Rheological Studies and Effect of Feeding Guar (Cyamopsis tetragonoloboa L.) Seeds on Histology of Some Organs of the Albino Rats

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ABSTRACT
In this study, the rheological properties of composite flour and their guar meal blends were studied by farinography. The water absorption percent, arrival time, development time, dough stability, mixing tolerance, ash, protein, falling number, baking strength, index of swelling and extensibility were estimated. The effect of feeding guar seeds powder on liver enzymes (GOT and GPT) and kidney functions (urea and creatinine) of rats were investigated. The results showed that diets with 5% guar seeds powder had the best effect on GOT (17.7 U L⁻¹), GPT (22.2 U L⁻¹), urea (26.0 mg dL⁻¹) and creatinine (0.83 mg dL⁻¹). Histopathological studies were also carried out in the kidney and liver of the albino rats.

Key words: Guar seeds powder, rheology, liver and kidney functions, in vivo, histopathology

INTRODUCTION
Guar bean (Cyamopsis tetragonoloboa L.) is an annual legume cultivated principally in India and Pakistan and to a lesser extent in the United States. Guar gum is a cold water soluble polysaccharide derived from the endosperm of guar beans by extraction. This polysaccharide consists of galactomannan at a ratio of 2:1 mannose to galactose (M/G) (Roberts, 2011). Generally, galactomannans are composed of a mannose backbone or main chain, with galactose residues or side chains. Therefore, with respect to guar gum, for every two mannose units there is one galactose side chain (Butt et al., 2007). Guar gum has a plethora of uses, mainly as an additive in the food industry as an infant formula prescribed for the treatment of gastroesophageal reflux (Freitas et al., 2006).

Typically guar gum is incorporated into foods at 1 g/100 g to facilitate gelling, firming and emulsification of food products (Flammang et al., 2006). Thickening or stabilizing agents for a wide range of processed foods (Zhang et al., 2010). Guar gum has a plethora of uses, mainly as an additive in the food industry. Guar gum may also be used as a source of soluble dietary fiber in food products and has been assessed to be safe at usage levels of 20 g per day (Grabitske and Slavin, 2009; Kawamura, 2008). In the course of present studying program of medicinal plants with nutritional values (Badr et al., 2013a, 2014), the guar (Cyamopsis tetragonoloboa) seeds were
investigated. The aim of this study is to evaluate the rheological properties of guar seeds powder and fortified wheat flour with guar. Additionally, the biochemical and histological effects of guar seeds on albino rats were also evaluated.

MATERIALS AND METHODS

Collection of samples: Guaran seeds (Cyamopsis tetragonoloba) were obtained from the Crops Research Institute, Agriculture Research Center, Giza, Egypt. The collected seeds were washed with tap water, dried and then crushed to very fine powder with the crusher at Department of Agricultural, Institute of Sufficient Productivity, Zagazig University. Samples were sieved through an 841 mm screen and kept frozen in polyethylene bags until the time of analysis.

Carbohydrate profile: Samples of guar flour and their extracts were analyzed for sugar by high performance liquid chromatography (HPLC) (AOAC., 2012).

Guar dough rheology: The effects of guar seed gum on the properties of dough were analyzed using farinography (Brabender, Duisburg, Germany) according to the AACC (2000), No. 54-21, 54-21B, 54-21 and 54-10. Commercially available all purpose wheat flour 72% (ElKawther, Milling North Cairo Co. Egypt) with a moisture of 11.7%, ash 0.66% and protein 12.1% was used to prepare Wheat Guar Gum seeds powder blends. Guar seeds powder “GSP” was added to wheat flour at 5, 10 and 15 g/100 g levels. The parameters tested for analyses were falling number index, baking strength (W), index of swelling (G) and extensibility (L) (mm). According to the constant flour weigh procedure, 300 g of flour were mixed at the optimum water absorption level and the farinography was centered on the 500 Brabender Unit (BU) line. The water absorption (WA%), arrival time (min), dough development time (min), dough stability time (min) and mixing tolerance index (BU) of the dough and its blends were obtained. These studies were carried out in Wheat and flour Lab. of Regional Center for Food and Feed “RCFF” at Agricultural Research Center “ARC”.

In vivo experiment

Animals and housing: The study was approved by Regional center for Food and Feed “RCFF” at Agricultural Research Center (ARC). Sixty male Sprague Dawley rats weighing (150±10 g) were purchased from animal house of National Research Center (NRC). They were divided into four groups, fifteen in each, were kept in their own cages at constant room temperature (25±2°C) and housed in stainless steel cages with wire-mesh bottoms under a 12 h light/dark cycle with free access to food and water (Badr et al., 2013a).

Diets and experimental designs: Experiments were initially designed to find out the effect of the three best composite diets (B, C and D) on kidney and liver organs of rats. All the other nutrients were added according to the Recommended Dietary Allowance (RDA) for each of the rat according to NRC (1995). The guar seeds powder was mixed well with the other ingredients of the diets B, C and D (Table 1). Basel diet (A) was used as a control experiment.

Rats were fed on the prepared diets and watered on daily basis for duration of 8 weeks divided into three intervals (zero time, after 4 and 8 weeks). The rats had free access to a barely and water for 3 days before starting the experiments as adaptation period. At zero time, three rats were withdrawn from each group. The rats of both zero time and each interval “4 weeks” were anaesthetized with CO₂ and blood samples were collected via the retro-orbital plexus (Saka et al., 2012; Badr et al., 2013b). Serum was obtained by centrifuging at 3000 rpm for 15 min as reported in Akhigbe et al. (2008). From rats of each group at zero time, end of first 4 weeks and end of
second 4 weeks, they were scarified. Their liver and kidney were harvested, weighed and rapidly kept in formalin solution (10%) at room temperature for histopathology examination.

Analysis and investigation: Diets analysis for determination of moisture content, crude protein, fiber, ash and fat were carried out according to the methods described by AOAC (2012), blood serum analysis of urea (Newman and Price, 1999) and creatinine (Friedman and Young, 1997) as functions of kidney were estimated. In addition, “GOT” and “GPT” were determined as liver functions of subjected rat groups for the three interval periods of the experiment and histopathological analysis for the collected organs “Kidney and liver” through the 3-interval periods were carried out (Bancroft and Stevens, 1996).

Statistical analysis: The data was analyzed in Completely Randomized Design (CRD) in factorial arrangement as outlined by Gomez and Gomez (1984). Least Significant Difference (LSD) at 5% level of probability was used for comparisons among treatment means. Additionally, Duncan’s Multiple-Range (DMRT) test was also done.

RESULTS AND DISCUSSION

In previous studies, chemical composition of guar seeds was investigated. Briefly, it was found that the seeds contain 4.53% ash, 3.32% fat, 11.06% fiber, 10.0% moisture and 33.25% protein. The most abundant minerals and fatty acids detected in the guar seeds were iron (465 ppm) and cis-linoleic acid (53.89%), respectively. Amino acids (e.g., glutamic acid, arginine and aspartic acid) were being realized (Badr et al., 2014).

Carbohydrate profile: Analysis of the carbohydrate profile of guar seeds powder by HPLC showed the presence of D-galactose (27.45%) and D-mannose (55.04%). The ratio of D-galactose and D-mannose were changed in GU1 and GU2 (Table 2). The variation in sugars percent is due to the formation of galactomannan which confirm the insertion of cationic moity on guar backbone. This result is in a good agreement with McCleary et al. (1985).

Rheological properties of guar and fortified wheat flour with guar: The rheological properties of composite flour and their guar meal blends are shown in Table 3 and Fig. 1. The water absorption of blend with 15% Guar Seed Powder (GSP) was higher compared to that of the control.
Fig. 1: Rheological evaluation of composite flour and their guar seed blends by farinography

Table 2: Profile of sugar compounds in guar seed flour and their extracted guar samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>D-Galactose</th>
<th>D-Mannose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guar seed flour (100%)</td>
<td>27.45</td>
<td>58.04</td>
</tr>
<tr>
<td>Extracted GR1</td>
<td>14.79</td>
<td>61.05</td>
</tr>
<tr>
<td>Extracted GU2</td>
<td>17.99</td>
<td>58.90</td>
</tr>
</tbody>
</table>

GR1: 1 g guar gum was dissolved in 85 mL distilled water (1:85). GU2: 2 g guar gum was dissolved in 60 mL of distilled water (2:60)

Table 3: Rheological evaluation of flour and their guar gum seed blends by farinography

<table>
<thead>
<tr>
<th>Test</th>
<th>WF (ER)*</th>
<th>WF:GU (95:5)</th>
<th>WF:GU (90:10)</th>
<th>WF:GU (85:15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water absorption (%)</td>
<td>55.70±0.20</td>
<td>50.70±0.80b</td>
<td>62.50±0.50b</td>
<td>69.90±0.40*</td>
</tr>
<tr>
<td>Arrival time (min)</td>
<td>1.00±0.10</td>
<td>0.60±0.11</td>
<td>0.50±0.20</td>
<td>0.40±0.00</td>
</tr>
<tr>
<td>Development time (min)</td>
<td>1.50±0.20</td>
<td>3.50±0.01</td>
<td>5.90±0.12</td>
<td>6.80±0.20</td>
</tr>
<tr>
<td>Dough stability time (min)</td>
<td>15.80±0.10</td>
<td>13.60±0.20</td>
<td>6.40±0.30</td>
<td>3.00±0.10</td>
</tr>
<tr>
<td>Mixing tolerance index (BU)</td>
<td>85.00±0.00</td>
<td>30.00±0.12</td>
<td>27.00±0.24</td>
<td>19.00±0.12</td>
</tr>
<tr>
<td>Ash</td>
<td>0.66±0.10</td>
<td>0.78±0.20</td>
<td>0.95±0.30</td>
<td>1.10±0.00</td>
</tr>
<tr>
<td>Protein</td>
<td>12.10±0.10</td>
<td>12.80±0.12</td>
<td>13.40±0.20</td>
<td>15.00±0.10</td>
</tr>
<tr>
<td>Falling number</td>
<td>383.00±0.16</td>
<td>415.00±0.24</td>
<td>423.00±0.12</td>
<td>427.00±1.02</td>
</tr>
<tr>
<td>Baking strength (W) 10E-4j</td>
<td>306.00±0.10</td>
<td>218.00±0.10</td>
<td>158.00±0.20</td>
<td>122.00±0.00</td>
</tr>
<tr>
<td>Index of swelling (G)</td>
<td>18.80±0.10</td>
<td>13.00±0.10</td>
<td>9.20±0.07</td>
<td>8.30±0.00</td>
</tr>
<tr>
<td>Extensibility (L) (mm)</td>
<td>79.00±1.00</td>
<td>34.00±3.30</td>
<td>14.00±1.40</td>
<td>17.00±0.20</td>
</tr>
</tbody>
</table>

WF: Wheat flour, GU: Guar gum seeds meal, *ER: Extraction ratio

This is expected due to increasing of the water absorption by the addition of the hydrophilic guar gum seeds. The dough stability time and mixing tolerance of the guar blend with wheat flour at 15% were lower compared to that of the control. By increasing the ratio of “GSP” to wheat flour, the development time, falling number and ratio of protein were increased. Generally, addition of GU at 15% resulted in a farinography parameter which resembled that of a control obtained by wheat flour, i.e., the development time was short, the consistency and elasticity had a maximum and then reduced with the mixing time. Similar characteristics had also been reported by Slavin and Greenberg (2003).

Interestingly, 15% “GSP” with 85% WF blend exhibited very poor dough stability, mixing tolerance, extensibility and baking strength. This result is in a good agreement with Hsu et al. (2004), who reported that increasing the substitution of wheat flour with other flour progressively reduced the quality of the whole. These has been attributed to a decrease in flour strength and gas retention due to lower gluten content and thereby bread volume and the sensory appeal of the most baked composition bread. Same trend has been reflected in the rheological evolution by farinography in the dough stability, mixing tolerance, extensibility and baking strength which was lower for ratio of guar at 5 and 10% with WF, when compared to control WF 100%.
Increase of “GSP” was significantly decreased the extensibility and baking strength. This reduction may be due to presence of gelling strength of guar gum flour which reduces the elastic nature of the dough. Fortification of “GSP” into wheat flour at 5% was good in properties of dough stability, mixing tolerance, extensibility and baking strength compared to control of WF.

The study on rheological behavior of the dough’s containing “GSP” showed that guar as hydrocolloid had the most pronounced effect on viscoelastic properties yielding strengthened dough’s. Addition of “GSP” at level not more than 5% to the gluten-free formulation resulted in a farinography curve typical of wheat flour dough’s. Moreover, the fortification of blends by “GSP” is produced gluten-free products that demand parallels to real increase in coeliac disease, or other allergic reactions/intolerances to gluten consumption. Coeliac disease is related to the inflammation of the small intestine leading to small absorption of several important nutrients and intestinal mucosal damage. The only effective treatment for coeliac disease is a strict adherence to a gluten-free diet throughout the patient’s lifetime which in time, results in clinical and mucosal recovery (Gallagher et al., 2004).

Biochemical and histological effects of guar seeds on rats
Diet analysis: The four subjected rats groups were taken the recommended dietary allowance for rats. According to Hamed et al. (2008) carbohydrate content for all diets were estimated by difference (Table 4).

Liver and kidney functions: A study was carried out to determine the effects of using different levels of Guar Seeds Powder (GSP) on performance and functions of liver and kidney organs of subjected rats. After 4 weeks of experimental period, G.O.T. and G.P.T. of liver functions for rats fed diets of 5% “GSP” were 17.7 and 22.2 U L⁻¹ as illustrated in Fig. 2a and 2b, respectively. The results showed no significant difference (p<0.05) as compared with that of rats fed normal diet 12.2 and 20.7 U L⁻¹, respectively. The G.O.T. and G.P.T. of blood serum for rats fed diets of 10% “GSP” (31.8 and 32.3 U L⁻¹) and 15% “GSP” (44.0 and 55.3 U L⁻¹) showed significant difference of (p<0.05) and (p<0.01) values, respectively. In the same interval of experimental period, urea and creatinine of kidney functions for rats fed with diets of 10% “GSP” (36.2 and 53.0 mg dL⁻¹) and 15% “GSP” (1.01 and 1.74 mg dL⁻¹), respectively. The urea and creatinine recorded significant difference of p<0.05 and p<0.01 values, respectively, as shown in Fig. 3a and 3b. At the end of experimental duration (eight weeks), the rats were fed with 10 and 15% “GSP”. The G.O.T (39.8 and 56.2 U L⁻¹) and G.P.T (40.2 and 53.7 U L⁻¹) were recorded for blood serum of rats (p<0.01) and illustrated at Fig. 2a and 2b. Additionally, urea and creatinine (Fig. 3a and b) were changed with highly significance levels (p<0.01), where their values were 51.5 and 66.8 mg dL⁻¹ and 1.3 and 2.5 mg dL⁻¹, respectively. The high concentrations of “GSP” rat’s diets deleteriously

<table>
<thead>
<tr>
<th>Diet components</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>20.4</td>
<td>20.9</td>
<td>22.2</td>
<td>23.6</td>
</tr>
<tr>
<td>Fat</td>
<td>5.1</td>
<td>5.4</td>
<td>5.6</td>
<td>5.6</td>
</tr>
<tr>
<td>Ash</td>
<td>4.9</td>
<td>5.2</td>
<td>5.2</td>
<td>5.4</td>
</tr>
<tr>
<td>Moisture</td>
<td>7.8</td>
<td>7.8</td>
<td>8.3</td>
<td>8.5</td>
</tr>
<tr>
<td>Fiber</td>
<td>5.2</td>
<td>6.0</td>
<td>6.4</td>
<td>6.9</td>
</tr>
<tr>
<td>Total carbohydrate</td>
<td>66.6</td>
<td>54.7</td>
<td>52.3</td>
<td>50.0</td>
</tr>
</tbody>
</table>

A: Based diet (control), B: Diet contains 5% guar seeds powder, C: Diet contains 10% guar seeds powder, D: Diet contains 15% guar seeds powder.

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Fig. 2(a-b): Changing of (a) G.O.T and (b) G.P.T of liver function according to change of interaction between "GSP" and periods

Fig. 3(a-b): Changing of (a) Urea of kidney function and (b) Creatinine according to change of interaction between GSP and periods

affect kidney and liver as well as, digest a viscosity. These effects are attributed to gum, hull and germ in the ‘GSP’. These results suggested that, gum was responsible for some deleterious effects.
The germ significantly increases the intestinal viscosity at 10 and 15% inclusion rates. The kidney and liver functions through all 10 and 15% "GSP" are changed with highly significance levels and this is in agreed with Lee et al. (2003). The results indicated that the upper feeding level of germ and hull fraction of guar in "GSP" was 5%. It was concluded that substitution with 5% "GSP" had the best effect on performance and functions for kidney and liver organs of subjected rat's diets.

**Effect of guar seeds powder on histopathology of kidney:** The kidney of rats at zero time shows the normal histological structure of renal parenchyma (Fig. 4).

After 4 weeks of experimental period, kidney of rats fed diets of 5% "GSP" and 10% (Fig. 5b, c), respectively showed no histopathological changes as compared with kidney of rats fed normal diet (Fig. 5a). Kidney of rats fed diets of 15% "GSP" (Fig. 5d), illustrated a congestion of

![Histopathological changes of kidney of rat at zero time](image1)

Fig. 4: Histopathological changes of kidney of rat at zero time

![Histopathological changes in kidney of rats](image2)

Fig. 5(a-d): Histopathological changes in kidney of (a) Control and experimental rats after four weeks, (b) Rats fed with 5% GSP, (c) Rats fed with 10% GSP and (d) Rats fed with 15% GSP
renal blood vessel. At the end of the experiment, kidney of rat group fed diet of 5% “GSP” (Fig. 6b) revealed no histopathological changes over that of the control group (Fig. 6b). Kidney of rat group fed diet of 10% “GSP” (Fig. 6c) showed vaculations of epithelial lining renal tubules. While kidney of rat groups fed diet of 15% “GSP” (Fig. 6d) showed small focal interstitial leucocytic cells infiltration and slight distension of Bowman’s space.

**Effect of guar seeds powder on histopathology of liver:** The liver of rat at zero time showed no histopathological changes Fig. 7. After 4 weeks of the experimental period, livers of rats fed diets of 5% “GSP” and 10% (Fig. 8b, c) showed no histopathological changes as compared with liver of rats fed normal diet (Fig. 8a). Livers of rats fed diets of 15% “GSP” (Fig. 8d), illustrated a slight activation of Kupffer cells.

![Histopathological changes in kidney of (a) Control and experimental rats after eight weeks, (b) Rats fed with 5% GSP, (c) Rats fed with 10% GSP and (d) Rats fed with 15% GSP](image)

**Fig. 6(a-d):** Histopathological changes in kidney of (a) Control and experimental rats after eight weeks, (b) Rats fed with 5% GSP, (c) Rats fed with 10% GSP and (d) Rats fed with 15% GSP

![Histopathological changes of liver of rat at zero time](image)

**Fig. 7:** Histopathological changes of liver of rat at zero time
Fig. 8(a-d): Histopathological changes in liver of (a) Control and experimental rats after four weeks, (b) Rats fed with 5% GSP, (c) Rats fed with 10% GSP and (d) Rats fed with 15% GSP

Fig. 9(a-d): Histopathological changes in liver of (a) Control and experimental rats after eight weeks, (b) Rats fed with 5% GSP, (c) rats fed with 10% GSP and (d) rats fed with 15% GSP

At the end of the experiment, liver of rat group fed diet of 5% "GSP" (Fig. 9b) showed no histopathological changes over that of the control group (Fig. 9a). Liver of rat group fed diet of 10%
“GSP” (Fig. 9c) revealed a cytoplasmic vacuolization of centrilobular hepatocytes. While liver of rat group fed diet of 15% “GSP” (Fig. 9d) showed dilation and congestion of hepatic sinusoids as well as vacillations of hepatocytes.

CONCLUSION
Guar seeds powder and fortified wheat flour with guar were investigated for their rheological properties. It was that the fortification of “GSP” into wheat flour at 5% was good in properties of dough stability, mixing tolerance, extensibility and baking strength compared to white flour. The biochemical and histological effects of guar seeds on albino rats were studied. The higher performance was obtained when rats were fed with 5% guar germ in the diet compared to the control. Treatments above 5% had negative effects on kidney and liver functions and pathology.

REFERENCES


