Fortification of White Bread with Guava Seed Protein Isolate

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Abstract: The aim of this study was to evaluate the effect of the fortification of guava seed protein isolate on nutritional properties in white bread. The guava seed protein isolate had functional properties similar to the wheat flour protein by lyophilized dry, to prepare 4 mixtures with wheat flour and guava seeds protein isolate: 100, 99.7, 99.6, 99.5 and 99.4% wheat flour with 0, 0.3, 0.4, 0.5 and 0.6% guava protein isolate. The tryptophan content in the guava seeds was 1.0%. The texture profile indicated that the hardness, elasticity, cohesiveness and adhesiveness of the dough increased relative to supplementation levels, although stability decreased relative to supplementation levels as well. The extensibility (based on the Kieffer and Dobrasckzky parameters) indicated that the R max and work of the dough increased relative to supplementation with the guava seed protein isolate. The supplemented bread (at 0.1 and 0.2% levels) had a greater volume compared to control; color was not affected at 0.1% levels. Appearance and flavor were not affected at levels up to 0.1, 0.2 and 0.3%, whereas color was affected. Supplementing bread with guava seed protein isolate results in an acceptable and gradual increase in protein level in bread.

Key words: Guava seed, fortification, white bread

INTRODUCTION

Guava (Psidium guajava L.) is a plant native of the American tropics and today is found in many tropical and subtropical regions across the world (Abd El-Aal, 1992; Fontanari, 2006; Fontanari et al., 2006). Guava fruit is consumed in Mexico primarily in juice and the seeds (12 g/100 g of fruit weight) are discarded; however, this vegetal material has a high protein content (9.73% dry matter basis) and the in vitro digestibility is higher than that of soybean isolate (94.8 vs. 89.9 g/100 g) (Bernardino et al., 2001). The essential amino acid profile of guava seeds is above that recommended by the FAO/WHO (1985) for adults, except for lysine (Adsule and Kadam, 1995; Bernardino et al., 2001). One of the most common problems in the food processing industry is the disposal of sub products produced, which results in ecological problems related to the proliferation of insects and rodents and an economical burden due to transportation to repositories. Therefore, strategies for the profitable use of these materials are needed. Several studies have found that guava waste has a high antioxidant potential since it is rich in compounds that can delay oxidation (Kanner et al., 2001; Melo et al., 2008; Norshazila et al., 2010). Shams El-Din and Yassen (1997) used guava seeds as an additional source of fiber in cookies. They found that using 9% guava seed meal produced an acceptable but comparatively inferior product; however, using an increased ratio of guava seed meal resulted in a reduction in water absorption, dough development, time and stability and increased dough weakening. The addition of guava seed meal to wheat flour improved volume, diameter and thickness of the cookies after baking. Abd El-Aal (1992) also studied the optimum conditions for preparing protein isolates from ground and found that defatted guava seed flour could be used as a value add to the product.

MATERIALS AND METHODS

Guava seeds protein isolate: Guava (Psidium guajava) was purchased at local fruit markets in Tulancingo Hidalgo, Mexico. Guava fruits were scalded with hot water (90°C) and seeds were collected in a Polinox fruit-pulping machine (Polinox SA, Mexico City). Seeds were washed and ground in an IKA A11 basic analytical mill (IKA Works, Wilmington). The guava seed protein isolate was obtained using the methodology previously reported by Bernardino et al. (2001). Ten gram of grounded guava
seeds were dispersed in 200 mL of water. The pH was adjusted to 11.5 with NaOH 0.1 N at 40°C for 30 min in order to solubilize proteins. Suspension was centrifuged at 2000 x g for 30 min; then the precipitate was collected, the supernatant pH was adjusted to 5.0 with HCl 0.1 N and centrifuged at 4000 x g for 30 min; both precipitants were mixed and lyophilized to obtain a fine dust.

**Experimental design:** Table 1 shows the different formulations employed to replace wheat flour with guava seed protein isolate. The results were subjected to an analysis of Variance (ANOVA) and regression analysis and treatment means were compared using Tukey’s HSD test at a significance level of p≤0.05. All analyses were performed using the SAS (2002) version.

**Dough and bread elaboration:** For dough elaboration, wheat (Triticum aestivum) flour (Molino Elizondo, Mexico City) was used. 9 different formulations and one control were used according to the mixtures listed in Table 1. The basic baking recipes included wheat flour, 0.4 g sugar 100/g flour, 0.8 g yeast 100/g flour, 0.8 g salt 100/g flour, 2 g pork lard 100/g flour, 4.8 g dry milk 100/g flour and 64 g water 100/g flour. Dry ingredients were mixed for 5 min with water before the fat was added; it was then mixed until total incorporation in a Kitchen Aid 600 Stand Mixer (KitchenAid, St. Joseph, MI). Dough was fermented at 30°C for 32 min.

**Wheat-guava seed flour blends:** Based on the amino acid profile results and the calculations of tryptophan in the 1.97 g amino acid/10 g content of the guava seed proteins, replacement percentages were determined for the enrichment of wheat flour. Guava seed flour was added at the following proportions: 0.7, 0.6, 0.5, 0.4 and 0.3%.

**Dough analysis:** Textural profile analysis 25 g of dough samples were compressed to 20% of their original height with a P25D 2.5 cm diameter acrylic probe in a TA-HDi texture analyzer (Texture Technologies/Stable Microsystems) equipped with a 5 kg load cell. From the force-deformation curves, the textural parameters were obtained as follows: hardness, defined and the force necessary to attain a given deformation; maximum force; cohesiveness, defined as the strength of the internal bonds making up the body of the product (Szczeniak, 1963; Bourne, 1978; Texture Technologies, 2003). Extensibility measurements at large deformations were performed for uniaxial (stretching) extension using a dough/gluten extensibility rig. Uniaxial extensibility was assessed using the Kieffer dough and gluten extensibility rig developed by Stable Micro Systems (Surrey, UK) for the TA-HDi texture analyzer. The resistance to extension (g) and extensibility (mm) were determined in tension mode by recording the peak force and the distance at the extension limit, calculating extensibility (distance to break) and determining maximum resistance (the maximum force) (Texture Technologies, 2003).

**Alveograph testing:** Alveograph measurements were obtained under conditions of constant dough water content and mixing times using the standard Method 54-30 (AACC, 2000). A computer software program automatically recorded the following alveograph parameters: the maximum over-pressure needed to blow the dough bubble (P index of resistance to extension), the average absissa at bubble rupture (L index of dough extensibility) and the deformation energy (W index of dough strength). Two curves were considered for each sample and the analysis were conducted in the Dobraszczyk-Roberts Dough and in the Inflation System model DR/DIS at the temperature of 18-22°C with a UR of 65±15% with 2.5% saline solution.

**White breads analysis:** Textural profile analysis 3 pieces 25 g bread were compressed to 20% of their original height using a P25D 2.5 cm diameter acrylic probe in a TA-XT2i texture analyzer (Texture Technologies/Stable Microsystems) equipped with a 5 kg load cell. The textural parameters were obtained as follows from the force-deformation curves: hardness (defined as the force necessary to attain a given deformation) and maximum force, cohesiveness (defined as the strength of the internal bonds making up the body of the product) and chewiness (defined as the energy required to masticate a solid food product to a state ready for swallowing) (Szczeniak, 1963; Bourne, 1978).

**Weight, volume and color**

**Weight:** The result is obtained from the initial weight of bread crumbs, given the amount that should be relative to 100; this test was performed in triplicate (Anderson et al., 1995).

**Volume:** This was calculated according to the method 10-05 (AACC, 2000) reported by Lainez (2006), in which loaf volume is determined by displacement of rapeseed in a cylindrical glass container 5 cm in diameter and 35 cm high (687, 225 cm³ volume) (Kilborn and Tipples, 1975).
Color: The CIE-Lab coordinate was determined using a Minolta 508D colorimeter reporting luminosity (L*), redness (a*) and yellowness (b*) (Guemes et al., 2012).

Image analysis of crumb quality: Total area, total number of cells, the number of cells smaller than 4 mm², total cell area, number of cells per centimeter square and the cell area/total area ratio were calculated (Paraskevopoulou et al., 2010).

Sensory evaluation: Sensory evaluation was carried out to determine the effect of guava seed on bread properties. An affective test assessing the degree of satisfaction was performed according to Anzaldúa (1994) who employed a hedonic scale of 7 points. 35 consumers were recruited and asked to evaluate the bread (the best two formulations were in agreement with rheological tests).

Bread chemical analysis: Chemical proximal analysis was performed on control bread and the bread with higher acceptance score. Moisture, ashes, fat, protein and crude fiber were determined according to AOAC (2002) methods 925.10, 923.03, 920.39 928.87 and 962.09, respectively.

RESULTS AND DISCUSSION
Dough texture: Table 2 lists the texture profile analyses of formulated dough. The results showed that the incorporation of guava seed protein isolate significantly reduced dough hardness (p<0.05); a greater amount of protein isolate (up to 0.6%) resulted in softer dough. The addition of this protein isolate also significantly (p<0.05) increased dough cohesiveness, as compared to control (no added protein). In a similar manner, dough adhesiveness in TPA increased significantly (p>0.05) relative to the increase in guava seed protein isolate. Similar effects were observed for dough cohesiveness, where the incorporation of guava seed protein isolate increased dough cohesiveness. This effect is in contrast to the findings of Armero and Collar (1998) who reported that dough hardness was less cohesive and it recovered its shape more quickly (higher resilience values); this is likely due to the amino acids in this protein, which affect the properties of the flour wheat dough. Guava seed protein isolate reduced dough hardness, resulting in a more adhesive and cohesive texture.

The resistance to deformation and the extensibility was significantly (p<0.05) reduced relative to the increase in guava seed protein isolate. The deformation energy increased concomitantly with these parameters, resulting in significantly (p<0.05) greater energy to inflate the dough (related to more cohesive TPA dough). The maximum resistance and extensibility resulted in a dough with a significantly (p<0.05) greater amount of guava seed proteins isolate (Table 2). Cauvain and

<table>
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<tr>
<th>Table 2. Texture profile analysis and rheology in dough with the addition of guava seed protein isolate to white bread formulation</th>
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</thead>
<tbody>
<tr>
<td>Dough hardness (N)</td>
</tr>
<tr>
<td><strong>Control</strong></td>
</tr>
<tr>
<td>T3 (0.70)</td>
</tr>
<tr>
<td>T4 (0.60)</td>
</tr>
<tr>
<td>T5 (0.50)</td>
</tr>
<tr>
<td>T6 (0.40)</td>
</tr>
<tr>
<td>T7 (0.30)</td>
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</table>

Means with same letter were not significantly different (p>0.05)
Young (2000, 2001) reported that the extensibility of dough is a property of significant interest to the baker. Resistance of the dough due to guava protein isolate requires further mixing and thus greater energy is required. Once the mixture is fully hydrated, a smooth developed dough is obtained. Continual mixing beyond this point results in the breakdown of gluten structure and considerable changes to the rheological characteristics of the dough.

Atheye Uchoa et al. (2009) showed that water absorption, arrival time and dough weakening increased and stability decreased the level of guava seed flour (with 100% semolina) was increased compared to control. Increased water absorption is mainly due to the strong water-binding ability of fibers. The greater length of dough development and lower dough stability could result from dilution of the gluten and the difficulty caused by mixing fibers and semolina homogeneously. The viscoelastic network plays a predominant role in dough macromolecular and affects the textural characteristics of the finished bread as well (Collar and Armero, 1996). This property of dough depends on both the quality and quantity of the proteins; guava seed protein contains both parameters.

Wieser et al. (2003) found that, in the elasticity of dough with guava protein isolate, the results of energy (W) produced by the physical movement of the flour-water mixture (which imparts energy) resulted in cross-linking flour proteins through the formation of S-S bonds at the terminal ends of the protein chains. A rheological study of 10 formulations using variance analysis differentiated 4 formulations; only 6 wheat flour and protein isolate mixes were similar to control and were developed for bread analysis based on their texture, color and crumb.

**Bread texture:** Guava seed protein isolate incorporation resulted in a softer bread texture; greater amounts of guava seed protein isolate significantly reduced (p<0.05) dough hardness. In contrast, bread cohesiveness and springiness were significantly reduced (p<0.05) when a greater amount of guava seed protein isolate was supplemented (Table 3). This effect could be due to the visco-elastic property of gluten. Elasticity alone is not a property that bakers require in bread and fermenting dough results in a degree of elastic recovery following molding and shaping operations. In order to compensate for this effect, bakers may increase the force applied to dough during shaping; however, this can lead to problems associated with damage to the relatively delicate gas-bubble structures present in the dough. Cauvain and Young (2000, 2001) reported examples of typical product-quality defects that may arise when excessive molding pressures are applied to dough.

Guava seed protein isolate could be incorporated into the bread-making stages of ingredient mixing, dough resting, dividing and shaping, proofing and baking, with great variations in the intermediate stages depending on the type of product used. According to Mac Ritchie et al. (1985), two factors contribute to dough strength: the proportion of proteins above a critical size and the size distribution of the proteins. The size, distribution, growth and failure of the gas bubbles released during proofing and baking have a major impact on the final quality of the bread in terms of both appearance (texture) and final volume (Cauvain, 2003).

**Bread weight and volume:** The weight of the supplemented bread was significantly (p<0.05) reduced compared to control (Table 4). These results are similar to those reported by Hussein et al. (2011). They reported that the volume (swelling%) of high fiber pasta (10-20%) was increased compared to control pasta (semolina 100%), while weight was reduced in all replacement levels of guava seeds fraction 1 compared with the control pasta sample. These results indicate that an increase in fiber level leads to a decrease in the weight of cooked pasta.

**Bread crumb quality:** The bread crumb image analysis results are listed in Table 4. Lower guava protein isolate incorporation significantly (p<0.05) increased total bread area. Control bread had significantly (p<0.05) lower air cells area per cm², indicating that the incorporation of guava seed protein isolate increased the number of air cells per cm². The relationship between area cells and total area ratio was significantly (p<0.05) lower for control bread, indicating that addition of guava seed protein increased the number and area of cells in the bread. In the same manner, control samples had a significantly (p<0.05) lower number of cells per cm². Guava protein isolate increased bread hardness, cohesiveness and gumminess, which resulted in reduced bread size (lower volume and weight) with a greater number of air cells.

**Bread color:** The addition of guava seed protein isolate resulted in significantly (p<0.05) darker colored bread. Both redness and yellowness parameters were significantly (p<0.05) lower compared to control samples (Table 5). Table 3 shows that there were significant differences in the color parameters in the
Table 4: Bread with guava seed protein isolate weight, volume and crumb quality

<table>
<thead>
<tr>
<th></th>
<th>Weight</th>
<th>Volume</th>
<th>Bread total area</th>
<th>Cells area (cm²)</th>
<th>Cells area/total area</th>
<th>No. of cell/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>53.5±0.50¹</td>
<td>4.0±0.08⁴</td>
<td>2139±725⁴</td>
<td>1642±58⁴</td>
<td>0.009±0.0001²</td>
<td>13.00±0.89⁶</td>
</tr>
<tr>
<td>T1 (0.70)</td>
<td>52.5±0.62²</td>
<td>3.65±0.04⁴</td>
<td>2188±704⁴</td>
<td>1653±53²</td>
<td>0.009±0.0001²</td>
<td>13.00±0.89⁶</td>
</tr>
<tr>
<td>T2 (0.60)</td>
<td>52.0±0.44¹</td>
<td>3.30±0.08⁴</td>
<td>2854±721³</td>
<td>2098±25²</td>
<td>0.007±0.0001²</td>
<td>13.50±1.40⁶</td>
</tr>
<tr>
<td>T3 (0.50)</td>
<td>51.8±0.48⁴</td>
<td>3.30±0.09⁴</td>
<td>2831±916³</td>
<td>2059±42²</td>
<td>0.008±0.0001²</td>
<td>16.50±0.86⁶</td>
</tr>
<tr>
<td>T4 (0.40)</td>
<td>52.0±0.53⁴</td>
<td>3.40±0.08⁴</td>
<td>2841±831³</td>
<td>1957±89³</td>
<td>0.007±0.0001²</td>
<td>18.50±0.65⁶</td>
</tr>
<tr>
<td>T5 (0.30)</td>
<td>51.2±0.45⁴</td>
<td>3.00±0.08⁴</td>
<td>3032±720³</td>
<td>2362±70⁷</td>
<td>0.007±0.0001²</td>
<td>19.50±1.07⁶</td>
</tr>
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</table>

*Means with same letter were not significantly (p>0.05) different

Table 5: Bread with guava seed protein isolate color

<table>
<thead>
<tr>
<th></th>
<th>Luminosity</th>
<th>Redness</th>
<th>Yellowness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>62.8±1.47¹</td>
<td>13.9±1.52²</td>
<td>35.8±2.89²</td>
</tr>
<tr>
<td>T1 (0.70)</td>
<td>61.9±1.81¹</td>
<td>10.8±0.27²</td>
<td>32.3±2.40²</td>
</tr>
<tr>
<td>T2 (0.60)</td>
<td>63.0±0.94⁴</td>
<td>10.28±1.54³</td>
<td>33.8±2.11¹</td>
</tr>
<tr>
<td>T3 (0.50)</td>
<td>64.3±1.14⁴</td>
<td>11.79±0.27³</td>
<td>32.9±1.80⁴</td>
</tr>
<tr>
<td>T4 (0.40)</td>
<td>65.8±0.93⁴</td>
<td>10.36±1.40³</td>
<td>33.7±2.47¹</td>
</tr>
<tr>
<td>T5 (0.30)</td>
<td>67.4±1.48⁴</td>
<td>9.17±1.25³</td>
<td>27.0±2.35³</td>
</tr>
</tbody>
</table>

*Means with same letter were not significantly (p>0.05) different

The bread supplemented with guava seed protein isolate. Increasing the levels from 0.3, 0.4, 0.5 and 0.6% led to a reduction in lightness (L*) to 67.41, 72.47 and 61.92, respectively; however, the redness and yellowness parameters increased. Table 3 showed that the supplemented bread had a significant increase in lightness (L*) and a reduction in redness (a*); the control bread (wheat flour 100%) was 62.56 (L*) and 35.87 (b*), while the bread with guava seed protein isolate was 9.17 (a*) and 27.02 (b*).

Bread chemical proximal analysis: Table 6 shows the results of chemical proximal analysis. Protein content did not increase when 0.3% protein isolate was added. This result differs from the findings of Shams El-Din and Yassen (1997) who showed that pasta containing guava seed flour had an acceptable, gradual increase in moisture, protein, fat, ash and crude fiber and an increase in carbohydrates. There were significant differences (p<0.05) in the protein level of the supplemented guava fruit cookies due to the level of protein powder added. This discrepancy in results could be related to the use of guava fruit powder, which contains seed storage protein, which could have increased the protein content. Bernardino et al. (2001) reported that guava seed could also be used as an alternative source of protein for human and animal consumption, although the addition of fruit powders produced slightly dark products. Larrea et al. (2005) reported an increase in the texture of cookies proportional to the amount of added orange pulp (Uchoa et al., 2009).

Sensory evaluation of bread: Consumers perceived the control bread as tasty and sweet and gave it high scores. Martinez-Anaya (1996) showed that free amino acids in wheat flour and dough play an important role in the generation of bread flavor precursors through the formation of Maillard compounds during baking. When leucine, proline, isoleucine and serine react with sugars, they create the flavors and aromas described as "toasty" and "bread-like," whereas excessive amounts of leucine produce bread with an unappetizing flavor. Consumers rated the bread fortified with 0.3% isolate protein isolate to be the most acceptable; it had significantly better quality compared to the control bread.

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