EFFECT OF FROZEN STORAGE AND CONCENTRATION METHODS ON SOME QUALITY PARAMETERS OF LIME JUICE AND CONCENTRATE
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ABSTRACT
Lime juice (Citrus aurantiifolia) was stored at -20°C for 12 months and concentrated using four methods. The effect of frozen storage and concentration methods on some physical, chemical and rheological properties as well as sensory evaluation of reconstituted juice was investigated.

The results showed that the frozen storage had insignificant effect on total soluble solids, pH value and total acidity percent of the juice. Color index, redness (a*) values and total changes in color (ΔE) of the juice gradually increased during storage period. Pectin content, lightness (L*), yellowness (b*), plastic and 10 rpm viscosities, and consistency coefficient values were decreased.

Concentration methods caused a significant increase in color index values of resultant concentrates. The changes in most tasted properties were low in concentrates produced by “cut-back” process. The differences between rheological properties of the concentrates referred to the differences in pectic fractions content of the concentrates. The overall acceptability scores of reconstituted juices prepared from concentrates produced by “cut-back” process were higher than those for the other concentrates.

Keywords: Lime juice, concentration, rheological properties, physical properties, chemical properties, sensory evaluation

INTRODUCTION
Lime fruits (Citrus aurantiifolia) are grown in large quantities in many countries including Egypt. The Egyptian limes production reached about 338,000 tons in 2004 (FAO, 2005). Lime fruits have the same structure and composition of lemon (Citrus limon), but they contain more citric acid (6-9%), ascorbic acid, flavor and juice yield. The lime fruits are smaller in size and have a very thin and yellow green peel (Saad-Allah and Melehy, 2003).

Limes and lemons contain unique flavonoid compounds that have antioxidant and anti-cancer properties. Among flavonoid compounds in limes, the flavonol glycosides, including many kaempferol related molecules are the most interesting. These flavonoids have been shown to stop cell division in many cancer cell lines. Also, they are perhaps most interesting for their antibiotic effects. Also, limes contain important compounds called limonoids, which have been shown to help fighting cancers of the mouth, skin, lung, breast, stomach and colon in laboratory tests (Sharagozoo and Ghaderi, 2001 and Cho, et al., 2004). In addition limes are an excellent source of one of the most important antioxidants vitamin C. Vitamin C can be helpful for preventing the development and progression of atherosclerosis and diabetic heart disease (Kurl et al., 2002 and Pattison et al., 2004). Moreover, limes are not commonly allergenic foods, and not known to contain measurable amounts of goitrogens, oxalates or purines.
Environmental protection Agency (EPA) allow a maximum amount of 8ppm of malathion to be present as a residue on specific crops used as foods, and 0.1mg/L to be present in drinking water for lifetime exposure of adults.

Due to the large scale proliferation of environmental pollution, search for agents capable of minimizing their toxicity on human health become very essential. Recently, there is considerable emphasis on identifying the potential of natural plant products as chemopreventive agents present in food consumed by human population (Wanger, 1990; Keiloff et al., 1994; Ahmed et al., 2003). Molokhia (Corchorus olitorius) is a common edible and famous vegetable in Egypt. It has a very high nutritive value as well as active compounds. Inami et al., (1995) found that the leaf powder of molokhia and its water-souble viscous solution led to decrease total serum and liver cholesterol and increased fecal extraction of bile acid, total neutral sterols and cholesterol. It was also found that molokhia leaves suppress elevation of postprandial blood glucose levels in rats and humans (Inami et al., 2005). Three cardenolides were isolated from molokhia seed-leaves and their cytoxic activities were evaluated against six cancer cell lines as found by Abdel Wahab et al., (1999). Flavonoid is one of the possible candidates of the active compounds in molokhia. It abundantly contains 5-caffeoylquinic acid, 3,5-dicaffeoylquinic acid, quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-(5-malonylglucoside), quercetin 3-(mangoxyglucoside), ascorbic acid, 9-tocopherol, and chlorophyll, and the content of quercetin glycosides is remarkable (Azuma et al., 1999). Molokhia specially suppressed aryl hydrocarbon receptor, induced by dioxins, suggesting that molokhia contains the unique or specific active compound(s) or the considerable amounts of general compounds possessing the antagonistic action (Nishiumi et al., 2006).

Therefore, it is highly possible that molokhia could also protect liver against malathion-induced toxicity via preventing or alleviating intracellular GSH depletion and oxidation damage. In view of this the purpose of study was to evaluate the in vivo protection from molokhia in malathion treated rats.

MATERIALS AND METHODS

Chemicals

All chemicals used in this study were obtained from Sigma Chemical Company (St. Louis, USA). Commercial kits were purchased from BioMerieux Company (L'Etolile/Flance) and from Eage Diagnostics (Dalllas, TX, USA).

Animals

Three to four-weeks old male albino rats, were obtained from the Animal House Colony, Giza, Egypt. Rats were housed on a 12h light-12h dark schedule, and fed with water ad-libitum, and rat standard diet containing by weight (g/100g): 64 starch, 23 protein, 3.5 fat, 5 fiber, 1 vitamin mixture and 3 salt mixture as mentioned by National Research Council (NRC, 1978).
Preparation of molokhia supplemented diet

Molokhia (Corchorus olitorius L.) leaves were purchased from the local market, pulverized and lyophilized using freez dryer system (Dura-Dry Freeze Dryer, Model PAC-TC-V4; FTS system, Inc., Stone Ridge, NY, USA). The dried molokhia was stored in a freezer until used.

Chemical analysis of molokhia

Moisture, content, protein, fat, carbohydrate, fiber and ash were determined according to AOAC (1980). Vitamin A and C were determined as described by Strong and Koch (1976). Total free phenols were measured using the Folin-Denis reagent as described by Swain and Hillis (1959). Chlorophyll was determined according to Wetlestein (1957).

Experimental design

Malathion containing diet was prepared and standardized as described by Banerjee and Hussain (1986). In brief, 20mg malathion was mixed in 30ml groundnut oil to produce feed containing 20ppm malathion. This was incorporated in 1 kg diet and thoroughly mixed to ensure even distribution. The rats were randomly divided into four groups of 10 animals each and treated for 4 weeks as follows: Group I-control: rats fed on normal diet. Group II-molokhia: rats fed on 100gm/kg bw molokhia. Group III-malathion: rats received 20ppm malathion along with normal diet and Group IV-malathion + molokhia: rats received 20ppm malathion along with 100mg/kg bw molokhia. Food consumption, general condition and any other symptoms were observed daily and body weights were recorded weekly.

Samples

At the end of the experimental period, fasting blood samples were collected from the retro-orbital sinus from all animals under ether anesthesia. Blood samples were left to clot and centrifuged at 5000g while cooling (5C) for 10 min to separate the serum. The clear serum was kept at -20C until analysis. The effect of malathion and molokhia on liver was assayed by assayed serum transaminases, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities according to the method of Reitman and Frankel (1957); alkaline phosphatase (ALP) by the method of Roy (1970); colorimetric determination of albumin using bromocresol green at PH 4.2 according to the method of Doumas et al., (1971); estimation of total protein (TP) according to the method described by Gornall et al., (1949), by using commercial test kits obtained from BioMerieux Company (L’Etoile/France). Glutathione (GSH) level activity in serum was measured by the method of Paglia and Valentine (1967) and serum glutathione-S-transferase (GST) was measured by the method of Habig et al., (1974), using commercial kits purchased from Eagle Diagnostics (Dellas, TX, USA).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) and computing using the SAS General Linear Model program (SAS, 1990). Differences with P < 0.05 were considered to be significant.
RESULTS AND DISCUSSION

The liver is one of the major organs for detoxification of xenobiotics. At present there is considerable interest in free radical mediated damage to biological systems due to pesticide exposure (Ahmed et al., 2000). A large number of xenobiotics have been identified to have potential to generate free radicals in biological system (Kehrer, 1993). Free radicals have become an attractive means to explain the toxicity of numerous xenobiotics. Standard hematological and serum chemistry panel tests have been widely used in clinics to monitor the adverse effects resulting from diseases or exposure to xenobiotics (Abou Zeid et al., 1993). A study of some commonly used plant antioxidants against xenobiotic therefore appeared to be of interest.

The results in Fig 1 illustrate the effect of different treatments on serum enzymes ALT, AST and ALP which is known as liver functions. Malathion treatment caused significant increase in ALT, AST and ALP activities (P<0.05) in rats fed the normal diet. However, the level of enzymes was significantly lower in rats fed molokhia supplemented diet as compared to control or malathion-treated group. The intakes of molokhia significantly alleviate the elevation of enzymes activities (P<0.05) in malathion-treated rats. The increased level of ALT, AST and ALP activities may indicate degenerative change and hypofunction of liver. In clinical diagnosis, increased in these enzymes indicates affected liver (Hsu et al., 2006). The affected liver function by malathion is typically those reported by Jabber et al., (1990) who found that short term (24h) and long term (4 weeks) of malathion-treated rats increased activities of ALT, AST and ALP.

As reported by others (Abou Zeid et al., 1993; Hazanka et al., 2003; Timur et al., 2003), malathion induced the level of TP and albumin in the rats serum. This study confirmed those previous studies. Serum TP and albumin were significantly decrease in animals treated with malathion (P<0.05), whereas animals treated molokhia alone or molokhia supplemented to malathion treated group show a significant improvement in both parameters (Fig 2). The reduced level of TP and albumin may be due to inhibition of tRNA-synthetase accompanied with a lower protein synthesis and /or protein catabolism accompanied in impaired production of functional protein in the organs, in liver affected by chemical compounds, and in protein losses from the organism, increased blood vessel permeability and affected kidney (Robert et al., 1993). Takao (1987) emphasized that human albumin has a significant high affinity in binding with alkylating agent and xenobiotics. Hence, it can be anticipated that human serum albumin and its other protein will be more susceptible and highly affected by the exposure to acute poisoning pesticide such as malathion (Abou Zeid et al., 1993).

As shown in Fig (3) malathion depleted GSH and increased GST activities. The decrease in GSH and increase in GST due to malathion treatment was significantly reduced (P<0.05) when diet supplemented with molokhia. The decrease in GSH content after malathion exposure was related to utilization of antioxidant in the detoxification of this pesticide through GST. The depletion of GSH and increased GST after malathion exposure was observed by (Ahmed et al., 2000; Timur et al., 2003).
Fig. 1. Effect of Molokhia on serum ALT, AST and ALP in rats fed malathion contaminated diet.
Fig. 2. Effect of Molokhia on serum total protein and albumin in rats fed malathion contaminated diet.
Fig. 3. Effect of Molokhia on serum GSH and GST in rats fed malathion-contaminated diet.

The GSH depletion, especially occurred in acute hypotocic necrosis, liver failure or death (Hau et al., 2006). However, the intake of molokhia alleviated malathion-induced depletion of GSH content and consequently reduced damage in liver.

Dietary supplementation of molokhia alleviated GSH depletion and improved liver functions in malathion-treated rats, suggesting that molokhia contains specific active compound(s) or the considerable amounts of general compounds possessing the antagonistic action. The chemical composition of
molokhia (Table 1) revealed that fresh and freeze dried molokhia leaves contained high nutritive value, chlorophyll as well as high amounts of active compounds (phenolic compounds and vitamins). Phenolics are known as potential chemopreventive agents (Urso and Clarkson, 2003). Six phenolic antioxidant compounds in molokhia were identified by Azume et al., (1999) and it was found that quercetin glycoside was the predominant phenolic antioxidant. Another candidate for the active compounds in molokhia is vitamin A and C. Ascorbic acid (vitamin C) widely known as antioxidant, interacts with free radicals and oxidative product to protect cells against the genotoxicity of various oxidants (Urso and Clarkson, 2003). Another important compound is vitamin A. It is an antioxidant and free radical scavenger, when supplements to the diet diminish symptoms of organochlorine pesticide DDT (Calabrese, 1980). Three cardenolids were isolated from molokhia and showed cytotoxic activities against six cancer lines (Abdel Wahab et al., 1999).

Table 1. Chemical composition of Molokhia.

<table>
<thead>
<tr>
<th>Chemical composition</th>
<th>Fresh</th>
<th>Freeze dried</th>
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<tbody>
<tr>
<td>Moisture%</td>
<td>80.15±8.0</td>
<td>5.21±0.26</td>
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<tr>
<td>Protein%</td>
<td>6.62±0.39</td>
<td>3.20±2.71</td>
</tr>
<tr>
<td>Fat%</td>
<td>0.30±0.01</td>
<td>1.94±0.69</td>
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<tr>
<td>Fiber%</td>
<td>7.50±0.42</td>
<td>33.52±2.62</td>
</tr>
<tr>
<td>Ash%</td>
<td>3.18±0.16</td>
<td>13.90±0.69</td>
</tr>
<tr>
<td>Carbohydrate%</td>
<td>2.39±0.14</td>
<td>15.29±0.78</td>
</tr>
<tr>
<td>Vitamin A (IU)</td>
<td>9565±1367</td>
<td>9565±1367</td>
</tr>
<tr>
<td>Vitamin C (mg/100g)</td>
<td>50.42±4.0</td>
<td>59.80±3.50</td>
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<tr>
<td>Total soluble phenolic compounds%</td>
<td>4.11±0.2</td>
<td>4.0±0.27</td>
</tr>
<tr>
<td>Total chlorophyll mg/100g</td>
<td>468.57±21.68</td>
<td>487.16±22.51</td>
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The other mechanism by which molokhia suppressed the toxicity of malathion may be attributed to the interaction of chlorophylls and fibers with malathion and inhibit absorption of malathion from intestine. Fukuda et al., (2004) found that chlorophyll suppressed transformation of the any hydrocarbon receptor induced by dioxin. On the other hand Nishiuni et al., (2005) found that molokhia extract administered rat liver revealed a tolerance to dioxin-induced any hydrocarbon transformation, suggesting that molokhia contains two classes of active compounds; the first class comprises direct inhibitor(s), which is able to permeate the intestinal cell and/or hepatocytes, and the second is the latent active compound(s), which reveals the suppressive effect after permeation and metabolic conversion in the intestinal cells and/or hepatocytes.

In conclusion, this study revealed that molokhia is a potential multiple-protective against malathion-induced hepatotoxicity and it is an attractive food for isolation and identification of a natural antagonist for pollutants and human diseases.
REFERENCES


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دور الملوحة الوقائي للإلكيد ضد السمية الناتجة عن مبيد الميثيون

عزة زهير

قسم الأمراض المزمنة كليية التربوية المبكرة - جامعة المنوفية

يربط الميثيون من مبيدات الوزن المركبة شايلة الاستخدام في جميع أنحاء العالم بهدف مكافحة المرض، ويدعى الوقاء والتحكم في الأمراض من خلال الرعاية، وتعزيز النباتات في الأغذية. للذكرين والذكور. وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكرين، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكريين، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكر، وللذكور، وللذكور، وللذكور، وللذكور، وللذكريين، وللذكور، وللذكور، وللذكور، وللذكر، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكور، وللذكريين، وللذكور، وللذكر، وللذكور، وللذكريين، وللذكور، وللذكور، وللذكريين، وللذكو