the economic feasibility and accessibility.

The yield of extracted pectin from baobab pulp (a) and avocado pulp (b) (Figure 1) were found 20.94±2.42% and 3.01±1.41% respectively on dry base. The reported baobab pectin yield was obtained more than 10%.

**Table 1**: Screening of cereals and fruits for crude fibers content in wet and dry base.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>%Moisture</th>
<th>%Fiber in Wet Base</th>
<th>%Crude Fiber in Dry Base</th>
<th>Reference Moisture</th>
<th>Reference Crude Fiber</th>
</tr>
</thead>
<tbody>
<tr>
<td>TW</td>
<td>10.78±0.02</td>
<td>2.82±0.21</td>
<td>2.93±0.42</td>
<td>10.90</td>
<td>2.00</td>
</tr>
<tr>
<td>BWB</td>
<td>10.27±0.29</td>
<td>2.72±0.40</td>
<td>3.03±0.02</td>
<td>9.10</td>
<td>2.20</td>
</tr>
<tr>
<td>WWW</td>
<td>10.95±0.06</td>
<td>3.08±0.74</td>
<td>3.46±0.02</td>
<td>9.50</td>
<td>2.30</td>
</tr>
<tr>
<td>WWB</td>
<td>10.91±0.03</td>
<td>4.85±0.02</td>
<td>5.44±0.03</td>
<td>11.30</td>
<td>3.00</td>
</tr>
<tr>
<td>BWW</td>
<td>11.27±0.17</td>
<td>3.97±1.47</td>
<td>4.47±0.69</td>
<td>7.70</td>
<td>2.00</td>
</tr>
<tr>
<td>AVP</td>
<td>4.83±0.05</td>
<td>5.16±0.69</td>
<td>5.42±0.01</td>
<td>8.12</td>
<td>3.10</td>
</tr>
<tr>
<td>ADP</td>
<td>8.73±0.22</td>
<td>8.53±1.47</td>
<td>9.35±0.18</td>
<td>10.40±0.1</td>
<td>16.2±0.09</td>
</tr>
</tbody>
</table>

**Figure 1**: Extracted pectin from (a) Baobab pulp, (b) Avocado pulp.

The yield (%) of dietary fibres TDF, IDF, SDF (p<0.05) for avocado and baobab pulp (Table 2) suggested baobab has been considered as a potential source for pectin.

**Table 2**: Dietary fiber composition of raw materials, avocado and baobab.

<table>
<thead>
<tr>
<th>%Yields</th>
<th>AVR</th>
<th>ADR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDF</td>
<td>13.09±0.80%</td>
<td>33.29±2.58%</td>
</tr>
<tr>
<td>IDF</td>
<td>5.38±0.16%</td>
<td>12.95±2.17%</td>
</tr>
<tr>
<td>SDF</td>
<td>7.71±0.96%</td>
<td>20.34±4.75%</td>
</tr>
</tbody>
</table>

The nutrient compositions, which indicate the food quality, was indicated by its proximate composition (Table 3).
The improved bread with avocado (AVB) indicated higher energy value due to its fat content however it reduced the carbohydrate composition lesser by improved bread by baobab (ADB).

### Table 3: Proximate value in dry base (g/100 g) for wheat flour, raw fruits and improved breads.

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>%Moisture</th>
<th>Ash</th>
<th>Protein</th>
<th>Fat</th>
<th>Crude Fiber</th>
<th>Carbohydrate</th>
<th>Energy (KCal/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WFR</td>
<td>12.68±0.22</td>
<td>2.05±0.46</td>
<td>10.47±0.65</td>
<td>2.10±0.51</td>
<td>8.70±0.71</td>
<td>3.63±0.37</td>
<td>318.81±2.89</td>
</tr>
<tr>
<td>AVR</td>
<td>13.19±0.33</td>
<td>3.01±0.40</td>
<td>9.00±0.69</td>
<td>2.50±0.28</td>
<td>16.72±0.34</td>
<td>5.95±0.51</td>
<td>405.03±2.89</td>
</tr>
<tr>
<td>ADB</td>
<td>8.59±1.27</td>
<td>5.63±0.79</td>
<td>10.40±0.69</td>
<td>2.50±0.51</td>
<td>9.70±0.71</td>
<td>3.65±0.37</td>
<td>318.81±2.89</td>
</tr>
<tr>
<td>ADB2</td>
<td>8.93±0.52</td>
<td>5.50±0.18</td>
<td>12.46±0.78</td>
<td>6.14±1.20</td>
<td>9.33±0.05</td>
<td>3.65±0.37</td>
<td>318.81±2.89</td>
</tr>
<tr>
<td>ADB3</td>
<td>8.42±0.29</td>
<td>6.55±1.78</td>
<td>12.46±0.78</td>
<td>6.14±1.20</td>
<td>9.33±0.05</td>
<td>3.65±0.37</td>
<td>318.81±2.89</td>
</tr>
<tr>
<td>AVB</td>
<td>4.97±0.07</td>
<td>3.25±0.82</td>
<td>3.65±0.19</td>
<td>9.41±1.15</td>
<td>5.27±0.89</td>
<td>3.65±0.37</td>
<td>318.81±2.89</td>
</tr>
<tr>
<td>AVB2</td>
<td>5.84±0.08</td>
<td>3.52±0.25</td>
<td>4.30±0.20</td>
<td>7.04±0.04</td>
<td>65.60±0.29</td>
<td>3.65±0.37</td>
<td>318.81±2.89</td>
</tr>
<tr>
<td>AVB3</td>
<td>6.03±0.64</td>
<td>3.68±0.42</td>
<td>4.30±0.22</td>
<td>16.41±0.30</td>
<td>5.85±0.70</td>
<td>3.65±0.37</td>
<td>318.81±2.89</td>
</tr>
<tr>
<td>WBD</td>
<td>6.26±0.32</td>
<td>2.80±0.15</td>
<td>9.71±0.69</td>
<td>4.92±0.43</td>
<td>10.51±0.67</td>
<td>3.65±0.37</td>
<td>318.81±2.89</td>
</tr>
<tr>
<td>AVR</td>
<td>13.19±0.33</td>
<td>3.01±0.40</td>
<td>9.00±0.69</td>
<td>2.50±0.28</td>
<td>16.72±0.34</td>
<td>5.95±0.51</td>
<td>405.03±2.89</td>
</tr>
<tr>
<td>WFR</td>
<td>12.68±0.22</td>
<td>2.05±0.46</td>
<td>10.47±0.65</td>
<td>2.10±0.51</td>
<td>8.70±0.71</td>
<td>3.63±0.37</td>
<td>318.81±2.89</td>
</tr>
</tbody>
</table>

Values are mean±SE, superscript letters a,b,c indicate significantly different at (p<0.05). **Table 4: Hydrolysis index and predicted GI for the samples and controls.**

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>HI</th>
<th>GI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADB1</td>
<td>64.41±0.98</td>
<td>348.67±4.37</td>
</tr>
<tr>
<td>ADB2</td>
<td>60.70±0.56</td>
<td>341.10±5.98</td>
</tr>
<tr>
<td>ADB3</td>
<td>54.67±0.24</td>
<td>326.84±4.06</td>
</tr>
<tr>
<td>AVB</td>
<td>75.46±1.56</td>
<td>393.19±5.78</td>
</tr>
<tr>
<td>AVB2</td>
<td>70.03±0.30</td>
<td>378.19±5.78</td>
</tr>
<tr>
<td>AVB3</td>
<td>65.60±0.29</td>
<td>341.10±5.98</td>
</tr>
<tr>
<td>WBD</td>
<td>53.46±1.56</td>
<td>293.19±5.78</td>
</tr>
<tr>
<td>AVR</td>
<td>419.76±8.51</td>
<td>393.19±5.78</td>
</tr>
<tr>
<td>WFR</td>
<td>286.92±2.71</td>
<td>318.81±2.89</td>
</tr>
</tbody>
</table>

Figure 2: Kinetic response of improved products.

These findings for estimation of in vitro analysis was compared with the report from Daou et al, Goni et al and Lee et al. The physical properties (structure, particle size, porosity and density) and chemical composition have an important effect on the soluble fractions due to the water holding capacity of dietary fibres. As reported by Lecumberry et al, soluble fibres like pectin have a high hydration capacity, holding water and swelling to form viscous solutions (Figure 3). Thus, it adsorbs and retains other substances like glucose, minerals, no polar molecules (i.e. fats, bile acids), etc.

Figure 3: White bread (WFB), avocado improved bread (AVB), baobab improved bread (ADB).

The sensory analysis for products were described by semi-trained analysts by whom the data was analyzed in SPSS-20 for different attributes. The result was indicated (Table 5) for the 3 products.

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Bread type</th>
<th>ADB</th>
<th>AVB</th>
<th>WB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>2.18±0.93</td>
<td>2.88±1.46</td>
<td>1.60±0.71</td>
<td></td>
</tr>
<tr>
<td>Texture</td>
<td>2.50±0.65</td>
<td>2.65±1.17</td>
<td>2.15±0.92</td>
<td></td>
</tr>
<tr>
<td>Flavor</td>
<td>3.15±1.37</td>
<td>2.40±1.11</td>
<td>2.00±0.88</td>
<td></td>
</tr>
<tr>
<td>Taste</td>
<td>3.10±1.30</td>
<td>2.60±1.06</td>
<td>2.25±0.84</td>
<td></td>
</tr>
</tbody>
</table>

Means±SE, superscript letters a,b,c indicates significantly different at (p<0.05). Keys: 1=like extremely, 2=like very much, 3=like moderately, 4=neither like nor dislike, 5=dislike moderately, 6=dislike very much, 7=dislike extremely. **Table 5: Overall acceptance of the sensory attributes between products.**

The degree of likeness for color and texture, there is no significant difference (p>0.05) between ADB and AVB, while WB indicates significantly different (p<0.05) from ADB and AVB. Declining the magnitudes of the degree of likeness for flavor and taste, all products (ADB, AVB and WB) have shown that there were significant differences among them. This may be from the habituated nature or alteration of users from normal circumstance unenhanced (unimproved) dietary fiber and this probably required longtime adaptation. The texture for AVB is significantly different from WB while insignificantly different from ADB. Probably, this may be from the moisture content of avocado and baobab and due to the water absorption or holding capacity of the product.
CONCLUSION

The study has argued for a hypothesis that improved white bread has no effect on estimation of GI. For this reason, analysis for estimation of hydrolysis, GI and sensory attribute was carried out for improved white bread with dietary fibre.

- As a result, the prediction for low GI in enhanced white bread was significantly differing from white bread. Hence, the present study is concluded as: The components of dietary fibre composition of selected avocado and baobab fruits pulp were found as an advantage to be integrated in a white bread.
- The potential composition of functional fibre (pectin) component in fruits indicates its physiological metabolic benefit as a nutraceuticals model.
- The dietary fibre (functional fibre) enhanced white bread suggested an apparent delay in releasing glucose concentration during hydrolysis and effects in slowing down the carbohydrate absorption in the management of blood glucose level.
- The overall acceptability for the improved white bread was liked moderately while for white bread was liked very much, however an adaptation to typical product natures interferes.

RECOMMENDATION

- Metabolic effect of enhancing dietary fiber in human subject has not been studied and an in vivo glycemic index or blood glucose level shall be conducted for future study.
- Purification and characterization of the soluble dietary component, physico-chemical characteristics and other most related parameters like available or resistance starch were not included in the study. Hence, the measurements of such parameters are suggested.
- Consumers shall use dietary fiber enhanced foods for the management of blood glucose.
- The findings of this study can be used as base line information or an indicator to food processors and related users.

ACKNOWLEDGMENT

AAU-Center for Food Science and Nutrition, Megazyme Co. Ltd (Dr. Barry) - Ierland, Eng. Wudeneh, Dr. Endale, Dr. Yehune, Dr. Kaleab/Dr. Ashagrie/Dr. Dawd.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

